Reduced folate carrier 1 (*RFC1*) gene polymorphisms among acute lymphoblastic leukemia patients

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Abstract

Reduced Folate Carrier1 (*RFC1*) gene's metabolism is crucial for DNA synthesis, epigenetic mechanisms, and cellular methylation events. Nonetheless, *RFC1* polymorphisms have drawn a lot of interest in current medical genetics studies. The objectives of the study were to ascertain the relationship between the risk of Acute Lymphoblastic Leukemia (ALL) in Sudanese patients and the genetic variant of *RFC1* (G 80A). Using Real-Time Polymerase Chain Reaction (RT-PCR) method, 150 ALL patients and 150 healthy control volunteers had their DNA collected and examined for the case-control study. Version 25 of the Statistical Package for Social Science (SPSS) software was used to analyze the data. There was no discernible difference in the age or gender of the patients compared to the controls. According to flow cytometry data, T-ALL accounts for 31 (21%) and B cell type accounts for 119 (79%). According to this study, there is a statistically significant correlation between the mean blast (p=0.050) and the mean total white blood cell count (p=0.000) in T-ALL cases. The genotype frequency of *RFC1* (G80A) was found to be GG 39 (26.0%), GA 41 (27.3%), and 70 (46.7%) for AA in the cases, while GG 41 (27.3%), GA 28 (18.7%), and AA 81 (54.0%) in the control group. While the homozygous genotypes AA and GG were more common in the control group, the heterozygous genotype GA was more common in the patient group. On the other hand, the genotype distribution among the research groups was not statistically significant with odds ratio: 0.745 (0.473-1.174), p=0.192. In conclusion**,** *RFC1* (G80A) polymorphism was not found to be associated with an increased risk of ALL, according to the findings of this investigation.

Introduction

Folate metabolism plays a vital role in DNA synthesis and methylation. The presence of a folate deficiency or an aberrant folate metabolism has been associated with several types of cancer, such as Acute Lymphoblastic Leukemia (ALL).[1–5] Insufficient dietary intake, alterations in cellular transportation, and genetic differences associated with folate can all lead to disruptions in folate metabolism.[6] The Reduced Folate Carrier 1 (*RFC1*), also known as SLC19A1, is widely expressed and is considered the primary transport route for folates in mammalian cells and tissues.[7] The *RFC1* gene, situated on chromosome 21, codes for a folate transport protein that is recognized as a key element of the folate transport system.[8,9] *RFC1* enables the transportation of 5-methyltetrahydrofolate from the bloodstream to cells located in the periphery.[10] The G80A polymorphism is characterized by the replacement of adenine with guanine at the 80th position. The protein structure at codon 26 in exon one exhibits a frequent polymorphism where histidine substitutes arginine. Genetic changes in the *RFC1* gene (rs1051266) can potentially modify the course of folate metabolism, leading to the onset and advancement of cancer.[11] There have been conflicting findings in several research addressing the correlation between this genetic variation and the likelihood of acquiring leukemia. Multiple studies have consistently demonstrated that the *RFC1* polymorphism leads to a decrease in the effectiveness of cellular uptake of folate and methyltetrahydrofuran, resulting in an increased risk of ALL,[6] Although there were just a few findings, they remained disputed. In order to provide additional clarification on the relationship between the *RFC1* (G80A) polymorphism and the risk of leukemia, we conducted a research investigation on this polymorphism in a suitable population from Sudan.

This study aimed to clarify the association between the *RFC1* polymorphism and the risk of ALL in the Sudanese population by identifying the polymorphism using Real-Time Polymerase Chain Reaction (RT-PCR) and assessing the prevalence of T-ALL and B-ALL among the case and control groups.

Materials and methods

Patients Data

This study was a case-control study conducted on patients with ALL. A total of three hundred blood samples were collected at the Flow Cytometer Center for Diagnosis of Leukemia and Lymphoma, Central Laboratory-Ministry of Higher Education, and National University Biomedical Research Institute (NUBRI) in Khartoum, Sudan. The study was conducted from May 2017 to April 2021. A total of 150 patients diagnosed with ALL were examined using flow cytometry. Out of the study groups, 100 individuals (66.7%) were men, while 50 individuals (33.3%) were women. Both groups had participants ranging in age from 2 to 78 years, with a mean age of 20.7 years and a standard deviation of 18.3 years. Out of the total participants, 86 individuals, accounting for 57.3% of the sample, were youngsters aged 16 years or less. The remaining 64 participants, making up 42.6% of the sample, were adults. Additionally, a control group consisting of 150 seemingly healthy volunteers was included, with matching gender and age distribution.

Hematological analysis

Hematological examinations were conducted with an automated blood counter known as the Sysmex KX 21 (Holliston, MA, USA). ALL was diagnosed by analyzing the White Blood Cell (WBC) count and examining the blood morphology. All cases were additionally validated by employing antigen-specific antibodies to examine the different subsets of immune cells using a flow cytometer (Coulter EPICS X-Mcl™, Miami, Florida, USA).

Analysis of *RFC1* polymorphism

The Spin-Column Extraction method has been used to isolate genomic DNA from peripheral leucocytes. The concentration and purity of DNA were evaluated using a NanoDrop™ Lite-UV Spectrophotometer (ThermoFisher Scientific, Waltham, MA, USA). The *RFC1* polymorphism was examined using the TaqMan Real-Time Polymerase Chain Reaction (qPCR) (qTOWER3G, Analytik, Jena, Germany). The primer and probe sequences utilized are listed in Table 1. PCR was run 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.

Statistical analysis

The data was analyzed to compare the genotype distributions between the ALL and the control groups according to the age and gender. This comparison was tested for significance using means, Standard Deviations (SD), Odds Ratios (ORs) and 95% Confidence Intervals (CIs), which were calculated through univariate logistic regression analysis using the Statistical Package for Social Science SPSS 25.0 software (SPSS Inc., Chicago, IL, USA). A p-value below 0.05 indicates statistical significance.

Results

Demographic data

There were no notable differences in terms of age, gender, and other characteristics between the study groups (ALL patients, and controls). Patients diagnosed with ALL had a greater average count of WBCs and lower average concentrations of hemoglobin, as well as lower counts of Red Blood Cells (RBCs) and Platelets (PLTs), in comparison to the control group. The average blast percentage across all patients was 62.5% (Table 2).

Immunophenotyping

Flow cytometer immunophenotyping validated the diagnosis of ALL and indicated that B-cell ALL (B-ALL) was more common, accounting for 119 cases (79%), compared to T-cell ALL (T-ALL), which accounted for 31 cases (21%). The B-ALL subtype was more prevalent in both the children and adult's groups compared to T-ALL. Additionally, it was more common in male than in female patients. However, no statistically significant correlation was seen between the ALL subtype and either age group or gender, as shown in Table 3.

When comparing the hematological characteristics among different subtypes of ALL, it was shown that patients with T-ALL had

Table 1. *RFC1* primer and probe sequences.

Primers	F- GGC CTG ACC CCG AGC T R- AGC CGT AGA AGC AAA GGT AGC A
Probes	G- CAC GAG GCG CCG C; A- CGA GGT GCC GCC AG

a substantially higher mean total WBCs count (p=0.000) and blast percentage (p=0.050) compared to those with B-ALL. The analysis of Table 4 did not reveal any statistically significant correlation between the mean counts of RBCs, hemoglobin, and PLTs.

Genotyping of *RFC1* (G80A) polymorphism

The *RFC1* homozygous AA genotype was prevalent in both study groups. The comparison of genotypes distribution among the study groups showed that the heterozygous genotype GA was more frequent in the patients' group than in the control group, while the homozygous genotypes AA and GG were more frequent in the control group. However, the distribution of genotypes among the study groups was not statistically significant. The regression analysis showed no statistically significant association between *RFC1* polymorphism and risk of ALL (Table 5).

The comparison of *RFC1* genotypes between youngsters aged 16 years or less (86 samples) and oldest patients (64 samples) revealed a higher frequency of all genotypes (homozygous AA and GG, and heterozygous GA) in children. However, the distribution of genotypes among the study groups did not show any statistically significant differences, as indicated in Table 6.

Table 2. Comparison of the hematological parameters among the studied population.

Parameter	Patients Mean± SD	Control Mean±SD
WBCs count (×10^9/L)	67.9±91.6	6.9±1.6
RBCs count (×10^{12}/L)	2.7±0.8	4.7±0.3
PLT count (×10^9/L)	46.6±45.8	275.4±69.6
Hemoglobin (g/dL)	8.7±5.6	13.6±0.9
Blast (%)	62.5±18.8	-

SD, Standard deviation.

Table 3. Association of acute lymphoblastic leukemia (ALL) types with patient's demographic data.

Variable		B-ALL (%)	T-ALL (%)	*p* value
Age group	Children	66 (44.0)	20 (13.3)	0.364
	Adults	53 (35.3)	11(7.3)	
Gender	Men	79 (52.7)	21 (14.0)	0.887
	Women	40 (26.7)	10 (6.7)	

Table 4. Comparison of the hematological parameters according to acute lymphoblastic leukemia (ALL) subtype.

Parameter	B-ALL Mean±SD	T-ALL Mean±SD	*p* value
Total WBCs count (×10^9/L)	43.3±52.1	162.2±139.4	0.000
RBCs count (×10^{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 (×10^9/L)	45.4±4.3	51.3±39.9	0.525
Blast (%)	60.9±18.3	68.3±19.6	0.050

SD, standard deviation.

Table 5. Frequency of *RFC1* polymorphic genotypes among the study groups.

Genotype	Patients (%)	Control (%)	*p* value	Odds Ratio (95% Confidence Interval)
GG	39 (26.0)	41 (27.3)		
GA	41 (27.3)	28 (18.7)	0.192	0.745 (0.473-1.174)
AA	70 (46.7)	81 (54.0)		

Table 6. Comparison of the RFC1 (G80A) genotype among children and adult populations.

Genotype	Children (%)	Adult (%)	*p* value	Odds Ratio (95% Confidence Interval)
GG	22 (25.6)	17 (26.6)		
GA	21 (24.4)	20 (31.2)	0.571	1.651 (0.860-3.173)
AA	43 (50.0)	27 (42.2)		

Discussion

Replication Factor C subunit 1 (RFC1) is a common transmembrane protein that transports 5-methyltetrahydrofolate from the bloodstream into cells. The malfunction of RFC1 has been demonstrated to be associated with various diseases, including neural tube abnormalities, congenital heart problems, Alzheimer's Disease, and homocysteinemia.[12–16] In addition, RFC1 facilitates the transfer of Methotrexate from the extracellular fluid to the intracellular fluid, hence contributing to its efficacy in treating rheumatoid arthritis and ALL. Chango and colleagues[15] found that persons with the AA genotype had elevated plasma folate levels compared to those with the GG genotype. Recent studies have underscored the importance of the *RFC1* G80A polymorphism in cancer development.[17–19]

This study aimed to investigate the genetic variants of the folate-related gene *RFC1* (G80A) as potential risk factors for the development of ALL, given their involvement in the metabolism of several environmental chemicals. The study included a total of 300 individuals of similar age and gender, 150 of them being patients diagnosed with ALL and serving as the case group, while the other 150 were healthy volunteers, serving as the control group. The majority of the ALL patients were men, with a men-to-women ratio of approximately 2:1. This data is consistent with the results recorded by Jawaid *et al.* in Pakistan, which indicated a higher prevalence of men compared to women.[20] In addition, the research conducted by Sultan *et al*. and Shahab and Raziq in Pakistan revealed that the majority of the studied populations were men, with a ratio men:women of 2:1.[21,22] Our findings are also in agreement with the results observed in Saudi Arabia and in USA.[23,24] The findings of the current study revealed that the majority of the patients (57.3%) were below the age of 16, which aligns with a report provided by the American Cancer Society,[25] Two-thirds of the patients observed were between the ages of 6 and 16, according to a study conducted in Sudan by Ebrahim *et al.*[26] The examination of the Complete Blood Count (CBC) in ALL patients showed leukocytosis, anemia, thrombocytopenia, and a high blast percentage. These results align with the findings of other studies conducted in Sudan, Mexico, Iran, Egypt, and Pakistan.[23–31]

In this study, the prevalence of B-ALL was higher than that of T-ALL. This finding is consistent with many studies conducted in Jordan, Nepal, Italy, Egypt, and Mexico;[32–36] all reports indicated a greater occurrence of B-ALL compared to T-ALL. However, our discovery contradicts a study conducted in Iran by Pahloosye *et al.*[37] Furthermore, in a study conducted by Mushtaq *et al.* in Pakistan[38] a higher occurrence of T-ALL than B-ALL was discovered. This variance may validate the diverse nature of the disease and seems to be influenced by a combination of environmental and biological factors, thus necessitating additional research to investigate potential contributing elements.

The current study found that patients with T-ALL had a significantly higher mean total WBC count (p value = 0.000) and mean blast percentage (p value = 0.050) compared to patients with B-ALL. However, there was no statistically significant difference in the mean values of other parameters such as RBCs count, PLTs count, and Hb concentration (p values = 0.843, 0.525 and 0.942, respectively). These findings are consistent with a study conducted in China by Dai *et al.*,[39] which also reported higher total WBC count and blast count in patients with T-ALL compared to those with B-ALL. Furthermore, our research aligns with two separate investigations conducted in Brazil by De Sousa *et al.*,[40] and in Iraq by Jaafar and Kadhom.[41] Both studies concluded that the total WBC count is significantly higher in T-ALL compared to B-ALL.

In relation to *RFC1* (G80A) polymorphism, the occurrence of the heterozygous GA genotype was greater (27.3%) in ALL patients compared to the control group (18.7%), whereas the homozygous AA and GG genotypes were lower in the patients (46.7% and 6.0%) than in the control group (54.0% and 27.3%, respectively). Nevertheless, the relationship between the polymorphism and the likelihood of developing ALL was not statistically significant when it is compared between children samples with adult samples (OR: 0.745, 95% CI: 0.473-1.174, p value = 0.192). Several research have investigated the associations between the *RFC1* (G80A) polymorphism and the risk of various diseases, including ALL. This finding is consistent with a limited number of studies that have found no significant connection between the *RFC1* genotypes of these polymorphisms and the likelihood of developing ALL. Yang *et al.* discovered that the *RFC1* (A80G) polymorphism did not have any impact on the risk of pediatric ALL in Han Chinese individuals. The OR was 1.29, with a 95% CI ranging from 0.81 to 2.07. The p value was 0.287.[42] However, other published research has refuted the present discovery and proposed that the *RFC1* polymorphism leads to a diminished effectiveness in the cellular absorption of folate and 2-Methyltetrahydrofuran, resulting in an elevated likelihood of developing ALL.[42–44] Yang *et al.* demonstrated that the *RFC1* 80AA mutation significantly increased the vulnerability to adult ALL by approximately two times (OR: 2.09, 95% CI 1.19–3.67; p value = 0.01).[42] De Jonge *et al.*, conducted a study in Western Europe,[44] which revealed that the *RFC1* variant had the most significant impact on the chance of developing leukemia. Specifically, those with the *RFC1* 80AA variant had a 2.1 times higher risk (95% CI, 1.3-3.2; p value = 0.002), while A-allelic carriers had a 1.5 times higher risk (95% CI, 1.1-2.1; p value = 0.02). In a study conducted by Vijayakrishnan in 2010 in the United Kingdom, a strong correlation was discovered between *RFC1* and a higher vulnerability to ALL. The OR was found to be 1.37, with a 95% CI of 1.10-1.72. The p value was determined to be 0.005.[45] A meta-analysis was conducted to reassess the relationship between the *RFC1* (G80A) polymorphism and the risk of cancer. The results of this analysis indicate that this particular polymorphism significantly increases the risk of ALL. Specifically, individuals with the GA genotype have a 1.13 times higher risk compared to those with the GG genotype (OR = 1.13, 95%; CI = 1.001-1.28, p value = 0.048). Furthermore, individuals with the AA or GA genotypes combined have a 1.28 times higher risk compared to those with the GG genotype (OR = 1.28, 95% CI = 1.13-1.46, p value < 0.001).[11]

The study found no statistically significant difference in mean RBCs count, hemoglobin concentration, total WBC count, platelet count, and blast percentage between ALL patients with wild and mutant types of *RFC1* (G80A). The p values for these comparisons were 0.355, 0.671, 0.255, 0.923, and 0.079, respectively. Additionally, there was no published data on the correlation between *RFC1* polymorphism and complete blood count.

Conclusions

As far as we know, there is no published evidence available on the link between *RFC1* polymorphism and complete blood count in Sudan. Our research findings indicate that ALL is more prevalent in children compared to adults. Additionally, according to the gender it seems that men are more susceptible to this condition than women, with a ratio of 2:1. Furthermore, the B-cell type of ALL is more prevalent than the T-cell type. Lastly, our study suggests that the *RFC1* polymorphism does not contribute to the risk of developing ALL. Further research with a substantial number of participants is necessary to examine the influence of these genetic variations on the Sudanese population. This would provide a more precise understanding of these findings.

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