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Methylenetetrahydrofolate Reductase (MTHFR C677T) Gene Polymorphism in Sudanese Patients with Acute Lymphocytic Leukemia

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Abstract

Background: Acute lymphocytic leukemia is one of the major types of leukemia that are found in Sudan. This study aimed to examine the associations of MTHFR c677T polymorphism with the risk of acute lymphocytic leukemia among patients in Sudan.

Methods: A total of 50 patients with myocardial infarction and 50 healthy controls who were matched by age and gender were included in the study. MTHFR C677T polymorphism was studied in both cases and healthy controls, 2 ml of blood samples were collected from both patients and controls into EDTA anticoagulated tubes, the extraction of DNA was done using the salting out approach, The primers, were used to amplify the MTHFR C677T fragment, the amplified fragment was detected on an agarose gel electrophoresis. And count the blood cells.

Results: low frequency of the mutants MTHFR C677T genotype with CT genotype being present in 6.7% of the patients and CC genotype representing 93.3% of the population. Among healthy controls, the frequency of CT genotype was 8% while that of CC genotype was 90%. There was no significant association between MTHFR gene polymorphism and the risk of ALL.

Conclusion: Our data suggest a low impact of MTHFRC677T gene polymorphism on the of developing ALL.

Key words: ALL, Acute Lymphoblastic Leukemia, Sudan, MTFHR gene.

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Introduction: Acute lymphoblastic leukemia (ALL) is heterogeneous a group of hematological malignancies that arise from clonal proliferation of immature lymphoid cells in the bone marrow, peripheral blood and other organs [1]. ALL is the most common pediatric malignancy as it represents about 25% of childhood Cancers and approximately 75% of all pediatric leukemias [2]. There has been a gradual Increase in the incidence of ALL in the past 25 years with the peak incidence of ALL being at 2 years of age [3][4]. Many genetic polymorphisms such as TEL-AML1 genes fusion [5], E2A-PBX (PBX1) genes fusion [6], BCR-ABL genes fusion [7], MLL-AF4 genes fusion [8], and IGH-MYC genes fusion [9] have been reported to be associated with ALL. MTHFR gene polymorphism was considered as a risk factor for ALL that is also associated with the disease outcome [10][11].

The MTHFR gene is located on the short (p) arm of chromosome 1 at position 36.3;
it spans approximately 2.2Kb and consists of 11 exons [12]. The MTHFR gene encodes for MTHFR enzyme which plays a central role in folate metabolism by irreversibly converting 5,10-methylenetetrahydrofolate to 5-methylenetetra-

hydrofolate, the predominant circulating form of folate. 5- Methylenetetrahydrofolate donates a methyl group to homocysteine in order to be converted further to methionine [13]. Two common single nucleotide polymorphisms in MTHFR have been reported, a C→T transition at nucleotide 677 in exon 4 and an A→C transition in exon 7 at position 1298. In C677T, the polymorphism occurs at nucleotide 677 (C- T) in the human MTHFR gene and subsequently results in an alanine to valine substitution at position 222 in the amino acid structure of the MTHFR protein. Individuals with two copies of 677C (677CC) have the "normal" or "wild-type" genotype while 677TT individuals (homozygous) are reported to have mild MTHFR deficiency. 677CT individuals (heterozygotes) are almost like normal individuals with normal enzymatic activity [14].

Studying the effect of different gene polymorphisms on developing ALL is important as it may provide data regarding the exact risk of developing the disease, disease outcomes, and possible treatment options. There is no enough and conclusive data regarding the role of MTHFR gene C677T polymorphism on the risk of developing AL and this study aim to provide evidence-based data regarding this issue in Sudan.

Materials and methods: This was a prospective case-control study that was conducted on 60 ALA patients and 50 healthy controls. ALL patients were recruited from Radiation and Isotopes Center-Khartoum (RICK) covering all patients who presented to the hospital with ALL during the period from 1st of January to 31st of September, 2015. Control samples were taken from the blood bank and matching by age and gender was applied. 5ml of venous blood samples were collected from patients in EDTA tubes and complete blood count test was performed using an automated cell counter (Sysmex-kx21) at Radiation and Isotopes Center-Khartoum (RICK). DNA analysis was performed at the department of hematology, faculty of medical laboratory sciences, Alneelain University. The DNA was extracted by salting out method. MTHFR C677T fragment was amplified using the forward primer:5`-TGAAGGAGAAGGTGTCTGCGGGA -3` and the reverse primer: 5`-AGGACGGTGCGG TGAGAGTG- 3`. The amplification was carried out in thermo-cycler (Techne TC-412, UK). An initial 5-minutes denaturation step that at 94°C was performed followed by 40 Cycles of 3 steps: 30-seconds denaturation step at 94°, 1-minute annealing step at 59°C, and 1-minute extension step at 72°C. Then a final 7-minutes extension step at 72 °C was done. The PCR reactions was performed in a final 20 µl solution containing 4 µl of premixed, ready to use, 5x FIRE Polmaster mix (Solis BioDyne, Russian), 11 µl of DNAase free DW, 3µl of genomic DNA and 1 µl of each primer). The amplified fragment was then digested with a 10 U Hinf1endonuclease (New England Biolab, UK) over night and then was visualized using agarose gel electrophoresis. Thin blood film was prepared and stained by lishman stain. Then it was examined under microscope cell morphology was determined.

Statistical analysis was performed using statistical package for social science (SPSS) software. Evaluation of patient´s data was performed using the t-test. Comparison of frequency distribution

between groups was made by means of the *X*2 test. All tests are two-sided and P-value less than 0.05 have been considered as statistically significant. Crude odds ratios (OR) were also calculated and given with 95% confidence intervals (CI).

Result: A total of 60 patients and 50 controls were included in the study. The median age of ALL patients was 5 years with males representing 50% (n = 30) and female representing the other 50% (n = 30). Similarly, 50% (n = 25) of the controls were male and 50% (n = 25) were females with a median age of 6 years.

Complete Blood Cells count was calculated for all ALL patients and control subjects and MTHFR C677T genotype status was determined. The mean hemoglobin (Hb) level for ALL patients was 8.42 ±1.99 g/dl Vs 12.45 ±1.26 g/dl, the meanTWBCs count was 62.8± 40.7×10^9/L Vs 7.95 ±1.94×10^9/L, and the mean platelets count was 44.3 ±34.4×109/L Vs 318.65 ±89.0×109/L, respectively (Table 1).

Table 1: Comparison of hematological characteristics between ALL patients and control subjects.

Parameters	Cases	Control	P value
Hb mean ±SD (g/L)	8.426 ±1.998g/L	12.454 ±1.26g/L	000
TWBCs mean±SD (*109/L)	62.8150 ± 40.72*109/L	7.952 ±1.945*109/L	
Platelets mean ±SD (*109/L)	44.333 ±34.426*109/L	318.65 ±89.01*109/L	
Plasts	12.7833 ±18.657		

Regarding MTHFR 677 polymorphism, 93.3% (n = 56) of ALL patients had MTHFR 677CC genotype while 6.7% (n = 4) had MTHFR 677CT genotype. On the other side, 92% (n = 46) of control subjects had MTHFR 677CC genotype while 8% (n = 4) had MTHFR 677CT genotype. However there was nog significant difference in genotype distribution between the two groups (OR = 0.8214, 95%CI = 0.194-3.4662, P =0.088) (Table 2).

Table 2: Comparison of MTHFR C677T Polymorphism between ALL patients and control subjects.

Genotype	ALL patients	Controls	OR	95% CI	*P* value
CC	56	46	0.8214	0.194 -3.4662	0.088
CT	4	4			

Discussion: This was a case-control study that aimed to evaluate the association between MTHFR C677T polymorphism and acute lymphocytic leukemia among a Sudanese population. In this study we found that the frequency of MTHFR C677T genotype was slightly higher among ALL patients (93.3% Vs 92%) with a 0.821 fold increase in the risk of developing ALL. However, this difference in frequency was not statistically significant (P = 0.088). Similarly, a marginal protection (OR = 0.90) from ALL has been reported in the presence of MTHFR C677T

polymorphism in a previous study (15). In a previous meta analysis of 51 case-control studies, no significant association was found between MTHFR C677T polymorphism and the risk of ALL (16), however, in another meta analysis of 37 case-control studies, a significant association between the two was reported among Caucasians (17).

This study was limited by the small number of sample size and inclusion of patients from only one centre, however, the lack of financial abilities and human resources was the reason for that.

Conclusion: In conclusion, we investigated whether the MTHFR C677T polymorphism and the risk of ALL were related. Our findings showed that the study group had a low prevalence of MTHFR C677T mutant genotypes with little effect on the risk of ALL. More studies are needed to formulate evidence-bases, conclusive data with this regard.

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