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Genetic Polymorphisms of MTRRA66G Genes and Susceptibility to Acute Lymphoblastic Leukemia in the Yemeni Population

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Background: Folic acid metabolism has a major role in DNA methylation and synthesis. Genetic Polymorphisms in folate may disrupt enzyme activities and maybe affect the malignant risk. Methionine synthase reductase <MTRR> are very important enzyme for the folate cycle.
Objectives: To study the possible relation between polymorphisms of MTRRA66G genes and susceptibility to acute lymphoblastic leukemia (ALL) in Yemeni patients.
Methods: A total of 115 patients with ALL attended oncology centers in Yemen and 140 unrelated healthy individuals as a control group was involved in a case-control study. DNA was extracted from collected EDTA venous blood samples and analyzed by polymerase chain reaction-restriction fragment length polymorphism assay [PCR-RFLP].
Results: The frequency of MTRR 66A-G heterozygous (AG), homozygous (GG) and wild type (AA) in cases was 52.2% (60), 22.6% (26), and 25.2% (29), respectively. Whereas, the frequency of MTRR 66A-G heterozygous (AG), homozygous (GG) and wild type (AA) in controls was 46.4% (65), 27.1% (38), and 26.4% (37), respectively. The difference in frequencies were statistically insignificant (P=0.471, OR = 1.065, 95% confidence interval (CI) 0.606–1.873).

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Conclusion: Our finding for MTRR A66G polymorphism does not associate with the development of acute lymphoblastic leukemia in Yemeni patients.

Keywords: Acute lymphoblastic leukemia; methionine synthase reductase; polymorphism; Yemen.

1. INTRODUCTION

"Folic acid metabolism has a major role in DNA methylation and synthesis. Genetic Polymorphisms in folate may disrupt enzyme activities and maybe affect the malignant risk. methionine synthase reductase <MTRR> are very important enzyme for the folate cycle" [1].

"The previous study shows the MTRR gene was associated with a significantly reduced risk of acute lymphoblastic leukemia (p = 1.21, OR = 0.55), which resulted mainly from the reduced risk of B-cell and hyperdiploid- acute lymphoblastic leukemia" [2]. "MTRR polymorphisms (66A>G, 524C>T, 1049G>T, and 2756A>G) could theoretically reduce the risk to develop childhood acute lymphoblastic leukemia by inhibiting MTR, resulting in DNA hypomethylation and reducing the risk of the silencing of genes, like tumor suppressor genes, such as TP53 and others" [3-4]. "For both MTRR 66AG and MTRR 66GG, a Korean study carried out by Kim et al. [5] found no significant difference in susceptibility to acute lymphoblastic leukemia". Gemmati et al. [6] evaluated "whether four common polymorphisms in methylene tetrahydrofolate reductase (MTHFRC677T and A1298C), methionine synthase (MSA2756G), and methionine synthase reductase (MTRRA66G) genes may have a role in altering susceptibility to adult acute lymphoblastic leukemia and non-Hodgkin's lymphoma (NHL). Individuals carrying the MTHFR677TT and MTRR66AG genotypes revealed a 4.2-fold acute lymphoblastic leukemia risk reduction (OR 0.24, 95% CI 0.06 – 0.81), whereas, those with the MS 2756AG/GG and MTRR66AG/GG genotypes revealed a2.2-fold acute lymphoblastic leukemia risk reduction (OR 0.45, 95% CI 0.10 – 0.85)".

Petra et al. [7] analyzed "common genetic polymorphisms of 5,10-methylenetetrahydrofolate reductase (MTHFR), thymidylate synthase (TS), methionine synthase (MS), and methionine synthase reductase (MTRR) in 68 children with acute lymphoblastic leukemia and 258 healthy controls to investigate their influence on the risk for acute lymphoblastic leukemia. They found no significant differences in frequencies of separate polymorphisms observed between both groups. Combined MTHFR 677CT/TT and MS 2756AG/GG genotypes showed a nonsignificant tendency to reduce the risk for acute lymphoblastic leukemia 2.24-fold (CI: 0.191 – 1.037, *P*: 0.061). The risk was significantly reduced in carriers of combined MTHFR 677CT/TT, MS 2756AG/GG, and MTRR 66AG/GG genotypes (OR: 0.312; CI: 0.107 – 0.907; *P*: 0.032). They suggested that gene-gene interactions may decrease the methylation capacity which may have a protective effect on the risk for childhood acute lymphoblastic leukemia".

Gra et al. [8] studied "genetic polymorphism of *GST, NAT2*, and *MTRR* and susceptibility to childhood acute leukemia in Russia. The frequency of *MTRR* genotype 66G/G was reduced in girls with acute leukemia as compared to healthy female donors (OR = 0.50, *P* =0.0015). The *GSTT1*and/or *GSTM1* null genotypes combined with *MTRR* genotype66A/G were considered to be a risk factor for acute leukemia in girls. Thus, the polymorphisms of *GSTT1, GSTM1, NAT2*, and *MTRR* proved to influence the risk of childhood acute leukemia in residents of European".

In the study performed by Koppen et al. [9] on "the folate-related gene polymorphisms (MTRR, MTR [MS] and susceptibility to develop childhood acute lymphoblastic leukemia, there is less clearly an association between these gene polymorphisms with susceptibility to acute lymphoblastic leukemia". "Also, folate-related gene polymorphisms associated with the risk of childhood leukemia were studied by Wagiman et al. [10] in the Malaysian population. They found MTRR 66 GG variant was associated with reduced risk of developing childhood leukaemia (OR = 0.05; 95 % CI, 0.01-0.29; p < 0.001)".

Amigou et al. [1] studied "the effect of folic acid supplementation, MTHFR, and MTRR polymorphisms, and the risk of childhood leukemia. They revealed that MTHFR and MTRR genetic polymorphisms were not associated with acute lymphoblastic leukemia".

Fang et al. [11] investigated "the association between methionine synthase reductase A66G polymorphism and leukemia risk in their meta-

analysis study. No significant associations were found for all comparisons in the overall pooled analysis. The results of stratified analyses revealed that *the MTRR* A66G GG genotype was associated with decreased leukemia risk in the Caucasian population, in children, and for acute lymphoblastic leukemia. In contrast, the increased risk was observed in the Asian population and for acute myeloid leukemia (AML). They suggested that *MTRR* A66G GG is associated with decreased risk of leukemia in a Caucasian population and children, especially for acute lymphoblastic leukemia".

Wang et al. [12] performed "a meta-analysis up to October 2016 to investigate the association of MTRRA66G polymorphism and cancer susceptibility and suggested that, the MTRR A66G polymorphism is associated with significantly increased cancer risk".

2. MATERIALS AND METHODS

2.1 Study Population

This study was conducted on 115 Patients attending the oncology centers who were diagnosed with ALL in the period from 2015-2018 were invited to take part in the study and sign an informed consent and 140 healthy controls matched to the cases in gender and age.

The study includes all Yemeni patients who have confirmed diagnosis of ALL, at any age, of both sexes, and from different areas, who attended oncology centers during the study period. The control group was of healthy individuals who matched to patients in gender and age.

Exclusion criteria: Non Yemeni patients and those who were refuse to participate in the study [13].

2.2 DNA Extraction

DNA was extracted from EDTA blood samples using a DNA purification kit (G-spin TM Total DNA extraction kit protocol intron biotechnology). DNA was quantified by nanodrop and stored at -20^{c} [13].

2.3 Genotyping of MTRR Gene (66A-G) Polymorphism

MTRR gene(66A-G)polymorphism was determined with a polymerase chain reaction-restriction fragment length polymorphism assay [PCR-RFLP]. PCR primers were: 5'-GCA AAG GCC ATC GCA GAA GAC AT-3'(F) and 5'-GTG AAG ATC TGC AGA AAA TCCATG TA3'(R). PCR was carried out in a total volume of 20 µl, consist 1µl of genomic DNA, 1µl of each primer, ready-to-load master mix (Maxime TM premix kit (i-Taq), and 17µl distilled water. PCR condition was initial denaturation at 95°C for 3 minutes, followed by 30 cycles at 94°C for 30 seconds, 60.5°C for 30 seconds, 72°C for 50 seconds, and the last extension at 72°C for 10 minutes. PCR products were analyzed on a 2% agarose gel stained with 0.3 µg/mL ethidium bromide, and visualized by a gel documentation system (to check the presence of 66 pb of MTRR). Then the PCR product was digested with the restriction endonuclease (Nde I) restriction enzyme {VIVANTIS product No RE1266} as follows: For each 5 µl of PCR product, 2 µl from 10X bufferV5 and 2µl from (Nde I) restriction enzyme (1:10 stock enzyme to diluent A) and 3µl of distal water were added, then incubated at 37˚C for 24 hrs, followed by incubation at 65˚C for 10 minutes to inhibit the enzyme activity. The products are then resolved on 4% agarose gel electrophoresis containing ethidium bromide, then visualized using UV trans illuminator. The amplified fragment after digestion with (Nde I) restriction enzyme, will give rise to 2 fragments at 44 bp and 22 bp indicating the presence of the A (isoleucine) allele but the PCR fragment of 66 bp remains uncut in the presence of the G (methionine) allele.

M, 50 bp ladder molecular weight marker .1ane 1-5, 66 bp PCR product.

M, 50 bp ladder molecular weight marker. wild type (66AA ,44,22), homozygous type (66GG ,66 bp un cut), heterozygous type (66 AG ,66, 44 and 22 bp).

2.4 Statistical Analysis

Demographic data were analyzed to obtain the mean, the standard deviation, and the probability (P value) between patients and the control group using the Statistical Packages of Social Science (SPSS) software program version 16. The Pearson's chi-square test was used to compare the genotype distribution between patients and control. P-values less than 0.05 were considered as statistically significant. Odd ratios were estimated for each variable. Logistic regression analysis was used to estimate the risk of developing ALL according to demographic

data. P value of <0.05 was considered significant.

3. RESULTS

115 patients with ALL included in this study shoed maled most effected 66(57.4%), compared with female 49(42.6%). Patients were grouped from one year to sixty years in four groups most of them were less than 10 years, and are statistically difference (P value =0.000 Table 1.

3.1 MTRRA66G Genotypes in Yemeni Acute Lymphoblastic Leukemic Patients and Controls

The frequency of MTRR 66A-G heterozygous (AG), homozygous (GG) and wild type (AA) in cases was 52.2% (60), 22.6% (26) and 25.2%(29), respectively. Whereas, the frequency of MTRR 66A-G heterozygous (AG), homozygous (GG) and wild type (AA) in controls was 46.4% (65), 27.1% (38), and 26.4% (37), respectively (Figs. 1, 2). The difference in frequencies were statistically insignificant (P=0.471, OR = 1.065, 95% confidence interval (CI) 0.606–1.873) (Table 2).

3.2 Relationship between MTRRA66G Genotypes and Risk Factors in Acute Lymphoblastic Leukemia Patients

The presence of MTRRA66G mutant genotypes showed a trend towards radiation, negative smoking, and infection showing an OR (95% CI) of 1.776(0.366-8.627), 1.147 (0.282-1.934), and 1.283(0.433-3.043) respectively. However, the association is not statistically significant (Table 3).

4. DISCUSSION

Acute lymphoblastic leukemia is the most common cancer seen in children [14-15]. This study is performed to detect if there is an association between polymorphism in MTRR gen and Acute lymphoblastic leukemia in the Yemeni population. A total number of 1522 cases of leukemia were evaluated over 5 years from June 2010 to December 2014. Previous studies indicated that ALL is the second most common adult leukemia in Yemen [16-20]. “Exogenous and Endogenous toxins may lead to alterations inside different genes, which may increase susceptibility to cancer development, like ALL” [21].

“There was little knowledge of MTRR gene polymorphisms, therefore, we examined this gene because the gene is important for the catalytic interaction with folate, homocysteine and cobalamin during embryonic development”. [22-24]. “Methionin synthase enzyme (MTR) encoded by the MTR gene, uses the methyl group from methyltetrahydrofolate for remethylation of homocystiene to methionine” [25]. “The MTRR is essential for the maintenance of MTR functions and is one of the key regulatory enzymes involved in the folate pathway” [26].

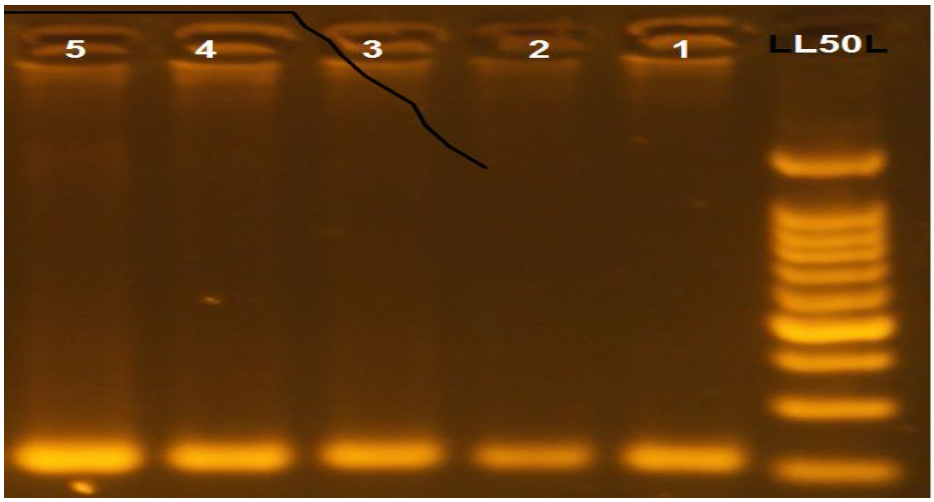

Fig. 1. PCR product of MTRR gene

Table 1. Distribution of demographic variables of the ALL patients and controls

		Cases (no =115)	Controls (no=140)	P value
Age group	<10year	70(60.9%)	30(21.4%)	
	11-20 year	29(25.2%)	98(70%)	
	21-30 year	13(11.3%)	11(7.9%)	0.000
	>30 year	3(2.6%)	1(.7%)	
Gender	Male	66(57.4%)	79(56.4%)	
	Female	49(42.6%)	61(43.6%)	0.574

Table 2. MTRRA66G genotypes in Yemeni acute lymphoblastic leukemic patients and controls

Gene	Genotype	Cases N=115	Control N=140	P value	OR	95%CI	
						Lower	Upper
MTRR	AG	60(52.2%)	65(46.4%)				
	GG	26(22.6%)	38(27.1%)	0.471	1.065	0.606	1.873
	AA	29(25.2%)	37(26.4%)				

Table 3. Relationship between MTRRA66G genotypes and risk factors in acute lymphoblastic leukemia patients

Risk factors		MTRRA66G genotype (N=115)			P value
		AG	GG	AA	
Family history	Yes	10	2	7	0.526
	No	50	24	22	
Radiation	Yes	6	4	2	0.781
	No	54	22	27	
Smoking	Yes	5	2	4	0.459
	No	55	24	25	
Negative smoking	Yes	15	8	7	0.982
	No	45	18	22	
Infection	Yes	31	13	19	0.267
	No	29	13	10	

There was no previous study on the prevalence of MTRR 66A-G polymorphisms and their association with acute lymphoblastic leukemic patients in Yemen. In the present study, we investigate ALL patients to identify associations with MTRR A66G polymorphisms compared with control. The results showed that the frequency of MTRR 66A-G heterozygous (AG), homozygous (GG), and wild type (AA) were 52.2% (60), 22.6%(26), and 25.2%(29), respectively in cases. In contrast, there were 46.4% (65) of control people with heterozygous (AG) type, 27.1% (38) with homozygous (GG), and 26.4% (37) with wild type(AA) (Table 4). The difference in frequencies were statistically insignificant (P=0.471, OR = 1.065, 95% confidence interval (CI) 0.606–1.873). Our finding for MTRR A66G polymorphism does not associate with the development of acute lymphoblastic leukemia in Yemeni patients. This results agree with previous study done by Wagiman et al. [10] in Malaysian population were MTRR66GG variant (OR = 0.05, 95% confidence interval (CI) 0.01–0.29), (Lautner-Csorba et al., 2013) (P value=1.21) [2]. In Korean study found no significant difference in susceptibility to ALL [5]. Another German study report that, a decrease in susceptibility to leukemia for MTRR genes heterozygous (AG) and homozygous (GG) [27]. Similarly, our results agreed with the meta-analysis study done by Fang et al. [11] performed for 2913 cases and 4764 controls. MTRR mutant genotype showed a trend toward the development of infections, however, the association is not statistically significant. Similar results were obtained in Filipino children [28].

Table 4. Relationship between MTRRA66G genotypes and risk factors in acute lymphoblastic leukemia patients

Risk factors		MTRRA66G genotype (N=115)		OR	Lower	Upper	P value
		Mutant	Non mutant				
Family history	Yes	12	7	0.510	0.179	1.452	0.161
	NO	74	22				
Radiation	Yes	10	2	1.776	0.366	8.627	0.373
	NO	76	27				
Smoking	Yes	7	4	0.554	0.150	2.049	0.286
	NO	79	25				
Negative smoking	Yes	23	7	1.147	0.282	1.934	0.351
	NO	63	22				
Infection	Yes	44	19	1.283	0.433	3.043	0.495

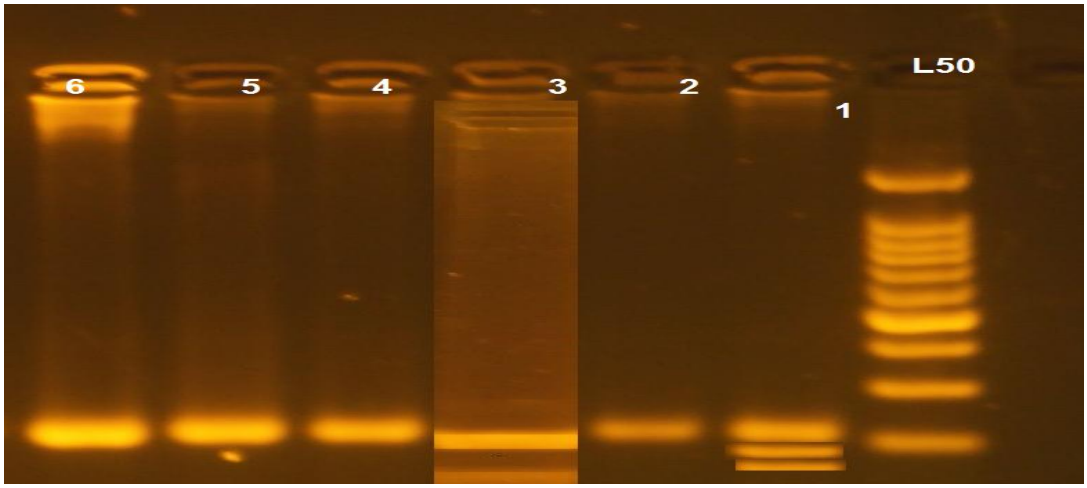

Fig. 2. MTRR A66G polymorphism digested by Ndel restriction enzyme

5. CONCLUSION

This study concluded there is no significant association between the MTRR A66G polymorphism and acute lymphoblastic leukemia in Yemeni patients.

CONSENT

As per international standard or university standard, patient(s) written consent has been collected and preserved by the author(s).

ETHICAL APPROVAL

Ethical clearance was obtained from the Ethical Review Committee of Faculty of Medical Laboratory Science, University of of Khartoum, Sudan.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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