International Journal of Biotechnology and Bioengineering

Volume 7 Issue 9,
November 2021

Citation

Tarig Altayb Mohammed et al. (2021), Association of Methylene Tetrahydrofolate Reductase Gene A1298C (rs1801131) Polymorphism with Myocardial Infarction among Sudanese Patients. Int J Biotech & Bioeng. 7:9. 156-161

ISSN 2475-3432

Published by
Biocore Group |
www.biocoreopen.org/ijbb/archive.php

Research Article

Association of Methylene Tetrahydrofolate Reductase Gene A1298C (rs1801131) Polymorphism with Myocardial Infarction among Sudanese Patients

Safaa Awad Mohammad[1], Tarig A. M. Hamid[*2], Mubarak Mustafa Elkarsany[3], Ahmed Elhadi Elsadig[4] , Abdelmohmoud .M.Bashir[5] , Nadia Madani Mohammed[6]

[1]*Department of Hematology and Immunohematology, Alyarmouk Collage, Khartoum, Sudan*
[2]*Department of Hematology and Immunohematology, Sharq El Nile Collage, Khartoum, Sudan*
[3,6]*Department of Hematology and Immunohematology, College of Medical Laboratory Science, Karary University, Khartoum, Sudan*
[4]*Department of hematology and blood transfusion , Alzaeim Al-Azhari University , Khartoum, Sudan*
[5]*Department of hematology, pathology laboratory blood bank , Zayed Military Hospital, Sudan.*

Corresponding author: Tarig Altayb Mohammed
Department of Hematology and Immunohematology, Sharq El Nile Collage, Khartoum, Sudan.
E-mail: tarig24@hotmail.com

Article History: **Received:** September 13, 2021;
Accepted: September 18, 2021;
Published: November 15, 2021.

Abstract

Background: Myocardial infarction (MI) is among the leading causes of mortality worldwide. Variations in folate metabolism including genetic polymorphisms in the key metabolic enzymes had showed influences in the MI process.
Objectives: To determine whether the C1298A transition in the Methylene Tetrahydrofolate Reductase (MTHFR) gene is associated with increased risk for MI among Sudanese patients.
Material and methods: This is a hospital based case control study in which a total of 140 Sudanese subjects were enrolled, 70 patients with myocardial infarction and 70 age- and sex matched healthy volunteers as a control group. Genomic DNA was extracted by (QIA gene, Korea) kits and the SNPs genotypes were determined using polymerase chain reaction followed by restriction fragment length polymorphism (PCR- RFLP). Data of this study were collected using a structured interview questionnaire and analyzed by statistical package for social sciences (version 21).
Results: The frequency of the AA genotype was higher in the patients group compared with control (96%, 71% respectively); while the AC genotype was higher in the control (20%, 5% respectively), while the CC genotype was not observed in this study population. The frequencies of A and C alleles were 0.68 and 0.02 respectively in MI patients while frequencies were 0.60 and 0.10 respectively in the control group. No statistically significant association was observed between MTHFR genotypes and MI (P. values = 0.4 and 0.1 for AA and AC genotypes respectively).
Conclusion: In this study population, the A1298C MTHFR polymorphism is not associated with the risk of MI among the Sudanese population.

Keywords

Myocardial Infarction, Methylene Tetra Hydrofolate Reductase

Declaration of Conflicting Interest

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Introduction

Coronary artery disease (CAD) is a group of disorders involved thrombotic lesion in coronary artery; these includes acute coronary syndrome (ACS) of acute myocardial infarction (AMI) and unstable angina, chronic coronary syndrome of chronic stable angina. Diagnosis of such disorders is still a challenge despite the considerable progress in the diagnostic modalities (Lippi G et al; 2006). Many risk factor are involved in the pathogenesis of coronary atherosclerosis, acting either in single way or in synergistic effect, these are -not limited to -smoking, obesity, diabetes mellitus, and hypercholesterolemia (Trip MD et al; 1990, Boos CJ et al; 2006).

Myocardial ischemia that results from a perfusion-dependent imbalance between supply and demand leads to myocytes necrosis which develops progressively depending on different factors (organ, species, cardiac work, duration of ischemia, collateral blood flow, etc.) (Alpert, J.S et al; 2000). MTHFR is an important enzyme involved in folate metabolism; it catalyzes the conversion of 5,10-methylenetetrahydrofolate (5,10-MTHF) in 5-methyltetrahydrofolate (5-MeTHF), the latter representing the active form of folate that is involved in re-methylation of homocysteine to methionine. MTHFR gene was mapped on human chromosome 1p36.3; it has 11 exons and exhibits multiple polymorphisms in general population, some of them with altered function in homozygous individuals. However, two polymorphisms of the MTHFR gene are found with higher frequency: 677C>T and 1298A>C; these polymorphisms are thermolabile variants of the normal gene and determine the accumulation of homocysteine in circulatory system and the decrease of folic acid concentration. The second MTHFR polymorphism involves an adenosine to cytosine substitution at base pair 1298 (1298A>C), causing a glutamate to alanine substitution in the MTHFR protein. The polymorphism is located in exon 7, within the presumptive regulatory domain (Goyette et al., 1998). The 1298A>C mutation results in decreased MTHFR activity, with a stronger effect in the homozygous than in the heterozygous state, yet with a lesser impact than that of 677C>T (Van der Put et al., 1998). Some previously published data show a frequency of 1298 CC genotype of 10% and an allele frequency of 1289C of 36% among distinct populations (Chadefaux et al 2002).The 677C>T and 1298A>C mutations are found in regions encoding the N-terminal catalytic and the C-terminal regulatory domains of the protein, respectively (Weisberg et al 2001). The effects of the 1298A>C mutation on plasma concentrations of homocystin remain controversial; as some studies showed significant influence of this polymorphism on plasma homocystin level (Kang SS et al; 1991), while others have either not found any effect (Lievers KJ et al; 2001) or observed an association with even lower levels of plasma homocystin in homozygous individuals (Friedman G et al; 1999).

Methodology

This is a hospital-based case-control study conducted at Sudan Heart Center, Khartoum, Sudan. A total of 140 subjects were enrolled for this study; among them 70 patients had a confirmed diagnosis of MI (based on results of serum Tropnin test: >99th percentile URL, and echocardiography) and 70 age- and sex-matched apparently healthy volunteers- as a control group. Venous blood samples were collected from all participants in Ethylene diamine tetra acetic acid (EDTA). Genomic DNA was isolated using QIA gene kits (KOREA) and stored at -30°C until genotyping is carried out. MTHFR A1298C polymorphism was analysed by Allele-Specific Polymerase Chain Reaction (AS-PCR) followed by restriction enzyme. For PCR amplification, A reaction mixture of 25µl was prepared for each sample, containing 5µl genomic DNA, 1µL of each of the forward (5'GCAAGTCCCCCAAGGAGG-3'), reverse (5'GGTCCCCACTTCCAGCAT-3'), (Nasiri et al 2014) (MACROGEN, KOREA), 5µL master mix (MAXIME PCR PRE-MIX KIT (I-TAQ), INTRON, KOREA), and 18µL sterile distilled water. The amplification program consists of initial denaturation at 95ºC for 1 minutes; then 30 cycles [each consists of denaturation at 95ºC for 30 second , annealing at 61ºC for 30 second, and extension at 72ºC for 30 second], and a final extension at 72ºC for 7 minutes. PCR products were incubated over night with MboII restriction enzyme (Thermo SCIENTIFIC) then the product was separated on 3% agarose gel electrophoresis containing ethidium bromide with a 100 bp DNA ladder (SOLIS BIODYEN, ESTONIA) run with each batch of samples and the size of the fragments was determined under UV transilluminator (SYNGENE, JAPAN).

Patient's data were collected using a structured interview questionnaire and analysed by statistical package for social science (SPSS), version 21. The qualitative data were presented as frequency and percentage. Quantitative data were presented as Mean±SD. Association between qualitative variable was tested by Chi-square (X2) and Fisher's exact tests. Multivariate logistic regression analysis was used for the prediction of MI risk as dependent variable with a set of predictors including MTHFR genotype and other MI risk factors. The allele frequencies and their accordance with Hardy Weinberg Equilibrium (HWE) were calculated using the conventional formulas.

The study was approved by the scientific research committee, faculty of medical laboratory sciences, Karary University Khartoum, Sudan. Written informed consent was taken from each subject before participation.

Results

The mean age of participants in this study is 58.4 ± 12.4 years, ranging from 40 to 88 years. The results of PCR amplification yielded amplicons of length 143bp for A1298C polymorphism Figure1. Post digestion results showed 77 and 28bp bands for AA genotype, 105, 77 and 28 bp for AC genotype as shown in Figures 2.

The AA genotype frequency is 94% in the MI group and 71% in the controls, while AC genotype frequency is 6% in patients group and 29% in controls. There was no subject with CC genotype in both patients and control group Figure 3. The frequency of A allele was 0.68 in the patients with MI and 0.60 in the control group, while the frequency of T Allele was 0.02 in the patients with MI and 0.1 in the control group. All genotypes frequencies are in accordance with HDW equation (P values > .05).

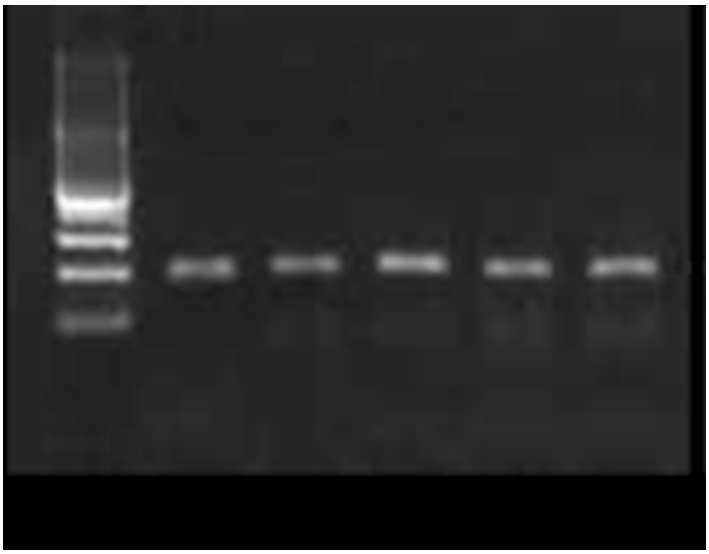

Figure1: shows the PCR fragment amplified by specific pair of primer designed for MTHFR A1298C polymorphic site and its flanking region yielded a 143 bp amplicon. (M: marker, 50 bp ladder, Fermentas, Germany)

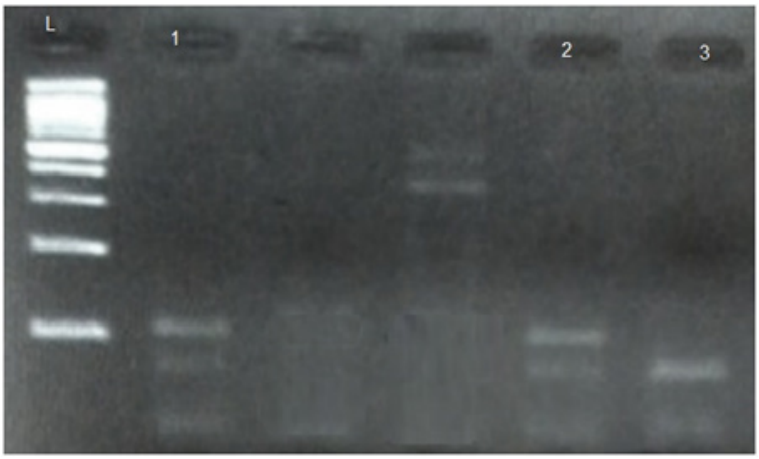

Figure 2 shows results of PCR-base restriction analysis of MTHFR A1298T polymorphism on 3% agarose electrophoresis. The digested fragment length 105,77, 28bp in lane 1,2 indicates (AC genotype), while fragment lengths 77, 28bp in lane 3 indicates (AA genotype). L: ladder, 100 bp ladder.

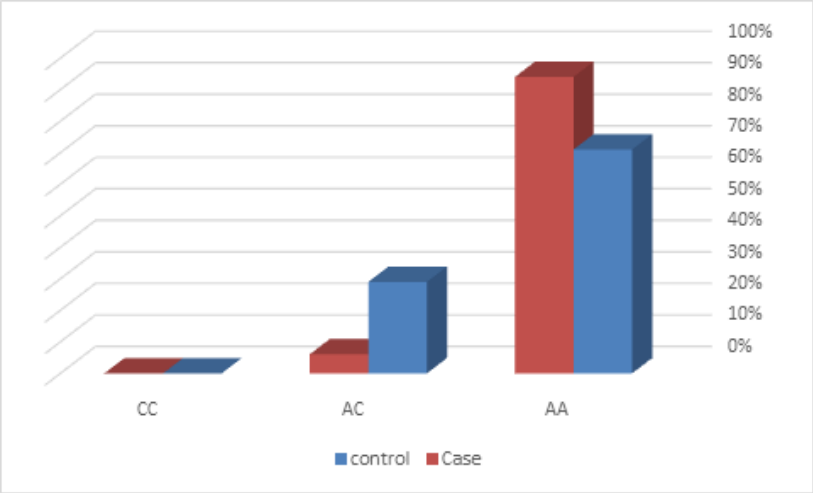

Figure 3 shows Frequencies of genotypes in patients and control (n=140)

The results of the current study showed no statistically significant difference in genotype between patients and controls (P. values: AA= 0.7 and AC= 0.9). Moreover, there were no statistically significant difference in the age group and gender of patients with genotype (P. values = 0.5 and 0.2 respectively). All the patients had at least one known risk factor for MI these include, 50 (71%) DM, HTN and obesity, 18(25%) had smoker and 10(14%) were alcohol users Figure 4. In addition the Multivariate regression analysis revealed no interaction between MI risk factors in case and control Table 1.

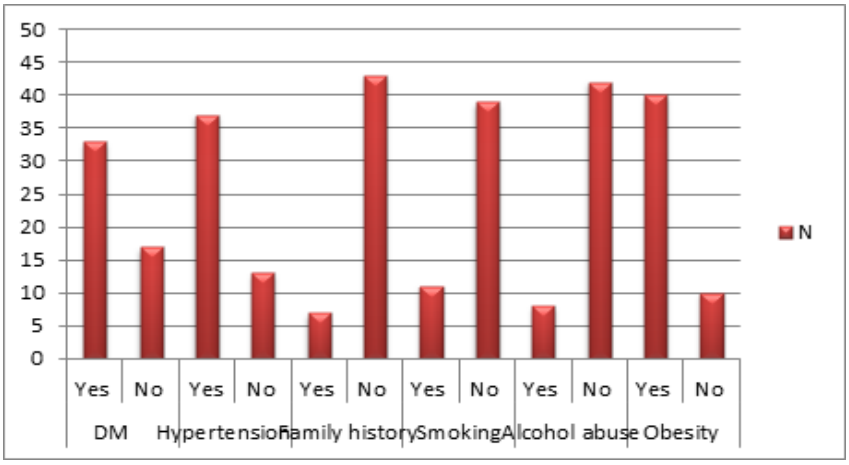

Figure 4 shows the distribution MI of risk factors in the cases group

Characteristic		Patients	Control	p. value
N		70	70	
Age		58.4 ± 12.4	58.6 ± 12.6	0.5
Sex(male: female)		35:35	35:35	0.2
Diabetic; no diabetic		50: 20	0: 70	0.287
Hypertensive non hypertensive		50: 20	0: 70	0.612
Alcohol abuse; non-alcohol abuse		10: 60	0: 70	0.447
Obese; non obese		50: 20	0: 70	0.824
Smoker: nonsmoker		18: 52	0: 70	0.558
Genotype	AA	94%	71%	0.4
	AC	6%	29%	0.1

Table1 Logistic regression showing interactions of MI risk factors

Discussion

Myocardial infarction is the consequence of atherosclerotic plaque disposition on the coronary artery wall. Its manifestation depends on interactions between environmental and genetic risk factors; the present study was conducted to investigate the association between MTHFR gene polymorphism (A1298C) and the risk of MI among the Sudanese population. The results showed that, the frequency of AA, genotype in patients was higher than controls (94%, 71% respectively); the frequency of AC genotype is lower in patients compared with controls (5%, 20% respectively), while CC genotype was not observed in this study population. There was no statistically significant association between MTHFR gene polymorphism (A1298C) and MI. Previous studies in different populations showed inconsistent results regarding the association of this polymorphism with risk of MI. In north Indian for A1298C locus (Butler et al., 2018), the AA was more frequent in patients than control (43%, 33%) respectively and AC was more frequent in controls than patients (35%, 53%). Also (Kerkeni et al 2006), MTHFR AA, AC and CC genotypes frequencies in the MI group were not significantly different from the control group. In addition according to (Eftychiou et al 2012), the existence and extent of disease are not significantly associated with MTHFR A1298C polymorphisms. Also logistic regression analysis done by (Nasiri et al 2014) showed that; A1298C locus is not associated with increased susceptibility to MI.
On the other hand, there are fewer studies disagree with our finding which reported a significant association between CC genotype and risk of MI (Butler et al., 2018).

The result of the present study showed no statistically significant difference in the age group of patients with different A1298C polymorphic variants, this indicating that MTHFR gene polymorphism does not affect the age of incidence of MI. Similar results were reported by Tripathi et al; (2010), Hmimech et al; (2016), Glue et al; (2001) and Anderson; et al (1997). All of them reported no association between age, and A1298C genotypes in patients with MI. In contrast, Butler et al; (2018) and Nishhama et al; (2007) suggested that the age of patients may be associated with MI occurrence.
The current study showed no statistically significant association between gender and A1298C genotype. Some studies agree with this finding like (Tripathi et al; (2010) and (Hmimech et al; 2016), while others disagree with this finding (Nishhama et al; 2007).

In the present study, there is no interaction between A1298C polymorphism and the conventional MI risk factors including (DM, HTN, smoking, obesity and alcohol abuse) was reported. This finding was in agreement with a study by (Anderson; et al 1997) who also reported no significant association between A1298C polymorphisms and any other risk factor among patients with acute coronary syndrome. Also, (Butler et al; 2018) found no statistically significant association of smoking with MI risk, in addition (Hmimech et al; 2016) no statistically significant association of diabetic, smoker, hypertension and obesity with MI risk. At the other hand, this finding disagrees with the finding of (Gluec et al; 2001) who observed strong statistically significant association of smoking, diabetes and hypertension with MI.

Conclusion

The present study concludes that there is no statistically significant association between MTHFR A1298C polymorphism and risk of MI among the Sudanese patients.

Acknowledgements

Authors acknowledges Dr. Salah gumaa for valuable help and support in samples genotyping, also acknowledgements are extended to the Sudan heart center staff for their great effort during sample collection.

References

[1] Alpert, J.S. (2000) Myocardial Infarction Redefined—A Consensus Document of The Joint European Society of Cardiology/ American College of Cardiology Committee for the Redefinition of Myocardial Infarction. Journal of the American College of Cardiology; 36: 959-969.

[2] Andresion et al (2003) Methylenetetrahydrofolate reductase gene C677T polymorphism, homocysteine, vitamin B12, and DNA damage in coronary artery disease. Hum Genet 112:171-177

[3] Boos CJ, Lip GY. (2006) Assessment of mean platelet volume in coronary artery disease. Thromb Res,:120: 11–13.

[4] Butler et al (2018) Association of methylenetetrahydrofolate reductase (MTHFR) C677T and A1298C polymorphisms with coronary artery disease (CAD) in a North Indian population. Cogent Medicine; 5: 1478477.

[5] Chadefaux-Vekemans .(2002) Methylenetetrahydrofolate reductase polymorphism in the etiology of Down syndrome. Pediatr. Res; 51: 766–767.

[6] Eftychiou et al (2012) Homocysteine Levels and MTHFR Polymorphisms in Young Patients with Acute Myocardial Infarction: A Case Control Study, Hellenic J Cardiol; 53: 189-194.

[7] Friedman G. (1999) A common mutation A1298C in human methylenetetrahydrofolate reductase gene: association with plasma total homocysteine and folate concentrations. J Nutr;129:1656–61.

[8] Goyette P. (1998) Chen Gene structure of human and mouse methylenetetrahydrofolate reductase (MTHFR).Mammalian Genome; 9: 652–656

[9] Gulec et al (2001) Methylenetetrahydrofolate Reductase Gene Polymorphism and Risk of Premature Myocardial Infarction .Clin. Cardio;. 24,: 281-284

[10] Hmimech et al (2016) Association of C677T MTHFR and G20210A FII prothrombin polymorphisms with susceptibility to myocardial infarction. BIOMEDICAL REPORTS; 5: 361-366.

[11] Kang SS. (1991) Thermolabile methylenetetrahydrofolate reductase: an inherited risk factor for coronary artery disease. Am J Hum Genet 48:536–45

[12] Kerkeni et al (2006) Hyperhomocysteinemia, methylenetetrahydrofolate reductase polymorphism and risk of coronary artery disease. Ann Clin Biochem ; 43: 200 206.

[13] Lievers KJ. (2001) A second common variant in the methylenetetrahydrofolate reductase (MTHFR) gene and its relationship to MTHFR enzyme activity, homocysteine, and cardiovascular disease risk. J Mol Med;79:522–8.

[14] Lippi G et al (2006) Potential value for new diagnostic markers in the early recognition of acute coronary syndromes. CJEM; 8: 27–31.

[15] Nasiri et al (2014)Association of Methylenetetrahydrofolate Reductase (MTHFR) Gene C677T and A1298C Polymorphisms with Myocardial Infarction from North of Fars Province. Res Mol Med; 2 (3): 38.

[16] Nishihama et al (2007) Association of gene polymorphisms with myocardial infarction in individuals with or without conventional coronary risk factors. International Journal of Molecular Medicine; 19: 129-14.

[17] Trip MD et al. (1990) Platelet hyperreactivity and prognosis in survivors of myocardial infarction, New Engl J Med; 322: 1549–1554.

[18] Tripathi et al (2010) Association of homocysteine and methylene tetrahydrofolate reductase (MTHFR C677T) gene polymorphism with coronary artery disease (CAD) in the population of North India. Genetics and Molecular Biology;33, 2, 224:228

[19] Van der Put. (1998) A second common mutation in the methylenetetrahydrofolate reductase gene: an additional risk factor for neural tube defects? Am. J. Hum. Genet; 62: 1044–1051.

[20] Weisberg IS. (2001) The 1298ARC polymorphism in methylenetetrahydrofolate reductase (MTHFR): in vitro expression and association with homocysteine. Atherosclerosis;156:409–15.