Original Article **Sudan Medical Laboratory Journal**

Frequency of Methylenetetrahydrofolate Reductase (C677T) genetic polymorphism among Sudanese patients with myocardial infarction

Maiada Kamal Ali Madani[1*] and Mahdi H. A. Abdalla[2]

[1]Department of Hematology, Faculty of Medical Laboratory Sciences, Alneelain University, Sudan
[2]Department of Hematology, Faculty of Medical Laboratory Sciences, Omdurman Ahlia University, Sudan

Abstract

Background: Myocardial infarction is a leading cause of mortality worldwide. Age, smoking, obesity, diabetes, hypertension, dyslipidemia, and low levels of physical activity are one of the known risk factors for the development of Ischemic Heart Diseases. However, the occurrence of Ischemic Heart Diseases in the absence of any of these factors raises the possibility of additional risk factors, such as genetic ones. Genetic risk factors for myocardial infarction have been established in the previous investigation and the 5,10-methylenetetrahydrofolate reductase (MTHFR) gene polymorphism was one of the most often examined ones. MTHFR C677T polymorphism increases serum homocysteine level which was believed to lead to a hypercoagulable state. Accordingly, this study aimed to examone the association between the risk of developing myocardial infarction and MTHFR C677T polymorphism 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: Ten percent (n = 5) of cases had the MTHFR C677T polymorphism while none of the healthy controls (0%) had it and there was a significant association between the polymorphism and having myocardial infraction in this study (P = 0.028).

Conclusion: this study revealed a statistically significant association between the risk of developing Myocardial infarction and having the MTHFR C677T gene polymorphism with none of the healthy controls reporting MTHFR C677T gene polymorphism.

Key words: Methylenetetrahydrofolate Reductase, C677T, genetic polymorphism, myocardial infarction, Sudanese.

Corresponding author: **Maiada Kamal Ali Madani** Email: mahdi_razig@hotmail.com

Introduction: Myocardial infarction (MI) is the death of cardiac cells due to insufficient blood supply resulting from coronary artery blockage. It is a leading cause of mortality worldwide and according to the World Health Organization, almost 50 million people die yearly due to Ischemic Heart Diseases (IHD), particularly myocardial infarction (1). Many modifiable and non-modifiable risk factors for the development of IHD such as age, smoking, Obesity, Diabetes, Hypertension, dyslipidemia, and low physical activity have been identified (2–4). However, the presentation of IHD without any known risk factor raises the question of the presence of other risk factors such as genetic ones (5). Previous studies have identified genetic risk factors for myocardial infarction and 5,10-methylenetetrahydrofolate reductase (MTHFR) gene polymorphism was

among the most commonly studied ones (6)(7).

The MTHFR gene is found at location 'Ip36.3' on chromosome 1 with its matching cDNA sequence that consists of 11 exons spanning 2.2 kb (8). The protein product of this gene contains 656 amino acids and functions as a catalyzing factor for the irreversible transformation of 5,10-methylenetetrahydrofolate (5,10-CH2-FH4) into 5-methyltetrahydrofolate (5-CH3-FH4), the main circulating form of folic acid that acts as a co-substrate for the re-methylation of homocysteine to methionine (9)(10). This 5-methyltetrahydrofolate is the form of folate that participate in the synthesis of nucleotides, the formation of S-adenosylmethionine, the re-methylation of homocysteine to methionine, and the methylation of DNA, proteins, and phospholipids (11).

The MTHFR gene's C677T polymorphism causes alanine to change to valine in the binding site of the flavin adenine dinucleotide, the cofactor of the MTHFR enzyme, by changing cytosine (C) to thymine (T) at position 677 (12). As a result, this MTHFR C677T allele makes it easier for the enzyme to become separated from its cofactor, which lowers its activity and thereby increases plasmatic homocysteine levels (13).

There is mounting evidence that patients with or without hypertension, diabetes mellitus, and other traditional risk factors are at a greater risk for cerebrovascular, peripheral vascular, and cardiovascular illness when their plasma Homocysteine level is elevated (14)(15). Several studies have examined the association between MTHFR C677T polymorphism and myocardial infarction before, however, different conclusions have been yielded. Accordingly, this study aimed to address this association in Sudan.

Materials and method: This was a case-control study that included 50 patients with myocardial infarction and 50 healthy controls who were matched by age and sex. MI patients were obtained from the Al-Shaab teaching hospital, in Khartoum, Sudan. Serum troponin I was tested in all MI patients, and all were positive. Before data collection and analysis, informed consent was taken from both cases and controls.

2 ml of blood samples were collected from both patients and controls into EDTA anticoagulated tubes and the laboratory work was done at the department of hematology, faculty of medical laboratory sciences, Alneelain University, Sudan. The extraction of DNA was done using the salting out approach. The forward primer, 5'-TGAAGGAGAAGGTGTCTGCGGGA-3', and the reverse primer, 5'-AGGACGGTGCGGTGAGTG- 3', were used to amplify the MTHFR C677T fragment. The thermocycler (Techne TC-412, UK) was used to do the amplification after a 5-minute denaturation stage at 94°C. After that, there were 40 cycles that each had three steps: a denaturation step at 94 °C for 30 seconds, an annealing step at 59 °C for 1 minute, an extension step at 72 °C for 1 minute, and the last extension step taking place at 72 °C for 7 minutes. A final 20 µl volume containing 4 µl of the pre-mixed, ready-to-use 5x FIREPolmaster mix (Solis BioDyne, Russian), 11 µl of DNAase-free DW, 3 µl of genomic DNA, and 1 µl from each primer was used for the PCR reaction. After being digested overnight with 10 U of

Hinf1endonuclease (New England Biolab, UK), the amplified fragment was detected on an agarose gel electrophoresis. An automatic cell counter was used to count the blood cells (Sysmex KX-21N).

Statistical analysis was performed using Statistical Package for Social Sciences (SPSS) version 25. Categorical data were presented as percentages and numbers (n (%)), and the frequency distribution between groups from different variables were compared using the Chi-square test. A P-value of less than 0.05 was considered statistically significant.

Results: A total of 50 patients with myocardial infarction were included in the study with their ages ranging from 35 to 65 years. Of them, 32 (64%) were males and 18 (36%) were females. Fifty healthy controls were matched to diseased subjects by age and gender. MTHFR C677T polymorphism was studied in both cases and healthy controls. Ten percent (n = 5) of cases had the MTHFR C677T polymorphism while none of the healthy controls (0%) had it and there was a significant association between the polymorphism and having myocardial infraction in this study (P = 0.028) (Table 1).

Table 1: Comparison of Methylenetetrahydrofolate Reductase (C677T) gene polymorphism frequency between patients and control

Genotype	Patients n (%)	Controls n (%)	Sig.
CC	45 (90%)	50 (100%)	**0.028***
CT	5 (10%)	0 (0%)	

Associations between myocardial infarction and known risk factors such as diabetes, hypertension, smoking, and obesity were tested in this study, including patients and controls. We found a significant association between all these factors and the disease (P = 0.000). Hb levels were measured for both patients and controls and no significant difference was reported between the two groups (P = 0.8010)

Discussion: This study is the first of its kind in Sudan to examine the association between MTHFR C677T gene polymorphism and the risk of developing MI. in this study we reported a significant association between the two and none of the healthy controls reported this polymorphism in their genes. Other previous studies have examined this association and the effect of this polymorphism on developing hypercoagulability state and thrombosis such as deep vein thrombosis (DVT) due to the increase in serum homocysteine level. Studies conducted in Morocco (7) and China (16) revealed no significant association between the risk of MI and MTHFR C677T polymorphism, however, in a previous systematic review, a significant association was reported between the two, particularly among young patients (17). Furthermore, a study conducted in Georgia, revealed a significant association between MTHFR C677T polymorphism and the risk of developing arterial thrombosis (18), while a previous Sudanese

study reported an insignificant effect of this polymorphism on DVT occurrence (19). All these suggest that there is inconclusive evidence regarding the effect of MTHFR C677T polymorphism on the development of MI and other hypercoagulable diseases. This study is limited by the small sample size and inclusion of patients from only one hospital. Moreover, the lack of financial ability and resources to measure serum homocysteine levels among both patients and controls is another limitation of this study.

In conclusion, this study examined the association between the risk of developing Myocardial infarction and having the MTHFR C677T gene polymorphism and a statistically significant association was reported between the two. Moreover, none of the healthy controls in this study reported MTHFR C677T gene polymorphism.

References

1. Lopez E and Jamison M: Global Burden of Disease and Risk Factors: Disease Control Priorities Project. World Bank and Oxford University Press, New York, NY, 2006.

2. Cheng X, Li W, Guo J, Wang Y, Gu H, Teo K, et al. Physical activity levels, sport activities, and risk of acute myocardial infarction: results of the INTERHEART study in China. Angiology. 2014 Feb;65(2):113–21.

3. Yusuf S, Hawken S, Ounpuu S, Dans T, Avezum A, Lanas F, et al. Effect of potentially modifiable risk factors associated with myocardial infarction in 52 countries (the INTERHEART study): case-control study. Lancet (London, England). 2004 Sep;364(9438):937–52.

4. Sarwar N, Gao P, Seshasai SRK, Gobin R, Kaptoge S, Di Angelantonio E, et al. Diabetes mellitus, fasting blood glucose concentration, and risk of vascular disease: a collaborative meta-analysis of 102 prospective studies. Lancet (London, England). 2010 Jun;375(9733):2215–22.

5. Ramtahal J, Duncan A. MYOCARDIAL INFARCTION IN A YOUNG PATIENT WITH METHYLENE TETRAHYDROFOLATE REDUCTASE (MTHFR) GENE MUTATION. EUR J GEN MED. 2004;1(4):78-80. https://doi.org/10.29333/ejgm/82251.

6. Sakowicz A, Fendler W, Lelonek M, Pietrucha T. Genetic variability and the risk of myocardial infarction in Poles under 45 years of age. Arch Med Sci. 2010 Apr;6(2):160–7.

7. Hmimech W, Idrissi HH, Diakite B, Baghdadi D, Korchi F, Habbal R, et al. Association of C677T MTHFR and G20210A FII prothrombin polymorphisms with susceptibility to myocardial infarction. Biomed Reports. 2016;5(3):361–6.

8. Goyette P, Pai A, Milos R, Frosst P, Tran P, Chen Z, et al. Gene structure of human and mouse methylenetetrahydrofolate reductase (MTHFR). Mamm Genome. 1998 Aug;9(8):652–6.

9. Rozen R. Genetic predisposition to hyperhomocysteinemia: deficiency of methylenetetrahydrofolate reductase (MTHFR). Thromb Haemost. 1997 Jul;78(1):523–6.

10. Kutzbach C, Stokstad EL. Mammalian methylenetetrahydrofolate reductase. Partial purification, properties, and inhibition by S-adenosylmethionine. Biochim Biophys Acta. 1971 Dec;250(3):459–77.

11. Rosenblatt DS: Inherited disorders of folate transport and metabolism. In: The Metabolic and

Molecular Bases of Inherited Disease. Scriver CR, Beaudet AL, Sly WS and Valle D (eds). McGraw-Hill Inc., New York, NY, pp3111-3128, 1995.

12. Skibola CF, Smith MT, Kane E, Roman E, Rollinson S, Cartwright RA, et al. Polymorphisms in the methylenetetrahydrofolate reductase gene are associated with susceptibility to acute leukemia in adults. Proc Natl Acad Sci U S A. 1999 Oct;96(22):12810–5.

13. Guenther BD, Sheppard CA, Tran P, Rozen R, Matthews RG, Ludwig ML. The structure and properties of methylenetetrahydrofolate reductase from Escherichia coli suggest how folate ameliorates human hyperhomocysteinemia. Nat Struct Biol. 1999 Apr;6(4):359–65.

14. Clarke R, Daly L, Robinson K, Naughten E, Cahalane S, Fowler B, et al. Hyperhomocysteinemia: an independent risk factor for vascular disease. N Engl J Med. 1991 Apr;324(17):1149–55.

15. Tripathi R, Tewari S, Singh PK, Agarwal S. Association of homocysteine and methylene tetrahydrofolate reductase (MTHFR C677T) gene polymorphism with coronary artery disease (CAD) in the population of North India. Genet Mol Biol. 2010 Apr;33(2):224–8.

16. Hou J, Zhong Z, Deng Q, Lin L, Zeng X. The role of MTHFR C677T and ALDH2 Glu504Lys polymorphism in acute coronary syndrome in a Hakka population in southern China. BMC Cardiovasc Disord. 2020;20(1):1–8.

17. Xuan C, Bai XY, Gao G, Yang Q, He GW. Association between polymorphism of methylenetetrahydrofolate reductase (MTHFR) C677T and risk of myocardial infarction: A meta-analysis for 8,140 cases and 10,522 controls. Arch Med Res [Internet]. 2011;42(8):677–85. Available from:
http://dx.doi.org/10.1016/j.arcmed.2011.11.009

18. Garakanidze S, Costa E, Bronze-Rocha E, Santos-Silva A, Nikolaishvili G, Nakashidze I, et al. Methylenetetrahydrofolate Reductase Gene Polymorphism (C677T) as a Risk Factor for Arterial Thrombosis in Georgian Patients. Clin Appl Thromb. 2018;24(7):1061–6.

19. Hana O, Abdalla M. Methylenetetrahydrofolate Reductase (MTHFR C677T) Polymorphism in Sudanese Patients with Deep Vein Thrombosis. Int J Biomed Res. 2015 May 30;6:323.