IJMPR 2021, 5(6), 05-09

International Journal of Modern Pharmaceutical Research
www.ijmpronline.com

ISSN: 2319-5878
IJMPR
Research Article
SJIF Impact Factor: 5.273

ASSOCIATION BETWEEN METHYLENETETRAHYDROFOLATE RUDACTASE ENZYME (RS1801113) POLYMORPHISM AND RISK OF MYOCARDIAL INFARCTION AMONG SUDANESE PATIENT'S

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Received on: 08/09/2021
Revised on: 27/10/2021
Accepted on: 17/11/2021

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ABSTRACT

Background: methylenetetrahydrofolate reductase (MTHFR) enzyme is a catalyst in the folate metabolism pathway, The MTHFR polymorphism associated with a 50% reduction of MTHFR enzyme activity, Variations in folate metabolism including genetic polymorphisms in the key metabolic enzymes had showed influences in the MI process. **Objectives***:* To determine association of Methylene tetrahydrofolate Reductase (*MTHFR*) gene C677T (rs1801113) Polymorphism among Sudanese patients with Myocardial Infarction**. Material and methods**: This is hospital based case control study, 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 kits and The SNPs genotypes were determined using polymerase chain reaction followed by restriction fragment length polymorphism method. (PCR-RFLP). **Results***:* The frequency of the CC genotype was higher in the control group compared with patients (100%, 94%), while the CT genotype was higher in the patients (6%) and absent in control; the TT genotype was absent in both study group. C and T allele frequencies were 0.97 and 0.04, respectively in the MI group, while the frequencies of C, T Allele were 1.0 and 0.00 in the control group. No statistically significant association was reported between MTHFR polymorphic genotypes and MI (*P.value*= CC (0.32), CT 0.09)). **Conclusion***:* C677T MTHFR polymorphism showed no association as genetic risk factor for MI among Sudanese patients

KEYWORDS: *Myocardial infarction; methylene tetra hydrofolate reductase enzyme-Sudan.*

INTRODUCTION

Coronary artery disease (CAD) is a major cause of death in the developed countries and its frequency is rising rapidly in developing countries (Tardif JC *et al*; 2010). Myocardial infarction (MI) is the most acute form of CAD (Um JY *et al;* 2004).CAD is classified as complex multifactorial disorders, which involved the contributions of many different genes, as well as environmental factors. A major current challenge is therefore to elucidate the genetic components that contribute to the pathogenesis of complex diseases (Pandey U *et al* ;(2011). Several recent studies focused on the effect of homocysteine as risk factor for MI and stroke (Eftychiou C *et al*; 2012, Klerk M *et al*; 2002, Wang W *et al*; 2013). Risk factors for MI are commonly classified into modifiable and non-modifiable factors. Modifiable risk factors involve smoking, Diabetes mellitus (DM), obesity, hypertension (HTN), inherited lipoprotein disorders, dyslipidaemia, sedentary lifestyle, stress, poor oral hygiene, and type of personality (Wilson *et al.,* 1998; Yusuf *et al.,* 2005), while non-modifiable risk factors include age, gender, and family history of coronary heart disease. However, about 50% to 60% of the risk factors for acute MI are determined by heritability (Dai *et al.,* 2019). The advancement in molecular genetics methods has led to the determination of many genetic variants that are correlated with an increased risk of MI (Erdmann *et al.,* 2010).

The *MTHFR* gene is located on chromosome 1 at the 'Ip36.3' position; the corresponding cDNA sequence comprises 11 exons spanning 2.2 kb (Goyette P R *et al*.; 1998). The main product of the *MTHFR* gene is a protein of 77 kDa with catalytic activity, contain of 656 amino acids (Rozen R *et al*; 1997). It is involved in folate metabolism by catalyzing the irreversible conversion of 5,10-methylenetetrahydrofolate (5,10-CH2-FH4) into 5-methyltetrahydrofolate (5-CH3-FH4), which is the main circulating form of folic acid and the co-substrate for remethylation of homocysteine to methionine (Kutzbach C *et al*; 1971, Ma J *et al;* 1997). The *C677T* allele of the *MTHFR* gene is the conversion of cytosine (C) to thymine (T) at position 677, which results in a conversion of alanine to valine at the binding site of the flavin adenine dinucleotide, the cofactor of *MTHFR* enzyme (Skibola CF *et al;* 1999). This allele is commonly known as 'labile'; it facilitates the separation of the enzyme from its cofactor, reducing the activity of the encoded enzyme at ≥37˚C. Thus, if the folate intake is decrease, the activity of the homozygote decreases by 50-60% at 37˚C, and by 65% at 46˚C; heterozygotes are in the intermediate range, and homozygotes tend to have slightly increased plasmatic homocysteine levels (Guenther BD *et al;* 1999).

Several studies have correlated MTHFR polymorphism with cardiovascular complications (Alam *et al.,* 2016) but studies concerning with the association of the polymorphism with MI risk among different populations showed controversial results, while some of them reported a significant association, others didn't (Mohsen et al; 2006, Hmimech *et al* 2016).

METHODOLOGY

This is an analytical case-control study, conducted at Sudan Heart Center, Khartoum, Sudan, in the period from January 2020 to August 2021. In which, 70 patients were diagnosed with MI (based on results of tropnin test (>99th percentile URL) and echocardiography) and 70 age- and sex-matched apparently healthy volunteers- as a control group- were enrolled. Venous blood samples were collected from all participants in Ethylene diaminetetraacetic acid (EDTA). Genomic DNA was extracted using AQI gene kits and stored at -30°C until PCR is carried out. MTHFR C677T polymorphism was detected by polymerase chain reaction followed by restriction fragment length polymorphism method (PCR-RFLP). A reaction mixture of 25µl was prepared for each sample, containing 5µl genomic DNA, 1µL of each of the forward (5'CAAAGGCCACCCCGAAGC-3'), reverse (5' AGGACGGTGCGGTGAGAGTG -3'), (MACROGEN, KOREA) (Elhadidy *et al*;2014).5µL master mix (MAXIME PCR PRE-MIX KIT (I-TAQ), INTRON, KOREA), and 18µL sterile distilled water. The amplification condition consists of initial denaturation at 94ºC for 5 minutes; then 34 cycles [each consists of denaturation at 94ºC for 45 second, annealing at 62ºC for 40 second, and extension at 72ºC for 50 second], and a final extension at 72ºC for 7 minutes. PCR products were incubated over night with Hinf1 restriction enzyme (restriction enzyme was prepared by 7.5 µl of DW, 2 µl of enzyme buffer and 0.5 µl l of enzyme, 10 µl of DNA was added) then the product was separated on 3% agarose gel electrophoresis and is visualized by ethidium bromide using a 100 bp DNA ladder (SOLIS BIODYEN, ESTONIA) The size of the fragments was determined under UV transilluminator (SYNGENE, JAPAN). PCR fragment of 245 bp indicates the presence of CC genotype, while a fragment of 245, 173, 72 bp indicates to CT genotype and TT genotype indicated by presence of 173 and 72 bp.

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 examination of interaction between the polymorphism and MI risk factors. The allele frequency and 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 and informed consent was obtained from each participant before sample collection. Patients' data was kept confidentially and only used for the purpose of the study.

RESULTS

Total of 140 subjects were enrolled in this study, 70 patients with MI and 70 age- and sex-matched healthy volunteers as a control group. 70 (100%) of the patients had- at least- one known risk factor for MI, 50 (71%) of them had DM, HTN and obesity, 18 (25%) had smoker and 10 (14%) were alcohol abuse. **Figure1**.

The mean age of the patients was (58.4 ± 12.4) (range from 40 to 88).

The results of PCR amplification yielded amplicons of 245bplength For C677T polymorphism, Post digestion documentation was done on gel using marker 100bp ladder (Fermentas, Germany), the result show, for C677T gene CC (245bp) and CT (245, 173, 72 bp)as shown in **Figures 2**, The frequency of C677T polymorphism, the CC genotype was 100 % in the control and 94% in the patient group, where CT genotype 6% in patients group only, there was no subject with TT genotype in both patients and control group. **Figure3**.

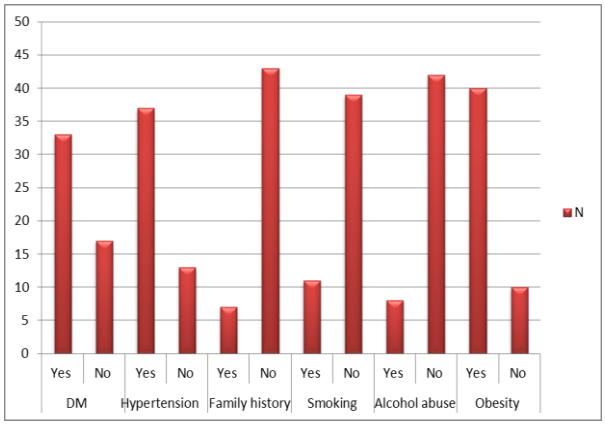

Figure 1: Frequency of risk factors in case group.

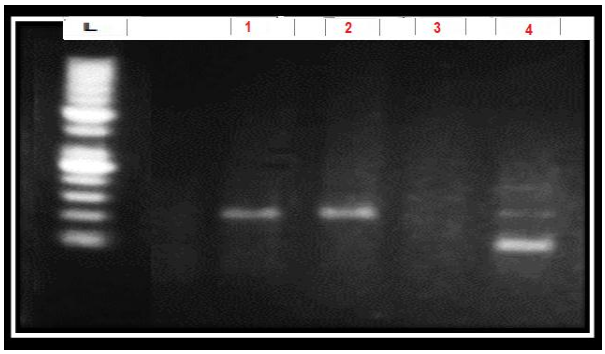

Figure 2: PCR-base restriction analysis of *MTHFR* C677T polymorphism shown on 3% agarose electrophoresis. The polymorphic region was amplified by PCR resulting in digestible fragment length 245 bp in lane 1, 2, (CC homozygote), other digestible fragment length 245, 173 in lane 4 (CT heterozygous)). M: marker, 100 bp ladder (Fermentas, Germany).

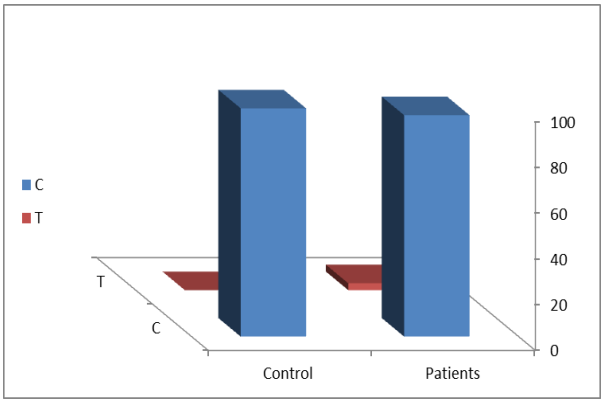

Figure 3: Frequency of genotype in patients and control.

There was no statistically significant difference in the age group of patients and CC, and CT genotype (*P.value*= 0.4). The results of the current study showed no statistically significant association between the genotypes and patients' gender (*P.value*= 0.73), also there was no statistically significant association when compare genotype in patients with control (P. value cc= 0.3, CT= 0.09), in addition the Multivariate regression analysis revealed no interaction between MI risk factors in case and control (Table 1).

The frequency of C allele was 0.97 in the patients with MI and 0.1 in the control group, while the frequency of T Allele was 0.03 in the patients with MI and 0.00 in the control group. No deviation from HWE observed when tested for the control group.

Characteristic		Patients	Control	p. value
N		70	70	
Age		58.4 ± 12.4	58.6 ± 12.6	0.4
Sex(male: female)		35:35	35:35	0.7
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	CC	94%	100%	0.3
	CT	6%	0%	0.09
	TT	00	00	

4. DISCUSSION

Involvement of MTHFR in hcy-methionine cycle makes it an important player to investigate the underlying 'T' allele/genotype frequency in MI. The prevalence of MTHFR 677C→T gene polymorphism varies substantially with ethnic and racial groups worldwide; the present study was conducted to investigate the association between MTHFR gene Polymorphism and the risk of MI among the Sudanese population. The results showed that, the frequency of CC, genotype was more frequent in both control and patients (100, 94%) respectively, the heterozygous genotype (CT)was (6%) in patients and (0,0%) absent in control samples, There was no statistically significant association between MTHFR gene polymorphism and MI. Many studies concerning with MTHFR gene polymorphism and risk of MI in different populations showed inconsistent results. The in Tunisian population by Kerkeni *et al; 2006* reported, no association between MTHFR gene polymorphism and the prevalence of MI, also in Maroco Hmimech *et al* 2016, no significant association was observed between the C677T MTHFR variant and

Myocardial infarction risk, in addition to study performed in Northern European by Anderson., *et al* 1997, no relation of the MTHFR C677T mutation with Myocardial infarction risk, also Lewis *et al* 2005 in Europe, North America, or Australia, no strong evidence exists to support an association of the MTHFR 677 C→T polymorphism and myocardial infarction, also a In a report from Egyptian population, by Ibrahiem *et al*; 2009, no significant association was shown between T allele and myocardial infarction, another study from Pandey *et al* (2011), which found no significant association between T allele and MI in Indian patients, also Eftychiou e*t al*;2017 in Nicosia General Hospital, the existence and extent of disease are not significantly associated with MTHFR C677T and MTHFR polymorphisms, in addition to study in china Lingli *et al*; 2017, no significant association between MTHFR C677T polymorphism and MI risk, also Schneider JA *et al* 1998 study from sub-Saharan Africa that included genotype information, no *C677T* homozygotes were identified among the 234 individuals tested. The *C677T* allele frequency was 7 percent, In another study of 89 Africans from four tribes in sub- Saharan Africa, the allele frequency was also 7 percent; the frequency of homozygosity was not reported. These estimates suggest that the *C677T* allele is less common in African Blacks than in some other ethnic groups. The frequency of *C677T* homozygosity among Blacks living outside of Africa (e.g., in Brazil and the United States) was also low, between 1 and 2 percent. (Schneider JA *et al* 1998).

On the other hand, there are many studies disagree with our finding, all of them reported a significant association between TT genotype and risk of MI, (Kerkeni *et al*; 2006, Lewis *et al*; (2005) &Nasiri et al; (2014)).

The result of the present study showed no statistically significant difference in the age group of patients with different C677T 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 a*l; (2001) & Anderson; *et al* (1997), all of them reported no association between age, and C677T genotypes in patients with MI. In contrast, Butler *et al*; (2018) & Nishhama *et al*; (2007*)* suggesting that the age of patients may be associated with MI occurrence.

The current study also showed, no statistically significant association was found between gender and C677T genotype, some study agree with the result (Tripathi *et al*; (2010), Hmimech *et al*; (2016)) and other dis agree with the present study (& Nishhama *et al*; (2007*)*).

In the present study, no interaction between C677T polymorphism and the conventional MI risk factors (DM, HTN, and smoking, obesity, alcohol abuse) was reported. This finding was in agreement with a study by Anderson; *et al* 1997 who also reported no significant association between C677T polymorphisms and any other risk factor among patients with acute coronary syndrome. Also, Butler *et al*; 2018 found no statistically significant association of smoker with Myocardial patients, in addition Hmimech *et al;* (2016) no statistically significant association in diabetic, smoker, hypertension and obesity with Myocardial risk. Our result disagrees with the finding of Glue *et a*l; (2001) strongly statistically significant association of smoker, diabetic, hypertension, and with Myocardial risk. Variations of results regarding the association of the C677T polymorphism and MI in different populations can be interpreted by what is reported by Lewis *et al*; 2005 that, differences in C677T polymorphism are due to folate intake, which lowering homocysteine, and has a role in prevention of cardiovascular disease is in some doubt also Jakó *et al*; 2017, in case report study, treated with folate supplements.

CONCLUSION

The present study conclude that the homozygous C/C genotype of MTHFR gene was more frequent in the healthy controls, while the heterozygous C/T genotype was more frequent in MI patients; the homozygous T/T genotype was completely absent in both study groups. However, there was no statistically significant association between MTHFR C677T polymorphism and risk of MI among the Sudanese population.

ACKNOWLEDGEMENTS

The authors thank all the participants for their cooperation, sudan heart center for given the samples for this study, Alneelin university for help and assist me in practice, all thanks for Dr: Salah Gmaa for their great effort.

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