Methylenetetrahydrofolate reductase gene (C677T and A1298C) polymorphisms and hyperhomocysteinemia as risk factors for Myocardial Infarction

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Conflicting data concerning the association between methylenetetrahydrofolate reductase gene *MTHFR* (C677T and A1298C) polymorphisms with or without hyperhomocysteinemia and myocardial infarction exists and data from developing countries is limited thus, a current study initiated to study whether any association exist between the above polymorphisms and myocardial infarction among Kurdish patients from Duhok province/Iraq.

A case-control study was performed in Azadi teaching hospital/Duhok/Iraq and included 75 patients with acute myocardial infarction and 75 age and sex-matched normal controls. All the patients and controls had their methylenetetrahydrofolate reductase gene analyzed for C677T and A1298C polymorphism using polymerase chain reaction/restriction fragment length polymorphism technique as well as their homocysteine level.

Methylenetetrahydrofolate reductase (*MTHFR*) gene polymorphism had been studied extensively and had been implicated in the pathogenesis of different diseases including coronary heart diseases, psychological diseases "Alzheimer's disease, depression, autism", various cancers and malignancy as well as in increasing fetal morbidity and mortality such as recurrent fetal loss and neural tube defect through mechanisms related directly or indirectly to hyperhomocysteinemia[1,2]. *MTHFR* (C677T) results in the production of thermolabile *MTHFR* variant with the half activity of the normal enzyme and is associated with mild hyperhomocysteinemia particularly in the presence of folic acid deficiency[3]. *MTHFR* (C677T) is a common variant occurs at increasing frequency in Iraq and surrounding Mediterranean countries[4].

Although the second common *MTHFR* variant (A1298C) show normal *MTHFR* enzyme activity and its associated with normal homocysteine level[5], it has been studied extensively and had been contributed to various disorders including coronary heart disease through its effect on the conversion of MTHF to BH4 (tetrahydrobiopterin, an important cofactor in the creation of neurotransmitters), and the production of nitric oxide[6,7].

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Hyperhomocysteinemia might arise from *MTHFR* (C677T) polymorphism as well as from other non-genetic conditions including renal failure, increasing alcohol consumption, vitamin B6 deficiency, folate, and vitamin B12 deficiency[7,8]. Although different mechanisms postulated in homocysteine-induced coronary heart disease including vascular endothelial dysfunction, nitrous oxide secretion inhibition, prostaglandin dysregulation and endothelium-derived hyperpolarizing factors (EDHF) inhibition[9]; a query still exists about the real mechanism and the exact pathogenesis. That is why the current study imitated to find any role of *MTHFR* gene polymorphisms and hyperhomocysteinemia in the pathogenesis of catastrophic arterial diseases-myocardial infarction among Kurdish patient from Duhok/Iraq.

Materials and Methods

The current study represents a case-control study. A total of 75 patients with acute myocardial infarction (AMI) attending the coronary care unit (CCU) of Azadi teaching hospital in Duhok/Iraq were enrolled. These patients were diagnosed as described by the universal definition of myocardial infarction[10]. Also, 75 age and sex-matched subjects with no history of myocardial infarction were recruited for the purpose of the current study and used as control. Individuals with hyperlipidemia, uncontrolled diabetes, renal failure, or morbid obesity were excluded[11]. At first,

informed consent was gained from all enrolled patients and the study was approved by the appropriate ethical committee at the Duhok directorate of health and Duhok College of Medicine.

Supportive information was collected from all enrolled individuals and then blood was collected in gel and K2- EDTA tubes from each enrollee and all had their DNA extracted by a modified salting-out extraction method adopted by Iranpur-Mubarakeh and his colleagues that yield high quantity with high purity DNA[12,13].

Genotyping was performed by polymerase chain reaction-restriction fragment length polymorphism (PCR–RFLP) method. For *MTHFR* (C677T) polymorphism, the DNA was subjected to amplification with primers: 677-F: 5′GCCTCTCCTGACTGTCATCC3′ and 677-R: 5′GGAGCTTATGGGCTCTCCTG3′ to produce a 254 bp fragment, then digested with *HinfI* restriction enzyme and finally subjected to electrophoresis. Following gel electrophoresis on 2% agarose (Fig. 1),

individuals with wild 677CC variant will show only 254 bp band; individuals with heterozygous 677CT variant will show three 254, 147, 107 bands pattern and individuals with homozygous 677TT will show 147 and 107 bp pattern[14].

For *MTHFR* (A1298C) polymorphism; amplification performed with primers 1298-F: 5′-CTTTGGGGAGCTG AAGGACTACTAC-3′ and 1298-R: 5′-CACTTTGT GACCATTCCGGTTTG-3′ to produce an 84 bp fragment, then digested with *MboII* restriction enzyme and finally subjected to polyacrylamide electrophoresis (Fig. 2). Individuals with wild 1298AA variant will show two bands 56 bp and 28 bp pattern; individuals with heterozygous 1298AC variant will show three 84, 56, and 28 bp bands pattern and individuals with homozygous 1298CC will show single 84 bp band pattern[14].

After completion of molecular work, statistical analysis done with allele frequencies calculation and comparisons of different allele frequencies between patients and control groups using $\chi2$ test for genotype and allele frequencies using SPSS windows version 23.0 software. A level of *P* <0.05 was considered statistically significant.

Fig. 1 — Different pattern was seen following amplification with specific primers for *MTHFR* C677T polymorphism and enzyme digestion with *HinfI* enzyme. Individuals with wild 677CC variant show only 254 bp band (lanes 3, 5); heterozygous 677CT variant show three 254, 147, 107 bands pattern (lanes 2, 4, 6, 7) and homozygous 677TT showing 147 and 107 bp pattern (lane 1). Electrophoresis was performed on 2% agarose gel electrophoresis, run at 95-100 volt/cm for 60-90 min. lane L: represents DNA Marker of (100-1500 bp).
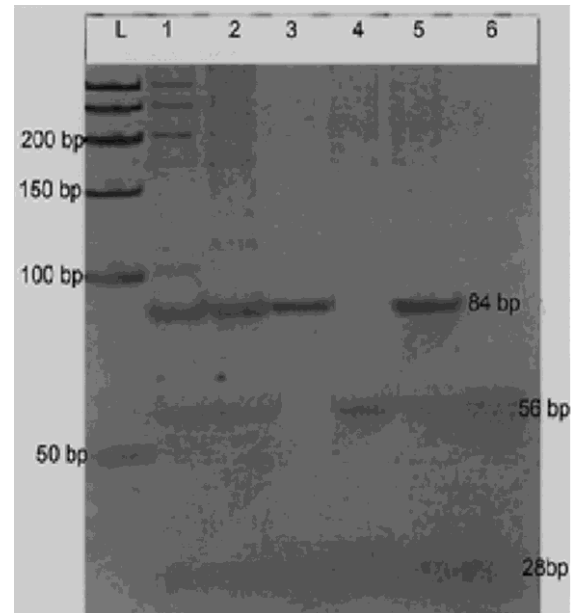

Fig. 2 — Different pattern was seen following amplification with specific primers for *MTHFR* A1298C polymorphism and enzyme digestion with *MboII* enzyme. Individuals with wild 1298AA variant show two bands 56 bp and 28 bp pattern (lanes 4,6); heterozygous 1298AC variant show three 84, 56, and 28 bp bands pattern (lanes 1,2,5) and homozygous 1298CC showing single 84 bp band pattern (lane 3). Electrophoresis was performed on Acrylamide gel electrophoresis, run at 95-100 volt/cm for 90-120 min. lane L: represents DNA Marker of (50 bp).

Results

The age of the enrolled patients ranged from 29 to 85 years with a median age of 59 years (mean ± SD = 57.96 ± 11.45 years). From these patients, 43 (57.3%) were males and 32 (42.7%) were females with male to female ratio of 1.34:1. While the control group had ages ranging between 36 to 75 years with a median age of 55 years (mean ± SD = 54.62 ± 10.33 years) with the same male: female ration as patients.

Serum homocysteine level for both patients and controls shown in (Table 1) and it reveals that in patient's group were ranged from 5.32-111.34 µM/L with a median 13.23 µM/L, while it was 4.11-52.1 µM/L with a median of 12.32 in the control group. No significant difference detected between both groups ($P = 0.271$).

Data analyses for genotypic and allelic frequency for this study are shown in (Table 2). For *MTHFR* C677T polymorphism they reveal that DNA from 42 patients and 36 controls showed the persistence of the same 254 bp segment following digestion with *Hinf I* enzyme, which was consistent with the 677CC variant. While 25 patients and 32 controls showed three bands (254, 147, and 107 bp) pattern of heterozygous 677CT state and the remaining 8 patients and 7 controls showed two bands (147 and 107 bp) pattern of the homozygous 677 TT state. Using the Chi-square test, no significant difference detected between both groups regarding *MTHFR* mutation with ($P = 0.4892$). Also when sex was taken into consideration no significant difference detected

between males of both groups regarding *MTHFR* mutation with ($P = 0.4761$) and the same is applied to female with ($P = 0.8456$).

For *MTHFR* A1298C polymorphism they reveal that DNA from 36 patients and 37 controls showed two band pattern (56 and 28 bp) following digestion with *MboII* enzyme, which was consistent with the 1298AA variant. While 31 patients and 28 controls showed three bands 84, 56, and 28 bp pattern of heterozygous 1298AC state and the remaining 8 patients and 10 controls showed single band (84 bp) pattern of the homozygous 1298CC state. Using the Chi-square test, no significant difference detected between both groups regarding *MTHFR* A1298C mutation with ($P = 0.8765$). Also when sex was taken into consideration no significant difference detected between males of both groups regarding *MTHFR* mutation with ($P = 0.9854$) and the same is applied to female with ($P = 0.9125$).

Discussion

Myocardial infarction is a multifactorial disorder that arises from the interaction of inherited genetic elements with the environmental factors that lead to atherosclerosis and coronary artery occlusion. The same is applied to hyperhomocysteinemia that might arise from the same previous interactions and large numbers of studies from different areas of the world have claimed hyperhomocysteinemia to be a risk factor for arterial diseases including myocardial infarction[15] and cerebrovascular accident[4]. However, despite this a discrepancy still exists for two reasons, firstly they fail to show any reduction in recurrence rate of such catastrophic disorders following therapy with B_{12} and folate that led to reduction in serum homocysteine level[16,17] and secondly almost all studies including the current study have shown that *MTHFR* (C677T) polymorphism were associated with mild to moderate elevation of serum homocysteine level and they fail to show any correlation of such polymorphism with increased risk of myocardial infarction[17].

The current study revealed none significant higher level of serum homocysteine among myocardial infarction patient in comparison to that of control and this contradictory result to the majority of previous studies may reflect different genetic and environmental factors including dietary folate load as well as the ethnic background of Kurdish people living in the region. The other data were consistent with previous studies including a positive correlation of *MTHFR* (C677T)

Table 1 — Homocysteine levels in the patient and control group

Group (no.)	Mean	SD	Median
Patients	17.48	20.22	13.23
Controls	15.36	10.43	12.32

Table 2 — Results of *MTHFR* gene polymorphisms among patients and control

Genotype	Patients	Patients	Controls
MTHFR C677T	CC	42 (56%)	36 (48%)
	CT	25 (33.3%)	32 (42.7%)
	TT	8 (10.7%)	7 (9.3%)
Allele frequency	C	109/150 (72.7%)	104/150 (70.3%)
	T	41/150 (27.3%)	46/150 (30.7%)
MTHFR A1298C	AA	36 (48.0%)	37 (49.3%)
	AC	31 (41.3%)	28 (37.3%)
	CC	8 (10.7%)	10 (13.3%)
Allele frequency	A	103/150 (68.7%)	102/150 (68.0%)
	C	47/150 (31.3%)	48/150 (32.0 %)

polymorphism with a higher level of homocysteine level and negative correlation of *MTHFR* (A1298C) with increased homocysteine level[18].

Both *MTHFR* (C677T) and *MTHFR* (A1298C) variants occur in the region at a polymorphic rate and this was consistent with the previous data from the surrounding countries, Middle East, European, North American, Australian and Asian countries[4,17]. The current study fails to show any correlation of the above two variants with myocardial infarction and this was consistent with most data from surrounding countries[17,19].

Lewis *et al*. (2005) have drawn a conclusion from previous meta-analyses and reach to the fact that folic acid, through lowering homocysteine, has failed to have any role in the prevention of cardiovascular disease and this may augment our study that refutes any role of hyperhomocysteinemia in the causation of myocardial infarction[20]. And finally, It is not clear that homocysteine, as well as *MTHFR* (C677T, A1298C) polymorphisms, has a causal role in atherosclerosis and current study do not support the pharmacological lowering of homocysteine with folate and B_{12} therapy.

Conclusion

No significant differences were found regarding the methylenetetrahydrofolate reductase (C677T and A1298C) polymorphisms and total homocysteine levels between patients with myocardial infarction and controls.

References

1   Trimmer E, Methylenetetrahydrofolate reductase: biochemical characterization and medical significance. *Curr Pharm Des*, 19 (2013) 2574.
2   Balaji G & Sinha S, Autism spectrum disorder (ASD): A current review of assessment, risk factor and prevention. *Indian J Biochem Biophys*, 55 (2018) 375.
3   Reilly R, McNulty H, Pentieva K, Strain J & Ward M, *MTHFR* 677TT genotype and disease risk: is there a modulating role for B-vitamins?. *Proc Nutr Soc*, 73 (2014) 47.
4   Al-Allawi N, Avo A & Jubrael J, Methylenetetrahydrofolate reductase C677T polymorphism in Iraqi patients with ischemic stroke. *Neurol India*, 57 (2009) 631.
5   Yakub M, Moti N, Parveen S, Chaudhry B, Azam I & Iqbal MP, Polymorphisms in *MTHFR*, MS and CBS genes and homocysteine levels in a Pakistani population. *PLoS One*,7 (2012) e33222.
6   Nagele P, Brown F, Francis A, Scott M, Gage B & Miller J, Influence of nitrous oxide anesthesia, B-bitamins, and *MTHFR* gene polymorphisms on perioperative cardiac events: the vitamins in nitrous oxide (VINO) randomized trial. *Anesthesiol*, 119 (2013) 19.
7   Mitchell ES, Conus N & Kaput J, B vitamin polymorphisms and behavior: Evidence of associations with neurodevelopment,

depression, schizophrenia, bipolar disorder and cognitive decline. *Neurosci Biobehav Rev*, 47 (2014) 307.
8   Oliva F, Coppola M, Mondola R, Ascheri D, Cuniberti F, Nibbio G & Picci RL, Blood homocysteine concentration and mood disorders with mixed features among patients with alcohol use disorder. *BMC Psychiatry*, 17 (2017) 181.
9   Ganguly P & Alam SF, Role of homocysteine in the development of cardiovascular disease. *Nutr J*, 14 (2015) 6.
10 Thygesen K, Alpert JS, Jaffe AS, Chaitman BR, Bax JJ, Morrow DA & White HD, The Executive group on behalf of the Joint European Society of Cardiology (ESC)/American College of Cardiology (ACC)/American Heart Association (AHA)/World Heart Federation (WHF) Task Force for the Universal Definition of Myocardial Infarction. Fourth universal definition of myocardial infarction. *Eur Heart J,* 138 (2018) e618.
11 Thygesen K, Alpert JS, Jaffe AS, Simoons ML, Chaitman BR & White HD, & Joint ESC/ACCF/AHA/WHF writing group, Third universal definition of Myocardial Infarction. *Circulation*, 126 (2012) 2020.
12 Iranpur-Mubarakeh V & Esmailizadeh AK, Rapid extraction of high quality DNA from whole blood stored at 4°C for long period. IOP Publishing protocol-online.org.2010. (http://www.protocol-online.org/prot/Protocols/Rapid-Extraction-of-High-Quality-DNA-from-Whole-Blood-Stored-at-4-C-for-Long-Period-4175.html). Accessed 14 March 2018.
13 Kashmoola M, Eissa A, Al-Takay D & Al-Allawi N, Molecular characterization of G6PD deficient variants in Nineveh province, Northwestern Iraq. *Indian J Hematol Blood Transfus*, 31 (2015) 133.
14 Aleyasin A & Mirakhorli M, Association of the *MTHFR* C677T polymorphism and fragile X syndrome in an Iranian population. *Neurol Asia*, 17 (2012) 347.
15 Skovierová H, Vidomanová E, Mahmood S, Sopková J, Drgová A, Červeňová T, Halašová E & Lehotský J, The molecular andcellular effect of homocysteine metabolism imbalance on human health. *Int J Mol Sci*, 17 (2016) 1733.
16 Martí-Carvajal AJ, Solà I, Lathyris D & Dayer M, Homocysteine-lowering interventions for preventing cardiovascular events. *Cochrane Database Syst Rev*, 8 (2017) 1.
17 Hmimech W, Idrissi H, Diakite B, Baghdadi D, Korchi F, Habbal R & Nadifi S, Association of C677T *MTHFR* and G20210A FII prothrombin polymorphisms with susceptibility to myocardial infarction. *Biomed Rep*, 5 (2016) 361.
18 Tanis BC1, Blom HJ, Bloemenkamp DG, van den Bosch MA, Algra A, van der Graaf Y & Rosendaal FR, Folate, homocysteine levels, methylenetetrahydrofolate reductase (*MTHFR*) 677C --> T variant, and the risk of myocardial infarction in young women: effect of female hormones on homocysteine levels. *J Thromb Haemost*, 2 (2004) 35.
19 Bahadır A, Eroz R & Türker Y, Does the *MTHFR* C677T gene polymorphism indicate cardiovascular disease risk in type 2 diabetes mellitus patients?. *Anatol J Cardiol*, 15 (2015) 524.
20 Lewis SJ, Ebrahim S & Smith GD, Meta-analysis of *MTHFR* 677C→T polymorphism and coronary heart disease: does totality of evidence support causal role for homocysteine and preventive potential of folate?. *BMJ*, 331 (2005) 1053.