Association Between Methylene tetrahydrofolate Reductase (MTHFR) Gene (C677T) Polymorphism and Stroke predisposition in Bahraini Population

Manal A. Fadl1*, Adel A. AlJishi2, Inas AlHarbi3, Safa M.Taha4, Moiz O. Bakhiet4

1Faculty of Science and Technology, Al Neelain University, Khartoum, Sudan.
2Salmanyia Medical Complex, 3Ministry of Health-Kuwait,
4Dept. of Molecular Medicine, CMMS, Princess Al-Jawhara Center, Arabian Gulf University, Manama, Bahrain

*Corresponding author email: manalfadl1@hotmail.com

Abstract

Background: The elevated plasma homocysteine level has been identified as a risk factor for cerebrovascular diseases. This Hyperhomocysteinaemia occurs "in part" as a result of polymorphism/s in methylenetetrahydrofolate reductase (MTHFR) gene. Of these polymorphisms is The C677T which may represent an important genetic risk factor in vascular diseases.

Aim: This study aim to assess the relationship between the presence of the C677T polymorphism of the MTHFR gene and the risk of stroke in Bahraini population.

Method: The study group consisted of 93 stroke patients and 105 age- and sex-matched control subjects free from stroke. The MTHFR gene mutation was detected by PCR followed by RFLP using HinfI restriction enzyme.

Results: Of the stroke cases; 77.4% were found to have the C677T wild type homozygous (CC) and 22.6% being heterozygous (CT) and homozygous (TT) for the mutant type.

The mutant genotypes (CT and TT) of the MTHFR did not differ between patients and control subjects (OR=0.49; 95% CI 0.26–0.92). This indicates that this mutation is unlikely to play a major role in stroke predisposition in our sample.

On the other hand, high frequency of hypertensive (71.4%), diabetics (67%), heart diseases (30.2%) and having previous stroke (28.9%) was observed among
stroke patients. Also high cholesterol level, sedentary life and smoking were common among patients (71.4%, 85.4% and 35.5% respectively).

Conclusions: Our results suggest that these common risk factors exert more influence on stroke predisposition than the MTHFR C677T polymorphism in Bahraini population.

Keyword: Bahraini population, MTHFR polymorphism, stroke risk factors,

INTRODUCTION

Stroke receives an increasing importance worldwide due to its high incidence of mortality and disabilities among large scale of survivors (Martin and Salim 2009; SCLAIBORNE ET AL., 2009). Over a third of stroke deaths occur in developing countries (Benamer and Grosset, 2009). Arab countries have witnessed a marked a change in lifestyle and diets (Benamer and Grosset, 2009) which are known to increase the risks of having metabolic syndrome such as diabetes, hypertension and heart diseases (all are known risk factors of stroke) (Steven et al., 1990).

In Bahrain, of the 2301 patients studied; 16.4% are hypertensive, 4.2% with heart attack and 0.4% stroke (Abeer, 2008). Also a recent study showed that the incidence of stroke in Bahrainis is rising over the last 16 years (110/100 000) (Al Banna et al., 2015) compared to the incidence of 57/100 000 reported 1995 (Al-Jishi and Mohan, 2000).

As the stroke is known as a multifactorial disease; Special interest was directed towards studying genes` polymorphisms that might contribute to the disease predisposition. Of the genes studied is the Methylenetetrahydrofolate reductase (MTHFR) gene (located in chromosome 1 p36.3) (Rosenblatt, 1995) which is known to play a vital role in folate and homocysteine metabolism by catalyzing the conversion of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, this inturns helps convert the amino acid homocysteine to another amino acid; the methionine. A common missense polymorphism in this gene (C677T, Ala --> Val at position 222) is associated with 50% reduction in enzyme activity.
(Kang et al., 1988) due to thermolability (Frosst et al., 1995) leading to an elevated plasma homocysteine (Ma et al., 1996); which results in vascular abnormalities including endothelial cell injury, increased platelets aggregations and abnormalities in clotting cascades, thereby The C677T of MTHFR polymorphism has been extensively investigated as a risk factor for several vascular disorders such as atherosclerosis, carotid artery disease (Kawamoto et al., 2001; Kadziela et al., 2003 and Ezzat et al., 2014), stroke (Huang et al., 2002; Simon et al., 2005; Alluri et al., 2005; Zhang et al., 2009; Almawi et al., 2009 and Fekih-Mrissa et al., 2013) however the results are controversial.

Given the important role of MTHR in the regulation of vascular functions (Charalambos et al 2009), we hypothesized that the genetic variant in MTHFR (C677T, Ala --> Val (rs1801133) might be associated with stroke predisposition in Bahrainis.

Materials and methods

Demography

The Bahraini population consists of an Arabian Peninsula population. The population consist of 1,314,089 individuals, the immigrants make up almost 55% of the total population and the Bahrain 45% (= 591,340). 97.3% of the population are ≤65 age and those above age 65 were 2.7%. The obesity - adult prevalence rate is 32.9% (Bahrain Demographics Profile 2014).

Sample collection and DNA extraction

A case-control study was conducted. Only Bahraini individuals were included, excluding expatriates and non Bahraini nationalities.

Blood was collected in EDTA tube from 93 of proven stroke patients and 105 unrelated controls without having history of stroke. Stroke cases were perfectly diagnosed by a consultant of neurology at Salmaniya Medical Complex (SMC); a co-author in this article. Brain CT scan was performed in all patients within 24 hours of onset of stroke.
INFORMED CONSENT WAS OBTAINED FROM PATIENTS AND CONTROLS. THE STUDY WAS APPROVED BY THE ETHICAL COMMITTEE OF THE ARABIAN GULF UNIVERSITY (AGU) AND (SMC), MINISTRY OF HEALTH, BAHRAIN.
Genomic DNA was extracted from EDTA buffy coat by the MagNA Pure Compact System. The quality and quantity of the DNA was checked on NanoDrop™ Spectrophotometer.

GENOTYPING OF THE MTHR POLYMORPHISM

In this study we conducted a (PCR-RFLP) analysis of MTHFR missense polymorphism (C677T, Ala -- Val (rs1801133)).

PCR amplification was performed in a 15μL reaction mixture containing 7μL of Promega mixture, 1 μL of 10 pmol/μL of each primer (F5’-CTC GCC TTG AAC AGG TGG AG-3’ and R5’-CTG GAT GGG AAA GAT CCC GG-3’), 3μL of ddH2O and 3.μL of 50ng/μL of template DNA. A negative control containing RNAase free water instead of genomic DNA was also prepared. The PCR was carried in an automated thermocycler with a PCR protocol consists of an initial denaturation for 4 min at 95°C, followed by 35 cycles of denaturing at 95°C for 30sec, annealing at 62°C for 1 min and extension at 72°C for 1min, then ended with final extension at 72°C for 10 min. 2ul of the amplicon of the polymorphism was digested with 0.5ul of HinfI restriction enzyme and 8.5ul buffer (Fermentas, thermoscientific), incubated for 3 hours at 37°C then the digested product was separated on 3% agarose gel stained with Gel Star or ethidium bromide stain and visualized with UVTEC CAMBRIDGE, TLC imaging system.

The HinfI restriction enzyme recognizes the sequence 5’-GANTC-3’, therefore, the product of MTHFR sequence that contains the wild type the C allele (5’- GAGCC -3’), was undigested and gave the size of 248bp. For the mutant type; the sequence contains the recognition site 5’-GAGTC-3’, thereby the product size was 130 bp and 118 bp for the mutant homozygous (TT) and 248bp, 118 bp and 130 bp for the mutant heterozygous (CT).
**Statistical Analysis**

SPSS version 21 statistical package was used to count the genotype, chi square, $p$ value, odds ratio (OR) and 95% confidence interval (CI). Statistical significance was determined at $p<0.05$. ORs with 95% CI was used to assess the strength of the association of the $MTHFR$ C677T polymorphism with the risk of stroke.

**Results**

Our results showed that about one third of stroke cases (32.3%) are at age≤60 and 28.9% of stroke patients have encountered previous attack. Of the 85 Ischemic cases, 49 were males compared to 36 were females, 7 were hemorrhagic and the one case of the transient ischemic attack TIA was male.

An insignificant association between the mutant genotypes (CT and TT) of the $MTHFR$ C677T polymorphism and stroke predisposition was observed (OR=0.49; 95% CI 0.26–0.92) (Table 1).

High frequencies of disease risks such as hypertension (71.4%), Diabetes (67%), heart diseases (30.2%) and atrial fibrillation (15.2%) was observed. Duration of more than 10 years of either hypertension and/or diabetes was reported in 45.8-51.9% of strokes

In addition high cholesterol level, sedentary life, smoking and alcohol consumption were also reported among strokes (71.4%, 85.4%, 35.5% and 4.5% respectively) (Table 1). The majority of the patients (888.4%) have normal body mass index.

**Table 1** Shows frequency of stroke risk factors in cases and controls

<table>
<thead>
<tr>
<th></th>
<th>Cases</th>
<th>Controls</th>
<th>P value/ OR</th>
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<tbody>
<tr>
<td>Age group≤60</td>
<td>32.3%</td>
<td>35.7%</td>
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</tr>
<tr>
<td>Age group≥60</td>
<td>67.8%</td>
<td>64.3%</td>
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<tr>
<td>Gender</td>
<td></td>
<td></td>
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<tr>
<td>Males</td>
<td>55.9%</td>
<td>54.3%</td>
<td></td>
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<tr>
<td>Females</td>
<td>44.1%</td>
<td>45.7%</td>
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<tr>
<td>Type of stroke:</td>
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<tr>
<td>Ischemic</td>
<td>91.4%</td>
<td>-</td>
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<tr>
<td>Hemorhagic</td>
<td>7.5%</td>
<td></td>
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<tr>
<td>TIA</td>
<td>1.1%</td>
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<tr>
<td>Genotype of MTHFR C677 polymorphism</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>CC</td>
<td>77.4%</td>
<td>62.9%</td>
<td></td>
</tr>
<tr>
<td>CT and TT</td>
<td>22.6%</td>
<td>37.1%</td>
<td></td>
</tr>
<tr>
<td>Previous stroke</td>
<td>28.9%</td>
<td>0%</td>
<td></td>
</tr>
<tr>
<td>Family History of stroke</td>
<td>9.7%</td>
<td>0%</td>
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<tr>
<td>Risk factor Diseases:</td>
<td></td>
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<tr>
<td>Hypertension</td>
<td>71.4%</td>
<td></td>
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<tr>
<td>Diabetes</td>
<td>67%</td>
<td>0%</td>
<td></td>
</tr>
<tr>
<td>Heart disease</td>
<td>30.2%</td>
<td>0%</td>
<td></td>
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<tr>
<td>Atrial fibration</td>
<td>15.2%</td>
<td>0%</td>
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<tr>
<td>Carotid disease</td>
<td>6.7%</td>
<td>0%</td>
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<tr>
<td>Sickle cell</td>
<td>1.1%</td>
<td>0%</td>
<td></td>
</tr>
<tr>
<td>No physical activities</td>
<td>85.4%</td>
<td></td>
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<tr>
<td>Cholesterol level≥5</td>
<td>71.4%</td>
<td></td>
<td></td>
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<tr>
<td>Smoking (cigare, tobacco, shisha)</td>
<td>35.5%</td>
<td>4.5%</td>
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<tr>
<td>Alcohol consumption</td>
<td></td>
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<tr>
<td>Body mass index BMI:</td>
<td>11.6%</td>
<td></td>
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<tr>
<td>Obese</td>
<td>88.4%</td>
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<td>Normal</td>
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In the samples examined, both cases and controls; the frequency of the mutant variants (C/T and T/T) is 30.3%. A slight gender differences were seen; in the distribution of this variants, with females showing more mutant variants (32.6%), compared to males (28.4%).

Figure 1 Shows PCR-RFLP of MTHFR C677T polymorphism: lane1 DNA 50bp marker, lane2 negative control, lane3,4,8,11and13 show undigested wildtype CC genotype, lane5,6 ,9,10,12 show mutant CT genotype. Lane 7and14 show mutant TT genotype
Discussion

Over the last few decades; Bahrain as many developing countries is experiencing rapid change in cultural and socio-economic status, which resulted in great changes from traditional to modernized lifestyle that is associated with over consumption of fatty foods, soft drinks and sedentary life. All these result in an increased incidence of many multifactorial diseases (Abdulrahman, 1998; Sokrab et al., 2002; Abeer, 2008) such as stroke. Beside the changes in lifestyle as stroke risks, there is now abundant evidence that genes’ polymorphisms in relation with numerous risk factors might increase the incidence of stroke in different ethnic groups (Flossmann et al., 2004; Razvi and Bone, 2006; James et al., 2011).

In this study we attempted to demonstrate the role of the C677T MTHFR gene polymorphism in the causation of stroke. To avoid the effect of population sub structuring, we select the Bahraini individuals excluding expatriates and non Bahraini residents.

Our results revealed that no significant association was found between MTHFR C677T mutant genotypes and stroke in Bahrainis (OR=0.49; 95% CI 0.26–0.92). This result is in accordance with (Kelly et al., 2002) finding who demonstrated; after a meta analysis of 19; small influence of MTHFR variant in determining susceptibility to ischemic stroke. Our result also agreed with (Meiklejohn et al., 2001) results which stated that the elevated plasma homocysteine concentrations" which is known to occur as a result of MTHFR C677T polymorphism" is not necessarily a causative and predate the stroke onset. Our result is also in accordance with (Brattstrom et al., 1998; Uçar et al., 2004; Zang et al., 2009; Nissar et al., 2015). On the other hand our result is inconsistent with (Alluri et al., 2005; Inusha et al., 2006; Almawi et al., 2009; Fekih-Mrissa et al., 2013) finding who demonstrated an influence of this polymorphism as a genetic stroke risk factor in different ethnic groups.
Stroke is known to have deleterious effects on the quality of life of survivors as it is associated with disabilities. Our results showed that about one third of stroke cases (32.3%) are at age ≤ 60 which is the reproductive age, indicating that stroke have negative impacts affecting work and income and also social relationships of the patients and imposing a heavy economic burden on the patients’ family.

High frequencies (30%-71.4%) of diseases risk factor of stroke such as hypertension, diabetes and heart diseases were observed and the frequency of other disease risks such as sedentary life, high cholesterol level and smoking was also high (71.4%, 85.4% and 35.5% respectively). These results are consistent with (Abdulrahman, 1998; Sokrab et al., 2002; Szolnoki et al., 2003; Benamer and Grosset, 2009) findings and indicate that these common risk factors could be claimed to influence stroke risks in Bahraini population rather than the MTHFR C677T polymorphism and also the reported high frequencies of these risk factors might explain the doubling of stroke cases reported recently by (Al Banna et al., 2015).

CONCLUSION

This result showed that the MTHFR C677T polymorphism is not a significant contributing factor to stroke predisposition in Bahraini population. Diseases` risk factor of stroke such as hypertension, diabetes and heart diseases and other stroke risks such as sedentary life, high cholesterol level and smoking could be significant contributing factors to stroke in this population.

To implement strategies for prevention of stroke in this population, a thorough management of these disease risk factors is needed.

COMPETING INTERESTS:

THE AUTHORS DECLARE THAT THEY HAVE NO COMPETING INTERESTS.
Acknowledgements

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