

PLASMA HOMOCYSTEINE IN PATIENTS OF MIGRAINE WITHOUT AURA

Faraz Ahmed Bokhari, Tania A. Shakoori*, Syed Ahmad A. Hassan**, Hamid J. Qureshi***, Ghulam A. Qureshi†

Department of Physiology, University of Health Sciences/Federal Postgraduate Medical Institute, *Faculty of Medicine, University of the Punjab, **Department of Neurology, Shaikh Zayed Medical Complex, ***Department of Physiology, Services Institute of Medical Sciences, †Department of Biochemistry, University of Health Sciences, Lahore, Pakistan

Background: Few studies have studied the role of homocysteine in migraineurs and have produced conflicting results. The MTHFR C677T genotype has been associated with increased risk of migraine in selected clinical samples. We assessed the association of the MTHFR C677T variant with migraine, the corresponding homocysteine levels and their correlation. **Method:** We studied 27 random adult migraineurs with aura (MWA), migraine without aura (MWOA), and 32 non-migraineurs (controls) from Lahore, Pakistan in this pilot study which is still under progress. **Results:** We found significant differences in homocysteine levels between various diagnostic groups (K-W test: $p=0.005$). One-way ANOVA, post-hoc tests revealed significant differences in homocysteine levels between Non-migraineurs, MWA ($p=0.002$, CI: 1.93 – 9.19) and MWOA ($p=0.002$, CI: -9.19 – -1.9). We found a significant association between the migraine group and C677T-MTHFR variant mutant allele (C/T) ($p=0.039$). We did not find a significant association between C677T-MTHFR variant and homocysteine levels. **Conclusion:** In this pilot study, we found plasma homocysteine levels to be significantly associated with MWOA. Additionally, plasma homocysteine levels were lower in MWA than in MWOA. Furthermore, we did not find a relationship between homocysteine levels and the MTHFR variant (SNP rs1801133). Lastly, there may be a relationship between the MTHFR variant (SNP rs1801133) and migraine in this population.

Keywords: Plasma homocysteine, Migraine

INTRODUCTION

Migraine is a benign and recurring syndrome of headache, nausea, vomiting, and other symptoms of neurological dysfunction in varying admixtures. Clinical diagnosis of migraine is based on International Classification of Headache Disorders-II (ICHD-II) criteria specified by the International Headache Society (IHS), which classifies migraine into two major groups; without aura (MWOA) and with aura (MWA). In contrast to MWOA, MWA patients experience distinguishing neurological disturbances known as 'aura', which may include visual and sensory symptoms in addition to speech disturbances.¹

Family and twin genetic studies demonstrate that migraine, especially MWA exhibits a genetic component.^{2,3} Migraine being a polygenic disorder, identification of exact genetic markers that cause or predispose to migraine is difficult due to many factors.⁴⁻⁶

Folate metabolism has been implicated in the pathogenesis of migraine. Two aspects are especially important in this regard; the role of homocysteine and the status of the C677T variant of the methylenetetrahydrofolate reductase (MTHFR) gene. Since migraine is a neurovascular disease⁷, the highly reactive amino acid, homocysteine has been postulated to play a role in migraine pathophysiology.^{4,8,9} A few studies have investigated the relationship between homocysteine and headache with conflicting results,

with some showing no association and others indicating there to be an association between MWA and homocysteine only.¹⁰⁻¹⁴ The conclusive role of homocysteinemia in migraine is therefore yet to be settled.¹⁵

The human MTHFR gene is on chromosome 1p36. A single nucleotide polymorphism, C677T; rs1801133) in exon 4 at codon 677 of the MTHFR gene (677C→T) causes cytosine to be replaced by thymidine, resulting in alteration in the thermolability of the MTHFR enzyme. In some populations, C677T-MTHFR variant is associated with migraine.^{12,18-22} It would be expected to exhibit significantly reduced MTHFR enzyme activity, leading to elevation in plasma homocysteine levels,^{16,17} thought to contribute towards migraine susceptibility. However, the relation between the C677T-MTHFR polymorphism (leading to altered MTHFR enzyme) and homocysteine (the product of this enzyme) does not seem to follow cause-and-effect rules. Many studies examining this association in various conditions have failed to confirm an association,²³⁻²⁷ and there is no conclusive data on this association in migraineurs. We investigated the role of homocysteine in this context in a pilot case-control study.

MATERIAL AND METHODS

Subjects were selected randomly from a tertiary care hospital in Lahore, Pakistan. Subjects were diagnosed as having migraine (with and without aura) based on a

questionnaire and examination by a hospital-based neurologist. Three diagnostic groups were specified as either MWA, MWOA (using the IHS criteria¹), or as being non-migraineurs (healthy individuals not exhibiting migraine symptoms). Based on medical records and history, subjects with known conditions affecting plasma homocysteine levels, such as coronary artery disease, chronic renal failure, malignancies and diabetes, were excluded in both cases and controls.²⁸ Participants were matched for sex and age within five years. Informed consent was obtained.

All subjects were asked to take a light meal the preceding evening and fast overnight (minimum 6 hours) before blood samples were drawn in the morning. 5 ml blood was drawn while the subject was sitting down. The blood was transferred into EDTA vacutainers, which were immediately either kept on ice or refrigerated at 4 °C. The samples were centrifuged at 5,000 rpm for 10 minutes within one hour to separate plasma from other blood constituents.²⁸ Two hundred L plasma was pipetted out into a sterile microcentrifuge tube for homocysteine estimation while the remaining blood was kept for DNA isolation.

Plasma homocysteine was determined by standard enzyme-linked immunoassay (EIA) using Axis® Homocysteine EIA (Ref: FHCY100, Axis-Shield Diagnostics Ltd. Dundee, United Kingdom).

The cellular portion of the collected samples was used to obtain DNA. Standard salting out procedure was used to extract DNA.²⁹ DNA fragments containing the C677T MTHFR variant were amplified by PCR using published oligonucleotides.¹² The sense primer sequence was 5'-TGA AGG AGA AGG TGT CTG CGG GA-3', while the antisense sequence was 5'-AGG ACG GTG CGG TGA GAG TG-3'. Restriction analysis was then performed (RFLP) using *Hinfl* and Agarose gel electrophoresis.

Fisher's exact test was used to test association of the C677T-MTHFR variant with migraine. Kruskal-Wallis test and one-way ANOVA (with post hoc test) were used to test the association of migraine with plasma homocysteine levels.

This research was approved by the Ethics Committee of University of Health Sciences, Lahore, in addition to being also approved by the Advanced Board of Studies and Research. Furthermore an approved consent form was administered and written consent obtained from all study participants.

RESULTS

There were 59 subjects, comprised of 32 controls and 27 migraineurs. Twenty (74%) had MWOA and 7 (26%) had MWA. There was no difference in mean

age or sex distribution between cases and controls ($p=0.51$ and $p=0.588$).

The difference between mean homocysteine levels in migraineurs and non-migraineurs was not significant (Table-1). There was significant association between homocysteine level and diagnosis by Kruskal-Wallis ($p=0.005$) and one-way ANOVA ($p=0.05$). Post-hoc testing (Tamhane) revealed significant differences in homocysteine levels between non-migraineurs, MWA ($p=0.002$, CI: 1.93 – 9.19) and MWOA ($p=0.002$, CI: -9.19 – -1.9), (Tables-2, 3). Multinomial regression of diagnosis against homocysteine levels and genotype show that homocysteine levels were significantly associated with MWOA ($p=0.025$, OR: 1.173, CI: 1.020–1.348).

There was an association between migraine and the C677T-MTHFR variant, with no control individuals carrying a T allele ($p=0.039$, Fisher's Exact Test) (Table-4), although the numbers studied were small.

Table-1: Mean Plasma Homocysteine level (moles/litre) in non-migraneurs

	Mean±SD	<i>p</i>
Non-Migraneurs (n=32)	23.43±5.61	Not significant
Migraneurs (n=27)	24.38±5.26	

Table-2: One-way ANOVA for homocysteine level and diagnosis (control, MWA, MWOA)

	Mean Value	<i>p</i>
Between groups	86.820	0.050*
Within group	27.390	

*Significant, plasma homocysteine level (moles/litre)

Table-3: Post Hoc Test (Tamhane) for homocysteine levels and diagnosis

Diagnosis (I)	Diagnosis (J)	<i>p</i>
MWA	Normal	0.053
	MWOA	0.002
MWOA	Normal	0.339
	MWA	0.002

Table-4: C677-TMTHFR Varian (SP: rs1801133) and Migraine

	Wild type C/C	Mutant (C/T)	<i>p</i>
Migraine	23	4*	Significant
Control	32	0	

DISCUSSION

We found plasma homocysteine levels to be significantly associated with MWOA. Additionally, plasma homocysteine levels were lower in MWA than in MWOA. Furthermore, we did not find a relationship between homocysteine levels and the MTHFR variant (SNP rs1801133). Lastly, there may be a relationship between the MTHFR variant (SNP rs1801133) and migraine in this population.

Homocysteine might have a direct role to play in migraine causation, especially keeping in view the role of homocysteine in terms of vascular

damage and migraine being regarded as a neurovascular disorder.^{7,17} There have been studies where hyperhomocysteinemia has been shown to be associated with MWA, while a similar association has not been noted in MWOA.¹¹ We have found increased homocysteine levels in MWOA as compared to MWA. This may indicate an ethnic difference.

An elaborate meta-analysis examining all major C677T-MTHFR related case-control studies around the world, concludes that there is no association between the said polymorphism and migraine, and that the studies included were deficient in their ethnic diversity.³⁰ Our study is the first in this regard in Pakistan. Our results show a significant association of the C677T-MTHFR variant in the migraineur group ($p=0.039$) in comparison with the control group. Additionally, this significance was lost when we looked within the migraine group. In our study we only found the C/T genotype. Since our sample size is small, we cannot rule out the presence of the T/T allele in our general population. These results however, can serve to form the basis of more extensive genetic studies in this population. This is important since T/T homozygosity is a recognized genetic risk factor for cardiovascular and cerebrovascular disorders.^{31,32}

There have been studies examining the role of the C677T-MTHFR variant and corresponding homocysteine levels in various disorders.¹⁷ It is interesting to note that though altered MTHFR activity is expected to affect corresponding homocysteine levels, yet this cause and effect relationship has not been seen in many studies on various diseases.³³⁻³⁵ In a population-based study, Scher *et al.* (2006) did not find a correlation between C677T-MTHFR variant and homocysteine levels in migraine patients.²⁰ In this study, we also did not find a significant correlation between the same parameters. Again this could mean that blood homocysteine level is independent of the MTHFR status and/or there are other metabolic considerations that influence this correlation. We will make available more results of this study as they come in.

CONCLUSION

There is a need to replicate this study in addition to studies elucidating other aspects of homocysteine metabolism in migraine.

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Address for Correspondence:

Dr. Faraz Ahmed Bokhari, Department of Physiology, UHS, Lahore/Federal Postgraduate Medical Institute, Shaikh Zayed Medical Complex Lahore. Cell: +92-333-4769344, Fax: +92-42-9230980.

Email: drfarazbokhari@yahoo.com