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Orignal article

Effects of MTHFR C677T gene polymorphism on migraine together with biochemical and clinical parameters in turkish population

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Abstract: To detect the relation between methylenetetrahydrofolate reductase (MTHFR) gene C677T polymorphism and both of clinical and biochemical parameters. One hundred sixty migraine patients and one hundred twenty control group were included in the current study. Pain intensity of individuals were measured, biochemical, clinical parameters and MTHFR C677T single nucleotide polymorphism were detected. Statistically higher occurrence MTHFR C677T genotype was detected in migraine group than control group. A statistically significant association between family history of migraine and MTHFR C677T genotypes was detected. Also, statistically higher BMI, homocysteine and the total cholesterol levels were detected in MO and MA groups than control groups. When we take into consideration of the clinical parameters, only statistically significant difference was detected between MA and MO for attack frequency (attack/ monthly). The triglyceride and homocysteine levels were significantly higher in males than females but HDL levels and folate were significantly higher in females than males. The frequency of CT genotype was significantly higher in cases with compression and allodynia than others in MA groups and cases with fatigue in both MA and MO subgroups. Also, cases without systemic complaints had significantly higher T allele frequency than cases with systemic complaints in the MA subgroup. Because the labor losses of patients with migraine may cause important economic losses, performed studies for the fully understanding of the disease including genetic and environmental factors are important for the prevention of negativity caused by the disease.

Key words: Migraine, methylenetetrahydrofolate reductase (MTHFR) gene, C677T polymorphism, clinical and biochemical parameters

Introduction

Migraine is an important neurological disorder causes primary headache and characterized by different (moderate or severe) recurrent and pulsating pain accompanied by nausea, vomiting, photo- and phonophobia (1). Migraine clinically diagnosed depending on the criteria depicted by the International Classification of Headache Disorders-II (ICHD-II) of the International Headache Society (IHS). The IHS subclassified into migraine with aura (MA) nearly 25 % of all migraines population and migraine without aura (MO) nearly70 % of all migraines. Both MA and MO subtypes have consequential symptomatic overlap, patients with MA have a clear phase of neurological disturbances, known as an "aura," which generally comes before the headache phase of an attack (2). Homocysteine is taken from the metabolism (demethylation) of the methionine via methionine cycle and folate cycle and it has been shown to be connected to the pathophysiology of migraine. The plasma homocysteine concentration was detected as higher in patients

with migraine than healthy controls (3). Therefore the severity and frequency of migraine may be decreased by the prevention of hyperhomocysteinemia with vitamin suplementation (4). Methylenetetrahydrofolate reductase(MTHFR) catalyzes the metabolism of homocysteine and has a key enzyme role in folate metabolism (5). To show whether is there a relation between MTHFR C677T and gender, migraine subtypes, clinical symptoms, and biochemical parameters, we performed the current study.

Materials and Methods

Study Population

One hundred sixty voluntary migraines patients (24 male and 136 female) (between 18 and 60 years) and one hundred twenty (49 male and 71 female) (between 18 and 60 years) voluntary control group consulted between January 2016- August 2019 were included in the current study after the approved by the local Ethics Committee (Erciyes University Ethic Number:2019/741). After the study, power analysis was performed and it was found that power is estimated at 0.9986 for alpha= 0.05 and effect size= 0.5923. All individuals were from Kayseri. All patients with migraine were evaluated according to a questionnaire based on the ICHD-II and classified as being MA or MO. All patients included in the study were headache-free for at least 7 days at the time of enrollment. The patients with vasculitis, diabetes mellitus, pregnancy (self-reported), myocardial infarction, stroke/transient ischemic attack (TIA) and systemic lupus erythematosus, use of non-steroidal anti-inflammatory drugs, anticoagulants (iv) or other antiplatelet agents in the week prior to testing were not included in the study.

The pain intensity of individuals was measured by a visual analog scale (VAS) (6). Pain score of each patients was defined as 0 = low pain (a score\10) and 1 = high pain (a score \10).

Total plasma homocysteine, vitamin B12, folate, LDL, HDL, triglyceride and total cholesterol levels were detected. Total genomic DNA was isolated from the peripheral blood samples of individuals and the detection of MTHFR C677T single nucleotide polymorphism (SNP) was performed via polymerase chain reaction–restriction fragment length polymorphisms (PCR–RFLP) using the Hinf I restriction endonuclease enzyme and the previously described pairs of primers (forward: 50-TGA AGG AGA AGG TGT CTG CGG GA-30 and reverse: 50-AGG ACG GTG CGG TGA GAG TG-30) (5). The PCR reaction protocol previously described by Kowa et al. (7) was used.

Statistical Analysis

Statistical analyses were carried out using the Statistical Package for Social Sciences (SPSS version 15.0). The data were given as mean ± SD and frequency. Kruskal–Wallis test was used for detection between the MTHFR C677T genotype and both biochemical and clinical parameters. Mann–Whitney U test was used for the evaluation of clinical and biochemical parameters according to gender. χ^2 test was used for the detection of the relation between migraine subgroups and the MTHFR and the relation between clinical parameters and MTHFR. The differences among all groups for clinical and biochemical parameters were detected using the One-Way ANOVA test. The $p<0.05$ value was accepted as statistically significant.

Results

The mean age of patients with migraine (MA:82 cases and MO:78 cases) and the control group were 38.1±8.7 and 36.3±7.4 years, respectively. Totaly 54 migraine cases (MO:26, MA:28) were CC genotypes, 63 cases (MO:31, MA:32) were CT genotypes and 43 cases (MO:21, MA:22) were TT genotypes. In the control group, 103 individuals were CC genotypes, 15 individuals were CT genotypes and 2 individuals were TT genotypes. In the migraine group, 171 cases have C alleles and 149 cases have T alleles. In the control group, 221 individuals have C alleles and 19 individuals have T alleles (Table 1). Statistically higher occurrence MTHFR C677T genotype was detected in the migraine group than the control group (p=0.000).

When we took into consideration the relation between MTHFR C677T genotypes (TT, CT, and CC) and both biochemical and clinical parameters, only a statistically significant association between family history of migraine and MTHFR C677T genotypes was

Table 1. Properties of individuals and frequencies of MTHFR C677T genotypes and alleles in migraine and control groups.

Group	Sub-group	Sex		N	Age (M ± SD)	Genotypes (%)			Alleles (%)	
		Male	Female			C/C	C/T	TT	C	T
Migraine	MO	13	65	78	38.1±8.7	26 (33.3)	31 (39.7)	21 (26.9)	83 (53.2)	73 (46.8)
	MA	16	66	82		28 (34.2)	32 (39)	22 (26.8)	88 (53.7)	76 (46.3)
Control		49	71	120	36.3.±7.4	103 (85.8)	15 (12.5)	2 (1.7)	221 (92.1)	19 (7.9)
Total		78	202	280						

N: Individual number, M:Mean, SD: Standart deviation, MA: Migraine with aura, MO: migraine without aura

detected (p=0.021, c2=7.645) (Table 2). In the control group, statistically significant differences were not detected among MTHFR C677T genotypes (TT, CT, and CC) for biochemical parameters (Table 3).

When we divided the patients as MA, MO, and control groups, a statistically higher BMI (Body mass index), homocysteine and the total cholesterol levels were detected in MO and MA groups than control groups (p=0.034, 0.038 and 0.021 respectively). When the group compared in pairs (as MO and MA), the BMI, homocysteine and the total cholesterol levels were not statistically significant between MA and MO groups (p>0.05). When we took into consideration the clinical parameters, only a statistically significant difference was detected between MA (2.424±2.428) and MO (3.928±3.764) (p=0.048) for attack frequency (attack/ monthly) (p=0.048) (Table 4).

In migraine groups, there were no statistically significant differences between males and females for age at onset of migraine, attack frequency, attack duration, pain intensity, LDL, total cholesterol, BMI and vitamin B12 levels (p>0.05). The triglyceride and homocysteine levels were significantly higher in males than females (p=0.001) but HDL levels and folate were significantly higher in females than males (p=0.002, p=0.004). The frequency of CT genotype was significantly higher in cases with compression and allodynia than others (p<0.05) in MA groups and cases with fatigue in both

Table 2. Association of MTHFR C677T genotypes and other parameters in migraine groups

PARAMETERS	MTHFR			p value	c2 value
	TT (Mean±SD)	CT (Mean±SD)	CC (Mean±SD)		
Biochemical parameters					
Vitamin B12 level (pg/ml)	331.503± 221.382	347.528 ± 320.412	361.113±275.213	0.743	0.589
Folate (ng/ml)	10.002 ± 4.381	10.875 ± 4.213	11.01± 3.1	0.315	2.415
Homocysteine (µmol/l)	15.875 ± 3.982	16.206± 4.105	16.678 ± 4.987	0.423	1.719
Triglyceride (mg/dl)	126.567 ± 56.124	137.715 ± 104.513	130.601 ± 87.231	0.427	1.785
Total cholesterol (mg/dl)	183.524 ± 57.205	190.231 ± 36.125	189.821 ± 41.351	0.483	1.114
HDL (mg/dl)	57.839 ± 15.412	56.542 ± 8.978	59.123 ± 25.274	0.121	3.562
LDL (mg/dl)	104.623 ± 28.389	111.217 ± 29.231	115.402 ± 31.023	0.142	3.682
BMI (Body mass index, kg/m^2)	26.813 ±4.796	27.201 ± 3.986	27.789 ± 4.235	0.915	0.298
Clinical parameters					
Pain severity (VAS)	8.546 ± 1.108	8.812 ± 1.285	8.757 ± 1.398	0.121	3.768
Attack frequency (attack/ mounthly)	3.123 ±2.985	3.215 ± 2.512	3.181 ± 3.789	0.831	0.365
Attack duration (hour)	32.321 ± 23.098	33.975 ± 19.987	34.102 ± 25.123	0.315	2.103
Age at onset of migraine (years)	21.974 ±6.867	23.743 ± 7.768	24.105 ±8.215	0.753	0.943
History family of migraine	Yes 28.00 ±0.00	43.00±0.00	26.00±0.00	**0.021**	**7.645**
	No 16.00±0.00	18.00±0.00	29.00±0.00		

HDL: High density lipoprotein, LDL: Low density lipoprotein, BMI: Body mass index, VAS: Visual analog scale, SD: Standard deviation, p<0.05 is statistically significant.

Table 3: Association of MTHFR C677T genotypes and other parameters in control groups

PARAMETERS	MTHFR			p value	c2 value
	TT (Mean±SD)	CT (Mean±SD)	CC (Mean±SD)		
Biochemical parameters					
Vitamin B12 level (pg/ml)	345.101± 2.203	352.102 ± 104.123	353.105±235.124	0.582	1.612
Folate (ng/ml)	12.582 ± 3.124	11.525± 5.102	11.322± 4.012	0.682	1.895
Homocysteine (μmol/l)	12.121 ± 4.213	12.764± 3.897	12.304 ± 4.101	0.416	1.693
Triglyceride (mg/dl)	127.312 ± 49.382	131.182 ± 75.954	132.531 ±91.125	0.293	2.324
Total cholesterol (mg/dl)	172.231 ± 68.282	174.576 ± 78.495	170.334 ± 52.233	0.541	1.387
HDL (mg/dl)	53.624 ± 20.121	49.231 ± 11.214	50.234 ± 23.142	0.132	3.211
LDL (mg/dl)	103.427 ± 30.102	105.285 ± 28.143	107.213 ± 33.127	0. 185	2.315
BMI (Body mass index, kg/m^2)	23.412 ±3.841	24.351 ± 4.102	24.615 ± 5.100	0.521	1.214

HDL: High density lipoprotein, LDL: Low density lipoprotein, BMI: Body mass index, VAS: Visual analog scale, SD: Standard deviation, p<0.05 is statistically significant.

MA and MO subgroups (p<0.05). Also, cases without systemic complaints had significantly higher T allele frequency than cases with systemic complaints in the MA subgroup (p=0.023). Conversely, there were no significant differences for other symptoms (sleeplessness, vomiting, nausea, vertigo, phonophobia, and photophobia, in both MO and MA subgroups (p>0.05). When we taken into consideration of migraine head localizations, there were no statistically significant differences among the MTHFR C677T genotype (TT, CT, and CC) for neck, overhead, forehead, nape, bilateral, unilateral, temple and around eyes (p >0.05).

Discussion

Migraine is a common neurological disorder related to symptoms like phonophobia, photophobia, nausea, vomiting (8). Different gene polymorphism such as Nitric Oxide Synthase Gene Polymorphisms (9) and MTHFR C677T (10) has been related to a range of diseases such as migraine. It was reported MTHFR genotypes are an indicator of migraine and this disease has a strong genetic component and the familial risk (10). According to our result, there is a statistically higher CT genotype in migraine patients

Table 4: Association of migraine subgroups and other parameters in migraine and control groups

PARAMETERS	Study groups			p value
	MA (Mean±SD)	MO (Mean±SD)	Control (Mean±SD)	
Biochemical parameters				
Vitamin B12 level (pg/ml)	349.123±231.548	344.198±28.325	350.113±270.251	0.678
Folate (ng/ml)	10.423±3.214	10.843±3.698	11.879±3.732	0.784
Homocysteine (μmol/l)	16.735±3.897	16.103±3.795	12.543±4.7398	**0.038**
Triglyceride (mg/dl)	132.318±114.223	130.609±91.118	131.945±87.436	0.742
Total cholesterol (mg/dl)	187.915±41.205	192.048±40.321	172.198 ±50.231	**0.021**
HDL (mg/dl)	58.512±23.875	57.214±15.3218	52.983±27.458	0.431
LDL (mg/dl)	109.387±39.324	111.546±39.102	105.246±32.651	0.361
BMI (Body mass index, kg/m^2)	27.121±5.423	27.328±5.102	24.865±4.542	**0.034**
Clinical parameters				
Pain severity (VAS)	8.493±1.521	8.845± 1.321	0.000±0.000	0.768
Attack frequency (attack/ mounthly)	2.424±2.428	3.928±3.764	0.000±0.000	**0.048**
Attack duration (hour)	32.025±26.213	34.658±28.312	0.000±0.000	0.685
Age at onset of migraine (years)	24.342±19.879	23.231±12.315	0.000±0.000	0.657

HDL: High density lipoprotein, LDL: Low density lipoprotein, BMI: Body mass index, VAS: Visual analog scale, SD: Standard deviation, p<0.05 is statistically significant.

with a family history than patients without a family history (p=0.021) (Table 3). Thus it may be said that migraine patients with family history have the familial risk of migraines.

CT and TT genotype carriers patients with a migraine for the MTHFR gene show from 40% to 70% decreased enzymatic activity than CC genotype (5). Decreased enzymatic activity may lead to higher plasma homocysteine levels (11). The significant relation between migraine and MTHFR C677T gene polymorphism was reported (10-13). Similarly to these studies, we also found a significant relationship between migraine and MTHFR C677T gene polymorphism. According to our results, the frequency of T alleles was higher in MO (%46.8) and MA (46.3) subgroups than control (%7.9) (Table 1).

Because homocysteine levels have an important role in vascular function, endothelial dysfunction associated with homocysteine may lead to the progression of migraine (14). Different results about the homocsyteine plasma levels such as significantly higher in migraine than control(3, 10, 14), significantly lower in migraine than control (15), and no statistically significant differences between migraine and control groups were detected in the literature (16). According to our results, significantly higher the plasma homocysteine levels were detected in patients with migraine than healthy controls (Table 4). Hyperhomocysteinemia may lead the reactive oxygen species (ROS) release, which causes oxidative damage to the vascular endothelium and can cause the initiation of migraine attacks (10, 17). According to our results, it may be said that hyperhomocysteinemia may lead to the initiation of migraine attacks.

When we took into consideration other biochemical findings (vitamin B12 HDL, LDL, total cholesterol, triglyceride, and folate), statistically significant differences were not detected among the MTHFR C677T genotype (CC, CT, and TT) for both migraine (Table 2) and control (Table 3) groups. Conversely, significantly higher total cholesterol and BMI were detected in migraine groups than control (p=0.021 and p=0.034, respectively) (Table 4).

It was reported that addition to aura status, attack frequency noted as a marker of migraine severity (18). When we took into consideration of the clinical parameters, while statistically significant difference was detected between MA (2.424±2.428) and MO (3.928±3.764) (p=0.048) for attack frequency (attack/ monthly) (p=0.048), the differences were not significant for age at onset of migraine (years), pain intensity (VAS), attack duration (hours) and attack frequency (attack/ monthly). So it may be said that different factors (genetic and environmental) may have a role in the occurrence of clinical parameters.

It was reported that the CT genotype was associated with osmophobia and nausea, and significantly higher in patients with compression and allodynia than patients without compression and allodynia. Significantly lower T allele frequency was detected in patients with systemic complaints than others (10). According to our results, the CT genotype frequency was significantly higher in patients with compression and allodynia than others (p<0.05) for MA subgroups and patients with fatigue for both MA and MO subgroups (p<0.05). Additionally, the frequency of CT genotype was significantly higher in patients with fatigue than others in both MO and MA subgroups. Also, significantly higher T allele frequency was detected in patients without systemic complaints than patients with systemic complaints in the MA subgroup (p=0.023). Conversely, for other symptoms (sleeplessness, vomiting, nausea, vertigo, phonophobia, and photophobia) significant differences were not detected in both MO and MA subgroups (p>0.05). When migraine head localizations to be taken into consideration, statistically significant differences among the MTHFR C677T genotype (TT, CT, and CC) were not detected for neck, overhead, forehead, nape, bilateral, unilateral, temple and around eyes (p >0.05). Therefore it may be said that patients with mutated type MTHFR C677T genotype carriers have an increased risk for migraines. When the gender to be taken into consideration, statistically significant differences were not detected between males and females for age at onset of the migraine, attack frequency, attack duration, pain intensity, LDL, total cholesterol, BMI and vitamin B12 levels (p>0.05). The triglyceride and homocysteine levels were significantly higher in males than females but HDL levels and folate were significantly higher in females than males (p<0.05). Because migraine mostly affects productive individuals, the labor losses of pa-

tients with migraine may cause important economic losses. Thus performed studies for the full understanding of the disease including genetic and environmental factors are important for the prevention of negativity caused by the disease.

Conflict of Interest: The authors declare that they have no conflict of interest.

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