Original Article
MTHFR polymorphism in patients with epilepsy in eastern Turkey

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Abstract: MTHFR gene C677T (rs1801133) and A1298C (rs1801131) polymorphisms are claimed to play a role in epileptogenesis. We aimed to define the prevalence of MTHFR gene C677T (rs1801133) and A1298C (rs1801131) polymorphisms in eastern Turkey and evaluated the possible relationships between polymorphism and epilepsy. A case-control study was conducted. Data for 81 adults diagnosed as epilepsy according to the International League Against Epilepsy (ILAE) criteria and were followed up in a neurology outpatient clinic of a tertiary hospital and 159 healthy controls were analyzed. Of the patients diagnosed as epilepsy, 54.3% (n=44) were females, and 45.7% (n=37) were males. In contrast, 47.2% (n=75) of the control group were females, and 52.8% (n=84) were males. There was no statistically significant difference concerning sex between the groups (P=0.295). The genotype distributions and allele frequencies of the MTHFR A1298C rs1801131 were statistically different between patients and the control group. A1298C rs1801131 polymorphism was significantly higher in patients with epilepsy. Additionally, the C allele in A1298C polymorphisms and T allele in C677T polymorphisms were also significantly higher in the patient group. However, there was no statistically significant difference concerning combined genotype analysis of MTHFR C677T and MTHFR A1298C polymorphisms or heterozygous/homozygous presentations. Polymorphism of MTHFR A1298C in eastern Turkey is higher in epileptic patients than in healthy controls. More studies are needed to investigate the significance of this condition in epilepsy.

Keywords: Epilepsy, genetic polymorphism, single nucleotide polymorphism

Introduction

Background/rationale

Epilepsy is a common neurological disease characterized by recurrent seizures resulting from increased excitability of nerve cells in the brain [1]. It is estimated that there are more than 65 million epilepsy patients in the world, more than 80% of which are in low and middle-income countries [2].

Epileptogenesis, on the other hand, is a chronic condition that can be triggered by genetic or acquired factors. This term was previously used to describe changes that occurred before the onset of the seizure. However, it refers today to a process that can continue even after the diagnosis of epilepsy [3, 4]. Epileptic seizures are caused by increased, rapid, and local electrical discharges in neurons as a result of changes in the ionotropic channels (Na, K, and Ca), as well as the inhibitor (GABA) and excitatory (AMPA, NMDA) receptors [5].

Studies in the literature claim that increased plasma homocysteine levels cause changes in these channels and receptors [6]. Lipton et al. [7] stated that homocysteine may cause excitotoxicity in neurons as an agonist on the NMDA receptor's glutamate site. Another study reported that homocysteine reduced seizure thresholds and could be used as a proconvulsant in experimental epilepsy models [8]. One reason for the increase in plasma homocysteine levels is MTHFR gene polymorphism in the first chromosome, which encodes the MTHFR enzyme and plays an essential role in folate-homocysteine metabolism [6].

In 2014, Yi-Le Wu and colleagues found a relationship between increased risk of epilepsy and MTHFR C677T polymorphism in a meta-analysis [9]. There is no study investigating the rela-
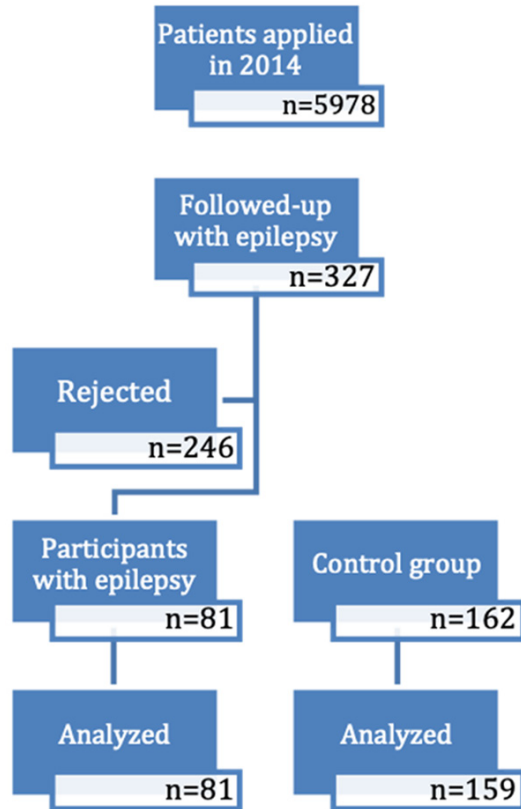
Figure 1. Participant flow diagram.

tionship between MTHFR polymorphisms and epilepsy in epileptic patient populations in Turkey, to the best of our knowledge.

Objectives

There is no information on the distribution of MTHFR gene C677T (rs1801133), A1298C (rs1801131) polymorphisms in epileptic patients in eastern Turkey. Therefore, we aimed to define the prevalence of these polymorphisms in our region and evaluated the possible relationships between polymorphism and epilepsy.

Methods

Study design

This research was designed as a case-control study. Study reporting was done per the STROBE guideline [10]. The study protocol was approved by the Local Ethics Committee at Kafkas University Faculty of Medicine (IRB number: 80576354-050-99/82, Date: 15 October 2014).

Setting

This research was performed at Kafkas University Health Research and Practice Hospital (Kars, Turkey), a tertiary-care reference center with 276 inpatient beds. The number of patients who applied to the neurology outpatient clinic in 2019 was 6266. Kars has a population of around 300 thousand inhabitants. However, the hospital serves to a broad region, including Artvin, Ardahan, Iğdır, and Ağrı provinces.

Participants

This study enrolled 81 patients (44 (54.3%) women, 37 (45.7%) men). Inclusion criteria of the study group were; being diagnosed as juvenile myoclonic epilepsy, focal epileptic syndrome, generalized tonic-clonic epilepsy, or secondary generalized tonic-clonic epilepsy, according to the International League Against Epilepsy criteria and followed-up at the Neurology Polyclinic of Kafkas University Medical Faculty Research Hospital. Also included were 159 healthy individuals (75 (47.1%) women, 84 (52.9%) men) as a control group (**Figure 1**). The healthy controls were selected from the patients' family members or caregivers and underwent a neurological examination to exclude epilepsy. Two control individuals were aimed for each patient.

DNA extraction

Two ml of venous blood taken from participants were stored in EDTA tubes at -20°C until the analysis day. For DNA extraction, Turbo-24 Nucleic Acid isolation device (Taigen Lab, Taipei, Taiwan) and Lab Turbo (Taigen Lab, Taipei, Taiwan) mini DNA isolation kit (Ct.No. LGD 480-220) were used with the membrane-column method and silica-membrane technology.

Detection of MTHFR polymorphisms

SNP genotyping was performed per the manufacturer's instructions, using the MassARRAY® system (Agena Bioscience, San Diego, California, USA) with the MALDI-ToF-MS method.

To detect rs1801133 and rs1801131, target pairs specific to target regions in genomic DNA and elongation probes for IPLEX Gold reaction (single base elongation reaction) were designed

using the Agena Bioscience's Assay Designer software (Agena Bioscience, San Diego, California, USA).

The target region in DNA was reproduced using primer pairs obtained by multiplex PCR due to the design. The obtained amplicons were subjected to SAP reaction and then hybridization of the probes, designed with the IPLEX Gold reaction with the target region. In the end, single mass-modified nucleotide elongation was performed. IPLEX reaction products have been cleaned with a cationic exchange resin to minimize background pollution by removing ions such as Na⁺, K⁺, and Mg⁺. After this stage, the products obtained were transferred to the matrix chip (384-element SpectroCHIP®) with nano dispenser and simultaneous detection after ionization by MassARRAY® mass spectrometry (Agena, San Diego, CA, USA). MassARRAY® TYPER 4.0 genotyping software was used to analyze the data obtained after the laser shot, to get the Spectro image and allele-specific peaks (IPLEX SpectroCHIP® II analysis).

Variables

The primary outcome variable of the study was "MTHFR C677T polymorphism". Also, data were collected on MTHFR A1298C polymorphism, age, and sex.

Study size

To compare MTHFR C677T polymorphism ratios between epilepsy and control groups with the Chi-square analysis, taking the effect size as 0.20 (small), α error as 0.05, power as 85%, and the degree of freedom as 1, the total number of participants required was calculated as 225 (G*Power, HHU, Düsseldorf, Germany).

Statistical analysis

The data were analyzed using the Statistical Package for the Social Sciences (SPSS) (SPSS for Windows, Version 15.0, Chicago, IC, USA). The study results were presented as frequencies and percentages for categorical variables and as means and standard deviations for numerical variables. Chi-square test was used to compare categorical data, and independent sample t-test was done to compare groups of numerical variables. Odds ratio (OR) and 95% confidence intervals (CIs) were calculated. The statistical significance threshold was taken as $P < 0.05$.

Results

Of the patients diagnosed with epilepsy, 54.3% (n=44) were females, and 45.7% (n=37) were males. In contrast, 47.2% (n=75) of the control group were females, and 52.8% (n=84) were males. There was no statistically significant difference concerning sex between the groups (P=0.295). On the other hand, a statistically significant difference was found regarding age (P < 0.001). The mean age of the patient group was 26.65±15.71 years, while the control group's mean age was 37.83±17.21 years. Age was not considered as a potential confounder for genetic polymorphism.

Most of the patients (73.8%; n=45) were diagnosed as generalized tonic-clonic epilepsy, followed by juvenile myoclonic epilepsy (13.1%, n=8), focal epileptic syndrome (11.5%, n=7), and secondary generalized tonic-clonic epilepsy (1.6%, n=1). The median duration of the disease in the study group was 4.0 years (min. 0, max. 40). Eight patients (13.1%) had a positive family history for epilepsy. On the other hand, the median number of seizures within the last year was 5 (min. 0, max. 120). Only 11 patients (18.0%) had no seizures within the last year. Of the patients, 45 (73.8%) were using single medications, while 16 (26.2%) were under combined antiepileptic therapy. The most commonly used antiepileptic medication was valproate (32.8%, n=20), followed by levetiracetam (18.0%, n=11), and lamotrigine (14.8%, n=9).

The genotype distributions and allele frequencies of the MTHFR A1298C rs1801131 were statistically different between the patients and the control group. A1298C rs1801131 polymorphism was significantly higher in patients with epilepsy (**Table 1**). Additionally, the C allele in A1298C polymorphisms and T allele in C677T polymorphisms were also considerably higher in the patient group (**Table 2**). However, there was no significant difference either concerning combined genotype analysis of MTHFR C677T and MTHFR A1298C polymorphisms or heterozygous/homozygous presentation (**Tables 3** and **4**, respectively).

DNA analysis for the MTHFR C677T polymorphism showed the CT genotype in 46%, CC in 35%, and TT in 17.8% of the cases.

Table 1. Comparison of polymorphism presence in the patient and control groups

		Patient n (%)	Control n (%)	χ^2	p
C677T rs1801133	CC	55 (67.9)	88 (55.3)	5,598	0,061
	CT	24 (29.6)	56 (35.2)		
	TT	2 (2.5)	15 (9.4)		
A1298C rs1801131	AA	14 (17.3)	53 (33.3)	6,945	0,031
	AC	50 (61.7)	81 (50.9)		
	CC	17 (21)	25 (15.7)		

χ^2: Chi-square test value.

Table 2. Comparison of MTHFR C677T and MTHFR A1298C allele frequencies in the patient and control groups

		Absent n (%)	Present n (%)	χ^2	p	OR	95% CI Lower	95% CI Upper
C allel in 677	Patient	2 (1.5)	134 (98.5)	4.380	0.036	0.231	0.052	1.025
	Control	15 (6.1)	232 (93.9)					
T allel in 677	Patient	55 (66.3)	28 (33.7)	5.605	0.018	1.920	1.115	3.306
	Control	88 (50.6)	86 (49.4)					
A allel in 1298	Patient	17 (17.9)	78 (82.1)	2.069	0.150	1.630	0.834	3.187
	Control	25 (11.8)	187 (88.2)					
C allel in 1298	Patient	14 (14.3)	84 (85.7)	7.441	0.006	0.412	0.215	0.789
	Control	53 (28.8)	131 (71.2)					

χ^2: Chi-square test value. OR: odds ratio. CI: confidence interval.

Table 3. Comparison of patient and control groups in terms of combined genotype alleles

		Patient n (%)	Control n (%)	χ^2	p
Combined genotype	CC/AA	6 (7.4)	20 (12.6)	9.403	0.094
	CC/AC	32 (39.5)	43 (27.0)		
	CC/CC	17 (21.0)	25 (15.7)		
	CT/AA	6 (7.4)	18 (11.3)		
	CT/AC	18 (22.2)	38 (23.9)		
	TT/AA	2 (2.5)	15 (9.4)		

χ^2: Chi-square test value.

the most commonly used antiepileptic medications being valproate, levetiracetam, and lamotrigine.

Limitations

This was a hospital-based study enrolling applicants in one hospital. Although we had a reasonable sample size, and the study center is the most prominent health institution in the area, we cannot claim to represent the whole region. Also, the genetic background of the control group was unknown.

Discussion

Key results

This study revealed two key findings: A1298C rs1801131 polymorphism was statistically higher in the epileptic group. Besides, the C allele in A1298C polymorphisms was significantly higher in the epileptic group.

On the other hand, the most common diagnosis of the patients was generalized tonic-clonic epilepsy. Of the patients, 13.1% had a positive family history for epilepsy, and only 18.0% had their disease under control with medications,

Interpretation

The prevalence of epilepsy varies widely between countries. In Turkey, the prevalence rate ranges from 0.08/1000 to 8.5/1000, while proportions of 0.9/1000 to 6.5/1000 were reported in Arab countries [11]. In 25% of all patients with seizures, the seizure type is tonic-clonic, the most frequent type of generalized epilepsy in adults [12]. From this perspective, our findings are conforming with previous reports. Furthermore, as in most ailments, epilepsy carries some familial risk too. Among relatives of patients with epilepsy, the risk in-

Table 4. Comparison of homozygous and heterozygous frequencies in the patient and control groups

		Homozygous n (%)	Heterozygous n (%)	χ^2	p	OR	95% CI	
							Lower	Upper
MTHFR C677T	Patient	57 (70.4)	24 (29.6)	0.755	0.385	1.291	0.725	2.300
	Control	103 (64.8)	56 (35.2)					
MTHFR A1298C	Patient	31 (38.3)	50 (61.7)	2.518	0.113	0.644	0.373	1.111
	Control	78 (49.1)	81 (50.9)					

χ^2: Chi-square test value. OR: odds ratio. CI: confidence interval.

creases by more than three times compared to the general population [13].

Although most of our patients were under monotherapy, a large-scale study from Italy reported polytherapy in 79% of adults and 75% of children [14]. Also, the types of medications used vary between studies. As an example, phenobarbital (21.7%), valproate (17.5%), and tiagabine (16.4%) are common medications used in Saudi Arabia [11], while levetiracetam (35%), carbamazepine (34%), and lamotrigine (30%) are reported as frequent medications used in epileptic adults in Italy [14]. Monitorization of antiepileptic drug consumption shows increasing trends for the use of new medicines [15].

The MTHFR gene is a functional gene that encodes the MTHFR enzyme, located in the telomere region (1p36.3) of chromosome 1 [16]. Single nucleotide polymorphisms (SNP), C677T (rs1801133) and A1298C (rs1801131) in the MTHFR gene have been shown to cause decreased enzyme activity and increased plasma homocysteine levels [17]. The methylenetetrahydrofolate reductase (MTHFR) gene C677T polymorphism is considered a risk factor because of its role in converting homocysteine to methionine in many diseases, including epilepsy [18, 19].

C677T polymorphism occurs in exon 4 of the MTHFR gene. This includes substitution from a C to T at position 677, which results from a conversion from a domain in the N-terminal catalytic domain to valine at codon 222. Individuals with a 677TT homozygous variant have up to 30% of the regular enzyme activity. On the other hand, the heterozygous CT genotype demonstrates 65% of the normal enzyme activity and increased thermal lability [20, 21]. In a meta-analysis made by Wu YL et al. [9], MTHFR C677T polymorphism was associated

with an increased risk of epilepsy. However, the author noted that more studies were needed in various regions to confirm the findings.

MTHFR A1298C, another polymorphism, occurs in exon 7. This polymorphism reduces the enzyme activity in the codon's C-terminal regulatory domain by changing from glutamic acid to alanine residue at codon 429 [22]. There are a 15% decrease in the enzymatic activity of heterozygous (AC) carriers and a 30% decrease in the enzymatic activity of homozygous (CC) carriers. Individuals with reduced MTHFR enzyme activity have been reported to have an increased risk of diseases such as spontaneous abortion, kidney failure, cardiovascular disease, breast cancer, and chromosomal anomalies [23, 24].

The distribution rates of the C677T and A1298C (MTHFR) polymorphisms in our study differed from a similar study previously conducted in Italy [25], which suggests that the genetic structure in epilepsy may be affected by regional differences. Similarly, our findings differed from a study where the relationship between homocysteine and epilepsy was confirmed [26]. In the DNA analysis for MTHFR C677T polymorphism, the CT genotype was shown in 46%, CC in 35%, and TT in 17.8% of the cases.

Although there are inconsistencies among studies investigating the relationship between epilepsy and MTHFR C677T polymorphism in the literature, most previous studies have claimed that there may be a relationship between C677T polymorphism and epilepsy [27-30]. Although the T allele frequency in MTHFR C677T polymorphism was higher in the epilepsy group than in the control group, it was not statistically related to epilepsy. Factors such as the number of patients and heterogeneity of the group with epilepsy may have affected this outcome.

In the current study, a significant *p*-value concerning the C allele in C677T rs1801133 polymorphism was found when the patient group with epilepsy was compared to the control group. However, this significance was not present regarding the odds ratio.

Another polymorphism that may be related to epilepsy is MTHFR A1298C. However, the findings of the studies on this variant are inconsistent [25, 27, 30]. The majority of previous studies indicated no significant relationship between MTHFR A1298C polymorphism and epilepsy [27, 30]. In contrast, in our research, we found a substantial connection between epilepsy and MTHFR A1298C polymorphism.

In a study on 95 cases with epilepsy and 98 controls, Caccamo et al. [25] found that the frequency of the C1298 polymorphic allele was significantly higher than the controls (30.5% vs. 19.4%, P < 0.05). In our study, we similarly determined the frequency of C1298 polymorphic alleles significantly higher in patients with epilepsy.

Conclusion

This study demonstrated for the first time that MTHFR A1298C polymorphism is higher in epilepsy patients in eastern Turkey compared to the healthy population. MTHFR gene polymorphisms in epilepsy patients in the sample differed from the literature. More studies are needed to investigate the significance of this condition in epilepsy cases seen in this region.

Acknowledgements

Disclosure of conflict of interest

None.

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References

[1] Manford M. Recent advances in epilepsy. J Neurol 2017; 264: 1811-1824.

[2] Fiest KM, Sauro KM, Wiebe S, Patten SB, Kwon CS, Dykeman J, Pringsheim T, Lorenzetti DL and Jetté N. Prevalence and incidence of epilepsy: a systematic review and meta-analysis of international studies. Neurology 2017; 88: 296-303.

[3] Pitkänen A, Lukasiuk K, Dudek FE and Staley KJ. Epileptogenesis. Cold Spring Harb Perspect Med 2015; 5: a022822.

[4] Terrone G, Pauletti A, Pascente R and Vezzani A. Preventing epileptogenesis: a realistic goal? Pharmacol Res 2016; 110: 96-100.

[5] Rogawski MA, Löscher W and Rho JM. Mechanisms of action of antiseizure drugs and the ketogenic diet. Cold Spring Harb Perspect Med 2016; 6: a022780.

[6] Škovierová H, Vidomanová E, Mahmood S, Sopková J, Drgová A, Červeňová T, Halašová E and Lehotský J. The molecular and cellular effect of homocysteine metabolism imbalance on human health. Int J Mol Sci 2016; 17: 1733.

[7] Lipton SA, Kim WK, Choi YB, Kumar S, D'Emilia DM, Rayudu PV, Arnelle DR and Stamler JS. Neurotoxicity associated with dual actions of homocysteine at the N-methyl-D-aspartate receptor. Proc Natl Acad Sci U S A 1997; 94: 5923-5928.

[8] Diaz-Arrastia R. Homocysteine and neurologic disease. Arch Neurol 2000; 57: 1422-1427.

[9] Wu YL, Yang HY, Ding XX, Zhao X, Chen J, Bi P and Sun YH. Association between methylenetetrahydrofolate reductase C677T polymorphism and epilepsy susceptibility: a meta-analysis. Seizure 2014; 23: 411-416.

[10] Cuschieri S. The STROBE guidelines. Saudi J Anaesth 2019; 13: S31-s34.

[11] Angalakuditi M and Angalakuditi N. A comprehensive review of the literature on epilepsy in selected countries in emerging markets. Neuropsychiatr Dis Treat 2011; 7: 585-597.

[12] Beghi E. Addressing the burden of epilepsy: many unmet needs. Pharmacol Res 2016; 107: 79-84.

[13] Thijs RD, Surges R, O'Brien TJ and Sander JW. Epilepsy in adults. Lancet 2019; 393: 689-701.

[14] Malerba A, Ciampa C, De Fazio S, Fattore C, Frassine B, La Neve A, Pellacani S, Specchio LM, Tiberti A, Tinuper P and Perucca E. Patterns of prescription of antiepileptic drugs in patients with refractory epilepsy at tertiary referral centres in Italy. Epilepsy Res 2010; 91: 273-282.

[15] Patsalos PN, Spencer EP and Berry DJ. Therapeutic drug monitoring of antiepileptic drugs in epilepsy: a 2018 update. Ther Drug Monit 2018; 40: 526-548.

[16] Ay A, Alkanli N, Sipahi T, Gulyasar T, Ustundag S, Guldiken S and Sut N. Investigation of the relationship between MTHFR, IRS and CALCA gene polymorphisms and development of diabetic nephropathy in patients with type 2 dia-

betes mellitus. Essays Biochem 2018; 32: 1257-1265.

[17] Weisberg IS, Jacques PF, Selhub J, Bostom AG, Chen Z, Curtis Ellison R, Eckfeldt JH and Rozen R. The 1298A → C polymorphism in methylenetetrahydrofolate reductase (MTHFR): in vitro expression and association with homocysteine. Atherosclerosis 2001; 156: 409-415.

[18] Rai V and Kumar P. Methylenetetrahydrofolate reductase C677T polymorphism and susceptibility to epilepsy. Neurol Sci 2018; 39: 2033-2041.

[19] Tanyıldız HG, Yeşil Ş, Bozkurt C, Çandır MO, Akpınar-Tekgündüz S, Toprak Ş, Yüksel D and Şahin G. Are the methylenetetrahydrofolate reductase 1298 and 677 gene polymorphisms related to optic glioma and hamartoma risk in neurofibromatosis type 1 patients? Turk J Pediatr 2016; 58: 152-158.

[20] Frosst P, Blom HJ, Milos R, Goyette P, Sheppard CA, Matthews RG, Boers GJ, den Heijer M, Kluijtmans LA and van den Heuvel LP. A candidate genetic risk factor for vascular disease: a common mutation in methylenetetrahydrofolate reductase. Nat Genet 1995; 10: 111-113.

[21] van der Put NM, Gabreëls F, Stevens EM, Smeitink JA, Trijbels FJ, Eskes TK, van den Heuvel LP and Blom HJ. A second common mutation in the methylenetetrahydrofolate reductase gene: an additional risk factor for neural-tube defects? Am J Hum Genet 1998; 62: 1044-1051.

[22] Weisberg I, Tran P, Christensen B, Sibani S and Rozen R. A second genetic polymorphism in methylenetetrahydrofolate reductase (MTHFR) associated with decreased enzyme activity. Mol Genet Metab 1998; 64: 169-172.

[23] Castiglia P, Sanna V, Azara A, De Miglio MR, Murgia L, Pira G, Sanges F, Fancellu A, Carru C and Bisail M. Methylenetetrahydrofolate reductase (MTHFR) C677T and A1298C polymorphisms in breast cancer: a sardinian preliminary case-control study. Int J Med Sci 2019; 16: 1089.

[24] Kim JW, Park HM, Choi YK, Chong SY, Oh D and Kim NK. Polymorphisms in genes involved in folate metabolism and plasma DNA methylation in colorectal cancer patients. Oncol Rep 2011; 25: 167-172.

[25] Caccamo D, Condello S, Gorgone G, Crisafulli G, Belcastro V, Gennaro S, Striano P, Pisani F and Ientile R. Screening for C677T and A1298C MTHFR polymorphisms in patients with epilepsy and risk of hyperhomocysteinemia. Neuromolecular Med 2004; 6: 117-126.

[26] Coppola G, Ingrosso D, Operto FF, Signoriello G, Lattanzio F, Barone E, Matera S and Verrotti A. Role of folic acid depletion on homocysteine serum level in children and adolescents with epilepsy and different MTHFR C677T genotypes. Seizure 2012; 21: 340-343.

[27] Dean JC, Robertson Z, Reid V, Wang Q, Hailey H, Moore S, Rasalam AD, Turnpenny P, Lloyd D and Shaw D. A high frequency of the MTHFR 677C > T polymorphism in scottish women with epilepsy: possible role in pathogenesis. Seizure 2008; 17: 269-275.

[28] Karatoprak E, Sozen G, Yılmaz K and Ozer I. Interictal epileptiform discharges on electroencephalography in children with methylenetetrahydrofolate reductase (MTHFR) polymorphisms. Neurol Sci 2020; 41: 631-636.

[29] Ono H, Sakamoto A, Mizoguchi N and Sakura N. Methylenetetrahydrofolate reductase 677C > T mutation and epilepsy. J Inherit Metab Dis 2000; 23: 525.

[30] Scher AI, Wu H, Tsao JW, Blom HJ, Feit P, Nevin RL and Schwab KA. MTHFR C677T genotype as a risk factor for epilepsy including post-traumatic epilepsy in a representative military cohort. J Neurotrauma 2011; 28: 1739-1745.