

Distribution of Different Genotypes MTHFR and GABRG2 Genes in Epileptic Population of Khyber Pakhtunkhwa Pakistan

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Abstract

Objectives: The present study determines the frequencies of different types of resistant epilepsies to carbamazepine and valproic acid therapy in population of Khyber Pakhtunkhwa. The study also determines the frequencies of MTHFR (C677T, A1298C) and GABRG2 (C588T, C315T) genes polymorphisms in population of KP as the said genes have role in management of epilepsies.

Materials and methods: Epileptic patients treated with carbamazepine and valproic acid therapy were enrolled in the study. DNA was extracted from the blood and genotyped for the MTHFR and GABRG2 genes polymorphisms using restriction fragment length polymorphisms (PCR-RFLP). Seizure control and adverse drug reactions were recorded during the treatment.

Results: There were 64% males and 34% females. Frequency of PGS was 79% of epilepsies. Heterozygous variants of MTHFR (C677T) gene was 50% in ATS and 40% in FS. Heterozygous variants of MTHFR (A1298C) and GABRG2 (C315T) genes were, respectively, 40% and 20% in FS. Heterozygous variant of GABRG2 (C315T) gene was 50% in ATS. Resistant heterozygous variants of MTHFR (C677T, A1298C) gene were respectively 41.5% and 15.1% with carbamazepine therapy. Resistant heterozygous variant of MTHFR (A1298C) gene was 34.8% to valproic acid therapy. Anemia associated with carbamazepine was 40% in heterozygous variants of MTHFR (C677T) and GABRG2 (C315T) genes.

Conclusion: Heterozygous variants of MTHFR (C677T, A1298C) gene were respectively resistant to carbamazepine and valproic acid therapy. While heterozygous variant of GABRG2 (C315T) was only resistant to valproic acid. Carbamazepine and valproic acid therapy was associated with anemia in heterozygous variant of MTHFR (C677T) gene.

Keywords: Pharmacogenomics •Carbamazepine •Valproic acid •Epilepsy •Single Nucleotide Polymorphisms •Methylene tetrahydrofolate Reductase •Gamma-Aminobutyric Acid Receptor

Background

Epilepsy is a neurological disorder manifested by repeated, unprovoked seizures [1]. Clinically observable phenomenology and underlying etiology are used to classify types of epilepsies [2,3]. Inconsistency exists in the prevalence of epilepsies in the world. However, the prevalence of epilepsy in the world is about 10 per 1,000 people [4]. The prevalence of epilepsy is high in Asia as compared to Europe which signifies that it is one of a major health issues in Asia. Biological variations and small physique may explain the differences in prevalence of epilepsies between Asian and Western population [5,6]. Prevalence of epilepsy is high in adolescence and childhood population of the world [7]. In Pakistan the prevalence of epilepsy is 10 per 1000 people [8,9]. GTCS is the most common types of seizures in Pakistani population [10].

Epilepsy has diverse and heterogeneous causes. Genetic abnormalities are considered one of a prominent causes of epilepsy [11,12]. Gene mutations, especially single nucleotide polymorphisms (SNPs) are

highly associated with channelopathies, brain malformations and various types of seizures that are sometime tagged as resistant epilepsies [13]. Approximately 20%-30 % of epilepsy cases are caused by acquired conditions, but the remaining 70%-80 % of cases are considered to be associated with one or more genetic factors [14]. However, there are more than 12 genes that have an established association with epilepsies [15]. So far, prevalence of homozygous genotypes (677CC) of MTHFR (C677T) and Heterozygous (1298AC) of MTHFR (A1298C) genes is high in previous studies conducted in cardiac patients of Pakistan. And in India, Korea and Puerto Rico the heterozygous genotypes of MTHFR (C677T and A1298C) gen have been commonly observed in different diseases [16-19]. Similarly, prevalence of homozygous genotypes of the C677T and A1298C of MTHFR gene is also high in Caucasians, Japanese and African population [20]. Likely, prevalence of homozygous genotype of GABRG2 (C588T and C315T) gene is predominant in India and Japan.

Whereas, GABRG2 gene encodes $\gamma 2$ subunit of GABA receptors present on the cell membrane of inhibitory neurons. As alteration in receptors binding domain change the response to administered antiepileptic drugs

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(AEDs). Hence, GABRG2 gene polymorphisms modulate response to drug therapy because of conformational changes in the structure of receptors that impair inhibitory function of GABA receptors [5]. On the other hand, ADRs of anti-epileptic drugs (AEDs) are a major cause of disability, morbidity that adversely affect the quality of life of patients [21,22]. Common ADRs to carbamazepine therapy is changes in hematological profile, and sedation that may affect 5%-17% of population [23]. Drug rash with eosinophilia and systemic symptoms (DRESS), Stevens-Johnson syndrome (SJS) and toxic epidermal necrolysis affect 1-10 in 10,000 with the use of carbamazepine [24]. However, predictability of ADRs is very difficult before the start of therapy. Advancement in the field of genetics has been considered as a hallmark for predictability of ADRs. More, changes in ethnicity are more related to genetics that ultimately affect pharmacological actions of drugs. Hence findings in one ethnic group may be different from another ethnic group. Therefore, the current study is aimed to determine the prevalence of different genotypes of MTHFR (C677T and A1298C) and GABRG2 (C588T and C315T) genes in different types of seizures in epileptic population of Khyber Pakhtunkhwa (KP). The current work also focused to determine the prevalence of different types of seizures as well as the frequency of most frequent ADRs among the target population of KP Pakistan.

Methodology

Target population

Newly diagnosed epileptic patients from Khyber Pakhtunkhwa were included in the study to whom carbamazepine and valproic acid therapy was advised by respective ward physicians/neurologists.

Inclusion criteria

Newly diagnosed epileptic patients were enrolled in the study to whom carbamazepine and valproic acid were prescribed. More, informed consents were taken from the patients to participate in the study.

Exclusion criteria

Patients having either co-morbidities or refused to participate in the study.

Study design and protocol

It was a hospital based longitudinal study. Epileptic patients were enrolled in the Out Patient Department (OPD) of neurology of Government Lady Reading Hospital Peshawar. Patients with epilepsy were followed in two phases. The first visit on the time of diagnosis, second visit after six months during treatment with carbamazepine and valproic acid (Figure 1). However, patients were observed for seizures controlled in between period as per wish and well.

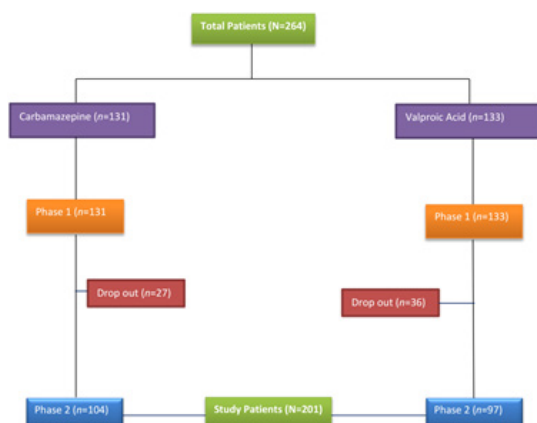


Figure 1. Schematic presentation of study.

Extraction of genomic DNA

Peripheral blood was obtained from the newly diagnosed epileptic patients. From one portion of the blood, genomic DNA was extracted using kit method (NucleoSpin® Blood, Germany). Briefly describing, the extraction of DNA was carried out through series of reactions which include deproteination of blood (proteinase K), lysis of blood cell (lysis buffer), purification of DNA (washing buffer), and finally elution of DNA (through elution buffer) according to a standard protocols provided by respective manufacturers. The extracted DNA was stored on -20°C for further analysis. Selected exons of MTHFR and GABRG2 genes were amplified using gradient thermo cycler. After amplification, the PCR products were run on 2% agarose gel and assessed their size with 50 bp ladder. Exon 4 of MTHFR gene was amplified by using primers 5-TTTGAGGCTGACCTGAAGCACTGAAGGAG-3 and primer 5-GAGTGGTAGCCCTGGATGGGAAAGATCCCG-3. The amplified product was digested using restriction enzyme Hinf I. Similarly, exon 7 of MTHFR gene was amplified by using primers 5-CTTTGGGGA GCTGAA GGACTACTAC-3 and 5-CAC TTTGTG ACCATTCCGGTTTG-3. The resulted product was digested using restriction enzyme MbolI. Similarly, exon 5 of GABRG2 gene was amplified by using primers 5- AATCACCTTTTATTCTAATGGTC-3 and 5-CAGTGAAGGCAACTTACTAAGA-3. The amplified product was digested using restriction enzyme ApeI. Similarly, exon 3 of GABRG2 gene was amplified by using primers 5- CAAATGTGGTGAATTAGTAAGTGG-3 and 5-TCACATTTTCTCTCAAACATGC-3. The PCR products were digested using restriction enzyme BamHI. The digested products of each exon were run on 5% agarose gel and respective fragments sizes were assessed with 50 bp ladder.

Drug therapy

Carbamazepine was prescribed in dose range of 200-1200 mg/day. Similarly, valproic acid was prescribed in dose range of 100-2000 mg/day. Dose was escalated till seizure control. More, high performance liquid chromatography (HPLC) was used to monitor and manage the plasma level of carbamazepine and valproic acid in their range of plasma levels.

Meteorology of seizures control

Seizures controlled in patients were recorded using proforma standardized in local context.

Adverse drugs reactions

Adverse Drug Reactions (ADRs) were documented according to the study recommended by Advance Study and Research Board of Khyber Medical University (AS&RB). ADRs were reported according to the American Society of Health-System Pharmacists (ASHP) criteria which are; when there is a temporal relationship between the onset of drug therapy and the adverse reaction, adverse reaction subside when the drug is withdrawn, laboratory tests that provide evidence for the reaction being an ADR, and the symptoms resume when the agent was re-administered. ADRs like nervousness, headache, tiredness, behavioral problems drowsiness, difficulty paying in attention, memory problems, dizziness, restlessness, tremor, hematological ADRs, nausea, vomiting, loss of appetite, disturbed sleep, weight gain, hyperactivity, disturbed vision, gingivitis and cutaneous adverse effects of carbamazepine and valproic acid were recorded during the course of therapy.

Statistical analysis

Graph Pad Prism 6 was used for analysis. Types of epilepsies were presented in the form of pie chart. Frequencies of genotypes were expressed in tables. ADRs are presented in the form of bar chart with its respective frequencies. Clinical outcomes of therapy were expressed in the form of tables against its respective genotypes.

Results

Demographic features of epileptic patients

Study population (N=264) were comprised of 64% male and 36% female patients. Majority of patients belong to urban areas (61%) as compared to rural areas. 42% patients have positive family history of epilepsy). Whereas, frequencies of consanguineous marriages were high in the targeted study population (Table 1). Primary generalized seizure (PGS) was the most frequent (79%) type of epilepsy followed by secondary generalized complex seizure (SGCS) (8%) (Figure 2). Generalized tonic clonic seizure was high (73%) among other types of GS (Figure 3).

Table 1. Demographic features of epileptic patients.

Demographic data	Patients n (%),(N=264)
Male	169 (64.39)
Female	95 (35.98)
Urban	160 (61)
Rural	104 (39)
Family history positive	112 (42.4)
Family history negative	152 (57.6)
Cousin marriages	113 (42.8)
Non cousin marriage	151 (57.2)

Frequencies of different genotypes of mthfr and gabrg2 genes polymorphisms

Frequencies of different genotypes of MTHFR and GABRG2 genes are presented in Table 2. homozygous genotypes (677CC, 1298AA and 588CC, 315CC) of MTHFR and GABRG2 genes were dominant in the targeted study population. Heterozygous genotypes (677CT, 1298AC and 588CT, 315CT) of MTHFR and GABRG2 genes were the second most prevalent genotypes in epileptic population of KP.

Table 2. Frequencies of different genotypes of MTHFR (C677T and A1298C) and GABRG2 (C588T and C315T) gene polymorphisms verses demography.

Study population (N=264)	MTHFR gene				GABRG2 gene			
	C677T n (%)		A1298C n (%)		C588T n (%)		C315T n (%)	
Male (n=169)	CC	134 (79.3)	AA	128 (75.7)	CC	135 (79.8)	CC	135 (79.8)
	CT	28 (16.6)	AC	23 (13.6)	CT	21 (12.4)	CT	23 (13.6)
	TT	7 (4.1)	CC	18 (10.7)	TT	13 (7.8)	TT	11(6.6)
Female (n=95)	CC	69 (72.6)	AA	70 (73.6)	CC	77 (81.0)	CC	79 (83.2)
	CT	16 (16.8)	AC	18 (18.9)	CT	13 (13.7)	CT	8 (8.4)
	TT	10 (10.6)	CC	7 (7.5)	TT	5 (5.3)	TT	8 (8.4)
Urban Area (n=160)	CC	122 (76.2)	AA	130 (81.2)	CC	130 (81.2)	CC	130 81.2)
	CT	27 (16.8)	AC	20 (12.5)	CT	10 (6.3)	CT	18 (11.3)
	TT	11 (6.8)	CC	10 (6.3)	TT	20 (12.5)	TT	12 (7.5)
Rural Area (n=104)	CC	81 (77.8)	AA	82 (78.9)	CC	82 (78.9)	CC	84 (80.7)
	CT	17 (16.3)	AC	14 (13.5)	CT	14 (13.5)	CT	13 (12.6)
	TT	6 (5.7)	CC	8 (7.7)	TT	8 (7.7)	TT	7 (6.7)
Cousin Marriages (n=113)	CC	82 (72.6)	AA	83 (73.4)	CC	93 (82.3)	CC	89 (78.7)
	CT	24 (21.2)	AC	19 (16.8)	CT	9 (7.9)	CT	16 (14.2)
	TT	7 (6.2)	CC	11(9.7)	TT	11(9.7)	TT	8 (7.0)
Non cousin marriages (n=151)	CC	121 (80.1)	AA	115 (78.2)	CC	119 (78.8)	CC	125 (82.8)
	CT	20 (13.2)	AC	22 (14.6)	CT	25 (16.5)	CT	15 (9.9)
	TT	10 (6.6)	CC	14 (9.3)	TT	7 (4.6)	TT	11(7.3)
Family history positive (n=112)	CC	89 (79.4)	AA	86 (76.9)	CC	94 (83.9)	CC	92 (84.1)
	CT	20 (17.8)	AC	13 (11.6)	CT	12 (10.7)	CT	16 (14.3)
	TT	3 (2.8)	CC	13 (11.6)	TT	6 (5.4)	TT	6 (5.3)
Family history negative (n=152)	CC	114 (75.0)	AA	112 (73.7)	CC	118 (77.6)	CC	122 (80.4)
	CT	24 (15.8)	AC	28 (18.4)	CT	22 (14.5)	CT	15 (9.8)
	TT	14 (9.2)	CC	12 (7.9)	TT	12 (7.9)	TT	15(9.8)

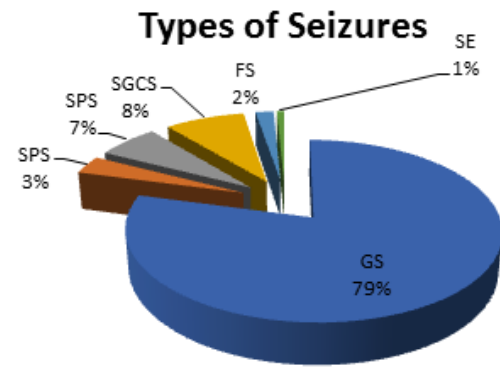


Figure 2. Types of Seizures in the study population.

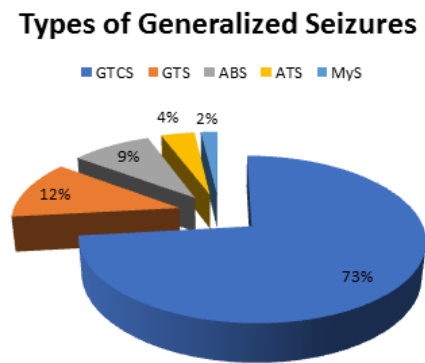


Figure 3. Types of generalized seizures.

Frequencies of different genotypes of mthfr and gabrg2 genes polymorphisms and types of seizures

On average, homozygous genotypes (677CC, 1298AA and 588CC, 315CC) of MTHFR and GABRG2 genes were predominant. Patients with different types of seizures and their genotypes are expressed in Table 3. However, heterozygous genotypes (677CT and 315CT) of MTHFR and GABRG2 genes were high (50%) in generalized atonic seizures than other types of seizures (Table 3).

MTHFR gene.

Adverse effect of carbamazepine in different genotypes of MTHFR and GABRG2 Genes

20 patients experienced adverse drugs reactions including anemia (n=15), Steven's Johnson syndrome (n=3), drowsiness (n=1) and confusion (n=1) in target study population (n=104) that were treated with carbamazepine. These ADRs mostly happened in wild genotypes of MTHFR (C677T and A1298C) and GABRG2 (C588T and C315T) gene (Figure 4).

Table 3. Frequencies of different genotypes of MTHFR (C677T and A1298C) and GABRG2 (C588T and C315T) gene polymorphisms verses types of seizures.

Study population (N=264)	Types of seizures	MTHFR gene		GABRG2 gene					
		C677T n (%)	A1298C n (%)	C588T n (%)	C315T n (%)				
CC	Generalized tonic clonic seizures (n=153)	CC	116 (78.8)	AA	112 (73.2)	CC	122 (79.7)	CC	124 (80.0)
		CT	25 (16.3)	AC	28 (18.3)	CT	22 (14.4)	CT	20 (13.1)
		TT	12 (7.8)	CC	13 (8.5)	TT	9 (5.8)	TT	9 (5.9)
CC	Generalized tonic seizures (n=25)	CC	17 (68)	AA	17 (68)	CC	21 (84)	CC	21 (84)
		CT	5 (20)	AC	4 (16)	CT	3 (12)	CT	2 (8)
		TT	3 (12)	CC	4 (16)	TT	1 (4)	TT	2 (8)
CC	Generalized atonic seizures (n=8)	CC	4(50)	AA	8 (100)	CC	7 (87.5)	CC	3 (37.5)
		CT	4 (50)	AC	0 (0)	CT	1 (12.5)	CT	4 (50)
		TT	0 (0)	CC	0 (0)	TT	0 (0)	TT	1 (12.5)
CC	Absence seizures (n=19)	CC	15 (78.9)	AA	15 (78.9)	CC	18 (94.6)	CC	17 (89.5)
		CT	3 (15.8)	AC	2 (10.5)	CT	1 (5.4)	CT	2 (10.5)
		TT	1 (5.4)	CC	2 (10.5)	TT	0 (0)	TT	0 (0)
CC	Myoclonic seizures (n=4)	TT	4 (100)	AA	3 (75)	CC	3 (75)	CC	4 (100)
		CC	0 (0)	AC	0 (0)	CT	1 (25)	CT	0 (0)
		CT	0 (0)	CC	1 (25)	TT	0 (0)	TT	0 (0)
CC	Simple partial seizure (n=9)	CC	7 (77.8)	AA	7 (77.8)	CC	7 (77.8)	CC	7 (77.8)
		CT	2 (22.2)	AC	2 (22.2)	CT	2 (22.2)	CT	1 (11.1)
		TT	0 (0)	CC	0 (0)	TT	0 (0)	TT	1 (11.1)
CC	Complex partial seizure (n=17)	CC	17 (100)	AA	16 (94.1)	CC	12 (70.6)	CC	14 (82.3)
		CT	0 (0)	AC	1 (5.9)	CT	2 (11.8)	CT	2 (11.8)
		TT	0 (0)	CC	0 (0)	TT	3 (17.6)	TT	1 (5.9)
CC	Secondary generalized complex seizure (n=22)	CC	18 (81.8)	AA	16 (72.7)	CC	19 (86.4)	CC	18 (81.8)
		CT	2 (9.1)	AC	2 (9.1)	CT	2 (9.1)	CT	3 (13.6)
		TT	2 (9.1)	CC	4 (18.2)	TT	1 (4.5)	TT	1 (4.6)
CC	Febrile seizure (n=5)	CC	3 (60)	AA	2 (40)	CC	5 (100)	CC	2 (40)
		CT	2 (40)	AC	2 (40)	CT	0 (0)	CT	1 (20)
		TT	0 (0)	CC	1 (20)	TT	0 (0)	TT	2 (40)
CC	Status Epilepticus (n=2)	CC	2 (100)	AA	1 (50)	CC	2 (100)	CC	2 (100)
		CT	0 (0)	AC	0 (0)	CT	0 (0)	CT	0 (0)
		TT	0 (0)	CC	1 (50)	TT	0 (0)	TT	0 (0)

Seizures control in different genotypes of mthfr and gabrg2 genes polymorphisms with carbamazepine therapy

It is noteworthy that patients with poor control seizures (n=53) also having high frequencies of homozygous genotypes (677CC and 1298AA) of MTHFR gene and (588CC and 315 CC) of GABRG2 gene (Table 4). However, 41.5% patients with poor seizure control having heterozygous genotypes (677CT) of MTHFR gene. These patients were treated with carbamazepine. Drug's plasma levels were found within therapeutic range (4-12 mg/L).

Seizures control in different genotypes of mthfr and gabrg2 genes polymorphisms with valproic acid therapy

Poor seizures controlled (n=46) patients were found in target study group (n=133) that were treated with valproic acid (Table 5). Drug's plasma levels were found within therapeutic range (40-100 mg/L). Majority of patients have homozygous genotypes of MTHFR (C677T and A1298C) and GABRG2 (C588T and C315T) gene. Although, 34.8% patients with poor seizures control patients have heterozygous genotypes (1298AC) of

Table 4. Frequencies of different genotypes of MTHFR (C677T and A1298C) and GABRG2 (C588T and C315T) gene polymorphisms verses poor controlled seizures in patients treated with carbamazepine.

Genes (N=53)	Genotypes	Poor seizure controlled	Percentage of poor seizure controlled
MTHFR (C677T) (n=53)	CC	23	43.4%
	CT	22	41.5%
	TT	8	15.1%
MTHFR (A1298C) (n=53)	AA	38	71.7%
	AC	8	15.1%
	CC	7	13.2%
GABRG2 (C588T) (n=53)	CC	42	79.2%
	CT	6	11.3%
	TT	5	9.5%
GABRG2 (C315T) (n=53)	CC	42	79.2%
	CT	6	11.3%
	TT	5	9.5%

Table 5. Frequencies of different genotypes of MTHFR (C677T and A1298C) and GABRG2 (C588T and C315T) gene polymorphisms verses poor controlled seizures in patients treated with valproic acid.

Genes (N=46)	Genotypes	Poor seizure controlled	Percentage of poor seizure controlled
MTHFR (C677T) (n=46)	CC	40	86.9%
	CT	4	8.7%
	TT	2	4.5%
MTHFR (A1298C) (n=46)	AA	22	47.8%
	AC	16	34.8
	CC	8	17.4%
GABRG2 (C588T) (n=46)	CC	36	78.2%
	CT	5	10.9
	TT	5	10.9
GABRG2 (C315T) (n=46)	CC	36	78.2%
	CT	8	17.4%
	TT	2	4.4%

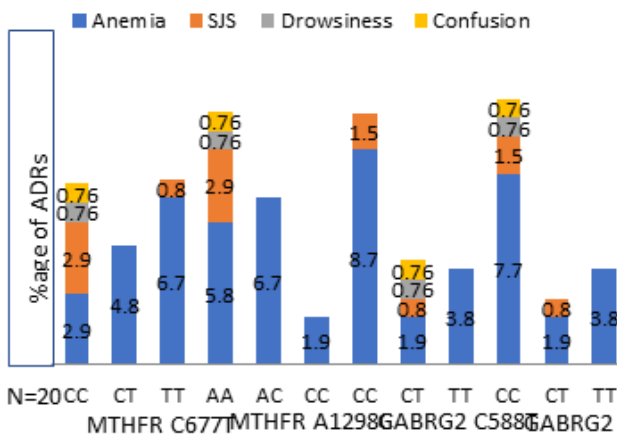


Figure 4. Adverse drugs reactions in carbamazepine treated patients. Number each sections represent the percentage of adverse drugs reactions (n=104).

Adverse effect of valproic acid in different genotypes of mthfr and gabrg2 genes

16 patients got ADRs including anemia (n=12) and tremor (n=4) in 95 patients treated with valproic acid. Similarly, majority of ADRs observed in wild genotypes of MTHFR (C677T and A1298C) and GABRG2 (C588T and C315T) gene (Figure 5).

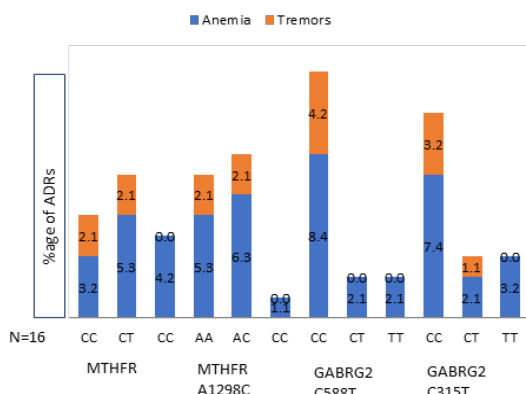


Figure 5. Adverse drugs reactions in valproic acid treated patients (n=95). Numbers inside each section represent the percentage of patients out of 95 patients.

Discussion

Prevalence of homozygous genotypes of MTHFR (C677T and A1298C) and GABRG2 (C588T and C315T) genes were high in gender and all types of seizures of the target population. Prevalence of heterozygous genotypes (1298AC) of MTHFR gene is high in female than male patients. However, the difference in the prevalence of heterozygous and homozygous mutant genotypes of (MTHFR C677T and A1298C) and GABRG2 (C588T and C315T) gene is not significant in gender. GTCS is more prevalent seizure followed by SGCS. More, it is found that the prevalence of different types of genotypes of MTHFR (C677T and A1298C) and GABRG2 (C588T and C315T) genes were in line to the previous studies conducted in India, Korea and Hispania. The prevalence of different genotypes of MTHFR (C677T and A1298C) of MTHFR gene is also comparable to the previous results of a study conducted in Pakistan which says that wild genotypes of MTHFR (C677T and A1298C) gene is predominant. However, minor difference in the frequencies of different genotypes of MTHFR gene is exists in different regions of the china as compared to our results. This is how ethnicity affects genotyping. However, meta-analysis corroborate that there is a considerable geographical and ethnic variations that affect the frequencies of MTHFR (C677T and A1298C) gene polymorphisms [25]. The prevalence of different genotypes of MTHFR (C677T and A1298C) gene is also comparable to whites, Japanese and Africans population [20]. Similarly, the prevalence of variant genotypes of GABRG2 (C588T and C315T) gene is high in epileptic patients of India, which is slightly different from our study in which wild genotypes (588CC and 315CC) of GABRG2 gene is predominant [26]. It has been also found that the frequency of different genotypes of GABRG2 (C588T and C315T) gene polymorphisms in Japanese population is similar to our study in which the prevalence of wild genotypes (588CC and 315CC) of GABRG2 gene is predominant [21]. Another study shows that variant genotypes (588CT and 588TT) of GABRG2 (C588T) gene were high in Tiwani population as compared to wild genotypes (588CC). The frequencies of variant genotypes (588CT and 588TT) of GABRG2 (C588T) gene is quite higher than the prevalence of wild genotype (588CC)of GABRG2 (C588T) gene in our study population [27]. Our data about the frequencies of GABRG2 (of C588T, C315T) gene polymorphisms show similarity with the study conducted in Germans, Italy , British, Irish , America and Egypt [28-32]. As it has been reported that variant genotypes (677CT, 677TT and 1298AC, 1298CC) of MTHFR and (588CT, 588TT and 315CT, 315TT) of GABRG2 (C588T and C315T) genes polymorphisms is responsible for drug resistant epilepsies [33,34].

We observed 53 patients who were resistant to carbamazepine therapy in the targeted population (n=104) despite the fact that their drug's plasma levels were in therapeutic range (4-12 mg/L) (data not shown). Similarly, 46 poor seizures controlled patients were found within valproic acid therapy population (n=97) although their plasma level was within therapeutic range (40-100 mg/L). Interestingly the frequencies of variant genotypes of MTHFR (C677T and A1298C) and GABRG2 (C588T and C315T) genes were high in these patients. It is evident from the literature that variant genotypes of MTHFR (C677T and A1298C) and GABRG2 (C588T and C315T) genes is responsible for drug resistant epilepsies in other population. We came across similar type of findings in our population. Hence, our observations can be supported by Schwahn study that polymorphisms in the MTHFR gene lead to altered MTHFR activity may affect the individual metabolome either in shape of vitamin B6 deficiency that may be one cause for weakness of inhibitory neurons in CNS as vitamin B6 helps in synthesis of GABA. Thus it can, therefore, be a pharmacogenetic effect that still to be confirmed via knock out model in experimental animals [23]. Similarly, Ulrich imagines that indirect effect of MTHFR polymorphisms is commonly associated with variation in gene-specific or general DNA methylation, leading to differential gene expression. These differential expressions of genes affect different transcriptome and proteome, which may lead to drug variable response. As mutation in $\gamma 2$ subunits of the GABAA receptor is also involved in the resistance to AEDs [34,35]. So, mutations in GABRG2 genes affect the

pharmacodynamics (GABA receptor, etc.) of drugs in animals models. Mostly AEDs exerts its action through binding with GABA receptors. Alteration in receptors binding site because of variant (588CT, 588TT and 315CT, 315TT) of GABRG2 gene may change the clinical outcomes of administered AEDs. Since the plasma levels of carbamazepine and valproic acid were in therapeutic range, hence we postulate that metabolism of drugs was not affected and the resistance to seizure control may be attributed to the said genetic polymorphisms. Though, other causes like plasma levels of vitamin B6 that helps in synthesis of GABA cannot be ruled out. Furthermore, anemia was more commonly observed with carbamazepine and valproic acid therapy in heterozygous genotypes (677CT) of MTHFR and (315CT) of GABRG2 genes. Other ADRs like, anemia, SJS, drowsiness, confusion and tremor were most common ADRs observed during carbamazepine and valproic acid therapy in wild genotypes (677CC, 1298AA) of MTHFR and (588CC, 315CC) of GABRG2 genes. According to the previous literature a slight decline in hemoglobin level is associated with the use of carbamazepine [36]. Moreover, it has been found that monotherapy of AEDs has no significant association with the changes in Hb level and some other blood parameters [37]. However, carbamazepine, valproic acid and other AEDs is responsible for bone marrow suppression lead to blood problem (s) like thrombocytopenia, leucopenia and aplastic anemia via anti-folate activity [38]. Baily et al study shows that patients using CBZ, VPA and other AEDs significantly reduce Plts count [39]. The anti-folate properties of carbamazepine result in decrease of Hb level and increase in homocysteine level which affect some metabolic pathways [40]. Ghaffarpou et al observed that hematological profile shift was mostly happened in early six months of therapy with antiepileptic drugs [41-43]. So far relations have been reported with MTHFR (C677T and A1298C) genotypes polymorphism and elevated levels of homocysteine due to vitamin B6, B12 and folic acid deficiency (13, 14). More, we have recently observed that heterozygous variant (677CT and 1298AC) of MTHFR gene was frequently experienced low level of vitamin B6, which helps in synthesis of GABA, inhibitory neurotransmitters in carbamazepine treated patients. Health professionals should be vigilant about the frequencies of resistant epilepsies and possible ADRs associated with carbamazepine and valproic acid therapy in our population within the context of MTHFR (C677T and A1298C) and GABRG2 (C588T and C315T) genes polymorphisms.

Conclusion

Frequency of heterozygous genotypes of MTHFR (C677T) gene polymorphism is high in population of KP that are resistant to carbamazepine therapy. While, heterozygous genotypes of MTHFR (A1298C) gene polymorphism are more common in our population that are resistant to valproic acid therapy followed by heterozygous genotypes of GABRG2 (C315T) gene polymorphism. Clinicians should consider alternate therapy for the said heterozygous variants of MTHFR and GABRG2 gene in the start of therapy to avoid possible precipitation of seizures during change of therapy either due to hematological profile shift or poor prognosis.

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List of Abbreviations: Absence Seizure (ABS), Anti-epileptic drugs (AEDs), Atonic Seizure (ATS), Complex Partial Seizure (CPS), Deoxyribonucleic Acid (DNA), Febrile Seizure (FS), Secondary Generalized Complex Seizure (SGCS), Gamma-Aminobutyric Acid Receptor (GABRG2), Generalized Tonic Clonic Seizure (GTCS), Generalized Tonic Seizure (GTS), Generalized Seizure (GS), Methylene Tetrahydrofolate Reductase (MTHFR), Myoclonic Seizure (MS), Simple Partial Seizure (SPS), and

Status Epilepticus (SE)

Ethics Approval and Consent to Participate

The study was approved by the Ethics Board of the Khyber Medical University, Peshawar via approval no: DIR/KMU-EB/AC/000047 that complied with Helsinki's declaration. Those patients were included in the study who were agreed to participate in the study and withdraw blood for genetic analysis through written consent, assent or surrogate assent form.

All treatments provided to patients were standard of care and provided to the patient regardless of this or any other study.

Consent for Publication

No details, images or videos relating to any of the study participants are included in this manuscript. The patients gave consent to publish their data. Their confidentiality is maintained.

Availability of Data and Material

The datasets during and/or analyzed during the current study available from the corresponding author on reasonable request.

Competing Interest

The authors have no competing interests.

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Authors' Contributions

SK carried out experimental work as Ph.D Scholar. Also prepared the 1st draft of manuscript. NA extensively revised the manuscript. He is also designed the study and wrote the research project. AK helped in diagnosis and patients follow up for their seizures control. SA helped in Clinical scoring of epilepsies. HN helped in experimental work. ZU helped in experimental work. SJ helped in supply of chemicals and reagents. WA helped in preparing the manuscript. All authors approved the final version of manuscript.

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