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SCN1A and SCN1B gene polymorphisms and their association with plasma concentrations of carbamazepine and carbamazepine 10, 11 epoxide in Iranian epileptic patients

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ABSTRACT

Objective (s): From a genetic point of view, epilepsy is a polygenic multifactorial syndrome. The SCN1A and B genes belong to a family of genes that provide instructions for making sodium channels. Understanding the relevance of SCN1A and SCN1B gene polymorphisms to plasma concentration of carbamazepine (CBZ) and its active metabolite carbamazepine 10, 11 epoxide (CBZE), may shed more light on inter-individual variations in response to CBZ.

Materials and Methods: In this cross-sectional study, genotype distribution and allele frequency of six non-synonymous exonic single nucleotide polymorphisms (SNPs) of the SCN1A and B genes were selected and determined using PCR-RFLP in 70 epileptic patients treated with CBZ for at least 6 months. The patients had no hepatic or renal diseases and received no medications known to have a major interaction with CBZ. Serum concentrations of CBZ and CBZE were measured using High-Performance Liquid Chromatography (HPLC).

Results: The AA, AG and GG alleles of SCN1A were found in 23, 37 and 10 patients, respectively. There were no statistically significant differences in the mean (± standard deviation) of plasma concentrations of CBZ (P=0.8) and CBZE (P=0.1) among these 3 groups. Likewise, there was no statistically significant relationship between SCN1A polymorphisms and CBZ concentration/dose ratio (P=0.7). A significant association was found between CBZ plasma level and CBZ concentration/dose with CBZ daily dose. All patients had the same genotype of SCN1B gene(CC). and no statistical analysis was performed.

Conclusion: No significant association between SCN1A gene polymorphisms and plasma levels of CBZ and CBZE were found.

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Introduction

Epilepsy is a common and chronic neurological disorder characterized by episodic and recurrent interruptions of normal brain function called epileptic seizures. In fact, epilepsy is a spectrum of clinical manifestations and electroencephalographic abnormalities that reflect various underlying etiologies (1). These epileptic attacks are unpleasant for patients and can disrupt their daily life. Moreover, epilepsy poses a significant economic burden on healthcare systems, which significantly depends on the severity of the disease and the response to treatment (2). Both genetic and environmental factors influence patients' responses to antiepileptic drugs (AEDs**) (**3**)** Among the genetic factors, the genes encoding the receptors and voltage-gated sodium channels are considered to play an important role (4). Voltage-gated sodium channel is a heteromeric protein that is composed of one alpha and one or more beta subunits. The alpha subunit is responsible for channel functions and the beta-1 subunits adjust the channel kinetics (5). SCNA

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gene family consists of nine genes (SCN1A, SCN2A, etc) and encodes the alpha subunit (3, 6). Several studies revealed the association between SCN1A polymorphism and different types of epilepsies including myoclonic, generalized, idiopathic, and febrile seizure plus epilepsies (3, 7-8). Some other studies indicate the association between this polymorphism and drug response. For instance, a significant association was found between SCN1A and retention rate on CBZ monotherapy in the Chinese population with focal seizure (9). In addition, Heinzen *et al*, (2007) revealed the role of SCN1A polymorphism as a determinant of AEDs response (10). Also, the association between SCN2B gene encoding sodium channel β1 subunits and different types of epilepsies such as epilepsy with febrile seizures plus and response to AEDs has been reported (5, 11-12).

Carbamazepine (CBZ), with a half-life of about 35 hr, is a widely prescribed drug for several types of epilepsy. It is extensively metabolized through oxidation, conjugation, and hydroxylation with just 1% remaining unchanged (13, 14). CBZE is the main active metabolite of CBZ, which is formed in the liver through CBZ metabolism by the CYP3A4 enzyme and is considered to have the same effect and toxicity as CBZ for epilepsy treatment (14). Some studies have also shown that CBZ affects voltage-dependent ion channels, especially alpha subunit of voltage-gated sodium channels, on the surface of neurons (3, 6, 15-16). CBZ inhibits these channels and reduces neural excitability. Beside its Ca^{2+} antagonist property, CBZ can decrease Ca^{2+} flow during the action potential. However, it is not clear whether this property plays a role in anticonvulsant effects, and sodium channels are still known as the main and determining target for CBZ (17).

The various side effects are associated with CBZ including dizziness, headache, diplopia, skin rash, and decreased level of consciousness (18). Therefore, determination of a proper concentration of CBZ and its metabolite in plasma may help reduce the drug side effects. In practice, Iranian patients are found to respond to lower doses of CBZ in comparison to international recommended doses (19). Therefore, we hypothesized that certain polymorphisms in sodium channel genes of Iranian patients may be involved in responsiveness of this channel, The present study as the first report on Iranian patients, evaluated the association between SCN1A and SCN1B single nucleotide polymorphisms (SNP) and demographic features and plasma concentrations of CBZ and CBZE in controlled epileptic patients in Shiraz, Iran.

Materials and Methods

Patients

In this cross-sectional study, a total of 136 adult patients (18–65 years) were recruited from the outpatient epilepsy clinic affiliated with Shiraz University of Medical Sciences, Shiraz, Iran, from April 2010 to March 2012. All patients met the specific diagnostic criteria for epilepsy, based on guidelines of the International League against Epilepsy and clinical grounds and EEG findings (20). Patients with secondary generalized tonic-clonic epilepsy and complex partial epilepsy were recruited (20). Only patients with controlled epilepsy whose CBZ regimen had not changed for at least six months were recruited. Only patients on monotherapy with the same brand of CBZ (Carbamazepine-Sobhan, Sobhan Pharmacy, Tehran, Iran) were included. Exclusion criteria were patients with abnormal liver function test (alanine aminotransferase and aspartate aminotransferase ≥ 3 times of upper limit of normal) (21) and kidney dysfunction (serum creatinine > 1.7 mg/dl) (22), and those receiving any medications known to have major pharmacodynamic and pharmacokinetic interactions with CBZ (23-25).

Demographic factors of patients including gender, age, BSA, BMI, and IBW were recorded. After personal interview, an informed consent was signed by each participant. This survey was approved by Shiraz University of Medical Sciences' Review Board and Ethics committee.

Polymerase chain reaction-restriction fragment length polymorphism (PCR- RFLP)

Five ml of peripheral blood was taken from each patient 30 min before receiving morning dose of CBZ. 3 ml of collected blood was centrifuged and the serum was separated and stored at -70 ˚C until measurement of the concentration of CBZ and CBZE using HPLC. The remaining 2 ml was poured into EDTA tubes to be used for DNA extraction. The genomic DNA was extracted from buffy coat using boiling protocol. PCR- RFLP was performed to determine SCN1A and SCN1B genotypes. The sequences of SCN1A and SCN1B primers are summarized in Table 1 (26).

The PCR procedure included 35 cycles at 95 ˚C for 30 sec, 60.6 ˚C for 35 sec and 72 ˚C for 40 sec for both SCN1A (SNP2298771), and SCN1B (SNP2305748). PvuII and NcoI restriction enzymes were used for SCN1A (SNP2298771) and SCN1B (SNP2305748), respectively, and the products were directly analyzed using 2% agarose gel electrophoresis. A/G polymorphism of *SCN1A* (SNP2298771), was identified as ("A" allele, 168 bp; "G" allele, 145 and 23 bp) and C/T polymorphism of SCN1B (SNP2305748) was identified as (C allele, 151 bp; T allele, 109 and 42 bp)

Statistical analysis

Statistical analyses were performed using the SPSS statistical analysis software, version 15.0 (SPSS Inc., Chicago, IL, USA). Continuous data are presented

Table 1. The sequence of SCN1A and SCN1B primers

gene	Primer
SCN1A	F: 5'-TGC ACA AAG GAG TAG CTT ATG-3' R:5'-AGT CAA GAT CTT TCC CAA TTT CAG-3'
SCN1B	R:5'-AGC CAC CCT ACT CAC GCA TT-3' R:5'-GAG ACA TGG CAT CCA TCG TGT-3'

as mean±standard deviation and 95% confidence intervals (CIs); categorical data are reported as proportions. One-sample Kolmogorov-Smirnov test was performed to assess normal distribution of CBZ and CBZE plasma levels and CBZ concentration/dose ratio. These were evaluated in relation to gender by independent sample t-test with data of normal distribution and Mann-Whitney U test with data of abnormal distribution. One-way ANOVA and Kruskal-Wallis tests were performed for data with normal and abnormal distributions, respectively. This established a statistical relationship between SCN1A polymorphism and CBZ and CBZE plasma levels and CBZ concentration/dose ratio. *A P*-value <0.05 was considered as significant.

Results

During the study period, 136 patients were referred to us by a neurologist according to inclusion criteria. Having measured the plasma levels of CBZ and CBZE using HPLC, only 70 patients were shown to be qualified for the study. The demographic, laboratory and clinical findings of these patients are demonstrated in Table 2.

Using PCR- RFLP analysis, A/A, A/G and G/G alleles of SCANIA gene were identified in 23 (32.8%), 37 (52.8%) and 10 (14.2%) of patients, respectively. The mean±SD of CBZ and CBZE plasma levels were 5±2.4 µg/ml and 0.7±0.7 µg/ml, respectively. Plasma levels of CBZ and CBZE in most of our patients were within the therapeutic level (65.7% and 62.9%, respectively).

Mean ±SD of CBZ plasma level in, A/A allele was 5±2.9 µg/ml [minimum= 1.5 µg /ml, maximum=10.5 µg /ml]; 95%CI= (3.8-6.3), while in patients with A/G and G/G alleles, the means±SD were 4.9±2.3 µg/ml [minimum= 0.5 µg /ml, maximum= 10 µg /ml]; 95%CI= (4.2-5.7) and 5.5±1.7 µg/ml [minimum= 3.2µg /ml, maximum= 8.5 µg /ml]; 95%CI= (4.3-6.7), respectively. Statistical analysis did not reveal any difference between them (*P*= 0.8).

Mean ±SD of CBZE plasma levels in A/A alleles ,was 0.9±0.7 µg/ml [minimum= 0.07 µg /ml, maximum= 2.6 µg/ml]; 95%CI= (0.6-1.2)], while in patients with A/G and G/G alleles, the means±SD were 0.6±0.7 µg/ml [minimum= 0.04 µg/ml, maximum=4.2 µg/ml; 95% CI= (0.4-0.9)] and 0.7±0.4 µg/ml [minimum= 0.1 µg/ml, maximum= 1.5 µg/ml]; 95%CI= (0.4-0.98)], respectively. These differences were not statistically significant (*P*=0.1).

Likewise, with regard to CBZ and CBZE plasma levels, there was no statistically significant association between SCN1A polymorphisms and CBZ concentration/dose ratio (*P*=0.7).

CBZ concentration/dose was (Mean±SD) 0.01±0.006 µg/ml [minimum= 0.00 µg/ml, maximum=0.03 µg/ml]; 95%CI= (0.009-0.01), 0.01±0.007 µg/ml [minimum=0.00 µg /ml, maximum= 0.04 µg/ml]; 95%CI= (0.009-0.01), and 0.01±0.003 µg/ml [minimum=0.01 µg/ml, maximum= 0.02 µg/ml]; 95%CI= (0.007-0.01) for A/A, A/G, and G/G alleles, respectively. Table 3 demonstrates associations between demographic characteristics and CBZ and CBZE plasma levels and associated CBZ concentration/dose ratio. There was no significant association between age, BMI, IBW, and BSA and plasma levels of CBZ and CBZE in our patients

Table 2. The demographic, laboratory and clinical characteristics of epileptic patients (N=70)

Age (years), Mean± Standard Deviation (range)	28±12.5 (18-60)
BSA[1], m^2, Mean± Standard Deviation (range)	1.6±0.2 (1.1-2.1)
BMI[2],Kg/m^2, Mean± Standard Deviation (range)	22.5±4.4 (13-34)
IBW[3], Kg, Mean± Standard Deviation (range)	57±11.3 (27-83)
Carbamazepine Plasma Level (µg /ml) Mean± Standard Deviation (range)	5±2.4 (0.5-10.4)
CBZE [4]Plasma Level (µg /ml) Mean± Standard Deviation (range)	0.7±0.7 (0.04-4.2)
Carbamazepine concentration/dose ratio, Mean± Standard Deviation (range)	0.01±0.006 (0.00-0.04)
Carbamazepine Daily Dosage (mg/day), Mean± Standard Deviation (range)	515±254 (200-1400)
Male (%)/Female (%)	27 (38.6%)/43 (61.4%)
Patients with Carbamazepine Plasma Level:	
-Under Therapeutic Level (<4 µg/ml)	24 (34.3%)
-In Therapeutic Level (4-12 µg/ml)	46 (65.7%)
-Above Therapeutic Level (>12 µg /ml)	--
Patients with CBZE Plasma Level:	
-Under Therapeutic Level (<0.4 µg/ml)	25 (35.7%)
-In Therapeutic Level (0.4-4 µg/ml)	44 (62.9%)
-Above Therapeutic Level (>4 µg/ml)	1 (1.4%)

1- Body Surface Area: BSA, 2- Body Mass Index: BMI, 3- Ideal Body Weight: IBW, 4- CBZE: Carbamazepine Epoxide

Table 3. Associations between demographic characteristics and CBZ plasma level, CBZE plasma level and CBZ concentration/dose ratio in 70 patients

Characteristics	Gender							Age	BSA	BMI	IBW	CBZ Daily Dosage
	Mean± SD		Min		Max		*P*	*P*	*P*	*P*	*P*	*P*
	M	F	M	F	M	F						
CBZ plasma level	4.7±2.3	5.2±2.5	1.06	0.52	9.9	10.5	0.4	1.00	0.4	0.8	0.7	0.01
CBZE plasma level	0.7±0.8	0.7±0.6	0.06	0.04	4.3	2.6	0.6	0.6	0.2	0.4	0.2	0.1
CBZ concentration/ dose ratio	0.01±0.006	0.1±0.006	0.00	0.00	0.03	0.04	0.8	0.8	0.2	0.7	0.4	<0.001

CBZ: Carbamazepine, CBZE: Carbamazepine Epoxide, SD: Standard Deviation, Min: Minimum, Max: Maximum, M: Male, F: Female, *P*: P-value, BSA: Body Surface Area, BMI: Body Mass Index, IBW: Ideal Body Weight

(*P*≥0.05). However, a significant association was found between the CBZ plasma level and CBZ concentration/dose with the CBZ daily dose (*P*< 0.05).

Table 4 shows that there is no significant association between the daily dose of carbamazepine and SCN1A gene polymorphisms (*P*=0.18).

All patients in the present study had the same genotype of SCN1B gene (CC), which did not allow evaluating its association with CBZ and CBZE plasma levels and CBZ concentration/dose ratio.

Discussion

Epilepsy is a common neurological disorder that can impair the individual's activity and quality of life. Due to the chronic nature of the disease, patients usually need lifelong treatment. The nature of the disease alongside annoying side effects of AED frustrate patients. Therefore, achieving the optimal dose with the best control and least side effects can guarantee patient cooperation followed by a better response to treatment (18, 27, 28).

In this study, we revealed that the CBZ and CBZE plasma levels are not significantly different between AA, AG and GG alleles of SCN1A. Likewise, no significant relationship was observed between SCN1A polymorphism and CBZ concentration/dose ratio. This lack of association between polymorphism of SCN1A and drug level in plasma of the Iranian population is not in accordance with the previous data from Lakhan *et al*, (2009) and Tate *et al*, (2005) SCN1A genotype and the maximum CBZ concentration in their patients. They revealed that SCN1A polymorphism was associated not only with epilepsy development but also with the response to treatment (3, 29). In Tate *et al*, (2005) study in the UK, 425 epileptic patients including AA allele=112 (26.3%), AG allele= 220 (51.7%) and GG allele=93 (21.8%), were analyzed in terms of SCN1A polymorphism and its association with the maximum dose of CBZ. They showed that maximum CBZ dose was significantly higher in AG allele, but unlike our study, they did not evaluate the CBZE level (29). Lakhan *et al*, (2009) also studied 336 epileptic patients selected from the Indian population with different types of seizures including AA allele=144, AG allele= 179 and GG alleles= 13. Unlike our study, they investigated the efficacy of CBZ therapy in different genotypes based on clinical response rather than CBZ and CBZE plasma levels (3). In addition, Heinzen and his colleagues (2007) evaluated the prevalence of different SCN1A genotypes in 43 patients with drug-resistant mesial temporal lobe epilepsy. Having recognized the association between SCN1A polymorphism and response to CBZ, they suggested that this association could be secondary to the association between polymorphism and severity of epilepsy. Indeed, the patients with the AA genotype developed more severe epilepsy, thus requiring higher doses of AEDs such as CBZ (10). Some other studies also confirmed this relationship and emphasized that SCN1A gene polymorphism can influence the CBZ efficacy (6, 30).

In contrast, studies of Zimprich *et al*, (2008) and Sterjev *et al*, (2012) did not find any significant association between SCN1A genotypes and CBZ dosage (15, 31). Sterjev *et al*, (2012) studied 147 adult Macedonian patients with AA allele= 41(28%), AG allele= 77(52%) and GG alleles=29(21%). 90 out of 147 patients with cryptogenic partial epilepsy, 48 patients with symptomatic partial epilepsy, 5

Table 4. The distribution of doses of carbamazepine in different SCN1A polymorphism groups (N=70)

GENOTYPES	Minimum dose (mg/day)	Maximum dose (mg/day)	MEAN±SD (mg/day)	*P*-value
AA	200	800	452±162	
AG	200	1400	524±295	0.18
GG	400	1000	620±239	

patients with general epilepsy, and 4 patients with other types of epilepsy were diagnosed. Contrary to our study, the intergroup comparisons were mainly based on patient's response to therapy. Although no significant difference between AA, AG or GG groups was found, they suggested a larger clinical study to confirm their findings. They also found no significant differences between the groups with regard to the distribution of CBZ dosage in CBZ responsive patients (15). Zimprich and colleagues, (2008) also evaluated this possible association in a cohort study on 369 Australian patients with focal epilepsy syndrome. Alleles frequencies for AA, AG, and GG alleles were 132 (35.7%), 170 (46.0%) and 67 (18.1%) with no significant association between CBZ plasma levels and the genotypes (31). Similarly, Haerian *et al*, (2013) reported that following gene polymorphism was not associated with AED-response (32). This controversy can be a result of the difference in ethnic background, measurement methods, sample size, and type of seizure among these studies. However, the allele frequencies of the SCN1A gene in our patients were similar to the European population, with AG being the most frequent (15).

Although SCN1B gene polymorphism is repeatedly reported to be involved in epileptogenesis and response to AEDs (5, 11), another study did not find a significant association between SCN1B polymorphism and CBZ resistance (4).

All patients had the same genotype of SCN1B (SNP2305748) gene (CC) in the present study. In general, the population diversity of the SCN1B (CC) genotype is extremely prevalent compared to the CT genotype. For instance, other studies reported the prevalence of the CC genotype in Asians (100%,) Europeans (97.2%) and Sub-Saharan Africans (99.1%) (33). Although our findings are compatible with the latter study, however, due to the lack of adequate information about the Iranian population, further studies on this issue will be helpful.

We also evaluated the association of demographic features alongside genetic factors with CBZ concentration in controlled epileptic patients. The results showed that the demographic characteristics of the patients did not have a significant association with CBZ plasma level, CBZE, and CBZ concentration/dose ratio. However, other studies found that age and weight had a possible impact on CBZ plasma concentration and CBZ dose (34-35).

Actually, the present study had some limitations: firstly, we evaluated only two SNPs (SNP2298771 and SNP2305748), and other SNPs in these genes with potential association with CBZ response were overlooked. Secondly, we employed CBZ and CBZE plasma levels to evaluate the association between SCN1A and the patient's response; however, the efficacy of this variable as diagnostic criteria seems to be controversial and needs more evaluation. Finally, the sample size in our study was not fit for a comprehensive statistical conclusion.

Conclusion

In summary, no significant association between SCN1A gene polymorphisms and demographic features of Iranian patients with plasma concentrations of CBZ and CBZE was found. Altogether, a larger clinical study, including more variables and genotypes, is suggested to be conducted to fit comprehensive statistical analysis. A functional *in vitro* study to silence and block the SCN1A gene and protein and analysis of CBZ and CBZE response genes and signaling pathways will provide insightful data on possible interaction of SCN1A and SCN1B genes and CBZ.

Conflict of interest

The authors declare no conflict of interest.

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References

1. Ghaem H, Borhani-Haghighi A. Validity and reliability of the Persian epilepsy quality of life questionnaire. Neurosciences (Riyadh) 2010; 15:249-253.
2. Asadi-Pooya AA, Emami M, Ashjazadeh N, Nikseresht A, Shariat A, Petramfar P, *et al.* Reasons for uncontrolled seizures in adults; the impact of pseudointractability. Seizure 2013; 22:271-274.
3. Lakhan R, Kumari R, Misra UK, Kalita J, Pradhan S, Mittal B. Differential role of sodium channels SCN1A and SCN2A gene polymorphisms with epilepsy and multiple drug resistance in the north Indian population. Br J Clin Pharmacol 2009; 68:214-220.
4. Jang S, Kim M, Nam T, Kim J, Choi S, Lee S, *et al*. Polymorphisms in the voltage-gated sodium channel genes and antiepileptic efficacy of carbamazepine. J Korean Neurol Assoc. 2009; 27:147-153.
5. Yu FH, Catterall WA. Overview of the voltage-gated sodium channel family. Genome Biol 2003; 4:207.
6.Thompson CH, Kahlig KM, George AL Jr. SCN1A splice variants exhibit divergent sensitivity to commonly used antiepileptic drugs. Epilepsia 2011; 52:1000-1009.
7. Miller IO, Sotero de Menezes MA. SCN1A-related seizure disorders. In: Pagon RA, Adam MP, Bird TD, Dolan CR, Fong CT, Stephens K, editors. Gene Reviews. Seattle (WA); 1993.
8. Toldo I, Bruson A, Casarin A, Salviati L, Boniver C, Sartori S, *et al*. Polymorphisms of the SCN1A gene in children and adolescents with primary headache and idiopathic or cryptogenic epilepsy: is there a linkage? J Headache Pain 2011; 12:435-441.

9. Zhou B, Zhou Q, Yin J, Li G, Xu X, Qu J, *et al.* Comprehensive analysis of the association of SCN1A gene polymorphisms with the retention rate of carbamazepine following monotherapy for new-onset focal seizures in the Chinese Han population. Clin Exp Pharmacol Physiol 2012; 39:379-384.
10. Heinzen EL, Woohyun Y, Tate SK, Sen A, Wood NW, Sisodiya SM, *et al.* Nova2 interacts with a cis-acting polymorphism to influence the proportions of drug-responsive splice variants of SCN1A. Am J Hum Genet 2007; 80:876-883.
11. Uebachs M, Albus C, Opitz T, Isom L, Niespodziany I, Wolff C, *et al.* Loss of β1 accessory Na+ channel subunits causes failure of carbamazepine, but not of lacosamide, in blocking high-frequency firing via differential effects on persistent Na+ currents. Epilepsia 2012; 53:1959-1967.
12. Scheffer IE, Harkin LA, Grinton BE, Dibbens LM, Turner SJ, Zielinski MA, *et al.* Temporal lobe epilepsy and GEFS+ phenotypes associated with SCN1B mutations. Brain 2007; 130:100-109.
13. Battino D, Estienne M, Avanzini G. Clinical pharmacokinetics of antiepileptic drugs in paediatric patients. Part II. Phenytoin, carbamazepine, sulthiame, lamotrigine, vigabatrin, oxcarbazepine and felbamate. Clin Pharmacokinet 1995; 29:341-369.
14. Egnell A, Houston B, Boyer S. *In vivo* CYP3A4 heteroactivation is a possible mechanism for the drug interaction between felbamate and carbamazepine. J Pharmacol Exp Ther 2003; 305:1251-1262.
15. Sterjev Z, Kiteva G, Cvetkovska E, Petrov I, Kuzmanovski I, Ribarska T, *et al.* Influence of the SCN1A IVS5N + 5 G>A polymorphism on therapy with carbamazepine for epilepsy. Balkan J Med Genet 2012; 15:19-24.
16. Ragsdalea DS, Avoli M. Sodium channels as molecular targets for antiepileptic drugs. Brain Res Rev 1998; 26:16–28.
17. Schirrmacher K, Mayer A, Walden J, Düsing R, Bingmann D. Effects of carbamazepine on membrane properties of rat sensory spinal ganglion cells *in vitro.* Eur Neuropsychopharmacol 1995; 5:501-507.
18. Namazi S, Borhani-Haghighi A, Karimzadeh I.Adverse reactions to antiepileptic drugs in epileptic outpatients: a cross-sectional study in Iran. Clin Neuropharmacol 2011; 34:79-83.
19. Samaei A, Nobahar M, Vafaei AA. An experimental design for finding of minimum dosage of carbamazepine and valproate in preventing of seizure attacks. Pak J Pharm Sci 2009; 22:180-183.
20. Panayiotopoulos CP. The Epilepsies: seizures, synd-romes and management. Oxfordshire (UK); 2005.
21. Guidance for Industry, drug-induced liver injury: Premarketing clinical evaluation. 2009.
22. Bagshaw SM, Mortis G, Doig CJ, Godinez-Luna T, Fick GH, Laupland KB. One-year mortality in critically ill patients by severity of kidney dysfunction: A population-based assessment. Am J Kidney Dis 2006; 48:402-409.
23. Fong SY, Gao Q, Zuo Z. Interaction of carbamazepine with herbs, dietary supplements, and food: A Systematic Review. Evid Based Complement Alternat Med 2013; 2013:898261.
24. Spina E, Pisani F, Perucca E. Clinically significant pharmacokinetic drug interactions with carbamazepine. Clin Pharmacokinet 1996; 31:198-214.
25. Uijla SG, Uiterwaalb CSMP, Aldenkampc AP, Carpayd JA, Doelmane JC, Keizerf K, *et al.* A cross-sectional study of subjective complaints in patients with epilepsy who seem to be well-controlled with anti-epileptic drugs. Seizure 2006; 15:242–248.
26. Chou IC PC, Tsai FJ. The lack of assossiation between febrile convulsions and polymorphisms in SCN1A. Epilepsy Res 2003; 54:53-57.
27. Kwan P, Brodie MJ. Neuropsychological effects of epilepsy and antiepileptic drugs. Lancet 2001; 357:216-222.
28. Asadi-Pooya AA, Shokouhyar S, Emami M, Sharifzade M. Perception and use of complementary and alternative medicine among patients with epilepsy. Arch Neurosci 2013; 1:60-66.
29. Tate SK, Depondt C, Sisodiya SM, Cavalleri GL, Schorge S, Soranzo N, *et al.* Genetic predictors of the maximum doses patients receive during clinical use of the anti-epileptic drugs carbamazepine and phenytoin. Proc Natl Acad Sci USA 2005; 102:5507-5512.
30. Hung CC, Chang WL, Ho JL, Tai JJ, Hsieh TJ, Huang HC, *et al.* Association of polymorphisms in EPHX1, UGT2B7, ABCB1, ABCC2, SCN1A and SCN2A genes with carbamazepine therapy optimization. Pharmacogenomics 2012; 13:159-169.
31. Zimprich F, Stogmann E, Bonelli S, Baumgartner C, Mueller JC, Meitinger T, *et al.* A functional polymorphism in the SCN1A gene is not associated with carbamazepine dosages in Austrian patients with epilepsy. Epilepsia 2008; 49:1108-1109.
32. Haerian BS, Baum L, Kwan P, Tan HJ, Raymond AA, Mohamed Z. SCN1A, SCN2A and SCN3A gene polymorphisms and responsiveness to antiepileptic drugs: a multicenter cohort study and meta-analysis. Pharmacogenomics 2013; 14:1153-1166.
33. Wang M, Sang S, Hwang LS, Ho C-T. Herbs, Challenges in Chemistry and Biology, ACS symposium series 925. 2006:xii, 369p.
34. Chan E, Lee HS, Hue SS. Population pharmacokinetics of carbamazepine in Singapore epileptic patients. Br J Clin Pharmacol 2001; 51:567-576.
35. Liu H, Delgado M. Influence of sex, age, weight, and carbamazepine dose on serum concentrations, concentration ratios, and level/dose ratios of carbamazepine and its metabolites. Ther Drug Monit 1994; 16:469-476.