Original Article

ASSESSMENT OF *CYP2D6*10* POLYMORPHISM WITH POST HERPETIC NEURALGIA PATIENTS UNDERGOING TRAMADOL TREATMENT

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ABSTRACT

Objective: To evaluate association of *CYP2D6*10* polymorphism with respect to demographic characteristics (age at onset, genders and weight), numerical rating scale (NRS) for measuring pain intensity in relation with resting and movement associated pain and adverse drug effects of PHN patients receiving tramadol therapy.

Methods: Total 246 patients of PHN (148 males and 98 females) were selected who fulfilled the inclusion/exclusion criteria. Clinicians were recorded numerical rating scores (at rest and with movement), and note down adverse drug side effects during the time of study. All samples were analyzed for *CYP2D6*10* polymorphism using PCR-RFLP method.

Results: We observed genotype distribution of *CYP2D6* 10* did not vary significantly with age at onset [non-responders (p=0.317) and responders (p=0.260)], genders[non-responders (p=0.317) and responders (p=0.949)], and weight [non-responders (p=0.298) and responders (p=0.279)] and also did not find significant role with respect to resting (p=0.428) and movement associated type of pain (p=0.178). In addition, *CYP2D6*10* was not associated with adverse effects such as somnolence (p=0.135), dizziness (p=0.178), local site reactions (p=0.535), headache (p=0.502), hypotension (p=0.567) and nausea and vomiting (p=0.268) of analgesic therapy. Therefore we conclude that, *CYP2D6*10* may not be a predictor of treatment outcomes of patients with PHN receiving tramadol.

Keywords: Post Herpetic Neuralgia, *CYP2D6*10* allele, Tramadol, PCR- RFLP, Clinical trial.

INTRODUCTION

Post herpetic neuralgia (PHN) is the most common complication of *herpes zoster* (HZ) and one of the most challenging to treat. Commonly prescribed medications for pain relief of PHN, opioids such as tramadol are receiving greater consideration for the treatment of PHN type of pain[1,2,3]. Tramadol is a weak μ -opioid agonist that also inhibits the reuptake of norepinephrine and serotonin. The results of randomized control trials in patients with PHN, painful DPN, painful polyneuropathies, PHN of different etiologies, and postamputation pain demonstrated that tramadol reduced pain and improved some aspects of health-related quality of life[1-3,5-7]. But it has developed lots of drug-induced adverse side-effects such as somnolence, dizziness, local site reaction, headache, hypotension, nausea and vomiting [8-10,3,4].

Tramadol is metabolized by the *CYP2D6* enzyme [11,3,4]. The *CYP2D6* polymorphism has been reported to significantly affect the pharmacokinetics of tramadol and also found to be associated with variability in opioids efficacy and toxicity [9,12-15]. The variation in *CYP2D6* activity may impact upon a patient's pain level and may contribute to interindividual variation in their response to opioids[16-17,3-4]. The *CYP2D6* polymorphisms were reported to be associated with specific phenotypes such as pain sensitivity [16-18]. This enzyme plays a vital role in deciding doses of tramadol in PHN patients.

The Indian population is interesting with regard to *CYP2D6*10* polymorphism, as India is located midway between the east and the west; two populations with clear geographic demarcations in terms of polymorphisms. With many drugs that are substrates of *CYP2D6*, the clinical significance may be important. There is, however, a dearth of data from India and this study describes a phenotyping study in Indian subjects [19]. To test phenotype–genotype associations, a larger number of subjects is needed. Owing to cost constraints, however, this is usually not possible. The more

commonly found genotype groups should probably be chosen to better infer clinical relevance. They could potentially take advantage of the distinctive characteristic of their populations in terms of the frequencies of *CYP2D6*4* and *CYP2D6*10*. Such groups may not be easily found in other populations in the east or the west [20].The prevalence of the PMs phenotype ranges from 0–1% in Asians to 10% in Caucasians, whereas the prevalence of the UMs phenotype ranges from 1–2% in Asians to 29% in some African populations [21-23]. The phenotyping studies on South Indians using dextromethorphan as a probe drug for the polymorphic drug-metabolizing enzyme *CYP2D6*, the proportion of PMs among this population was identified. The CYP2D6*10* frequency in Tamilnadu was significantly higher compared to that in Kerala, the frequencies in Tamilnadu, Kerala, Karnataka, and Andhra Pradesh were 12.9%, 7.0%, 11.2% and 9.0% respectively[24].

Currently, in the Indian scenario, has not been published that establish the clinical utility of *CYP2D6*10* genotyping in determining treatment choice or dose, in relation to tramadol therapy with respect to PHN patients. In the present study, we investigated the genotype and phenotype frequency of *CYP2D6*10* polymorphism in tramadol receiving PHN patients.

MATERIALS AND METHODS

Study design

The study was a prospective, non-responders versus responders in the treatment of PHN and consisted of oral administration of tramadol (short acting) for 4 weeks with day 0 (baseline) considered as a baseline. A total of 270 patients were initially enrolled for the treatment of which 15 patients did not fit the inclusion criteria and 9 patients did not receive tramadol therapy, according to the study design. This prospective study included 246 patients (age group 20-80 years) of PHN patients reported with less than 50% pain relief were categorized as "non- responders" (72

males and 51 females), and patients reported with 50% pain relief after14 day of tramadol treatment were categorized as "responders" (76 males, and 47 females). The present study was carried out with the help of Pain Clinic, Department of Anesthesiology, Department of Dermatology and all molecular biology analysis were carried out in Environmental Biochemistry and Molecular Biology Laboratory, Department of Biochemistry and Department of Pharmacology at University College of Medical Sciences (University of Delhi) & Guru Teg Bahadur Hospital, New Delhi- 110095, India during the period January 2009 to January 2012. Prior approval of Institutional Ethics Committee –Human research was received and patients consent was taken in written in the printed Performa.

In our previous published papers we have already discussed the duration of oral tramadol treatment were 4 weeks from day 0 (inclusion visit) to day 28 (the day before the end visit), dose incrementation, inclusion criteria, exclusion criteria, and rescue analgesia (topical cream containing 3.33% doxepin and 0.05% capsaicin) [2-4].

Numerical Rating Scores (NRS)

On every visit, the intensity of spontaneous pain, including both resting and movement associated pain was measured over the past 24 h on an 11-point NRS. The NRS scoring was entered directly into the case record form for each patient [25].

PCR-RFLP for *CYP2D6*10* polymorphism

Blood (peripheral lymphocytes) 5 ml was collected from each volunteer in ethylenediaminetetraacetic acid (EDTA) coated vials and DNA was extracted using Hi- Media mini-preparation kit. Genotyping for *CYP2D6*10* was performed by Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) method using specific primer sequences: forward; 5' GTGCTGAGAGTGTCCTGCC 3'and reverse; 5' CACCCACCATCCATGTTTGC 3'. Briefly, the PCR mixture (25 μl) was prepared using 50—500 ng of DNA, 2.5 μl of 1X PCR reaction buffer (500 mMKCl; 100 mM Tris–HCl; pH 8.3; 5 mM MgCl2), (as supplied by the manufacturer), 1 μl of the 2.5 mm dNTPs, each of the respective primers at 10 pmol and 0.5U of *Taq* polymerase (Supratherm). All reactions were performed in a thermal cycler (Eppendorf Thermal Cyclers). The reaction conditions for *CYP2D6*10* allele was 2 min at 94°C for 1cycle; 30 s at 94°C, 30s at 56°C, and 1 min at 72°C for 30 cycles; and 7 min at 72°C for 1 cycle. The fragment of *10 allele (325bp) was analyzed in 2% agarose gel (Fig. 1).
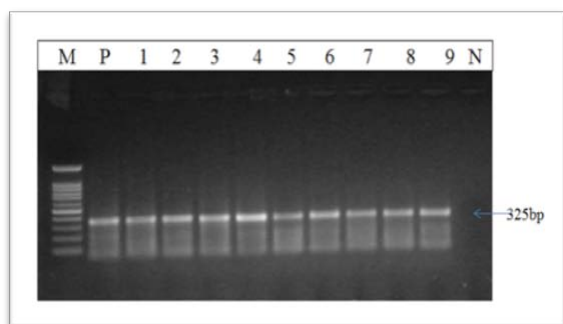
Fig. 1: Amplification of *CYP2D6*10* allele (2% agarose gel)

Lane M: 100bp Molecular marker; Lane P: Positive control; Lanes: 1-9: *CYP2D6*10* allele (325bp) from samples of PHN patients; Lane: N: Negative control

After the PCR amplification, using *Hphl* restriction digestion enzyme for four hours, samples were analyzed in 1.2% agarose gel and stained with EtBr(0.5μg/ml). 100bp DNA molecular weight marker was used as a marker to compare the amplimer size of the PCR products (**Fig. 2**). The UMs, EMs, IMs and PMs patients were categorized based on genetic analysis (PCR-RFLP Method) [3-4,26-27].
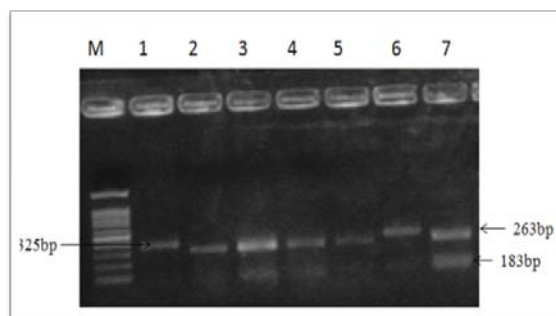
Fig. 2: PCR-RFLP of *CYP2D6*10* allele after restriction digestion (1.5% agarose gel)

M=100 bp Molecular marker ladder; Lane 1 = PM genotype; Lane 6,= IM genotype; Lane 2,3, 4,5,7 = EM genotypes

Statistical analysis

The descriptive statistics were expressed as mean ± SD. The unpaired t- test was used to compare all mean differences among the two groups on day 14. Three factors repeated measure ANOVA was applied, taking time as a repeated factor and group and metabolizers as a fixed factor. We report multivariate (Wilks' Lambda test) analysis since the Mauchly's test of Sphericity was found, to the significant in NRS scores. We report multivariate (Wilks' Lambda test) analysis since the Mauchly's test of Sphericity was found to significant in all NRS variables. The Chi - square test was used to find the association between onset at ages, genders, weight, adverse events with different metabolizers of *CYP2D6*10* polymorphism. Odds ratios were calculated to test the significance of genotype association with the occurrence of PHN. p-values <0.05 were considered as significant. The frequency of EMs genotype was calculated by adding the total of the EMs genotypes and half of the IMs genotypes, which was divided by the total number of individuals, the PMs genotype allele frequency was calculated by subtracting E allele frequency from 1 (P =1-E).

RESULTS

Patient data

Both the groups of PHN patients were comparable with respect to sex, age, weight, duration of disease and gender ratio were found no significant (p=0>0.05). In non-responders mean was (males 53.94±13.24; females 52.45±11.35) and in responders mean was (males 53.50±12.72; females 50.17±10.79.33). The mean age (in years) of patients in non-responders was 53.33±12.47 and in responders was 52.23±12.08. The mean weight (in kg) in non-responders was 56.28±10.95 and in responders was 51.23±11.45. The mean duration of disease (in months) of patients in non-responders was 4.79±3.48 and in responders was 4.23±4.47. The gender ratio (M: F) in non-responders and responders was 72:51 and 76:47 respectively.

Demographic charactestics with respect to *CYP2D6*10* polymorphism

Age

The primary age of onset for PHN patients were categorized according to agewise distibutions (20-40 years), (41-60 years) and (61-80 years) respectively. In order to examine the distribution of EMs in age wise groups, EMs were found in higher numbers in the age group of (41-60 years) [NR- 41 (50.6%) and R- 45 (51.1%)]. IMs were also observed higher in numbers of the age group (41-60 years) [NR- 12(40.0%) and R- 18 (60.0%)]. The age groups (20-40 years) and (61-80 years) a reduced number of EMs and IMs were found as compared with age (40-60 years). The PMs were found in all age groups. PMs were observed in higher at the age group (41-60 years) but less numbers were found in (20-40 years) and (61-80 years). Hence, a significant linear trend was not observed between age of onset of the PHN patients. The metabolizers (EMs, IMs and

PMs) with respect to the *CYP2D6*10* polymorphism was insignificantly (p>0.05) between ages at onset in both groups. Further, no significant differences were observed in EMs and PMs genotype allele frequencies (Table 1).

Sex

Although sex differences in the *CYP2D6*10* genotype distribution in both groups was not evident, male (non-responders) patients had an increased frequency of both EMs and IMs genotypes compared with female (non-responders) patients, whereas in the responder group same observation observed, frequency of EMs and IMs genotypes was higher in males than in females. The PMs genotype allele frequency was found to be increased in females compared with males in both responders and non-responders. The *CYP2D6*10* polymorphism did not vary significantly between gender in both groups (p>0.05) (Table 1).

Weight

According to weight, stratification of the study group was done on the basis of the agewise distribution, i. e., agewise groups (20-40 years), (41-60 years) and (61-80 years) respectively. Significant linear trend was not observed between weight of the PHN patients. The metabolizers (EMs, IMs and PMs) compared to the *CYP2D6*10*

polymorphism was not significantly (p>0.05) associated with weight (Table 1).

NRS Scores with respect to *CYP2D6*10* polymorphism

Resting Pain Intensity

The non- responders group shows EMs (n= 81), IMs (n= 30), PMs (n= 12) whereas responders having EMs (n= 87), IMs (n= 31), PMs (n= 5) respectively. Three factors repeated measures ANOVA was carried out to find out interaction between time, group and metabolizers. Insignificant (p=0. 428) interaction was found among time, group and metabolizers. Similarly with time and metabolizers insignificant (p=0. 934) interaction was observed in **10* polymorphism. Interaction between the groups was found to be significant (p<0.001) indicate that NRS resting scores changes with time. (Table 2).

Movement Associated Pain Intensity

Three factor repeated measures analysis ANOVA was carried out which shows significant interaction with time that means NRS movement score changes with the time. Significant interaction between the time and metabolizers were found (p=0.098) whereas insignificant results were observed between the time, group and metabolizers (p=0.178) (Table 2).

Table 1: Demographic characterstics and *CYP2D6*10* polymorphism

Charactestics	Groups	Distributions	Metabolizers			Allele frequencies		Pearson's Chi Square	P-value
			EM n(%)	IM n(%)	PM n(%)	EM genotypes	PM genotypes		
Age	NR	20–40 years	16(19.8%)	7(23.3%)	2(16.7%)	0.78	0.22	2.299	0.317
	(n=	41–60 years	41(50.6%)	12(40.0%)	6(50.0%)	0.796	0.203		
	123)	61–80 years	24(29.6%)	11(36.7%)	4(33.2%)	0.782	0.269		
	R	20–40 years	18(20.5%)	5(16.7%)	2(40.0%)	0.82	0.18	1.270	0.260
	(n=	41–60 years	45(51.1%)	18(60.0%)	3(60.0%)	0.796	0.18		
	123)	61–80 years	25(28.4%)	7(23.3%)	0(0%)	0.782	0.109		
Genders	NR	Males	44(61.1%)	23(31.9%)	5(6.9%)	0.778	0.229	2.299	0.317
	(n=	Females	37(72.5%)	7(13.7%)	7(13.7%)	0.794	0.840		
	123)								
	R	Males	55(72.4%)	19(25.0%)	2(2.6%)	0.848	0.151	1.299	0.949
	(n=	Females	33(70.2%)	11(23.4%)	3(6.4%)	0.205	0.202		
	123)								
Weight	NR	20–40 years	16 (47.1%)	7(58.3%)	2(50.0%)			2.422	0.298
	(n=	41–60 years	41(47.7%)	12(40.0%)	6(66.7%)				
	123)	61–80 years	24(49.0%)	11(61.1%)	4(10.0%)				
	R	20–40 years	18(52.9%)	5(41.7%)	2(50.0%)			2.550	0.279
	(n=	41–60 years	45(52.3%)	18(60.0%)	3(33.3%)				
	123)	61–80 years	25(51.0%)	7(38.9%)	0(0.0%)				

NR- Non-responders; R- Responders; All values are expressed in numbers and percentages; N- Numbers; EM-Extensive metabolizers; IM-Intermediate metabolizers; PM-Poor metabolizers

Table 2: Numerical rating scales (NRS) scores and *CYP2D6*10* polymorphism

NRS Scales	Group	Metabolizers	0 Day (Baseline)	3 Day	7 Day	14 Day	28 Day	P- value
Resting pain intensity	NR (n=123)	EM(n=81)	7.90±1.281	6.79±1.403	5.85±1.314	4.86±1.081	2.89±1.084	**p=0.001[a]**
		IM(n=30)	7.87±1.252	6.77±1.331	5.63±1.299	4.77±1.278	2.80±1.095	**p=0.001[b]**
		PM(n=12)	7.83±1.337	7.08±1.311	5.83±1.115	4.75±.866	2.67±.985	p=0.934[c]
	R (n=123)	EM(n=87)	7.36±1.438	5.90±1.463	4.68±1.289	3.23±1.042	1.66±.790	p=0.428[d]
		IM(n=31)	7.63±1.426	6.07±1.507	4.90±1.373	3.37±.999	1.73±.944	
		PM(n=5)	5.80±1.304	4.40±.894	3.60±0.894	2.40±1.140	0.80±.837	
Movement associated pain	NR (n=123)	EM(n=81)	7.52±1.441	6.52±1.582	5.64±1.399	4.69±1.068	2.57±1.193	**p=0.001[a]**
		IM(n=30)	7.57±1.591	6.70±1.579	5.63±1.671	4.63±1.520	2.67±1.348	**p=0.001[b]**
		PM(n=12)	7.17±1.642	6.83±1.801	5.75±1.422	4.58±1.311	2.75±1.215	p=0.098[c]
	R (n=123)	EM(n=88)	7.05±1.604	5.65±1.612	4.45±1.381	3.02±.994	1.47±1.028	p=0.178[d]
		IM(n=31)	7.37±1.520	5.80±1.495	4.77±1.524	3.20±1.126	1.53±1.137	
		PM(n=5)	5.00±1.225	4.00±.707	2.80±.837	2.00±.707	0.60±.548	

NR- Non- Responders; R-Responders; EM- Extensive metabolizers; IM- Intermediate metabolizers; PM- Poor metabolizers; All values are expressed in mean and standard deviation P>0.05- Non significant; a-interaction with time; b-interaction with group (non-responders versus responders);c-interaction with metabolizers and group; d- interaction with group (non-responders versus responders), metabolizers and time; Analysis was performed by Three Way Repeated measure ANOVA using Wilks's Lambda test.

Table 3: Adverse effects and *CYP2D6*10* polymorphism

Adverse Effects	Yes/No	Metabolizers			Total
		EM, N (%)	IM, N (%)	PM, N(%)	
Somnolence	No(n=181)	127(70.2%)	45(24.9%)	9(5.0%)	181
	Yes(n=65)	42(64.6%)	15(23.1%)	8(12.3%)	65
	Total(n=246)	169(68.7%)	60(24.4%)	17(6.9%)	246
	Pearson Chi-Square Test	p=0.135			
Dizziness	No(n=192)	127(66.1%)	52(27.1%)	13(6.8%)	192
	Yes(n=54)	42(77.8%)	8(14.8%)	4(7.4%)	54
	Total(n=246)	169(68.7%)	60(24.4%)	17(6.9%)	246
	PearsonChi-Square Test	p=0.178			
Local site reaction	No (n=197)	133(67.5%)	51(25.9%)	13(6.6%)	197
	Yes(n=49)	36(73.5%)	9(18.4%)	4(8.2%)	49
	Total(n=246)	169(68.7%)	60(24.4%)	17(6.9%)	246
	Pearson Chi-Square Test	p=0.535			
Headache	No (n=196)	134(68.4%)	50(25.5%)	12(6.1%)	196
	Yes (n=50)	35(70.0%)	10(20.0%)	5(10.0%)	50
	Total(n=246)	169(68.7%)	60(24.4%)	17(6.9%)	246
	Pearson Chi-Square Test	p=0.502			
Hypotension	No(n=181)	127(70.2%)	41(22.7%)	13(7.2%)	181
	Yes(n=65)	42(64.6%)	19(29.2%)	4(6.2%)	65
	Total(n=246)	169(68.7%)	60(24.4%)	17(6.9%)	246
	Pearson Chi-Square Test	p=0.567			
Nausea and vomiting	No (n=178)	122(68.5%)	41(23.0%)	15(8.4%)	178
	Yes(n=68)	47(69.1%)	19(27.9%)	2(2.9%)	68
	Total(n=246)	169(68.7%)	60(24.4%)	17(6.9%)	246
	Pearson Chi-Square Test	p=0.268			

All values are expressed in numbers and percentages; N- Numbers; UM-Ultra metabolizers; EM-Extensive metabolizers; IM-Intermediate metabolizers; PM-Poor metabolizers

Adverse effects with respect to *CYP2D6*10* polymorphism

In this study, somnolence was noticed in sixty five patients, out of which 42(64.6%) in EMs, 15 (23.1%) patients belong to IMs group and 8 (12.3%) PMs both groups. Fifty four patients dizziness was observed, out of which 42(77.8%) in EMs, 8(14.8%) in IMs patients and PMs was 4(7.4%) in a both groups which lasted from 2-3 days and then subsided. Local site reactions found fourty nine and when compared to *CYP2D6*10* polymorphism showing non-significant (p=0.535) using the Chi - square test. The headache was fifty patients in both groups. Hypotension was also observed in sixty five patients in both groups. The sixty eight patients were observed in nausea and vomiting, out of 246 patients in both groups. In all, adverse events compared to *CYP2D6*10* allele with EMs, IMs and PMs shows none significant(p >0.05). The adverse effects for all evidence (somnolence (p=0. 135), dizziness (p=0. 178), and local site reactions (p=0. 535), headache (p=0. 502), hypotension (p=0. 567), nausea and vomiting (P=0.268) were showing non-significant for all the patients (p>0.05) (Table 3).

Rescue analgesia with respect to *CYP2D6*10* polymorphism

The one hundred and thirty five patients required rescue analgesia. In all, rescue analgesia were compared to *CYP2D6*10* polymorphism with metabolizers (EMs, IMs and PMs) showing insignificant (p>0.05). The *CYP2D6*10* allele did not find significant (p=0. 179) with respect to rescue analgesia **(**data not shown).

DISCUSSION

In the current study genotype relationship of *CYP2D6*10* has not been studied in PHN patients in Indian population undergoing tramadol treatment. In our previous study, clinically evaluated safety and efficacy of oral tramadol therapy using 50 mg to 200 mg per day for 4 weeks in PHN patients were studied and results observed significant pain reduction in terms of enhanced pain relief, reduced sleep interference, greater global improvement, diminished side-effect profile, and improved quality of life[2]. Previous genetic studies also showed *CYP2D6*4*, and *CYP2D6*2* polymorphisms may not be a predictor of treatment outcome in patients with PHN receiving tramadol therapy [3,4].

The striength of the current study is that *CYP2D6*10* polymorphism was not significantly associated onset at ages, genders and weight may not be a predictors of PHN patients. Our previous results showed that the *CYP2D6*4* and *CYP2D6*2* genotype distributions and haploid frequencies did not vary significantly among the onset at ages, genders, and weight may not be a predictor of PHN [3,4]. Wang et al.[28] was observed that, age, gender distribution, duration of surgery, height as well as body weight of the patients among the postoperative tramadol analgesia in a Chinese population. There was no difference in the total analgesic consumption of tramadol between patient groups and also there was no significant difference in the satisfaction among the *CYP2D6* genotypes (p>0.05). Similarly Dworkin et al.[29] did not find sex differences to be associated with the various aspects of HZ, with the only exception being the intensity of acute pain, which is higher in females than in males. Gan et al.[9] also found that no difference in terms of gender between groups. Volpi et al.[30] have suggested that the female gender is associated with more severe acute HZ pain; it was shown in multivariate analysis that gender difference was observed by depression, which was found to be more severe and more frequent in women compared with men. Finally, female sex has been proposed as a predictor of PHN. However, this has not yet reached a convincing level of evidence [31-34]. There is evidence that PHN is more common in women than in men [35,36]. HZ incidence was more common in women than men, a finding supported by several other studies, albeit not all [37-41].

In this study, *CYP2D6*10* genotype–phenotype distribution did not vary significantly between ages-at-onset groups. Earlier reported *CYP2D6*4 and CYP2D6*2* genotype–phenotype distribution did not vary significantly between ages-at-onset groups [3,4]. The present study has some potential limitations, such as the relatively low number of patients examined compared with the number of studied predictors [42]. In addition, the results reported in this study support the use of analgesic drug (tramadol) prescription based on age but also on clinical findings. PHN is an immunocompetent disease and at an older age, the immune system weakens. The HZ virus attacks the immune system and because of old age damages the nerves; this remains to be the best indicator of developing PHN. On the other hand, age is the predominant predictor of PHN [43,44]. In a UK primary-care study, the prevalence of PHN increased markedly with age: from 8% between the ages of 50 and 54 years to

21% at the age of 80–84 years[35].PHN has been repeatedly associated with older age [30,33,40,45-47].

The effect of a drug on the body depends on the combination of pharmacokinetic factors. Women have a different volume of distribution and clearance than men, which could result in differences in effective drug concentrations [48-51]. Female patients, being generally lighter in weight and smaller in build than their male counterparts but usually receiving the same drug doses have been demonstrated to be more prone to ADRs in some studies [52-54]. This is most probably attributable to the exposure to higher dose per kg body weight for the females.

The present study demonstrated that, the *CYP2D6*10* polymorphism did not vary significantly among resting and movement stages on the NRS between the groups. The same type of results also found previous reported not significantly related to *CYP2D6*4* and *CYP2D6*2* alleles but clinically significant reduction related to resting and movement associated pain scale scores [2,3,4]. Jensen *et al.*[55] and McCarthy *et al.*[56] has shown that, the visual analogue scale (VAS) measurement for detecting effects in postoperative pain treatment was superior to the verbal rating scale (VRS), or their combination. The VAS used in this study, can thus be considered a valid score for the assessment of intensity of pain and the analgesic efficacy of tramadol.

Regarding the adverse events encountered in our study, due to tramadol therapy in both groups, the most common adverse events were somnolence, dizziness, local site reactions, headache, hypotension and nausea and vomiting. In the present study, no significant association was found between adverse events compared with the *CYP2D6*10* polymorphism. Also previous genotype studies related to adverse events did not find any significant related to *CYP2D6*4* polymorphism and *CYP2D6*2* polymorphism [3,4]. In term of adverse effects, UMs were more sensitive to tramadol than EMs. They found that UMs volunteers experienced quicker analgesic effects but were prone to higher mu-opioid-related toxicity after tramadol in experimental pain setting using a cold pressure test when they studied 11 carriers of a *CYP2D6* duplication allele (UMs) and compared with 11 carriers of two active *CYP2D6* (EMs). Pharmacokinetics and pharmacodynamics effects (pain threshold and pain tolerance, miosis and adverse events) were monitored after a single dose of 100 mg racemic tramadol, rapid release formulation [27] and they observed differences in tramadol adverse effects with a higher frequency of nausea (50.0 vs.9.0%) in the UMs compared with the EMs. Stamer *et al.* [14]suggested that, the PMs, non-response rates to tramadol treatment increased four fold compared with the other genotypes. Thus this genotype was associated with poor efficacy of tramadol analgesia. Li *et al.* [57] observed association between *CYP2D6*10* genetic polymorphisms and the pharmacokinetics of tramadol in Chinese volunteers. Wang *et al.*[28]investigated that, *CYP2D6 * 10* alleles had an impact on the postoperative analgesic effect of tramadol in 70 Chinese patients after gastrectomy. Gan *et al.* [9]was also observed that the incidence of vomiting, the IMs were found to have a statistically higher incidence of adverse drug reactions when compared with the groups that metabolize tramadol faster (UMs and EMs). This shows that the slower metabolizers of tramadol tend to experience more adverse effects of the drug and also found that there were significant differences in the adverse-effect profiles amongst the various genotype groups, with the IMs group experiencing more adverse effects than the EMs, and the EMs having more adverse effects than the UMs. It also *CYP2D6* activity may play an important role in determining the pharmacokinetics of tramadol and in predicting it's adverse. Other studies found no difference in term of adverse events such as nausea and vomiting between patients with *CYP2D6* UMs phenotype, PMs phenotype or reduced *CYP2D6* activity and EMs [15,28,58].

CONCLUSION

In conclusion, to the best of our knowledge, this is the first study reporting genotype- relationship with PHN patients in Indian population. Assessment of *CYP2D6*10* metabolic status before initiation of therapy may not help to identify patients at risk for no response to therapy or toxic drug effects and is needed to ensure optimal dosing recommendations.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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