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Original Article

THE IMPACT OF PHARMACOGENETICS ON ADVERSE DRUG REACTIONS TO PREDICT THE EFFICACY OF TRAMADOL MONOTHERAPY FOR THE TREATMENT OF POST HERPETIC NEURALGIA PATIENTS

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ABSTRACT

Objective: To evaluate the potential role of tramadol treatment with respect to *CYP2D6* polymorphism in reducing the incidence of adverse drug reactions.

Methods: The study comprised 246 patients of PHN receiving tramadol treatment. Adverse drug events during the time of the study were recorded by the physician. All samples were analyzed for *CYP2D6* (*2, *4 and *10) polymorphism using PCR-RFLP method.

Results: The *CYP2D6* polymorphism did not find significantly among onset at age, genders and weight in non-responders and responders. The *CYP2D6*4* polymorphism was significantly associated with somnolence (p=0.009), dizziness (p=0.007), local site reactions (p=0.015), headache (p=0. 039), and nausea and vomiting (p=0. 017). The dizziness (p=0. 029) and headache (p=0. 004) were found associated with *CYP2D6*2* both the groups. No associations were observed between adverse events compared with *CYP2D6*2* and *CYP2D6*10* polymorphisms (p>0.05).

Conclusion: *CYP2D6*4* polymorphism may be as important drug toxicity marker predictors of experiencing adverse drug reactions as PHN patients undergoing tramadol treatment.

Keywords: Adverse drug reactions, Tramadol, *CYP2D6*, Post herpetic neuralgia.

INTRODUCTION

Adverse drug reactions (ADRs) are a significant cause of morbidity and mortality. The person-to-person variability of drug response is a major problem in clinical practice and drug development [1]. The potential risk factors for drug in efficacy or toxicity include drug–drug interactions, the patient's age, sex, or other disease factors, and lifestyle variables such as smoking and alcohol consumption. All these factors, should be evident for the physicians. In addition, it has become clear in recent years, that genetic factors may also significantly modify drug responses or increase the risk for ADRs[2]. The neuropathic pain is very challenging to manage because of the heterogeneity of its aetiologies, symptoms and underlying mechanisms [3]. It is often difficult to treat, because it is resistant to many medications and/or because of the adverse effects associated with effective medications. A number of drugs are used to manage neuropathic pain, including antidepressants [4], anticonvulsant drugs [5], opioids especially tramadol [6,7,8,9] and topical treatments such as capsaicin[10] and lidocaine [11]. Many people require treatment with more than one drug but the correct choice of drugs and the optimal sequence for their use have been still unclear.

Tramadol is an analgesic drug which is used for neuropathic pain like post herpetic neuralgia (PHN) [6-9]**.** Consequently, there is a subset of patients who develop drug-induced adverse side-effects such as somnolence, dizziness, the local site reaction, headache, hypotension, nausea and vomiting [8,12-16]. Therefore, safe and effective treatments are needed for PHN patients. Tramadol is metabolized by *CYP2D6* to generate a pharmacologically active product, the analgesic opioid receptor agonist O-desmethyltramadol. Variability in response is, therefore, closely related to the *CYP2D6* genotype [17]. The polymorphic drug metabolism in a population identifies, the proportion of individuals differing inability to metabolize certain drugs and who therefore, are likely to react differently or adversely to drugs [18]. According to the activity of the *CYP2D6* enzyme, the polymorphism of the enzyme results in poor metabolizers (PMs), intermediate metabolizers (IMs), extensive metabolizers (EMs), and ultra extensive metabolizers (UMs) of *CYP2D6* drugs [19]. Thus, elevated drug levels in PMs and UMs might lead to increased side effects during pharmacological treatment.

The goal of our study is to predict *CYP2D6* response with respect to tramadol treatment and improves drug response in the personalized medicine in individual patients and reduce adverse drug effects. Here, the aim of the current study, tramadol treated PHN patients having adverse drug events comparing with *CYP2D6* (*2,*4 and *10) polymorphism.

MATERIALS AND METHODS

Study design

The study was a prospective, non-responders versus responders in the symptomatic treatment of PHN, and consisted of oral administration of tramadol (short acting) for 4 weeks with day 0 (baseline) considered as a baseline. According to the study design, 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. Patients those reporting less than 50% pain relief categorized as "non-responders" (72 males and 51 females) and patients who had achieved 50% pain relief 14 days of tramadol treatment as "responders" (76 males and 47 females). The study was conducted in association with the Pain Clinic of Department of Anesthesiology, Department of Dermatology, 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. The study was approved by the Institutional Ethics Committee –Human Research. Written informed consent was obtained from all patients.

The duration of oral tramadol treatment was 4 weeks from day 0 (inclusion visit) to day 28 (the day before the end visit). At 0 days (baseline phase), patients rated the intensity of PHN pain over the previous 24 hours using an 11-point NRS ranges from 0 as no pain in 10 as worst possible pain. The daily dose of tramadol was increased, depending on the therapeutic response and on treatment acceptability, from one tablet (50mg) per day to four tablets (200mg) per day. Dose incrementation was performed stepwise in accordance with the following schedule: at least 48 h between step 1 (one tablet =50mg per day) and 2 (two tablets = 100mg per day), at least 72 h between step 2 and 3 (three tablets= 150mg per day) or between step 3 and 4 (four tablets = 200mg per day) in patients aged at the maximum 70 years. On the other hand, the dose incrementation schedule for patients aged more than 70 years consisted of a time interval of 72 h between step 1 and 2, and at least 120 h between step 2 and 3. In patients, not responding to oral medications rescue analgesia was given in the form of topical application of capsaicin 0.05% and/or doxepin 3.33% cream, four times a day after two weeks in the painful affected dermatomes. The adverse drug reaction was recorded by the physician.

Inclusion criteria

The study participants included men and women (in age group of 20-80 years) of Indo-Aryan ethnicity and had PHN defined as pain present for more than 3 months after healing of a herpes zoster skin rash. Patients were considered eligible if their pain was at least 4 on the 11 point numerical rating scale (NRS) which includes three of the constituent symptoms of neuropathic pain namely severe burning, shooting pain, paresthesia and precipitation of pain on touch of a pledget of cotton (allodynia) during the base-week preceding randomization. All patients of PHN were having sharp, shooting pain that is unresponsive to non-steroidal anti-inflammatory drugs (NSAIDs). Patients receiving NSAIDs were instructed to stop these drugs three days prior to the commencement of treatment with tramadol.

Exclusion criteria

Patients who presented with symptoms or past history of depression, immune-depression, seizures, illicit drug abuse or central nervous system depressant drug abuse, severe hepatic, renal, cardiac or respiratory pathology, hypersensitivity to tramadol or to opioids were excluded from the study. Patients likely to receive any treatment known to interfere with the studied drug or with the study design such as neurological surgery, anesthetic blocks, local treatments of pain, antidepressants, anticonvulsants, anti-vitamin K, (enzymatic inducers), were also excluded. Patients with any history of diabetes mellitus, HIV, malignancy, haematological or liver disease, psychiatric illness, alcohol abuse or those receiving corticosteroids and immunosuppressive drugs were not included. In addition, patients having WBC <2500 mm^3, neutrophil count <1500 mm^3 or platelet count <100 x 10^3 mm^3 were also excluded. Pregnant or breast- feeding women and women who risked becoming pregnant during the study period were not included.

Genotyping

5 ml of blood was taken out from each patient and collected in EDTA coated vials. DNA was extracted using commercially available DNA extraction kit (Hi- Media Mini preparation kit, Hi- Media Laboratories Pvt. Ltd. Mumbai, India). The Polymerase Chain Reaction-Rescticon Fragment Lengh Polymorphism (PCR-RFLP) was done by digesting PCR product with their respective restriction enzyme that determines the polymorphic site depending on the presence or absence of its recognition sequence (Table 1). The UMs, EMs, IMs and PMs patients were categorized based on genetic analysis (PCR-RFLP Method) [20,21,8,9].

Table 1: Primer sequences of *CYP2D6* alleles and PCR-RFLP detection method using their respective restriction enzymes

S. No.	*CYP2D6* alleles	Primer Sequences	Detection Method
1	*2	5'GCTGGGGCCTGAGACTT'3	PCR-RFLP
		5'GGCTATCACCAGGTGCTGGTGCT3'	using *Hhal*
3	*4	5' TGCCGCCTTCGCCAACCACT3'	PCR-RFLP
		5'TCGCCCTGCAGAGACTCCTC3'	using *BstNI*
4	*10	5'GTGCTGAGAGTGTCCTGCC3'	PCR-RFLP
		5' CACCCACCATCCATGTTTGC3'	using *HphI*

Statistical analysis

The descriptive statistics were expressed as number and percentage. The Chi - square test was used to find the association between an onset at ages, genders, adverse events with different metabolizers of *CYP2D6* polymorphism. Odds ratios were calculated to test the significance of genotype association with the occurrence of PHN. p value <0.05 was taken 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

The study group consisted of 246 patients of PHN (148 males and 98 females) who fulfilled the inclusion/exclusion criteria.

Demographic characterstics

Both the groups (123 non- responders and 123 responders) of PHN patients compared with respect to sex, age, weight, duration of disease and gender ratio were found no significant (p >0.05). The total gender means non-responders were 53.33±12.47 (males 53.94±13.24; females 52.45±11.35) and total mean responders was 52.23±12.08 (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 were 52.23±12.08. The mean weight (in kg) in group 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 were 4.79±3.48 and in responders were 4.23±4.47. The gender ratio (Male: Female) in non-responders and responders were 72:51 and 76:47 respectively.

Adverse events in non responders and responders

Clinically, somnolence was noticed 38 (30.8%) in non- responder patients and 27 (21.95%) responder patients. Dizziness was experienced by 18 (14.63%) patients in responders and 36 (29.2%) patients in non- responders lasted from 2-3 days and then subsided. Thirty one non-responders (25.2%) and 18 (14.63%) patients in responders suffered local site reaction. Headache was seen in 30 (24.3%) non- responders and 20 (16.26%) patients in responders. However, hypotension was observed 46 (37.3%) in non- responders and 19 (15.44%) patients in responder. Fifty (40.6%) patients in non- responders and 18 (14.63%) patients in responders had nausea and vomiting (Table 2).

Onset at age with respect to *CYP2D6* polymorphism

The primary age of onset for PHN patients was categorized according to age wise groups (20-40 years), (41-60 years) and (61-80 years) respectively. UMs were found in *2 and *4 alleles and not in *10 allele. Higher numbers and percentage of UMs were found in the age (41-60 years) [Non responders - 3 (75%) and Responders (2 (66.7%)] in *2 allele whereas in *4 allele in the responders group 5 (62.5%) UMs were observed. Hence, a significant linear trend was not found between the age of the PHN patients and the UMs with respect to the *CYP2D6* polymorphism (p>0.05). EMs were higher in numbers and percentage with respect to *2, *4 and *10 alleles

in both the group of PHN patients. In the age wise groups (20-40 years) and (61-80 years) a reduced number of EMs were found as compared with age (40-60 years). IMs also showed the same pattern just like EMs. The PMs was found in all age groups. Higher numbers of PMs were found in the age group (41-60 years) in *2 allele but very less numbers were observed in *4 and *10 alleles. No linear trends were observed between the age wise groups of PHN patients and PMs with respect to *CYP2D6* polymorphism. None of the values was found to be statistically significant (p>0.05). Further, no significant differences were observed in EMs and PMs genotype allele frequencies (p>0.05) (Table 3).

Table 2: Adverse events in non responders and responders

Adverse events	Non-responders(n=123)	Responders(n=123)
Somnolence	38(30.8%)	27(21.95%)
Dizziness	36(29.2%)	18(14.63%)
Local site reaction	31(25.2%)	18(14.63%)
Headache	30(24.3%)	20(16.26%)
Hypotension	46(37.3%)	19(15.44%)
Nausea and vomiting	50(40.6%)	18(14.63%)

N= numbers; All adverse events expressed in numbers and percentage

Table 3: Onset at age and *CYP2D6* polymorphism

Ages	Metabolizers	*CYP2D6*2* allele		*CYP2D6*4* allele		*CYP2D6*10* allele	
		NR	R	NR	R	NR	R
20-40 years	**UM n(%)**	1(25%)	1(33.3%)	0(0%)	1(12.5%)	0(0%)	0(0%)
41-60 years		3(75%)	2(66.7%)	0(0%)	5(62.5%)	0(0%)	0(0%)
61-80 years		0(0%)	0(0%)	0(0%)	2(25.0%)	0(0%)	0(0%)
20-40 years	**EM n(%)**	11(19.6%)	15(23.1%)	14(16.9%)	20(21.7%)	16(19.8%)	18(20.5%)
41-60 years		26(46.4%)	34(52.3%)	36(46.8%)	47(51.1%)	41(50.6%)	45(51.1%)
61-80 years		19(33.9)	16(24.6%)	28(36.4%)	25(27.2%)	24(29.6%)	25(28.4%)
20-40 years	**IM n(%)**	8(26.7%)	7(17.1%)	10(29.4%)	4(19.0%)	7(23.3%)	5(16.7%)
41-60 years		13(43.3%)	19(46.3%)	18(52.9%)	13(61.9%)	12(40.0%)	18(60.0%)
61-80 years		9(30.0%)	15(36.6%)	6(17.6%)	4(19.0%)	11(36.7%)	7(23.3%)
20-40 years	**PM n(%)**	5(15.2%)	2(14.3%)	2(18.2%)	0(0%)	2(16.7%)	2(40.0%)
41-60 years		17(51.5%)	11(78.6%)	4(36.4%)	1(50.0%)	6(50.0%)	3(60.0%)
61-80 years		11(33.3%)	1 (7.1%)	5(45.5%)	1(50.0%)	4(33.2%)	0(0%)
	Total	123	123	123	123	123	123
Pearson's Chi-square Test		0.763	0.268	0.230	0.906	0.894	0.542
Allele Frequencies							
EM genotypes							
20-40		0.6	0.36	0.72	0.88	0.78	0.82
41-60		0.652	0.449	0.762	0.810	0.796	0.796
61-80		0.602	0.397	0.794	0.843	0.782	0.782
PM genotypes							
20-40		0.74	0.26	0.28	0.12	0.22	0.18
41-60		0.659	0.310	0.226	0.189	0.203	0.18
61-80		0.734	0.265	0.205	0.159	0.269	0.109

UM- Ultra metabolizers; EX- Extensive metabolizers; IM- Intermediate metabolizers; PM- Poor metabolizers; NR- Non responders; R- Responders; All values are expressed in numbers and percentage

Table 4: Genders and *CYP2D6* polymorphism

		CYP2D6 Polymorphism					
Genders	**Metabolizers**	***CYP2D6*2* allele**		***CYP2D6*4* allele**		***CYP2D6*10* allele**	
		NR	R	NR	R	NR	R
Males	UM n(%)	2(2.8%)	2(2.6%)	0(0%)	3(3.9%)	0(%)	0(%)
Females		2(3.9%)	1(2.1%)	0(0%)	5(10.6%)	0(%)	0(%)
Males	EM n(%)	30(41.7%)	38(50.0%)	43(60.6%)	60(78.9%)	44(61.1%)	55(72.4%)
Females		26(51.0%)	27(57.4%)	34(27.9%)	32(68.1%)	37(72.5%)	33(70.2%)
Males	IM n(%)	19(26.4%)	26(34.2%)	20(28.2%)	11(14.5%)	23(31.9%)	19(25.0%)
Females		11(21.6%)	15(%)	14(27.9%)	10(21.3%)	7(13.7%)	11(23.4%)
Males	PM n(%)	21(29.2%)	10(13.2%)	8(11.3%)	2(2.6%)	5(6.9%)	2(2.6%)
Females		12(23.5%)	4(8.5%)	3(5.9%)	0(0%)	7(13.7%)	3(6.4%)
Total		123	123	123	123	123	123
Pearson's Chi-square test		0.723	0.818	0.567	0.214	0.048	0.590
Allele Frequencies							
Males	EM genotypes	0.548	0.671	0.746	0.875	0.778	0.848
	PM genotype	0.451	0.328	0.253	0.151	0.229	0.151
Females	EM genotypes	0.671	0.734	0.803	0.787	0.794	0.840
	PM genotype	0.328	0.265	0.196	0.212	0.205	0.202

UM- Ultra metabolizers; EX- Extensive metabolizers; IM- Intermediate metabolizers; PM- Poor metabolizers; NR- Non responders; R- Responders; All values are expressed in numbers and percentage

Number of PMs was observed in male patients as compared to females. Difference in both EMs and PMs genotype was observed in males and females in both the groups i. e. Non-responders and responders (Table 4).

Table 5: Adverse effects (somnolence, dizziness and local site reactions) and *CYP2D6* polymorphism,

Group		*CYP2D6*2* allele					*CYP2D6*4* allele					*CYP2D6*10* allele				
Somnolence		**UM**	**EM**	**IM**	**PM**	**Total**	**UM**	**EM**	**IM**	**PM**	**Total**	**UM**	**EM**	**IM**	**PM**	**Total**
NR	Count	2	13	10	13	38	0	24	10	4	38	-	23	10	5	38
	%within group	5.3%	34.2%	26.3%	34.2%	100.0%	.0%	64.9%	27.0%	8.1%	100.0%	-	60.5%	26.3%	13.2%	100.0%
	% within allele	50.0%	50.0%	52.6%	81.2%	58.5%	.0%	58.5%	71.4%	100.0%	57.8%	-	54.8%	66.7%	62.5%	58.5%
R	Count	2	13	9	3	27	6	17	4	0	27	-	19	5	3	27
	%within group	7.4%	48.1%	33.3%	11.1%	100.0%	22.2%	63.0%	14.8%	.0%	100.0%	-	70.4%	18.5%	11.1%	100.0%
	% within allele	50.0%	50.0%	47.4%	18.8%	41.5%	100.0%	41.5%	28.6%.	0%	42.2%	-	45.2%	33.3%	37.5%	41.5%
Pearson chi square		***p=0.206***					***p=0.009***					**p=0.702**				
Dizziness																
NR	Count	1	12	10	13	36	0	23	6	2	31	-	27	7	2	36
	%with in group	2.8%	33.3%	27.8%	36.1%	100.0%	.0%	74.2%	19.4%	6.5%	100.0%	-	75.0%	19.4%	5.6%	100.0%
	% within allele	50.0%	52.2%	62.5%	100.0%	66.7%	.0%	69.7%	66.7%	100.0%	63.3%	-	64.3%	87.5%	50.0%	66.7%
R	Count	1	11	6	0	18	5	10	3	0	18	-	15	1	2	18
	%with in group	5.6%	61.1%	33.3%	.0%	100.0%	27.8%	55.6%	16.7%	.0%	100.0%	-	83.3%	5.6%	11.1%	100.0%
	% within allele	50.0%	47.8%	37.5%.	0%	33.3%	100.0%	30.3%	33.3%.	0%	36.7%	-	35.7%	12.5%	50.0%	33.3%
Pearson Chi-square		***p=0.029***					***p=0.007***					***p=0.338***				
Local site reactions																
NR	Count	1	14	10	6	31	0	23	6	2	31	-	23	6	2	31
	%with in group	3.2%	45.2%	32.3%	19.4%	100.0%	.0%	74.2%	19.4%	6.5%	100.0%	-	74.2%	19.4%	6.5%	100.0%
	% within allele	50.0%	58.3%	58.8%	100.0%	63.3%	.0%	69.7%	66.7%	100.0%	63.3%	-	63.9%	66.7%	50.0%	63.3%
R	Count	1	10	7	0	18	5	10	3	0	18	-	13	3	2	18
	%with in group	5.6%	55.6%	38.9%.	0%	100.0%	27.8%	55.6%	16.7%.	0%	100.0%	-	72.2%	16.7%	11.1%	100.0%
	% within allele	50.0%	41.7%	41.2%	.0%	36.7%	100.0%	30.3%	33.3%	.0%	36.7%	-	36.1%	33.3%	50.0%	36.7%
Pearson chi-square		***p=0.258***					***p=0.015***					***p=0.838***				

UM- Ultra metabolizers; EX- Extensive metabolizers; IM- Intermediate metabolizers; PM- Poor metabolizers; NR- Non responders; R- Responders; All values are expressed in numbers and percentage

Genders with respect to *CYP2D6* polymorphism

Both the group of PHN patients were compared with *CYP2D6* (*2,*4 and *10 alleles) polymorphism according to genders**.** UMs was observed in *2 and *4 alleles whereas it was not found in *10 allele. Higher numbers of males (EMs and IMs metabolizers) were found as compared to females of *CYP2D6* polymorphism. The PMs was found in both groups with respect to *2, *4 and *10 alleles. Non-responders group consisted of higher numbers of PMs as compared with the responders group in *2, *4 and *10 alleles in both the genders. Higher

Adverse effects with respect to *CYP2D6* polymorphism

Somnolence, dizziness and local site reactions

PHN patients experiencing the various adverse effects. The somnolence was observed in 65 patients and did not find an association with *2 (p=0. 206) and *10 (p=0. 702) polymorphism whereas it was strongly associated with *4 (p=0. 009) polymorphsim. The total 54 patients had dizziness, it was experienced by 36 patients in the non repsonders and half the number (18) in the responders group. The dizziness types of adverse effect was observed strongly associated with *2 (p= 0.029) and *4 (p= 0.007) polymorphism but not with 10* polymorphism (p= 0.338). The local site reactions were observed in 31 non-responders and only 18 responders**.** Only *4 polymorphism was (p=0. 015) significantly associated with local site reactions (Table 5).

Headache, hypotension and nausea and vomiting

The headache was significantly associated with the *2 (p=0.004) and *4 (p=0.030) polymorphism whereas no significant association (p=0.343) was observed with *10 polymorphsim. Hypotension was not significantly associated with *CYP2D6* polymorphism. The *4 allele showed significant (p=0.017) association with nausea and vomiting whereas *2 (p=0.360) and *10 (p=0.608) polymorphisms were not associated with this adverse effect (Table 6).

Rescue analgesia with *CYP2D6* polymorphism

The one hundred and thirty five patients were required rescue analgesia showed significant association with *2 (p=0. 023) and *4 (p=0. 002) polymorphism whereas *10 (p=0. 179) polymorphism having insignificant association with rescue analgesia (p>0.05) (Table 7).

Table 6: Adverse effects (headache, hypotension and nausea and vomiting) and *CYP2D6* polymorphism

Group		*CYP2D6*2* allele					*CYP2D6*4* allele					*CYP2D6*10* allele				
Headache		**UM**	**EM**	**IM**	**PM**	**Total**	**UM**	**EM**	**IM**	**PM**	**Total**	**UM**	**EM**	**IM**	**PM**	**Total**
N	Count	1	14	3	12	30	0	16	8	5	29	-	19	8	3	30
R	%within group	3.3%	46.7%	10.0%	40.0%	100.%	.0%	55.2%	27.6%	17.2%	100.0%	-	63.3%	26.7%	10.0%	100.0%
	% within allele	50.0%	56.0%	27.3%	100.0%	60.0%	.0%	64.0%	61.5%	83.3%	59.2%	-	54.3%	80.0%	60.0%	60.0%
R	Count	1	11	8	0	20	5	9	5	1	20	-	16	2	2	20
	%within group	5.0%	55.0%	40.0%	.0%	100.0%	25.0%	45.0%	25.0%	5.0%	100.0%	-	80.0%	10.0%	10.0%	100.0%
	% within allele	50.0%	44.0%	72.7%	.0%	40.0%	100.0%	36.0%	38.5%	16.7%	40.8%	-	45.7%	20.0%	40.0%	40.0%
Pearson's chi square		***p=0.004***					***p=0.030***					*p=0.343*				
Hypotension																
N	Count	1	24	10	11	46	0	25	13	7	45	-	31	12	3	46
R	%within group	2.2%	52.2%	21.7%	23.9%	100.0%	.0%	55.6%	28.9%	15.6%	100.0%	-	29.7	13.4	2.8	46.0
	% within allele	100.0%	72.7%	62.5%	73.3%	70.8%	.0%	69.4%	65.0%	100.0%	70.3%	-	67.4%	26.1%	6.5%	100.0%
R	Count	0	9	6	4	19	1	11	7	0	19	-	11	7	1	19
	%within group	.0%	47.4%	31.6%	21.1%	100.0%	5.3%	57.9%	36.8%	.0%	100.0%	-	57.9%	36.8%	5.3%	100.0%
	% within allele	.0%	27.3%	37.5%	26.7%	29.2%	100.0%	30.6%	35.0%.	0%	29.7%	-	26.2%	36.8%	25.0%	29.2%
Pearson's Chi-square		*p=0.789*					*p=0.132*					*p=0.686*				
Nausea and vomiting																
N	Count	3	22	13	12	50	0	29	15	6	50	-	35	13	2	50
R	%within group	6.0%	44.0%	26.0%	24.0%	100.0%	.0%	58.0%	30.0%	12.0%	100.0%	-	70.0%	26.0%	4.0%	100.0%
	% within allele	75.0%	71.0%	65.0%	92.3%	73.5%	.0%	64.4%	93.8%	100.0%	73.5%	-	74.5%	68.4%	100.0%	73.5%
R	Count	1	9	7	1	18	1	16	1	0	18	-	12	6	0	18
	%within group	5.6%	50.0%	38.9%	5.6%	100.0%	5.6%	88.9%	5.6%	.0%	100.0%	-	66.7%	33.3%	.0%	100.0%
	% within allele	25.0%	29.0%	35.0%	7.7%	26.5%	100.0%	35.6%	6.2%	.0%	26.5%	-	25.5%	31.6%	.0%	26.5%
Pearson's chi-square		*p=0.360*					***p=0.017***					*p=0.608*				

UM- Ultra metabolizers; EX- Extensive metabolizers; IM- Intermediate metabolizers; PM- Poor metabolizers; NR- Non responders; R- Responders; All values are expressed in numbers and percentage

Table 7: Rescue analgesia and *CYP2D6* polymorphism

Rescue Analgesia	Metabolizers	*CYP2D6*2* allele		*CYP2D6*4* allele		*CYP2D6*10* allele	
		NR	**R**	**NR**	**R**	**NR**	**R**
No	UM n(%)	0 (0%)	2(1.8%)	0(0%)	7(6.3%)	0(0%)	0(0%)
Yes		4(3.3%)	1(8.3%)	0(0%)	1(8.3%)	0(0%)	0(0%)
No	EM n(%)	0(0%)	57(51.4%)	0(0%)	82(73.9%)	0(0%)	79(71.20%)
Yes		56(45.5%)	8(66.7%)	77(63.1%)	10(83.%)	81(65.90%)	9(75.00%)
No	IM n(%)	0(0%)	39(35.1%)	0(0%)	21(18.9%)	0(0%)	28(25.20%)
Yes		30(24.4%)	2(16.7%)	34(27.9%)	0(0%)	30(24.40%)	2(16.70%)
No	PM n(%)	0(0%)	13(11.7%)	0(0%)	1(0.9%)	0(0%)	4(3.60%)
Yes		33(26.8%)	1(8.3%)	11(9.0%)	1(8.3%)	12(9.80%)	1(8.30%)
Total		123	123	123	123	123	123
Pearson's Chi square test		**p=0.023**		**p=0.002**		p=0.179	

NR- Non responder; R- Responders; All values are expressed in number and percentage.

DISCUSSION

In the current study, adverse events of tramadol relationship with *CYP2D6* polymorphsim has not been studied in PHN patients in Indian population. In our previous study, reported that treatment with tramadol 50 mg to 200 mg per day was associated with significant pain reduction in terms of enhanced pain relief, reduced sleep interference, greater global improvement, diminished side-effect profile, and improved quality of life in PHN patients [7] 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 [8,9].

In the present study found that adverse events encountered in our study, due to tramadol therapy in both groups most common adverse effects such as somnolence, dizziness, local site reaction, headache, hypotension and nausea and vomiting. Dizziness and somnolence were the most common side effects of tramadol treatment, but could be tolerated by patients, even though they may have persisted throughout the major portion of the treatment period. The other side effects to be observed were mainly dry mouth and constipation mainly due to the effect of oral tramadol, which were relieved symptomatically. All the possible side effects were asked for which are enumerated in adverse drug effects. The greater improvement in endpoint means pain scores of the tramadol-treated PHN patients remained significant in responders as compared non-responders. Our previous prelimanry results also same type of clinically adverse events observed after the treatment of tramadol of PHN patients [8,9].

The most common adverse effects of tramadol are nausea, vomiting, dizziness, fatigue, sweating, dry mouth, drowsiness and orthostatic hypotension. In a summary of data from phase II–IV and post-marketing studies, as well as from spontaneous reports including over 21,000 patients, the frequency of side effects was estimated to be 1–6% [12]. In an open study of 7,198 patients with chronic pain, adverse effects were noted in 16.8% of patients; 68.9% of patients had mild adverse effects, 22.1% had adverse effects of severe intensity, and there was no data for 9% of patients. The most frequent adverse effects were unspecific CNS irritation and signs of coordination disorders (7.1%), dizziness (5.3%), nausea (4.8%), sedation (2.4%), dry mouth (2.2%) and vomiting (0.8%). At least some of the adverse effects might have been evoked by tramadol's interaction with concomitant drugs, which were administered to 45% of patients receiving tramadol [22]. In a post-marketing study, controlled release tramadol was administered to 3,153 patients with chronic pain of different origins; adverse effects were noted in 6.5% of patients, with the most frequent effects being nausea (3.4%), dizziness (1.5%) and vomiting (1.1%). Boureau *et al.* [6] was found that nausea (12.5%) and constipation (4.7%) in the tramadol group and nausea (3.2%) in the placebo group. The side effects are reported of tramadol are nausea, vomiting, sweating, dry mouth, dizziness and sedation [23]. According to Lehmann *et al.* [13] adverse events occurring during the treatment period were dizziness (3%), headache (3%), nausea (6%), vomiting (6%), sweating (21%), and dry mouth (18%). In a study of hospitalized patients, Follin and Charland [14] reported that higher incidence of adverse effects was found: dizziness (26–33%), headache (18–32%), nausea (24–40%), vomiting (9–17%), sweating (6–9%), and dry mouth (5–10%).

In this study, we did not find an onset of age, genders and weight related adverse events when compared with *CYP2D6* polymorphism. Earlier reported, *CYP2D6*4 and CYP2D6*2* genotype–phenotype distribution, all adverse events did not correlated with ages-at-onset, genders and weight [8,9]. ADRs occur by a number of mechanisms, few of remain unclear, but several risk factors have been identified. Older age has often been identified as important risk factors [24-26]. A sevenfold increase in an occurrence of ADRs from 3% to 21% has been shown to occur between patients aged 20–30 years and 60–70 years [27]. Patients at the extremes of age are at increased risk of ADRs for several reasons. In the elderly, multiple medications are commonly taken to treat co-existing pathologies. Therefore, the risk of an ADR arising per se or from drug interactions is increased. Although older patients are generally at higher risk of adverse drug reactions, due to age-associated differences in pharmacokinetic and pharmacodynamic drug properties will change in older age.

Sex is an important determinant of drug use and drug response. Women tend to have a higher risk of adverse drug reactions with a 1.5 to 1.7-fold higher risk as compared with men [28-30]. Sex differences in pharmacology are complicated by the large variety of drugs, indications for use, and pharmacokinetic and pharmacodynamic differences between the sexes [31-34]. More data on drug response in women are needed. Although the authorities emphasized the importance of including more women in clinical trials as early as 1986, women are still under-represented in clinical research nowadays [35-37]. The policies and guidelines, set up by the National Institute of Health (NIH), Food and Drug Administration (FDA), and the European Medicines Agency (EMA), have unfortunately not resolved this inequality [38-40].

The effect of a drug on the body weight 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 [31-34,41]. Female patients, being generally lighter in weight and smaller in build than their male counterparts but usually receiving the same drug doses, had been demonstrated to be more prone to ADRs in some studies [42,28]. This is most probably attributable to the exposure to higher dose per body weight for the females. Gan *et al.* [15]found that, the metabolizers (UMs, EMs, IMs and PMs), there was no difference in terms of age, body weight and duration of study. Using Fisher's Exact test, there was also no difference in terms of sex, smoking status among the group, indicating that these factors did not confound the pharmacokinetics analysis.

In the present study, no significant association was found between the adverse events (somnolence, local site reactions, hypotension, nausea and vomiting) when compared with the *2 and *10 polymorphism. The adverse events such as (dizziness, and headache) founded a significant association with *2 polymorphism. All adverse events were found highly significant with *4 polymorphism. In our four weeks, the randomized trial study was found that, no serious adverse effects were observed. In our previously reported that, based on genetic model *CYP2D6*4* polymorphism using the adverse events after treatment of tramadol. All adverse events found when comparing the *CYP2D6*4* allele in EMs, IMs and PMs were nonsignificant ($p>0.05$). This may be due to the less sample size [8] and also all, adverse events compared with *CYP2D6*2* polymorphism with EMs, IMs, and PMs show none to be significant ($p>0.05$). This may be diiference in total numbers of patients calculated based on yes or no adverse events/in both the grups of non-responders and responders [9].

Kim *et al.* [35] found that, with respect to *CYP2D6* activity levels, EM having activity score 2.0 or 1.5 and IM having 1.0 or 0.5. IMs participants had 3.4-fold lower odds of nausea/vomiting than EM participants. Because the plasma concentration of *O*-desmethyltramadol is correlated with *CYP2D6* activity level [17]. The risk of nausea/vomiting could be attributed to *O*-desmethyltramadol. Gan *et al.* [15] was observed that twenty-seven percent of the patients were IM and 2.9% were UMs; the remaining 70% were EMs. However, the analgesic effects of tramadol were not measured adequately among postoperative patients to establish its full therapeutic effect. 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. Generally in case of UMs, Leppert *et al.*[43] are avoided tramadol administration in patients with UM genotypes and renal impairment. Patients being *CYP2D6* UMs are at risk for toxic responses to tramadol treatment. Thus, a case report described a man with renal insufficiency and a *CYP2D6* UMs genotype that developed postoperative respiratory depression while receiving tramadol [16]. *CYP2D6* UMs patients have high plasma levels of O-desmethyltramadol and better pain control than EMs, but UMs also experience a higher frequency of nausea [44]. The UMs genotype is thought to be present in approximately 5% of people in North America and Middle Europe, 7–12% of people in the Mediterranean, 21% of people in Saudi Arabia and 29% of people in Ethiopia[16].

The frequency of PMs has been reported to be as low as 0–1% in South-East Asian populations [45-46]. A higher incidence of adverse effects is expected among the PMs group, as reported by Rau *et al.*[47] who have investigated antidepressants and *CYP2D6* polymorphism. Earlier reports which found, no relationship between *CYP2D6 *1, *3, *4, *5, *6* and **7* genotypes and the number and severity of adverse drug effects [48-49]. Blonk *et al.* [50] have also observed that *CYP2D6*4* allele is not associated with drug related falls in elderly people. Gan *et al.* [15] 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. It was 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 was also observed the *CYP2D6* activity may play an important role in determining the pharmacokinetics of tramadol and in predicting it's adverse.

In the present study, all patients in non-responders and 11 patients in responder used rescue analgesia. The significant improvement in pain intensity scores and quality of life in non-responders is mainly attributed to the use of rescue analgesia for 28 days rather than recommended tramadol therapy. The *CYP2D6*2* and *4 alleles having significant impact with rescue analgesia whereas no significant impact on *10 polymorphism. The use of doxepin [51-53] and capsaicin, as an analgesic has been recommended for long in the pain management of PHN [54-57], diabetic neuropathy and surgical neuropathic pain[56]. A number of studies involving low-concentration capsaicin creams (0.025% and 0.075%) as a unimodal modality have demonstrated efficacy in the treatment of PHN [51-52,54]. Topical capsaicin, the doxepin cream or a combination of both have been shown to significantly reduce chronic neuropathic pain [58]. Recently in a prospective, randomized, double blind, placebo controlled trial, Indu *et al.*[59] (unpublished data) observed that a multimodal approach comprising of topical application of capsaicin (0.05 %) and Doxepin (3.33%) combination cream (4 times a day for 4 weeks), continuous thoracic epidural infusion (for 72 hours) and oral pregabalin (75 mg bd for 4 weeks) provided significant reduction in VAS scores, GPE scores and NPSI scores of PHN patients at days 4, 14 and 28 days follow up. Similar results were observed in the present study through the latter involves single treatment modality i. e. Tramadol.

In the present study, *CYP2D6*4* polymorphism may be as important drug toxicity marker predictor of experiencing adverse drug reactions as PHN patients undergoing tramadol treatment. More research is required to explain the clear *CYP2D6*4* polymorphism related to tramadol treatment. It is also suggested that the newer opioid, tapentadol, which is independent of *CYP2D6*-mediated metabolism, and hence devoid of any inter-individual variation, should also be evaluated for its safety and efficacy in PHN patients.

CONCLUSION

The current approach to the identification of genetic predisposition to ADRs is limited. The *CYP2D6*4* polymorphism strongly associated with development of adverse drug reactions whereas none of *CYP2D6*2* allele and *CYP2D6*10* allele did not find any associations with ADRs undergoing tramadol treatment of PHN patients.

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COMPETING INTERESTS

The authors declare that they have no competing interests.

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