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Original Research Article

CYP2D6*4 Polymorphism on Tramadol Analgesia in Acute Osteoarthritic Knee Pain in South Indian Population.

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ABSTRACT:

Context: Inadequate pain relief is commonly experienced by patients on pain medications for chronic diseases. Tramadol, an opioid analgesic is metabolized by CYP2D6 to active metabolite, responsible for analgesic activity. The presence of non-functional CYP2D6*4 allele may affect pain relief. Hence the aim of our study is to investigate the influence of CYP2D6*4 on the analgesic effect of tramadol in acute osteoarthritic knee pain

Methods and Material: Seventy six patients visiting the orthopaedicians of MGMCRI with acute osteoarthritic knee pain and 49 fulfilling the study criteria were included. Pain intensity was recorded using visual analogue scale (VAS) at baseline and after 5 days. 2mL blood was collected for genotyping. Patients received Tab.Tramadol 50mg BD for five days. Patients were categorized as responders if patients experience 50% or more pain relief from the baseline. Genomic DNA was extracted using Qiagen Kit. Genotyping was performed using TaqMan SNP Assay on Real Time system. Chi Square test was used to study the association between metabolizer phenotype determined by CYP2D6*4 and pain relief.

Results: The frequency of Extensive metabolizers(EM), Intermediate Metabolizers(IM) and Poor Metabolizers(PM) were 90.6%, 5.3% and 4% respectively. The allele was in Hardy Weinberg Equilibrium (p=0.1062). There was a statistically significant association between the metabolizer phenotype and pain relief (p=0.0349). There were significantly more number of responders with EM phenotype.

Conclusion: The genotype of CYP2D6*4 non-functional allele influences the analgesic effect of tramadol. However, studies on larger samples are required to extrapolate it.

Keywords : Cytochrome P450 oxidoreductase, Opioid analgesia, Osteoarthritic Pain Management, Pharmacogenomics.

INTRODUCTION:

Osteoarthritis of the knee is a common disabling disorder of the elderly, characterized by progressive inflammation and painful swelling of the knee joint.[1] Though NSAIDs are the common drug options, the gastrointestinal and cardiovascular risks along with their ceiling effect on long term use limits their use in treating the painful knee. Tramadol is regarded as one of the first line drugs in treating OA knee pain. It is an opioid analgesic approved for treating moderate to severe pain according to step two of WHO analgesic ladder.[2] It is mainly a μ opioid receptor agonist and also inhibits norepinephrine and serotonin reuptake. It is metabolized by CYP2D6 to an active metabolic O-Desmethyl tramadol (M_1) having 700 times higher affinity for μ receptor than the parent compound and mainly responsible for the analgesic effect. The parent compound is responsible for the inhibition of neurotransmitter reuptake. Pharmacogenomics plays an important tool in identifying the genetic polymorphisms so that dosage can be titrated individually or alternate drug can be chosen. Genetic polymorphisms in CYP2D6 results in varied metabolizing status of the individual.[3] CYP2D6 is the enzyme responsible for the metabolism of 25 – 30% of the medications including tramadol. CYP2D6 has the largest phenotype variation of CYP_{450} enzymes and hence highly polymorphic with more than 120 allelic variants.[4] CYP2D6*1,*2 are the common wild type alleles, whereas CYP2D6*3,*4,*5,*6 are non-functional alleles and CYP2D6*9,*10,*11,*17,*41 have diminished enzyme activity. Based on the genotype, the metabolizing phenotype status of the individual can be classified as follows. Poor Metabolizers(PM) who have two non-functional or absent alleles, Intermediate Metabolizers(IM) who have one normal and one variant allele causing diminished enzyme activity, Extensive Metabolizers(EM) who have two alleles coding for normal enzyme function and Ultra Rapid Metabolizers(UM) who have extra copies of the normal allele.[5] Large ethnic differences exist in the frequency of these alleles. CYP2D6*10 is the most common recessive allele found in the Asian population with a frequency of 39.4%.[6] Previous studies reveal the high incidence of CYP2D6*10 among Chinese population and their impact on the analgesic effect of tramadol in post-operative pain causing reduced analgesic effect.[7,8]The frequency of poor metabolizer phenotype CYP2D6*4/*4 in Indian population is 4% which is higher compared to other population.[9,10] The presence of non-functional alleles is clinically more significant as the patient will be unresponsive to tramadol and will require alternate drug therapy. Moreover, side effects may set in. Hence we planned to study the influence of CYP2D6*4 genetic polymorphisms on tramadol analgesia in osteoarthritic knee pain in South Indian population.

MATERIALS AND METHODS:

The study was approved by the Institute Human Ethics Committee and written informed consent was collected from the patient. An observational study was performed on Patients attending Out Patient Department of Mahatma Gandhi Medical college & Research Institute, Pondicherry and suffering from acute pain due to osteoarthritic knee as diagnosed by the clinicians in the age group of 20 - 65 years of both sexes. Patients with history of known hypersensitivity to the study drug tramadol, Pregnant & breast feeding women, Patients with hepatic dysfunction, Patients on anticonvulsants, patients with history of any concomitant medication which may interfere with CYP2D6 metabolism namely antidepressants, antipsychotics, beta blockers, amiodarone and quinidine, Patients who are already receiving any other pain medications like NSAIDs, Patients who receive any rescue pain medications apart from tramadol due to intolerable side effects like vomiting, constipation and dizziness were excluded from the study. Based on the previous studies with 18.35% prevalence rate of non-responders to tramadol analgesia, the sample size was calculated to be 70 based on the formula $\eta = (Z\alpha 2pq) / d2$, where $Z\alpha=1.96$ @ 95% confidence interval, p = prevalence rate = 18.35%, q = 1-p, d = 10% absolute precision.

Demographic details like name, age, sex, ethnicity, medical history, history of concomitant medications and duration of pain were recorded in a case proforma sheet. The diagnosis of acute exacerbation of osteoarthritis was made by the clinician and those fulfilling the inclusion criteria participated in the study. Patient's pain intensity[18] were recorded by the visual analogue scale (VAS) as a baseline assessment after which patients received Tab.Tramadol 50mg BD, procured from Neon laboratories limited under the trade name of Tablet Supridol 50 mg. If pain did not subside during the treatment period, dose was increased to TDS or QID to a maximum of 400 mg/day. The patient were advised about the treatment plan and about the side effects of the drugs like vomiting, giddiness and to report to the hospital at any time if pain is not subsiding or side effects noted. The importance of adherence to treatment were explained to each patient and checked during the follow up visit by pill count method. Any side effects like vomiting, dizziness, constipation occurring were noted and treated. Patients were assessed for pain using VAS again after three days and were categorized as responders or non-responders based on the following criteria.[19]

Responders: Patients who receive 50% or more pain relief from the baseline pain assessment.

Non-responders: Patients who receive less than 50% pain relief from the baseline pain assessment.

Determination of genotype: 2ml of EDTA blood was withdrawn from the patient. Genomic DNA was extracted from whole blood using QIAamp Blood Mini Kit. Extracted DNA was electrophoresed on 1% agarose gel and the product bands were visualized under UV light using gel documentation system. Genotyping was performed using TaqMan SNP Assay (ID: C_2710 2431) on Real Time PCR system (CFX96TM Real-Time System, Bio Rad,Corp, USA). Reaction mixture contained Taq Master Mix – 40X, primers & probes – 20X, exonuclease free water and 100ng of genomic DNA making up to a volume of 20 µl The PCR cycling conditions include denaturation at 95´C for 10 min, annealing at 95´C for 15 sec and extension at 60´C for 90sec for 50 cycles. Samples were randomly re-run to confirm genotyping results.

Outcome variables:

The frequency of ultra-rapid metabolizers (UM), extensive metabolizer (EM), Intermediate metabolizers (IM) and poor metabolizer (PM) in responders and the same in non-responders were recorded and analyzed by statistical tests. All data are expressed as mean $\pm$ SD. Hardy-Weinberg equilibrium was tested using the chi-square goodness of fit test. Fisher's exact test was carried out to test significance of association between drug response and metabolizer status. $p<0.05$ was considered statistically significant. All statistical analyses were performed using Graphpad Prism Software.

RESULTS:

Our study analysed the influence of CYP2D6*4 genotyping on 80 patients suffering from acute pain episode of osteoarthritis. The percentage of patients who responded to tramadol in our study was 57.33%. Demographic variables were comparable between the groups with a slight preponderance of disease in females and showed no significant difference between responders and non-responders (Table 1). No significant difference was observed in the mean baseline VAS scores between responders and non-responders (Table 1). The observed genotypes GG, GA and AA were in Hardy-Weinberg equilibrium. The minor allele (T) frequency was found to be 6,66%. Odds ratio was calculated to be 11.04 (1.282 to 95.02) at 95% CI with $p = 0.02$ and was considered statistically significant. Statistically significant association was found between genotypes and the response to tramadol.(Table 2). Figure 1 reveal the images procured from gel doc on agarose gel electrophoresis.

Table 1: Demographic characteristic between responders and non-responders

Demographic variables		Responders (n = 43)	Non-Responders (n = 32)	p value
Age[a]		52.49 (10.42)	52.12 (7.89)	0.8641
Sex[b]	Male n(%)	13 (17.33)	10 (13.33)	0.9247
	Female n(%)	30 (40.00)	22 (29.33)	
BMI[a]		25.56 (4.106)	25.03 (3.018)	0.5432
Duration of illness[c] (in weeks)		49.43 (54.03)	55.71 (104)	0.2233
Baseline VAS score[a]		5.744 (1.62)	5.625 (1.621)	0.7536

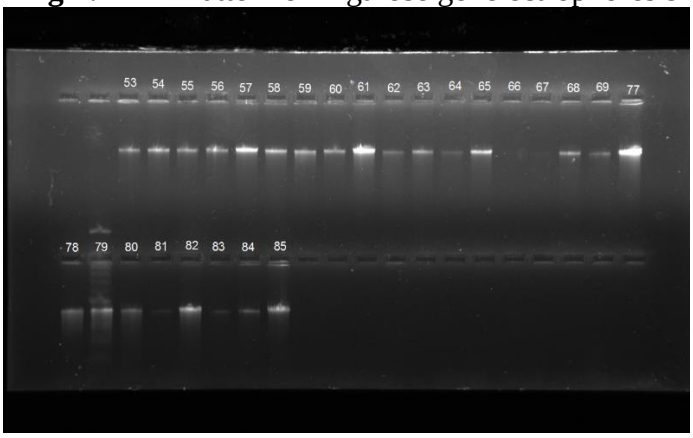
Data expressed as mean(SD)
[a] Student t-test was performed.
[b] Chi-square test was performed.
[c] Mann-Whitney U test was performed.
VAS - Visual Analog Scale

Table 2: Association between CYP2D6*4 carriers and tramadol response

	CYP2D6*1/*1 GG n (%)	CYP2D6*1/*4 GA n (%)	CYP2D6*4/*4 AA n (%)	OR (95%CI)	p value
Responder	42 (32)	1 (14.7)	0 (10.7)	11.04 (1.282 to 95.02)	0.0185
Non-responder	26 (16)	3 (22.7)	3 (4)		

Chi-square test performed between GG and GA + AA (CYP2D6*4 carriers)

Fig 1: DNA Pattern on Agarose gel electrophoresis



DISCUSSION:

The study was the first one to identify the impact of CYP2D6*4 polymorphisms on the clinical outcome of tramadol efficacy in patients with acute osteoarthritic knee pain in South Indian Population. The frequency of CYP2D6*4 genotypes were 90.6%, 5.3% and 4% in our study on South Indian population. The minor allele frequency was 6.6% which was consistent with the study by Adithan et al.[9] The percentage of non responders to tramadol in our study was 42.26%. This non responsiveness would be due to inter individual variations in the pharmacokinetic and pharmacodynamics response to tramadol that occurs due to inter-individual variation in the metabolic activity of tramadol. Moreover the influence of demographic factors on the analgesic effect of tramadol was insignificant in our study. Also the study revealed statistically significant influence of CYP2D6*4 genotype on the analgesic effect of tramadol in South Indian patients

suffering from acute painful episode of osteoarthritic knee pain. Though the majority of the Indian population has been found to exhibit intermediate metabolizer phenotype, CYP2D6*4 is the predominant null allele in the Indian population. A null allele prevents the formation of a functional CYP2D6 enzyme. The presence of 2 null alleles leads to poor metabolizer phenotype. In this study, as *4 was found at a lower frequency, *1/*4 and *4/*4 were combined for analysis and compared with *1/*1 genotypes between responders and non-responders. Though there was a significant association between response and extensive metabolizer phenotype, the results of this study, need to be analysed with caution due to a small sample size, which is also reflected in the wide confidence interval.

A study by Namina et al in Indian population found that CYP2D6 genotype did not influence tramadol analgesia in post herpetic neuralgia.[19] Moreover, the genetic polymorphisms of other CYP2D6 alleles that metabolise tramadol slowly can also influence the analgesic effect of tramadol. Studies by Gan et al revealed that the presence of CYP2D6*3,*4,*10 alleles can reduce CYP2D6 enzyme activity. CYP2D6*5 resulted in loss of enzyme activity.[20] Lansen et al stated that polymorphisms in transporter genes like OCT1 & P gp could also affect the therapeutic response.[2] GuoXiang et al investigated the effect of CY2D6*10 C188T polymorphism on post operative tramadol analgesia in Chinese population on 70 patients who underwent gastrectomy and found that the allele frequency in Chinese population was 52.4% was very high with the total consumption of tramadol in the post operative period being higher among the patients homozygous recessive for CYP2D6*10.[7] Similar study by HonDong et al in patients undergoing nephrectomy revealed that the allele frequency in Chinese population was 57.7% which was very high.[8] Moreover the total consumption of tramadol in the post-operative period was higher among the patients homozygous recessive for CYP2D6*10 which reflects clearly that it has a major influence on the analgesic effect of tramadol. Also other disease dependent factors like age dependent cartilage degeneration, obesity, deficiency of calcium and vitamin D, altered bone mineral density and patient factors like irregular pill intake, patient's belief on the doctor, pain perception can also influence the therapeutic response of tramadol.

Our study analysed the effect of CYP2D6*4 genotyping on tramadol response by VAS scoring which is highly subjective. As osteoarthritis is a chronic disorder requiring long term treatment and the percentage of non-responders to tramadol analgesia is also pretty high as revealed by our study, CYP2D6 genotyping of various alleles will reveal the patient's metabolizing status which in turn will help the physician prescribe alternative drug and move towards personalized medicine. The limitations of our study include a small sample size and subjective VAS score as measure of clinical outcome instead of measurement of plasma concentration of tramadol and its metabolite.

CONCLUSION:

The results of our study reveal that genetic factors do affect the clinical outcome of tramadol, CYP2D6*4 pharmacogenetic testing will help the physician to prescribe right drug for the right patient and prevent adverse outcomes. However, larger sample size studies taking in to account other potential non-genetic factors along with other polymorphisms of CYP2D6 are needed to extrapolate the results and enable the implementation of personalized medicine

Conflict of Interest: Nil

Acknowledgement: Nil

Ethical issues – IEC approved study

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Contributorship: SC- concept and design., SK and MM: data collection., MA-manuscript preparation., CA – overall supervision**.**

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