

# Association between the Severity of Tramadol Toxicity and Some CYP2D6 Allelic Variants in Egyptian Tramadol Intoxicated Patients

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## Abstract

Cytochrome P450 gene polymorphism is involved in the metabolism of tramadol. We investigated the effect of CYP2D6 gene polymorphism on tramadol metabolism by studying CYP2D6\*1, CYP2D6\*DUP, CYP2D6\*4, and CYP2D6\*10. CYP2D6 genotyping was performed using the xTAG CYP2D6 Kit v3 which incorporates multiplex PCR (Luminex kit). Tramadol levels were assessed using enzyme immunoassay. Our Study was done on 100 Egyptian patients with acute tramadol intoxication and 100 healthy control subjects. The patients were admitted to NECTR center. CYP2D6\*1 was the most presented allelic variant in both groups The CYP2D6\*DUP is associated with severe intoxication. There was a significant association between CYP2D6 allelic variants and tramadol metabolites level. In conclusion, our data suggests that studying CYP2D6 allelic variants may help clinicians to predict the severity of tramadol intoxication and individualize the drug treatment.

**Keywords:** Tramadol; CYP2D6 allelic variants; Intoxication; O-demethyltramadol (M1); Clinical severity

## Introduction

Tramadol is an orally-active and centrally-acting opioid analgesic used for the treatment of moderate to severe pain [1].

Tramadol was approved for marketing as a non-controlled analgesic in 1995 under the trade name of Ultram. Although the producing company claimed that this substance produced weak narcotic effects, researches demonstrated that opioid activity is one of the drug's pharmacological activity. Many physicians felt that this drug was safe to prescribe, because tramadol's products had inadequate labeling and established abuse potential. As a consequence, numerous reports of abuse, dependence and side effects had been received [2].

An increasingly alarming phenomenon of Tramadol abuse has been heavily demonstrated in the Egyptian community in the last four years as it is provided at cheap cost despite of it being scheduled [2].

The human cytochrome P450 (CYP) plays an important role in the metabolism of some endogenous compounds, therapeutic drugs, and other xenobiotics [3]. CYP2D6, one of the most important polymorphic cytochrome P450, metabolizes many drugs such as antidepressant and opiates including Tramadol [4].

Polymorphic CYP2D6 converts tramadol via hepatic phase I O - demethylation into its active metabolite O-demethyltramadol (M1) [5]. This active metabolite (M1) has potent opioid properties through inhibition of reuptake of monoamines and furthermore has an elimination half-life of nine hours [6]. There is little information regarding the toxicokinetic properties of tramadol such as the relationship between CYP2D6 polymorphisms and tramadol toxicity [7].

The pharmacokinetics and pharmacodynamics of tramadol is determined by the polymorphic CYP2D6 activity. The individual CYP2D6 genotype determines the level of his enzyme activity ('phenotype'). Individuals that carry either two functional wild-type alleles, or one functional wild-type allele e.g. CYP2D6\*1 are considered extensive metabolizers and have normal enzymatic activity, individuals that carry duplications or multiplications of the CYP2D6 gene are considered Ultrarapid metabolizers (UMs) and have increased enzymatic activity. In addition, individuals with inactivating alleles e.g.

CYP2D6\*4 or CYP2D6\*10 are considered poor metabolizers (PM) and have decreased enzymatic activity [8].

The clinical effects of the CYP2D6 poor metabolizer (PM) genotype on pharmacokinetics and pharmacodynamics of tramadol have been observed in healthy volunteers and patients [9]. Poor metabolizers didn't respond at all or very poorly to postoperative tramadol. In contrast, individuals with ultra-rapid CYP2D6 metabolizers had the highest concentrations of the active tramadol metabolite and were at risk for exaggerated effects or even intoxication [10].

Although the frequencies of CYP2D6 mutant alleles have been studied extensively in different populations, limited information is available for those of the Middle East populations and Egyptians [11].

The objective of the current study was to determine the frequencies of the CYP2D6\*1, Dup, \*4 and \*10 variants among Egyptian tramadol intoxicated cases and compare with control healthy individuals.

## Subjects and Methods

As a guide for a future wide scale genetic analysis of Tramadol intoxicated cases, a pilot study was done on 100 tramadol intoxicated (overdosed) Egyptian patients (90 males and 10 females). They were admitted to National Environmental and Clinical Toxicology Research center (NECTR); their mean age was 27.6 ± 6.6 years. For comparison, 100 healthy control subjects (90 males and 10 females), whose mean age was 24.5 ± 5.1 years, were included in this study. The study protocol was approved by the Ethical committee-Faculty of Medicine, Cairo University to assume confidentiality and privacy.

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## Patients' classification

The intoxicated patients gave history of ingesting a large amount of tramadol tablets. The toxic dose that was taken by the patient was calculated from his/her self-reporting.

The patients were arranged into 3 groups according to their clinical manifestations and laboratory tests results:

1. Normal group: intoxicated group presented with neither clinical nor laboratory changes

2. Mild to moderate intoxicated group presented with mild-moderate manifestations e.g. toxic dose less than 5 gm, drowsy, vital signs shows respiratory rate from 10-12 cycle/ minute, mild nausea or vomiting, no cardiovascular affection, oxygen saturation more than 75%, mild arterial blood gases changes and received supportive management e.g. oxygen, activated charcoal and antiemetic.

3. Severe group presented with severe manifestations e.g. toxic dose more than 5gm, coma or convulsions, vital signs shows respiratory rate less than 8 cycles/ minute, severe vomiting and/or hematemesis, presence of cardiovascular affection, oxygen saturation less than 70%, severe arterial blood gases changes and received antidotal therapy (naloxone) and supportive management e.g. activated charcoal and antiemetic.

The patients whether ingested any other drug with tramadol or have any other health problem were excluded from the study.

## Samples collection

Urine and blood samples were collected from all patients after taking their consent.

## CYP2D6 genotyping

Blood samples were collected in EDTA coated sample collection tubes and preserved at -20°C. For genotyping, genomic DNA was isolated from whole blood using the Puregene DNA Isolation Kit (Gentra Systems, Minneapolis, MN). CYP2D6 genotyping was performed using The xTAG CYP2D6 Kit v3 which incorporates multiplex PCR and multiplex Allele Specific Primer Extension (ASPE) with Luminex's Universal TAG sorting system on the proven Luminex® 100/200™ platform. CYP2D6 genotyping was identified according to the kit software. A first PCR reaction was performed to amplify a 1,700-bp fragment of CYP2D6, using primers specifically designed to avoid amplification from the pseudogenes CYP2D7 and CYP2D8. The 1,700-bp fragments were used as template for allele specific primer PCR were presented in Table 1.

## Urine samples analysis

To confirm the overdosed tramadol ingestion, urine samples were collected in sterilized cups at the time of presentation or 4 hours post ingestion and were preserved at -20°C.

Using urine samples for detection and quantitation of tramadol has many advantages such as usefulness especially its ability to detect tramadol up to 4 days after exposure; noninvasive, large volumes and large number of samples can be easily collected [12].

These samples were assessed using (Thermo Scientific Indiko Enzyme Immunoassay kit) which was simple, reproducible and fast. The tramadol assay kit is a homogenous enzyme immunoassay using ready liquid reagents and monoclonal antibodies which can detect O-demethyltramadol (M1), the major metabolite of tramadol, in human urine with detection level up to 200 mg/ml. The sample is added

*Alleles	Single nucleotide polymorphisms	Predicted Enzyme activity
*1	None	Normal
*4	100C>T, 1661G>C, 1846G>A, 4180G>C, 2850C>T	Reduced
*10	100C>T, 1661G>C, 4180G>C	Reduced
DUP	Present /absent	Ultrarapid

Table 1: xTAG CYP2D6 Kit v3 (Luminex) Alleles and Single Nucleotide Polymorphisms.

Group Variable	Study Group N=100 Mean ± SD	Control Group N=100 Mean ± SD	P-Value
Age (years)	27.6 ± 6.6	24.5 ± 5.1	0.56
Sex (female/male)	10/90	10/90	0.58

Table 2: Personal data among the studied groups.

Group Variable	Study Group N=100 Mean ± SD	Median	Range
Delay time (hours)	2.5 ± 1	2	1-5
Total dose (g)	4.2 ± 3.2	3	1.5-14

Table 3: Description of the total dose and the delay time among the studied groups.

to a mixture of reagents. The immunological reaction occurs between analyzed and antibody in homogenous solution then tramadol level is measured.

## Statistical analysis

Personal data, toxic dose, delay time, distribution, the frequency of the CYP2D6 allelic variants and tramadol level among the studied groups were statistically described in terms of mean, standard deviation, for quantitative data and frequencies (number of cases) and relative frequencies (percentages) for qualitative data. Comparison of quantitative variables was done using non-parametrical Kruskal-Wallis test and Mann-Whitney test. For comparing qualitative data, Chi square ( $\chi^2$ ) test was performed. A probability value (P value) less than 0.05 was considered statistically significant. All statistical calculations were done using SPSS (Statistical Package for the Social Science; SPSS Inc., Chicago, IL, USA) version 21.

## Results

### Personal data

The 100 Egyptian tramadol intoxicated patients (90 males and 10 females) were admitted to (NECTR); their mean age was 27.6 ± 6.6 years. 100 healthy subjects (90 males and 10 females), their mean age was 24.5 ± 5.1 years were studied as a control group Table 2.

The patients were exposed to oral tramadol doses ranged from 1.5 up to 14 grams (25-280 mg/kg). They were delayed before admitting to center; their median delay time was 2 hours Table 3.

We classified the intoxicated patients according to their clinical manifestations and laboratory results into three groups as mentioned earlier.

According to this classification, severity of tramadol intoxication was nil in 55 patients, mild to moderate in 30 patients and severe in 15 patients. The distribution of each CYP2D6 allelic frequencies among

intoxicated patients was presented in Table 4. The allelic distribution among the tramadol intoxicated subgroups was statistically significant. The Duplication was presented more frequently in severely intoxicated subgroup.

Regarding the distribution of each CYP2D6 allelic among the intoxicated patients and healthy control, CYP2D6\*1, Duplication, CYP2D6\*4 and CYP2D6\*10 were presented in 60%, 23%, 14% and 3% of patients respectively. While CYP2D6\*1, Duplication CYP2D6\*4 and CYP2D6\*10 were presented in 75%, 17%, 5% and 2% of the control subjects respectively. The control group had a higher frequency of the wild type allele \*1(75%) with lower frequency of the mutant nonfunctioning alleles (7%) compared to the intoxicated group. This difference in allelic distribution had no significant difference among them Table 5.

Our results displayed a significant association between the studied CYP2D6 alleles and tramadol's metabolite level as shown in Table 6. Higher levels of O-demethyltramadol (M1) were noticed with CYP2D6 DUP followed by CYP2D6\*1.

### Discussion

The role of CYP2D6 in tramadol metabolism is well known and the toxicogenomic importance of genetic alterations in CYP2D6 is being currently investigated to understand the inter-individual enzymatic activity variations. Actually the impact of CYP2D6 polymorphisms on the clinical outcome of tramadol intoxication hadn't been extensively published in several literatures. Moreover, identification of patient CYP2D6 genotypes can help clinicians to individualize the drug treatment by selecting appropriate therapies [13].

This study highlights the CYP2D6 allelic variants' distribution among Egyptian tramadol intoxicated patients. Depending on which

alleles are present in an individual, a wide range of clinical manifestations and tramadol level were observed.

60% of the patients had a wild type of CYP2D6 allelic variants (\*1) and the most common inactive allelic variants was (\*4). The severe tramadol intoxication was observed with CYP2D6 DUP allelic variants.

We are in the same line with Ali et al. [11] and Eyada et al. [14] who studied CYP2D6 allelic variants among the Egyptian cases.

Regarding the CYP2D6 allelic variants among Malaysian patients, Gan et al. [15] stated that 60% of the included patients had the wild-type allele (CYP2D6 \*1), while the CYP2D6 \*10 allele accounting for most of the rest.

The frequencies of CYP2D6\*4 and \*10 alleles among the studied groups were 14% and 3% respectively. Whereas, the frequencies of CYP2D6\*4, allele is described among different ethnic populations: 17.84% in Greeks [16], 20.7% in Germans [17], 15.3% in Italians [18], 33.4% in Faroese population [19]. This difference could be due to different population and the sample size in their studies.

We found that clinical toxic manifestations in patients carrying the CYP2D6 \*4 or \*10 were mild to moderate compared to that in patients with CYP2D6 Dup. This could be explained by that the CYP2D6\*Dup is associated with increased the activity of CYP2D6 enzyme which is responsible for production of active tramadol's metabolite and tramadol toxicity [9].

Ma et al. [20] stated that individuals with PM alleles (e.g.\*3,\*4,\*4xn,\*10,\*17or\*5) had minimal or absent enzymatic activity and less clinical severity of tramadol intoxication even with using high doses.

Niesters et al. [21] also reported that Tramadol is metabolized by

CYP2D6 allele distribution Patients' manifestations	Ultrarapid metabolizers	Extensive metabolizers	Poor metabolizers		Significance value
	DUP	*1	*4	*10	P value
No (n=55)	5(0.09%)	38(69%)	10(18%)	2(0.04%)	0.004*
Mild/moderate (n=30)	5(16.7%)	20(66.7%)	4(13.3%)	1(0.3%)	
Severe (n=15)	13(87%)	2(13.3%)	-	-	
Total (%)	23 (23%)	60 (60%)	14(14%)	3(3%)	

**Table 4:** The distribution of each CYP 450 allele and its frequencies in the intoxicated groups.

\*significant

CYP2D6 allele distribution Groups	Ultrarapid metabolizers	Extensive metabolizers	Poor metabolizers		Significance P value
	DUP	*1	*4	*10	0.32
Intoxicated patients	23(23%)	60(60%)	14(14%)	3(3%)	0.32
Control subjects	17(17%)	75(75%)	5(5%)	2(2%)	

**Table 5:** The distribution of each CYP 450 allele and its frequencies in intoxicated and control groups.

Allelic distribution	Tramadol's metabolite level			Significance P value
	Mean± SD	Minimum	Maximum	
CYP2D6*1	295±48	225	365	0.000*
CYP2D6*Dup	568±42	500	615	
CYP2D6*4	214±17	200	255	
CYP2D6*10	208±7.5	200	222	

**Table 6:** The tramadol metabolite level among CYP2D6 allele.

\*significant P value

CYP2D6 into active forms. In poor metabolizers, a reduced analgesic and toxic effects were observed due to reduced or absent metabolite formation.

The CYP2D6 high metabolizers' phenotype had been associated with quicker tramadol analgesic effects and higher mu-opioid-related toxicity [22,23].

A statistically significant difference in tramadol metabolites (M1) concentrations was found among different allelic variants.

This is coinciding with what mentioned by Halling et al. [10] who studied the impact of the CYP2D6 polymorphism on the pharmacokinetics of tramadol in a group of patients treated with tramadol. They found that the concentrations of (M1) were higher in the extensive or high metabolizers than in the poor metabolizers. Khosrojerdi et al. [7] found that the tramadol half-life in Iran overdosed patients was  $9.24 \pm 2.310$  hours and it was dose dependent.

## Conclusion

CYP2D6 polymorphism is positively affecting the clinical severity of Tramadol intoxicated patients. This study represented that CYP2D6\*1 (wild type) was the most common variant among Egyptian population and CYP2D6 Dup was found in the severely tramadol intoxicated cases. We found a significant relation between tramadol metabolite (M1) level and studied CYP2D6 allelic variants. Actually some caution-in the interpretation of the data-is warranted because of the relatively small number of studied patients, especially in subgroup analysis. In this respect, we recommend a wider scale analysis recruiting a large sample of Tramadol overdosed patients, involving a wide genome association with studying tramadol toxicokinetics in each specific phenotype and search for other important interactive genomic markers.

## References

1. Scott LJ, Perry CM (2000) Tramadol: a review of its use in perioperative pain. *Drugs* 60: 139-176.
2. Fawzi MM (2011) Some medico-legal aspects concerning tramadol abuse: The new Middle East youth plague 2010. An Egyptian overview. *Egyptian journal of forensic sciences* 1: 99-102.
3. Zhou SF (2009) Polymorphism of human cytochrome P450 2D6 and its clinical significance: part II. *Clin Pharmacokinet* 48: 761-804.
4. Sistonen J, Sajantila A, Lao O, Corander J, Barbujani G, et al. (2007) CYP2D6 worldwide genetic variation shows high frequency of altered activity variants and no continental structure. *Pharmacogenet Genomics* 17: 93-101.
5. Pedersen RS, Damkier P, Brosen K (2005) Tramadol as a new probe for cytochrome P450 2D6 phenotyping: a population study. *Clin Pharmacol Ther* 77: 458-467.
6. Slanar O, Nobilis M, Kvetina J, Mikoviny R, Zima T, et al. (2007) Miotic action of tramadol is determined by CYP2D6 genotype. *Physiol Res* 56: 129-136.
7. Khosrojerdi H, Alipour Taleh G2, Danaei GH3, Shokoo Saremi S4, Adab A5, et al. (2015) Tramadol half life is dose dependent in overdose. *Daru* 23: 22.
8. Cascorbi I (2003) Pharmacogenetics of cytochrome p4502D6: genetic background and clinical implication. *Eur J Clin Invest* 33 Suppl 2: 17-22.
9. Enggaard TP, Poulsen L, Arendt-Nielsen L, Brøsen K, Ossig J, et al. (2006) The analgesic effect of tramadol after intravenous injection in healthy volunteers in relation to CYP2D6. *Anesth Analg* 102: 146-150.
10. Halling J, Weihe P, Brosen K (2008) CYP2D6 polymorphism in relation to tramadol metabolism: a study of faroese patients. *Ther Drug Monit* 30: 271-275.
11. Ali AA, Wassim N M, Dowaidar M M, Yaseen A E (2013) Genetic polymorphism of CYP2D6 gene among Egyptian hypertensive cases. *The Journal of Basic & Applied Zoology* 66: 228-233.
12. Ismaiel O A and Hosny MM (2012) Development and Validation of a Spectrophotometric Method for the Determination of Tramadol in Human Urine Using Liquid-Liquid Extraction and Ion Pair Formation. *International Journal of Instrumentation Science* 1: 34-40.
13. Becquemont L (2009) Pharmacogenomics of adverse drug reactions: practical applications and perspectives. *Pharmacogenomics* 10: 961-969.
14. Eyada TK, El Ghonemy EG, El Ghoroury EA, El Bassyouni SO, El Masry MK (2007) Study of genetic polymorphism of xenobiotic enzymes in acute leukemia. *Blood Coagul Fibrinolysis* 18: 489-495.
15. Gan SH, Ismail R, Wan Adnan WA, Zulmi W (2007) Impact of CYP2D6 genetic polymorphism on tramadol pharmacokinetics and pharmacodynamics. *Mol Diagn Ther* 11: 171-181.
16. Arvanitidis K, Ragia G, Iordanidou M, Kyriaki S, Xanthi A, et al. (2007) Genetic polymorphisms of drug-metabolizing enzymes CYP2D6, CYP2C9, CYP2C19 and CYP3A5 in the Greek population. *Fundam Clin Pharmacol* 21: 419-426.
17. Sachse C, Brockmüller J, Hildebrand M, Müller K, Roots I (1998) Correctness of prediction of the CYP2D6 phenotype confirmed by genotyping 47 intermediate and poor metabolizers of debrisoquine. *Pharmacogenetics* 8: 181-185.
18. Sachse C, Brockmüller J, Hildebrand M, Müller K, Roots I (1998) Correctness of prediction of the CYP2D6 phenotype confirmed by genotyping 47 intermediate and poor metabolizers of debrisoquine. *Pharmacogenetics* 8: 181-185.
19. Scordo MG, Caputi AP, D'Arrigo C, Fava G, Spina E (2004) Allele and genotype frequencies of CYP2C9, CYP2C19 and CYP2D6 in an Italian population. *Pharmacol Res* 50: 195-200.
20. Halling J, Petersen MS, Damkier P, Nielsen F, Grandjean P, et al. (2005) Polymorphism of CYP2D6, CYP2C19, CYP2C9 and CYP2C8 in the Faroese population. *Eur J Clin Pharmacol* 61: 491-497.
21. Ma MK, Woo MH, McLeod HL (2002) Genetic basis of drug metabolism. *Am J Health Syst Pharm* 59: 2061-2069.
22. Niesters M, Overdyk F, Smith T, Aarts L, Dahan A (2013) Opioid-induced respiratory depression in paediatrics: a review of case reports. *Br J Anaesth* 110: 175-182.
23. Kirchheiner J, Keulen JT, Bauer S, Roots I, Brockmüller J (2008) Effects of the CYP2D6 gene duplication on the pharmacokinetics and pharmacodynamics of tramadol. *J Clin Psychopharmacol* 28: 78-83.