

Treatment of Iraqi People with Hyperkinetic Movement Disorders by Tetrabenazine in Relation to Genetic Polymorphism of CYP450 2D6

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Abstract

Genetic polymorphism is defined as the inheritance of a trait controlled by a single genetic locus with two alleles in which the least common allele has a frequency of about 1% or greater. The Cytochrome P450 (CYP2D6) enzyme metabolizes about 25% of clinically used drugs from many different drug classes including antidepressants, antipsychotics, antihypertensives, and analgesics. The CYP2D6 is a highly polymorphic gene locus with more than 75 allelic variants, thus subjects can be classified into poor metabolizers (PM), intermediate metabolizers (IMs), extensive metabolizers (EM), or ultra-rapid metabolizers (UM) of a given CYP2D6 substrate. By using pharmacogenomics, the pharmacotherapy can be optimized, thereby increasing the treatments overall efficacy and decreasing the incidence of adverse events.

The present study is aimed to predicted phenotypes of CYP2D6 as : poor metabolizers, intermediate metabolizers, extensive metabolizers, and ultra rapid metabolizers for tetrabenazine in patients with hyperkinetic movement disorders compared to healthy subjects in Iraq .

The study was carried on 75 subjects participated (30 male, 45 female) ; fifty of them were with hyperkinetic movement disorders(25 dystonia , 25 chorea) the reminder 25 were healthy . Genotyping of CYP2D6 gene was performed by polymerase chain reaction (PCR) conventional (allele specific method). Plasma concentration of Tetrabenazine was measured by High-performance liquid chromatography (HPLC) . Data were collected through direct interview with the subjects besides the assessment of genetic polymorphism of CYP 450 2D6 enzyme for Iraqi people.

The results of this study show there were a significant CYP 450 2D6 enzyme polymorphism. the number (percentage) of subjects with CYP450 2D6 gene polymorphisms was 17 (22.66%) of the total study population. The number (percentage) of subjects with CYP 450 2D6*2 was 58 (76.32%), whereas, subjects with CYP450 2D6 *10 was 17 (22.66%). However , no subjects were observed with CYP 450 2D6 *4 polymorphisms in this study. The tetrabenazine concentration with mean \pm SD was 3.08 ± 0.231 and 2.506 ± 0.235 ng/ml for chorea and dystonia patients respectively , while alpha and beta dihydrotetrabenazine range was $37.72 \pm (2.18 \text{ ng/ml} _ 38.13 \pm 2.00 \text{ ng/ml})$ and $(21.376 \pm 0.583 \text{ ng/ml} _ 18.252 \pm 0.874 \text{ ng/ml})$ respectively .

Keywords: CYP 450 2D6 polymorphism, poor metabolizers (PM), intermediate metabolizers (IMs) , extensive metabolizers (EM), tetrabenazine.

Introduction

Genetic polymorphism is defined as the inheritance of a trait controlled by a single genetic locus with two alleles in which the least common allele has a frequency of about 1% or greater^[1]. In other words, it is a alteration

in DNA sequence among individuals, groups, or populations. Polymorphisms can be in the coding regions (where they may be synonymous or nonsynonymous) or, more commonly, in the non-coding regions, and often vary by ethnicity^[2].

Subjects and Method

Subjects

The Cytochrome P450 (CYP2D6) enzyme metabolizes about 25% of clinically used drugs from many diverse medication classes including antidepressants, antipsychotics, antihypertensives, and analgesics. The CYP2D6 is a extremely polymorphic gene locus with more than 75 allelic variants, and subjects can be categorized into poor metabolizers (PM), extensive metabolizers (EM), or ultra-rapid metabolizers (UM) of a given CYP2D6 substrate^{[3],[4]}. EMs carry at least one CYP2D6 allele viewing normal enzyme function, IMs carry one CYP2D6 allele showing reduced function and another with no CYP2D6 function, and PMs carry two nonfunctional CYP2D6 alleles^[5]. ultra-rapid metabolizers, however, carry several copies of functional CYP2D6 genes^[6]. The CYP2D6*9, CYP2D6*10, CYP2D6*17, and CYP2D6*41 are the greatest vital intermediate metabolizer (IM) alleles^[7], in which CYP2D6*10 (49.5%)^[8], CYP2D6*1 (40.1%–43.5%)^{[9],[10]}, CYP2D6*2 (11.9%–26.2%)^[11], and CYP2D6*4 (0.2%– 2.8%)^{[7],[9]} are the most common CYP2D6 alleles in Asians. About 13%–50% of Asian people may be homozygous for the CYP2D6*10 allele^[12]

A cytosine-to-thymine replacement (C>T) at nucleotide 100 in the CYP2D6*10 allele, resultant in a change of proline-to-serine at codon 34, is public between Asian people^[13] and is related to reduced metabolism of numerous CYP2D6 substrates^[12]. There is a greatly conserved proline-rich zone among microsomal P450s. The proline at codon 34 perhaps performances a center among the lipophilic membrane anchor and the active site (heme moiety) of the enzyme. Alteration of an amino acid in this area may well reduce the activity of the enzyme^{[14],[15]}. The CYP2D6*4 (G1846A) allele results in a splicing defect with nonfunctioning enzyme activity due to change of guanine (G) by adenine (A) at position 1,846. This single base substitution in the intron 3/exon 4 boundary result in move of the consensus acceptor splice site, yielding a spliced messenger RNA with extra base, thus resultant in a early termination codon^[16].

Aim of the study: The present study is aimed to predicted phenotypes of CYP2D6: poor metabolizers, intermediate metabolizers, extensive metabolizers, and ultra-rapid metabolizers in Iraqi people with Hyperkinetic Movement Disorders treated with tetrabenazin and relation between the genotypes and level of tetrabenazin.

This is a prospective case control study ,that was carried on 75 subjects participated in this research (30 male, 45 female) , attending Baghdad medical city during the period from September 2018 to June 2019. Data were collected through direct interview with the subjects with mean age of 38.81±1.31years . Blood samples were collected at the beginning of the study to measure genetic polymorphism of CYP 450 2D6 enzyme for Iraqi people . The study was approved by the local committee of ethics .

DNA was extracted from whole blood samples using DNA Extraction kit, to be utilized for genotyping of CYP2D6 gene was performed by polymerase chain reaction (PCR) conventional (allele specific method) according to method described by Taimour Langaee, Issam Hamadeh, Arlene B. Chapman, et al^[17]. Polymerase Chain Reaction (PCR) was applied using MultiGENE OPTIMAX (Labnet international, Inc.,USA).

The following primers were used for PCR amplification.

1.CYP2D6 *2 (2850 C>T) rs16947

Forward 5' GGCCCCTGCACTGTTTCC 3'

Reverse 5' AAGGGGAACCCTGAGAGC 3'

2. CYP2D6 *4 (1846 G>A) rs3892097

Forward 5' TGCCGCTTCGCCAACCACT 3'

Reverse 5' GCAGAGACTCCTCGGTCTCTC 3'

3. CYP2D6 *10 (100 C>T rs1065852)

Forward 5' TGTCAGAGGAGCCCATTT 3'

Reverse 5' GTCGAAGCAGTATGGTGTGTTCT 3'

The agarose gel electrophoresis used to check the integrity and presence of the extracted DNA, using Mupid-ONE - ADVANCE/Japan. Agarose solution (1.0 %) heated to boiling till all agarose particles melted, and then 1.0 µL of ethidium bromide dye (10 mg/mL) added to agarose solution, then agarose allowed to solidifying

at room temperature onto the gel chamber ,then DNA samples were mixed with three μL of DNA loading dye and loaded in agarose, gel wells .Gel electrophoresis finished at 70 volts for 30 minutes then DNA bands were visualized under UV ray.

Measure Plasma Level of Tetraberazine by HPLC

Plasma about (20 μl) was injected in to the liquid chromatographic system involving of a C18 μ Bondapak column and fluorescence detector. The mobile phase was acetonitrile-1% acetate buffer, pH 4.5 (50: 50) at a flow-rate of 1 ml/min. The fluorescence of the eluent was measured using an excitation wavelength of 265 nm and an emission filter (KV418)^[18].

(Table -1): Demographic data of the subjects .

Data	subjects
Age (yrs.)	38.81 \pm 1.31
No. of subjects	75
With Chorea	25
With Dystonia	25
Healthy	25
Gender	45 female 30 male
BMI	21.987 \pm 0.476

Data are expressed as : Numbers, Mean \pm SE

Distribution of subjects with (CYP 450 2D6)gene Polymorphism:

Figure (1) shows that the numeral (percentage) of subjects with CYP 450 2D6 gene polymorphisms was 17 (22.66%) of the total study population. The number (percentage) of subjects with CYP 450 2D6*2 was 58 (76.32%), whereas, subjects with CYP450 2D6 *10 was 17 (22.66%).No subjects were observed with CYP 450 2D6 *4 polymorphisms in this study.

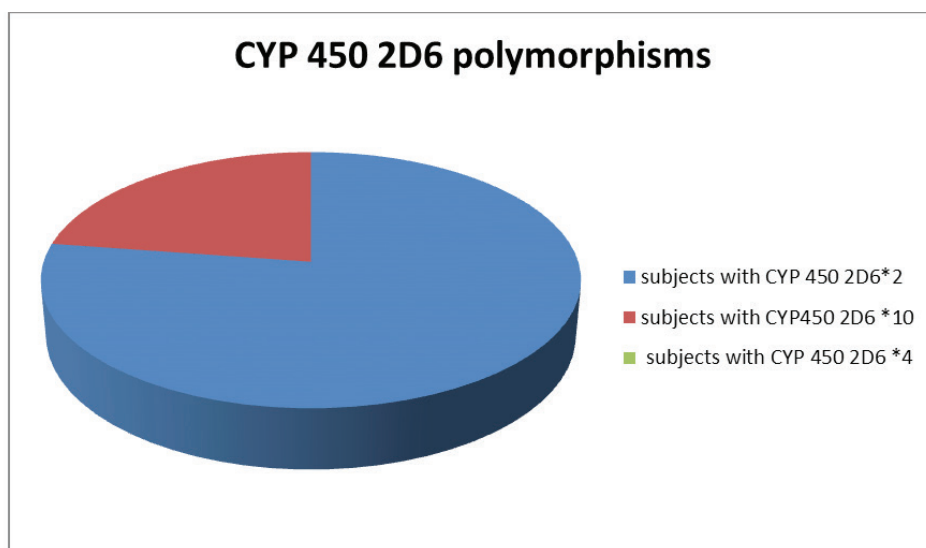
Statistical Analysis

Data will analyze by using SAS (Statistical Analysis System) (version 25.0) program (SPSS Inc., Chicago, Illinois, USA) and Minitab version 17 software. In all comparisons, a p-value <0.05 was reflected statistically significant.

Results and Discussion

1. Demographic data of the study population

Seventy five subjects included in this study. Demographic data of the subjects was shown in (Table-1).



(Figure-1) Histogram showing the distribution of subjects with (CYP 450 2D6) gene Polymorphism

Effects of CYP 450 2D6 gene Polymorphism on Tetrabenazine and main metabolites concentrations in plasma :

Table 2 shows that the plasma concentration tetrabenazine was ranged between 1.2–5.9 ng/ml with mean \pm SD 3.08 ± 0.231 and 2.506 ± 0.235 ng/ml for chorea and dystonia patients respectively , while alpha and beta dihydrotetrabenazine range was 28.1–68.3 ng/ml and 13.65–29.18ng/ml respectively .

(Table- 2) Plasma concentration of tetrabenazine alpha and beta dihydrotetrabenazine in patient group

Substance	Substance concentration for Group A	Substance concentration for Group B
Tetrabenazine	3.08 ± 0.231 ng/ml	2.506 ± 0.235 ng/ml
Alpha-dihydrotetrabenazine	37.72 ± 2.18 ng/ml	38.13 ± 2.00 ng/ml
Beta-dihydrotetrabenazine	21.376 ± 0.583 ng/ml	18.252 ± 0.874 ng/ml

Data expressed as Mean \pm SEM. Group A for chorea and group B for dystonia Patients

plasma concentration of tetrabenazine, alpha and beta dihydrotetrabenazine for patients with CYP 450 2D6 polymorphism versus the patients without CYP 450 2D6 polymorphism in chorea patients using tetrabenazine:

Table-3 shows that the mean tetrabenazine, alpha and beta dihydrotetrabenazine concentration were increased significantly in patients with CYP 450 2D6 polymorphisms compared to patients without CYP 450 2D6 polymorphisms using tetrabenazine.

(Table-3) plasma concentration of tetrabenazine, alpha and beta dihydrotetrabenazine for patients with CYP 450 2D6 polymorphism versus the patients without CYP 450 2D6 polymorphism in chorea patients using tetrabenazine

Substance	Group	Mean± SD	P-value
Tetrabenazine (ng/ml)	Patients without CYP 450 2D6 polymorphism	2.467±0.086	0.001*
	Patients with CYP 450 2D6 polymorphism	4.657± 0.37	
Alpha-dihydrotetrabenazine (ng/ml)	Patients without CYP 450 2D6 polymorphism	31.91± 0.56	0.001*
	Patients with CYP 450 2D6 polymorphism	52.69± 3.7	
Beta-dihydrotetrabenazine (ng/ml)	Patients without CYP 450 2D6 polymorphism	19.96± 0.34	0.002*
	Patients with CYP 450 2D6 polymorphism	25.01± 0.97	

*Significant difference

5. plasma concentration of tetrabenazine, alpha and beta dihydrotetrabenazine for patients with CYP 450 2D6 polymorphism versus the patients without CYP 450 2D6 polymorphism in dystonia patients using tetrabenazine:

Table 4 shows that the mean tetrabenazine, alpha and beta dihydrotetrabenazine concentration were increased significantly in the patients with CYP 450 2D6 polymorphisms compared to patients without with CYP 450 2D6 polymorphisms using tetrabenazine.

(Table-4): plasma concentration of tetrabenazine, alpha and beta dihydrotetrabenazine for patients with CYP 450 2D6 polymorphism versus the patients without CYP 450 2D6 polymorphism in dystonia patients using tetrabenazine.

Substance	Group	Mean± SD	P-value
Tetrabenazine (ng/ml)	Patients without CYP 450 2D6 polymorphism	1.932± 0.10	0.001*
	Patients with CYP 450 2D6 polymorphism	4.325±0.34	
Alpha-dihydrotetrabenazine (ng/ml)	Patients without CYP 450 2D6 polymorphism	33.59± 0.58	0.010*
	Patients with CYP 450 2D6 polymorphism	52.5± 4.7	
Beta-dihydrotetrabenazine (ng/ml)	Patients without CYP 450 2D6 polymorphism	18.25± 0.87	0.001*
	Patients with CYP 450 2D6 polymorphism	24.85± 1.1	

*Significant difference

Cytochrome P450 (CYP) 2D6 is one of the most examined CYPs in relative to genetic polymorphism, however it accounts for only a small fraction of all hepatic CYPs (around 2-4%). However, there is a great inter-individual variation in the enzyme action of CYP2D6. The enzyme is mainly non-inducible and metabolizes about 25% of present medications. Typical substrates for CYP2D6 are basically lipophilic bases and include certain antidepressants, antipsychotics, antiarrhythmics, antiemetics, beta-blockers and opioids^[19].

The CYP2D6 activity varies noticeably within a population and consists of ultrarapid metabolizers (UMs), extensive metabolizers (EMs), intermediate metabolizers (IMs) and poor metabolizers (PMs). There is a significant variability in the CYP2D6 allele spreading amongst diverse racial groups, resulting in variable fractions of PMs, IMs, EMs and UMs in a given population. The allele *10 provides a substrate-dependent reduced action. It is reported that alleles *3, *4, *5, *6 and *7 have no enzyme activity^{[20],[21]}. Furthermore, no patients were observed with CYP 450 2D6 *4 polymorphisms in this study.

There has been promoting from monitoring a drug level in plasma of tetrabenazine and its metabolites for hyperkinetic movement disorders and a taking precaution in the polymorphism of CYP 450 2D6 enzyme. The plasma concentration of tetrabenazine after one and half hour for therapeutic efficacy suggested as less than 2.5 ng/ml and Alpha and Beta dihydrotetrabenazine 40.5 ng/ml and 25.7 ng/ml respectively^[22].

In the presence of low CYP2D6 activity, dealkylated decreased, and excess intermediate metabolites are stayed in plasma. High alpha-DTBZ concentrations related to increased adverse effect like sedation, insomnia and depression. It is proposed that active of the drug and its metabolism. The range of plasma level of the tetrabenazine found in all patients subject was 3.08 ± 0.231 ng/ml with mean \pm SD equal to 2.506 ± 0.235 ng/ml, were due to the 26% of patients having CYP2D6 polymorphism. This polymorphism significantly affects the plasma concentration of alpha-DTBZ due to change in enzyme activity. Plasma concentration of tetrabenazine was narrow in comparison to its metabolite, which it may be due to the timing of assessment or the small data about

the short half-life of the drug about the long half-life of its metabolite. There was a significant increase in plasma concentration of alpha and beta-dihydrotetrabenazine in patients with CYP2D6 polymorphism in comparison to those with nonmutant gene-phenotype that is due to the low activity of the enzyme (31.91 ± 0.56 vs. 52.69 ± 3.7) (19.96 ± 0.34 vs. 25.01 ± 0.97) respectively for chorea patients, while (33.59 ± 0.58 vs. 52.5 ± 4.7) (18.25 ± 0.87 vs. 24.85 ± 1.1) for dystonia patients, which results in elevation in the plasma concentration of tetrabenazine (2.467 ± 0.086 ng/ml vs. 4.657 ± 0.37) for chorea and (1.932 ± 0.10 vs. 4.325 ± 0.34) for dystonia.

Conclusions

According to the data of the present study, we can conclude that:

There is a relatively high incidence (22.66%) of CYP 450 2D6 gene polymorphisms in Iraqi population and mainly CYP 450 2D6*10 which is intermediate metabolizers (IMs).

Higher plasma concentration of tetrabenazine among patient with CYP 450 2D6*10 polymorphism in comparison to patient without CYP 450 2D6 polymorphism (2.467 ± 0.086 ng/ml vs. 4.657 ± 0.37) for chorea and (1.932 ± 0.10 vs. 4.325 ± 0.34) for dystonia.

Depending on the results acquired in this study: Determination a genotype of the CYP 450 2D6 genes in Iraqi patients in relative to the safety and efficacy of drugs that metabolite and to determine the suitable dose for the patient depending on the genotype of the CYP 450 2D6 from one side and the concentration of the drug in the plasma from other side.

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Conflict of Interest: The authors declare that they have no conflict of interest.

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