

The prevalence of CYP2C19 mutations in Turkish patients with dyspepsia

Dispeptik yakınmaları olan Türk hastalarda CYP2C19 mutasyonlarının prevelansı

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Background/aims: We aimed to determine the distribution of cytochrome P450 2C19 (CYP2C19) genotype frequencies in Turkish patients with dyspepsia. **Methods:** CYP2C19 genotype was determined in 100 Turkish patients with dyspepsia. DNA of the patients was isolated from whole blood and genotypes were detected by specific probes in real-time polymerase chain reaction (PCR). **Results:** The frequencies of heterozygous CYP2C19*2 and CYP2C19*3 genotypes were 13% and 1% in dyspeptic patients, respectively. Homozygous mutant CYP2C19*2 was detected at a rate of only 1% in the study population, and homozygous mutant genotype of CYP2C19*3 was not found. The frequencies of homozygous CYP2C19*2 and CYP2C19*3 genotypes were 86% and 99% in dyspeptic Turkish patients, respectively. **Conclusions:** This is the first study investigating CYP2C19 polymorphism in dyspeptic Turkish patients. Our investigation revealed that the most common CYP2C19 genotype was wild type CYP2C19 in dyspeptic Turkish patients. Dyspeptic Turkish patients are extensive metabolizers for proton pump inhibitors. This finding might have impact on the clinical consequences for the treatment strategies in dyspepsia.

Key words: CYP2C19*2, CYP2C19*3, Turkish patients, dyspepsia

INTRODUCTION

The cytochrome P450 2C (CYP2C) subfamily of enzymes metabolizes approximately 20% of drugs commonly used in clinical practice (1). Proton pump inhibitors (PPIs) are metabolized by the cytochrome P450 system in the liver mainly through the pathway using the S-mephenytoin 4-hydroxylase enzyme, which is mediated by the CYP2C19 genotype. Genetic polymorphisms have been identified for the CYP2C19 enzyme, resulting in decreased metabolism of PPIs (2).

The most frequent of these genetic polymorphisms are CYP2C19*2, containing a G681A point muta-

Amaç: Dispeptik yakınmaları olan Türk hastalarda cytochrome P450 2C19 enziminin genotipik dağılımını belirlemek. **Yöntem:** Dispeptik yakınmaları olan 100 Türk hastada CYP2C19 genotipi belirlendi. Hastaların DNA'sı kandan ayrıldı ve genotipler real-time PCR ile spesifik problerle saptandı. **Bulgular:** Dispeptik hastalarda heterozigot CYP2C19*2 ve CYP2C19*3 genotipi sırasıyla %13 ve %1 saptandı. Çalışma hastalarının sadece birinde (%1) homozigot mutant CYP2C19*2 saptandı. Dispeptik hastalarda homozigot mutant CYP2C19*3 bulunmadı. Dispeptik Türk hastalarda homozigot CYP2C19*2 ve CYP2C19*3 genotipleri sırasıyla %86 ve %99 olarak bulundu. **Sonuç:** Bu araştırma dispeptik Türk hastalarda CYP2C19 polimorfizminin araştırıldığı ilk çalışmadır. Çalışmamız dispeptik Türk hastalarda en sık görülen CYP2C19 genotiplerinin homozigot CYP2C19*2 ve CYP2C19*3 olduğunu göstermektedir. Dispeptik Türk hastalar Proton Pompa İnhibitörleri için hızlı metabolize edicidirler. Bu bulgular dispepsi tedavisinin klinik sonuçlarında etkili olabilir.

Anahtar kelimeler: CYP2C19*2, CYP2C19*3, Türk hastalar, dispepsi

tion in exon 5 resulting in a splicing defect, and CYP2C19*3, containing a G636A transition in exon 4, which produces a premature stop codon. Together, they are responsible for more than 85% of poor metabolizers (PMs) in Caucasians and 99% in Orientals. However, other variants have also been identified and characterized. Many of these mutations give rise to the PM phenotype, owing to insufficient or inactive enzyme. The frequency of PMs shows wide inter-racial variation, ranging from 1–6% in Caucasians, 12–23% in Orientals and 4–8% in Africans (2,3).

The metabolizers may be classified into three groups: the wild type, so-called homozygous extensive metabolizer (HomEM), which contains two non-mutated alleles, the heterozygous extensive metabolizer (HetEM), which contains one mutated and one wild-type allele, and finally, the PMs, which have two mutated alleles. In patients who carry the HetEM and especially the PM genotype, metabolism of PPIs is much slower, which results in greater bioavailability of the PPI (2, 4).

Proton pump inhibitors are the most commonly used drugs for dyspepsia. The dependence of PPIs on the cytochrome P450 activity for its metabolism may have important reflections in the management of dyspepsia. It is noteworthy that determination of the prevalence of CYP2C19 mutations in a group of patients with dyspepsia may form a reference point for the PPI treatment dose used for dyspepsia.

The aim of this study was to assess the distribution of the CYP2C19 mutations in dyspeptic Turkish patients. To our knowledge, this is the first study committed to revealing the prevalence of CYP2C19 mutations in Turkish patients with dyspepsia.

MATERIALS AND METHODS

The patients (n=100) were recruited from the gastroenterology outpatient clinic. Written informed consent was obtained from all the participants. The study protocol was approved by the Local Research Ethics Committee.

Dyspepsia was defined according to the criteria of Rome III Working Teams formulation (5). The patients with resistance to PPI therapy were excluded from the study in order to prevent bias in determining the prevalence of CYP2C19 mutations.

Venous blood samples (5 ml) were collected from each patient in EDTA tubes. DNA was extracted from the leukocytes using a high-pure template preparation kit (Roche Diagnostics, GmbH, Mannheim, Germany). CYP2C19 alleles were detected by specific probes in 'Lightmix for the detection of human CYP2C19*2 and CYP2C19*3 Detection Reagent' (TIBMOBOL, GmbH, Berlin, Germany) by real-time polymerase chain reaction (PCR) (LightCycler, Roche Diagnostics, GmbH, Mannheim, Germany), according to the manufacturer's recommendations.

The G681A point mutation in exon 5 of CYP2C19*2 and G636A transition in exon 4 of

CYP2C19*3 were detected. The allele frequencies were estimated by melting curve analysis, and genotypes were scored.

RESULTS

One hundred dyspeptic patients (59 F, 41 M) were enrolled in the study. Their mean age was 41 years (range 20-64).

The frequency distribution of CYP2C19*2 and CYP2C19*3 in 100 dyspeptic patients was determined using real-time PCR. Specific melting points were obtained for the allelic variations. Melting curve analysis demonstrated the gradual reduction in fluorescence as temperature increased. The falls at 48°C for CYP2C19*2 mutant and at 55°C for the wild type alleles indicated the specific products melt at this temperature, on channel 530 in the LightCycler detection system (Figure 1). Similar data were obtained at 53°C for CYP2C19*3 mutant and at 61°C for the wild type alleles, on channel 640 (Figure 2).

The frequencies of heterozygous CYP2C19*2 and CYP2C19*3 genotypes were 13% and 1% in dyspeptic patients, respectively. Homozygous mutant CYP2C19*2 was determined at a rate of only 1% in the study population, and the mutant genotype of CYP2C19*3 was not found. The frequencies of homozygous CYP2C19*2 and CYP2C19*3 genotypes (wild type) were 86% and 99% in dyspeptic Turkish patients, respectively.

DISCUSSION

The difference in CYP expression can result in variability in drug metabolism and it is therefore important to understand the genetic factors that influence CYP levels and activities. The finding of prevalences for CYP2C19 mutations may provide

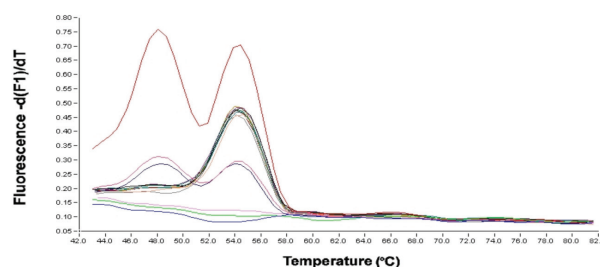


Figure 1. Melting curve analysis demonstrated the gradual reduction in fluorescence as temperature increases. The falls at 48°C for CYP2C19*2 mutant and at 55°C for the wild type alleles indicated the specific products melt at this temperature (channel 530 in the LightCycler detection system).

a reference point for dose regimens of the drugs metabolized by the CYP2C19 enzyme. Searching for the presence of CYP2C19 mutations in dyspeptic patients may serve as an objective base for dosing PPIs in the management of dyspepsia.

A comparison of the ratio of the area under the plasma-concentration curve (AUC) for PPIs is HomEM: HetEM: PMs = 1: 3.7: 20 (2). This data means that PMs have more than five times the PPI available of intermediate metabolizers and 20 times that of rapid metabolizers. Thus, PMs have more striking gastric inhibition compared to HomEM and HetEM.

The distribution of CYP2C19 mutations show variation between the eastern and western parts of the world (2,6). In the present study, we found that only 13 subjects out of 100 dyspeptic patients had heterozygous CYP2C19*2 mutation and 1 patient homozygous CYP2C19*2 mutation. In addition, 1 patient had CYP2C19*3 heterozygous mutation. Thus, for PM phenotypes, the prevalence was found as 1% in our study. A search of the literature revealed that prevalences of PM phenotypes are Caucasians 2.8%, African American 3.9%, Chinese 14.3%, Koreans 14%, and Japanese 22.3% (2). Whether or not the striking difference in geographic distribution of CYP2C19 mutations has important clinical consequences for patients using PPIs warrants further studies due to the heterogeneous results of the related trials in the literature.

The management of dyspeptic patients includes two options: (i) test and treat for *Helicobacter pylori* (*H. pylori*) using a validated noninvasive test and a trial of acid suppression if eradication is successful but symptoms do not resolve, or (ii) an empiric trial of acid suppression with a PPI for 4-8 weeks. The test-and-treat option is preferable in populations with a moderate to high prevalence of *H. pylori* infection ($\geq 10\%$); empirical PPI is an initial option in low prevalence situations (7). The current standard therapy for *H. pylori* eradication is the PPI-based triple therapy including PPI, clarithromycin, and amoxicillin. It is known that PPI-based therapy achieved high eradication rates, reduced the impact of primary resistance, and decreased the risk of secondary resistance compared with regimens containing only two antibiotics (8). Discrepant data exist regarding whether CYP2C19 polymorphisms alter the benefit ratio of PPI therapy. In order to assess the relation of CYP2C19 polymorphisms with the effectiveness of PPI, the first step should be a statement of

CYP2C19 distribution in the study population. From the point of reference for the treatment of dyspepsia, this is the first study related with the prevalence of CYP2C19 mutations conducted in Turkish patients with dyspepsia.

The frequency of PMs in Asian populations is approximately 20%. On the other hand, this phenotype is less common ($<5\%$) in the western part of the world. Thus, the dose regimens for the drugs metabolized by CYP2C19 should differ from each other in two populations based on the fact that low activity of CYP2C19 in PMs results in a higher level of active drug in the plasma. Elucidation of the issue about the relationship between the CYP2C19 polymorphisms and clinical effectiveness is still needed and warrants further studies in our society. The data for CYP2C19 mutations may exclude the patient-related bias in clinical studies and serve as a reference for cost-effective treatment.

Functional dyspepsia may represent a “Bermuda Triangle” for the physician, patient and drug manufacturers in clinical practice. As the precise etiology of functional dyspepsia is not known, empirical therapies such as antidepressant with uncertain efficacy are reserved for difficult cases as options (9,10). Although the clinical implications of the CYP2C19 mutations have not been clearly established, it may be anticipated that the PMs are more likely to develop adverse effects to antidepressants that are substrates of the enzyme if given at normal doses. It is estimated that antidepressant treatment with amitriptyline, clomipramine, doxepin, imipramine, trimipramine, citalopram, and moclobemide would benefit from a CYP2C19 genotype-based dose adjustment with approximately 60%, less than 100%, and 110% of the current drug dosage for PMs, HetEM, and Ho-

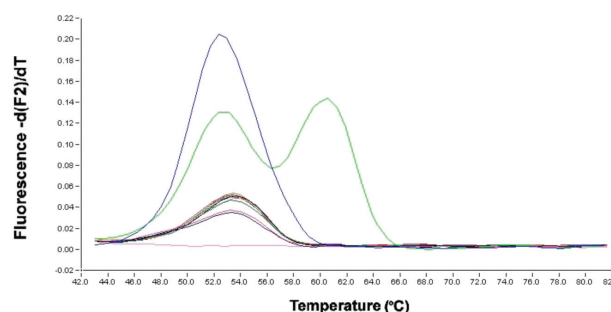


Figure 2. Melting curve analysis demonstrated the gradual reduction in fluorescence as temperature increases. Falls at 53°C for CYP2C19*3 mutant and at 61°C for the wild type alleles are seen (channel 640 in the LightCycler detection system).

mEM individuals, respectively (11). Thus, without taking the clinical manifestation of CYP2C19 mutations into consideration, the assessment of the clinical efficacy of antidepressants in functional dyspepsia may be misleading. The diverse results of the trials regarding the effectiveness of antidepressants in the management of dyspepsia may be related to the varied distributions of CYP2C19 mutations. This speculation needs to be investigated with further trials.

Our study revealed that the frequency of CYP2C19 mutations in Turkish dyspeptic patients was similar to that of other European populations. Turkish patients with dyspepsia are extensive metabolizers of PPIs and antidepressants. Additional studies are required to assess the clinical significance of CYP2C19 mutations on treatment outcome and the optimal dosage of PPIs, antidepressants, and antibiotics, which are used for HP eradication in dyspeptic patients.

REFERENCES

1. Goldstein JA. Clinical relevance of genetic polymorphism in the human CYP2C subfamily. *Br J Clin Pharmacol* 2001; 52: 349-55.
2. Dickson EJ, Stuart RC. Genetics of response to proton pump inhibitor therapy. *Am J Pharmacogenomics* 2003; 3: 303-15.
3. Rosemary J, Adithan C, Padmaja N, et al. The effect of the CYP2C19 genotype on the hydroxylation index of omeprazole in South Indians. *Eur J Clin Pharmacol* 2005; 61: 19-23.
4. Van Zanten SV, Thompson K. Should the presence of polymorphisms of CYP2C19 enzymes influence the choice of the proton pump inhibitor for treatment of *Helicobacter pylori* infection? *Am J Gastroenterol* 2006; 101: 1476-8.
5. Drossman DA. Rome III: the new criteria. *Chin J Dig Dis* 2006; 7: 181-5.
6. Chong E, Ensom M. Pharmacogenetics of the proton pump inhibitors: a systematic review. *Pharmacotherapy* 2003; 23: 460-71.
7. Talley NJ, Vakil N. Practice Parameters Committee of the American College of Gastroenterology. Guidelines for the management of dyspepsia. *Am J Gastroenterol* 2005; 100: 2324-37.
8. Lind T, Megraud F, Unge P, et al. The MACH2 study: role of omeprazole in eradication of *Helicobacter pylori* with 1-week triple therapies. *Gastroenterology* 1999; 116: 248-53.
9. Talley NJ. Therapeutic options in nonulcer dyspepsia. *J Clin Gastroenterol* 2001; 32: 286-93.
10. Tanum L, Malt UF. A new pharmacologic treatment of functional gastrointestinal disorder. A double-blind placebo-controlled study with Mianserin. *Scand J Gastroenterol* 1996; 31: 318-25.
11. Kirchheiner J, Nickchen K, Bauer M, et al. Pharmacogenetics of antidepressants and antipsychotics: the contribution of allelic variations to the phenotype of drug response. *Mol Psychiatry* 2004; 9: 442-73.