

Influence of CYP2C19 functional polymorphism on *Helicobacter pylori* eradication

CYP2C19 fonksiyonel polimorfizminin *Helicobacter pylori* eradikasyonu üzerine etkisi

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Background/aims: Proton pump inhibitors are mainly metabolized by cytochrome P450 2C19 in the liver. Recently, some studies have shown that the acid-suppressing effect of proton pump inhibitors are influenced by a functional polymorphism of cytochrome P450 2C19. The aim of the present study was to investigate the effect of cytochrome P450 2C19 polymorphism on *Helicobacter pylori* eradication in patients who received proton pump inhibitors-based triple therapy. **Methods:** We determined the incidence of cytochrome P450 2C19 genotypes and the effect of cytochrome P450 2C19 genotypes on *Helicobacter pylori* eradication rates in 105 patients with *Helicobacter pylori*-positive chronic gastritis. Upper endoscopic procedure and gastric biopsies were performed in all patients. *Helicobacter pylori* was demonstrated histologically. Lansoprazole, amoxicillin and clarithromycin twice a day for 14 days were prescribed for those found to be infected with *Helicobacter pylori*. More than one month after the medication, a ¹³C urea breath test was conducted to examine the success or failure of the eradication treatment. Cytochrome P450 2C19 polymorphism was analyzed by the polymerase chain reaction–restriction fragment length polymorphism method. **Results:** The genotypes of cytochrome P450 2C19 were classified into the three groups, as rapid extensive metabolizer, intermediate metabolizer and poor metabolizer. In our patient population, the frequencies of rapid extensive metabolizer, intermediate metabolizer and poor metabolizer were 72%, 23% and 5%, respectively. The eradication rate was 70.0% for rapid extensive metabolizer, 92% for intermediate metabolizer and 80% for poor metabolizer. The eradication rate was highest in intermediate metabolizer patients. **Conclusions:** The present study confirmed the low eradication rate for rapid extensive metabolizer. Our findings provide evidence that the cytochrome P450 2C19 genotype is useful to predict the success of treatment. For the rapid extensive metabolizer group, alternative regimens can be tried to increase the *Helicobacter pylori* eradication rates.

Key words: *Helicobacter pylori*, eradication, CYP2C19

INTRODUCTION

Genetic variation of drug-metabolizing enzymes has been recognized as one of the major causes of individual variability in drug response (1, 2). Cytochrome (CYP) P450 2C19 (CYP2C19) is an impor-

Amaç: Proton pompa inhibitörler, büyük ölçüde karaciğerde bulunan sitokrom P450 2C19 (CYP2C19) enzimi tarafından metabolize edilirler. Sitokrom P450 2C19 enziminin fonksiyonel polimorfizmi proton pompa inhibitörleri asit-baskılayıcı etkilerini değiştirebilir. Bu çalışmada, sitokrom P450 2C19 polimorfizminin proton pompa inhibitörler–temelli tedavilerde *Helicobacter pylori* eradikasyonu üzerinde etkisini araştırmayı amaçladık. **Yöntem:** Bu çalışmada, *Helicobacter pylori*'ye bağlı kronik gastrit tanısı olan 105 hastada sitokrom P450 2C19 genotipinin sıklığını ve bu genotipin *Helicobacter pylori* eradikasyon oranını belirledik. Üst gastrointestinal endoskopi ve gastrik biyopsi her hastada uygulandı. *Helicobacter pylori* tanısı histolojik olarak konuldu. Hastalara lansoprazol, amoksisilin ve klaritromisin günde 2 kez olmak üzere 14 gün süreyle uygulandı. *Helicobacter pylori* eradikasyonunu belirlemek üzere tedaviden bir ay sonra tüm hastalara ¹³C üre–solunum testi yapıldı. sitokrom P450 2C19 polimorfizmi polimeraz zincir reaksiyonu temelli–RFLP yöntemi ile saptandı. **Bulgular:** Sitokrom P450 2C19 polimorfizmleri 3 gruba ayrıldı; hızlı metabolize edici, orta derecede metabolize edici ve yavaş metabolize edici olmak üzere. Hastalarımız arasında, hızlı metabolize edici %72 oranında, orta derecede metabolize edici %23 oranında ve yavaş metabolize edici ise %5 oranında belirlendi. *Helicobacter pylori* eradikasyon oranı hızlı metabolize edici grupta %70 oranında, orta derecede metabolize edici grupta %92 ve yavaş metabolize edici grupta ise %80 oranında saptandı. **Sonuç:** Bulgularımız hızlı metabolize edici grupta *Helicobacter pylori* eradikasyon oranının düşük olduğunu doğrulamaktadır. Tedavi öncesi sitokrom P450 2C19 genotipinin belirlenmesi tedavi başarısının öngörülmesi açısından yararlı olur. Hızlı metabolize edici hastalarda *Helicobacter pylori* eradikasyon oranını yükseltmek amacıyla alternatif tedaviler denenebilir.

Anahtar kelimeler: *Helicobacter pylori*, eradikasyon, CYP2C19

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inactivation of potential carcinogens, or the bioactivation of some environmental procarcinogens to reactive DNA-binding metabolites such as nitrosamine (3-5). This enzyme shows genetic polymorphism. The genotypes of CYP2C19 are classified into three groups, as rapid extensive metabolizer (RM), intermediate metabolizer (IM) and poor metabolizer (PM) (5, 6). Several alleles of the CYP2C19 gene have been identified and have been shown to cause phenotypic variability. To date, more than 10 alleles have been described, and CYP2C19*2 and CYP2C19*3 are the most common of these variants. CYP2C19*2 arises from G→A mutation at base pair (bp) 636 in exon 5 of wild-type (wt) CYP2C19*1 that creates an aberrant splice site. CYP2C19*3 involves a G→A mutation at bp 636 in exon 4 that creates a premature stop codon and a truncated protein (7, 8).

Proton pump inhibitors (PPIs), such as omeprazole, lansoprazole, rabeprazole, esomeprazole and pantoprazole, undergo the hepatic metabolism by the CYP P450 system (9). The principle enzyme involved in the metabolism of PPIs is CYP2C19. CYP3A4 is also involved in the PPI metabolism. Omeprazole is partially first metabolized by CYP3A4 to omeprazole sulfone, then metabolized to 5-hydroxyomeprazole sulfone by CYP2C19 (10). Polymorphism of CYP2C19 affects pharmacokinetics and pharmacodynamics of PPIs (9-11). The analysis of genetics associated with the enzyme activity and phenotype of CYP2C19 enzyme has been classified into the three genotype groups as the RM group, the IM group and the PM group (11). Each genotype group corresponds to the homozygous extensive metabolizer (homEM) and PM in several previous reports. In RMs, neither allele has mutations, and the enzyme can be generated from both of the non-mutated (wild-type: wt) alleles (11, 12). In IMs, one allele has a mutation in the coding region of CYP2C19. However, the other allele has no mutation and normal enzyme can be generated from this allele. In PMs, both of the alleles have mutations in the CYP2C19 genes, and therefore, normal enzyme can not be generated from either of the two mutated alleles, thereby resulting in the deficiency of the enzyme activity (11, 12).

Many studies from different populations have suggested that the polymorphism of enzyme activity and the distribution of the alleles of CYP2C19 show significant geographic variation. For example, the prevalence of PM is 11–12% in Orientals, and the two most common alleles, CYP2C19*2 and

CYP2C19*3, account for almost all PMs. In Caucasians, however, only 3–5% are PMs and CYP2C19*3 is extremely rare (7, 13, 14). Genetic polymorphism of CYP2C19 is relevant to the disposition of drugs and may affect their efficacy and toxicity (14). Eradication of *H. pylori* is now an important treatment strategy for the cure of a variety of upper gastrointestinal disorders, such as peptic ulcer and mucosa-associated lymphoid tissue lymphoma (7, 14). Current regimens for the eradication of *H. pylori* consist of a PPI plus one or two antibacterial agents, such as amoxicillin, clarithromycin and metronidazole. A recent meta-analysis showed that the impact of CYP2C19 polymorphism on *H. pylori* eradication rates appears to be clinically relevant in patients receiving PPI as a component of dual or triple-drug therapy (7). Differences in plasma antibiotics and PPI levels among different CYP2C19 genotype groups result in different cure rates for *H. pylori* infection among the respective different CYP2C19 genotype groups (15).

In this study, we conducted a prospective, open trial to demonstrate the influence of CYP2C19 polymorphism on PPI-based eradication therapy of *H. pylori*.

MATERIALS AND METHODS

Patients

A total of 105 *H. pylori*-positive patients who underwent upper gastrointestinal endoscopy at Çukurova University Balcalı Hospital, Department of Gastroenterology, between September 2005 and December 2008, and who had not previously received *H. pylori* treatment were enrolled in this study. Lansoprazole (30 mg), amoxicillin (1000 mg), and clarithromycin (500 mg) twice a day for 14 days were used for patients infected with *H. pylori*. More than one month after the treatment, a ¹³C-urea breath test was performed. All patients provided informed consent, and the study was approved by the Committee for Ethics of Medical Experiments on Human Subjects, Faculty of Medicine, Çukurova University, Adana.

CYP2C19 Genotyping

A 5 ml sample of venous blood was collected from each subject into a test tube containing EDTA as anticoagulant. Genomic DNA was extracted from peripheral whole blood using High Pure PCR Template Preparation Kit (Roche Diagnostics, GmbH, Mannheim, Germany) according to the

manufacturer's protocol. Genotyping procedures for identifying the CYP2C19*1 (wt) allele and two mutated alleles, CYP2C19*2 in exon 5 and CYP2C19*3 in exon 4, were performed by means of the polymerase chain reaction–restriction fragment length polymorphism (PCR–RFLP) method with the use of allele–specific primers, as described by de Morais et al. (5) with minor modifications. PCR amplification of CYP2C19*2 was done using the forward and reverse primers: 5'–CAAC-CAGAGCTTGGCATATTG–3' and 5'–CACAAA-TACGCAAGCAGTCAC – 3'. The primers used for analysis of the CYP2C19*3 mutant allele were: 5'–CACCTGTGATCCCACCTTC–3' and 5'–ACT-TCAGGGCTTGGTCAATA–3'. Amplification was performed in GeneAmp PCR System 9700 thermocycler (Applied Biosystems, Singapore) with 100 ng of genomic DNA, 25 pmol of each primer, 200 μ M total dNTP, 1.5 mM MgCl₂, 1X PCR buffer, and 2.5 U Taq DNA polymerase (Promega, Madison, WI, USA). For both, the *2 and *3 PCR reactions consisted of the initial denaturation for 5 min at 94°C followed by 30 cycles of denaturation at 94°C for 1 min, annealing at 55°C for 1 min and extension at 72°C for 1 min. The terminal elongation was performed at 72°C for 10 min. The amplified PCR products of 300 bp (for CYP2C19*2) and 247 bp (for CYP2C19*3) were digested overnight with SmaI (Promega, Madison, WI, USA) and BamHI (Promega, Madison, WI, USA) enzymes, respectively, and then separated

and analyzed on 2% agarose gel with ethidium bromide staining (Figure 1.). Since the restriction site is absent in the mutant alleles (CYP2C19*2 and CYP2C19*3), the PCR products are not digested by restriction enzymes. Samples containing mutant alleles were reanalyzed to ensure the accuracy of the method. There was 100% reproducibility. Subjects were genotypically classified into the following three groups on the basis PCR–RFLP analysis for CYP2C19: homozygous (CYP2C19*1/*1) extensive metabolizers (homEMs) group, heterozygous (CYP2C19*1/*3, *1/*2) extensive metabolizers (hetEMs) group and PMs (CYP2C19*2/*2, *3/*3) group (Figure 1.).

Statistical Analysis

Data analysis was performed using the computer software Statistical Package for Social Sciences (SPSS) for Windows (version 10.0). For statistical analysis, the significance of differences in cure rates among genotype groups was determined by using the chi-squared (χ^2) test. A value of $p < 0.05$ was considered statistically significant. The observed genotype frequencies were compared with expected values calculated from Hardy–Weinberg equilibrium theory ($p^2 + 2pq + q^2 = 1$; where p is the frequency of the wt allele and q is the frequency of the mutated allele) by using a χ^2 -test with degree of freedom equal to 1. The odds (ORs) and 95% confidence intervals (95% CIs) were adjusted for sex and age using logistic regression model.

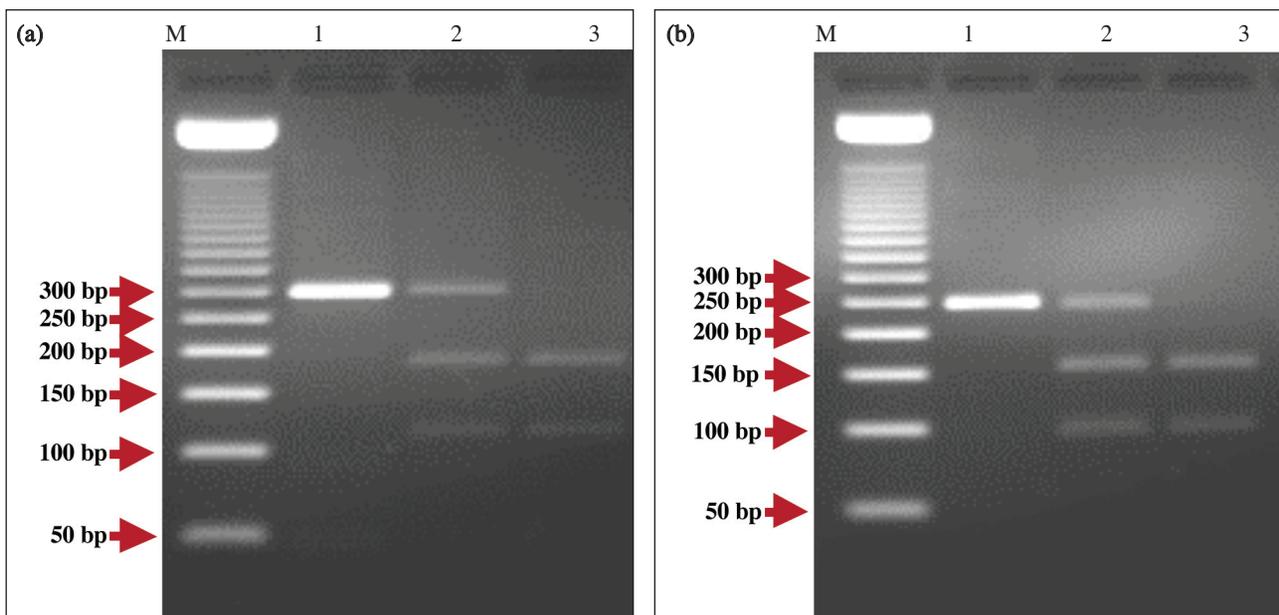


Figure 1. Gel electrophoresis photograph showing the analysis of CYP2C19*2 and CYP2C19*3. PCR-RFLP of (a) exon 5 digested with SmaI for CYP2C19*2 (M: 50-bp DNA ladder, Lane 1: *2/*2, Lane 2: *1/*2, Lane 3: *1/*1) and (b) amplification of exon 4 digested with BamHI for CYP2C19*3 (M: 50-bp DNA ladder, Lane 1: *3/*3, Lane 2: *1/*3, Lane 3: *1/*1).

RESULTS

The mean age of the patients was 46 ± 13.8 years (mean \pm S.D, range: 17–80), and there were 42 (40%) men and 63 (60%) women.

The genotypic frequencies of the CYP2C19 (CYP2C19*1, CYP2C19*2 and CYP2C19*3) were in Hardy-Weinberg equilibrium, suggesting that there was no population stratification and no sampling bias. Five different allelic patterns were noted in the 105 patients. A total of 76 were homozygous for wt alleles (*1) in exons 4 and 5 (*1/*1), 17 were heterozygous for the CYP2C19*2 polymorphism without the CYP2C19*3 polymorphism (*1/*2), 8 were heterozygous for the CYP2C19*3 polymorphism without the CYP2C19*2 polymorphism (*1/*3), and 3 were heterozygous for both CYP2C19*2 and the CYP2C19*3 polymorphisms (*2/*3). A total of 2 were homozygous for the CYP2C19*2 polymorphism without the CYP2C19*3 polymorphism (*2/*2). The overall *H. pylori* eradication rate for the HomEM, HetEM and PM groups were 70%, 92% and 80%, respectively ($p < 0.05$) (Figure 2). Logistic regression analysis showed that HetEM genotypic status of CYP2C19 was significantly associated with an increased eradication rate of *H. pylori* when compared with HomEM genotypic status (OR: 9.33; 95% CI: 1.2-73.2). In addition, compared with the HomEM genotypic status, PM cases have a 1.64-fold increase in eradication rate of *H. pylori* (95% CI: 0.17-15.4). The combination of CYP2C19 HetEM and PM had a significantly high rate relative to HomEM (OR: 5.49; 95% CI: 1.2-25.1). Age, gender and smoking habits did not affect the *H. pylori* eradication rate ($p > 0.05$) (Table 1).

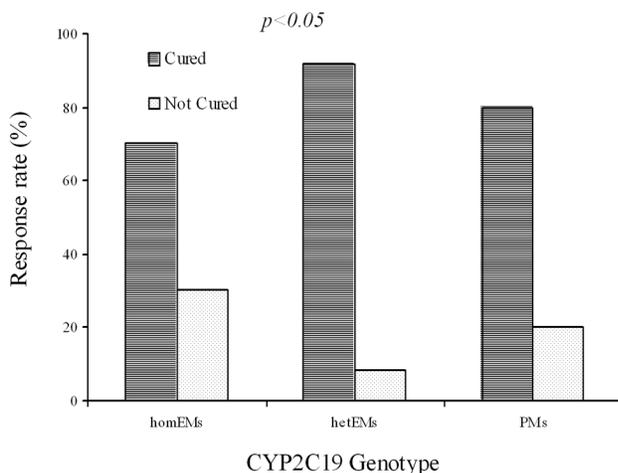


Figure 2. Eradication rate of *H. pylori* and cytochrome P450 2C19 (CYP2C19) polymorphism.

DISCUSSION

In this study, we have investigated the incidence of CYP2C19 polymorphism and the effect of this polymorphism on PPI-based eradication therapy of *H. pylori*. CYP2C19 is a major enzyme that degrades omeprazole and lansoprazole (16, 17). The effects of omeprazole and lansoprazole on intragastric pH have been shown to be dependent on the CYP2C19 gene polymorphism (17). Patients without mutations of the CYP2C19 gene metabolize PPI very extensively, and may not achieve enough acid suppression for amoxicillin, an acid-sensitive antibiotic, to be effective (17,18). This problem has been suggested to be one of the causes for treatment failure, and the cure rate in the dual therapy with omeprazole and amoxicillin has been correlated with the CYP2C19 polymorphism (17).

A PPI is one of the key drugs in *H. pylori* eradication therapy for the following reasons: first, a PPI makes antibiotics more stable and bioavailable in the stomach by raising intragastric pH levels to neutral levels (19). Second, neutralization of intragastric pH levels by a PPI allows *H. pylori* to reach the growth phase and it thus becomes more sensitive to antibiotics, such as amoxicillin (20). Third, suppression of acid secretion by a PPI increases the concentration of an antibiotic in the stomach (21). Fourth, PPI have per se an anti-*H. pylori* effect (22). Therefore, a PPI is indispensable in *H. pylori* eradication therapy. As a matter of fact, the cure rates achieved by treatment with two anti-bacterial agents without a PPI were lower than those achieved using treatment with the same anti-bacterial agents plus omeprazole, a representative prototype PPI (23).

Influence of the CYP2C19 polymorphism on triple PPI/amoxicillin/clarithromycin therapy for *H. pylori* infection at the usual dose has been investigated in many studies (12, 16, 17). Differences in plasma clarithromycin and PPI levels among the different CYP2C19 genotype groups result in different cure rates for *H. pylori* infection among the respective different CYP2C19 genotype groups. In a previous study, *H. pylori* eradication rates by a triple therapy with daily doses of omeprazole 40 mg or lansoprazole 60 mg, amoxicillin 1500 mg, and clarithromycin 600 mg for 1 week were 72.7% in RMs, 92.1% in IMs, and 97.8% in PMs (24). The incidence of the RM genotype was higher in the group without eradication, while the incidence of the PM genotype in patients without eradication was very low (24). Aoyama et al. (25) reported that

Table 1. The rate and odds ratios (ORs) of the eradication among the participants in the polymorphism study who were examined for the success/failure of eradication

Factors	Treated (%)	Eradicated n (%)	OR 95% CI	p-values	
Total, n	105	81 (77.1)			
Age	<30 years	14 (13.3%)	13 (16%)	>0.05	
	31-49 years	45 (42.9%)	28 (34.6%)		
	50-69 years	42 (40.0%)	36 (44.4%)		
	>70 years	4 (3.8%)	4 (4.9%)		
Sex	Males	42 (40%)	31 (38.3%)	>0.05	
	Females	63 (60%)	50 (61.7%)		
Smoking	Ever	38 (36.2%)	26 (32.1%)	>0.05	
	Never	67 (63.8%)	55 (67.9%)		
CYP2C19	homEMs	76 (72.4%)	54 (70%)	1 (Reference)	0.04
	hetEMs	24 (22.8%)	23 (92%)	9.33 (1.2-73.2)	
	PMs	5 (4.8%)	4 (80%)	1.64 (0.17-15.4)	
	hetEMs+ PMs	29 (27.6%)	97 (93%)	5.49 (1.2-25.1)	

cure rates by triple omeprazole/amoxicillin/clarithromycin therapy were 81% in RMs, 94.5% in IMs, and 100% in PMs. Tanigawara et al. (26) also reported similar results. Another study published by Dojo et al. (27) revealed that cure rates by triple omeprazole/amoxicillin/clarithromycin therapy were 73.3% in RMs, 86.1% in IMs, and 85.0% in PMs. We have shown that cure rates by triple lansoprazole/amoxicillin/clarithromycin were 70% in RMs, 92% in IMs and 80% in PMs. Taken together, these reports demonstrate that one of the reasons for the eradication failure of *H. pylori* by triple therapies is considered due to the insufficient dose of a PPI (omeprazole or lansoprazole) in RMs.

The incidence of RM among our patients infected with *H. pylori* was similar to that of the European population. The incidence of RM has been reported to be 81.1% of 143 patients in Italy (28) and 73.3% of 60 patients in Germany (29). The percentage of RM among our patients infected with *H. pylori* was much higher than in Japanese populations. A study published by Furuta et al. (16) from Japan reported that the incidence of RM was 32.6%. Take et al. (30) reported that the percentage of RM among Japanese patients infected with *H. pylori* was found to be 32.5%. The incidence of RM has ranged between 38.1% and 41.3% in other studies published by Japanese investigators (16).

The effect of smoking on *H. pylori* eradication treatment has been controversial. In our study, smoking during the non-medication period did not affect the eradication rate. It has been well documented that smokers had a lower success probability of eradication (31). On the other hand, those who quit smoking during the medication may have a higher eradication rate (32). A study published by Miyoshi et al. (33) showed that smoking is

strongly associated with treatment failure for *H. pylori* eradication in the dual therapy. In that study, the cure rate of smokers was more than 20% lower than that of non-smokers, irrespective of the CYP2C19 genotype. Several mechanisms may be responsible for the effect of smoking on the efficacy of eradication therapy against *H. pylori*. First, higher gastric secretion is found in patients who smoked compared with those who did not. Second, smoking decreases gastric blood flow and may reduce local delivery of drugs to gastric mucosa. Third, smoking induces a variety of CYP isoforms.

The limitation of the present study is that it was a hospital-based study, and patients were selected at a single institution (Çukurova University, Balcalı Hospital). However, the agreement between the observed distribution of CYP2C19 genotype frequencies according to the expected Hardy-Weinberg equilibrium model suggested no selection bias. Second, in the present study, we observed significant differences between the *H. pylori* eradication rate and CYP2C19 genotype frequencies, but the number of subjects in this study was relatively small. Thus, further studies with larger sample sizes are needed to clarify this issue. Third, we did not check for the presence of clarithromycin resistance, due to previous studies reporting that the eradication rate for clarithromycin-resistant strains was significantly lower than for clarithromycin-sensitive strains.

In conclusion, our study confirmed the low eradication rate among the RM patients receiving triple therapy. The incidence of RM among patients infected with *H. pylori* was similar to that of European populations. Whether smoking decreases the cure rate in patients receiving triple therapy remains controversial.

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