Genetic Polymorphisms of CYP2C19 and Resistance to Clopidogrel Therapy among Iranian Patients Suffering from Ischemic Heart Disease

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Abstract

Background: Clopidogrel is a standout amongst the most ordinarily recommended medications to avoid ischemic occasions taking after coronary disorder or stant position. However, impaired responses the therapy as well as resistance to the therapy have also been reported. Genetic variants play an important role in clopidogrel biotransformation of its active metabolite that may subsequently influence the antiplatelet effect of clopidogrel. The objective of this study was to evaluate the prevalence of the cytochrome P450 (CYP450) 2C19 enzyme (CYP2C19) genotypes which are involved in the activation of clopidogrel in a random Iranian population of various ethnic groups (Persian, Azari, Kurd, etc.). Molecular analysis of CYP2C19 polymorphisms may be helpful in the determination of optimal antiplatelet therapy.

Materials and Methods: CYP2C19 (*1/*2/*3) variants were assessed by Polymerase Chain Reaction-Restriction Length Polymorphism (PCR–RFLP) assays in a representative sample of 154 Iranian patients with ischemic heart disease.

Results: The frequencies of CYP2C19 *1 (normal genotype), *2 (heterozygote) and *3 (homozygote) were 112 (72.7%), 36 (23.4%) and 6 (3.9%), respectively.

Conclusion: The United States Food and Drug Administration (FDA) recommendations are more useful to be practiced in our country compared with other countries. Physicians should identify poor metabolizers for consideration of other antiplatelet medications or alternative dosing strategies.

Keywords: Clopidogrel; Ischemic heart disease; CYP2C19 polymorphisms; PCR–RFLP

Introduction

Clopidogrel and its combination with low-dose aspirin are currently recommended as an anti-platelet therapy to prevent ischemic events following coronary syndromes or stent placement (1, 2). However, 3-40% of the patients treated with clopidogrel show an impaired response to this treatment (3). It is reported that clopidogrel resistance has been recognized in patients exhibiting less platelet aggregation inhibition, with the value of dependence on the loading dose of clopidogrel and the

methods used to assess non responsiveness (3, 4). Clopidogrel is a prodrug that its clinical efficacy looks to be a function of the amount of enzymatically derived thiol metabolite (5, 6). Clopidogrel is activated in two steps and CYP2C19 protein plays a dominant role in this chemical way. CYP2C19 protein is encoded by the CYP2C19 gene, which is located in chromosome 10. Variations of this gene may lead to the creation of the loss-of-function alleles, CYP2C19*2 and CYP2C19*3, and subsequently

to the complete absence of the enzyme (8).

Genetic variants of CYP2C19 can occur as eitherheterozygous or homozygous alleles that cause the prevention of clopidogrel biotransformation to its active metabolite form. This seems to be an important indication of clopidogrel antiplatelet effect (9, 10) since it leads to reduction in in antiplatelet effect of clopidogrel.

Regarding the importance of CYP2C19 polymorphisms in clopidogrel therapy and considering its diverse prevalence rate in different geographical regions, the United States Food and Drug administration (FDA) has advised to consider CYP2C19 genotyping before administration of clopidogrel. The frequencies of these two alleles (*2 & *3) have been established in various countries. According to a number of studies, the frequency of CYP2C19*2 allele varies between 0.11 and 0.15 percent (11). Additionally, some other studies showed different frequencies of CYP2C19*3 alleles as 0.004 and 0.006 (12, 13).

In one of the studies done on Indian population, the frequencies of certain CYP2C19 genotypes, namely CYP2C19*1/*1, *1/*2, *2/*2,*1/*17,*17/*17, and *2/*17, were evaluated as 16.1, 31.0, 18.4, 20.7, 1.2, and 12.6%, respectively. In this population, the prevalence of the defective CYP2C19*2 allele as well as the gain-of-function CYP2C19*17 allele, were found to be 40.2% and 17.9 %, respectively (14). In another study performed on 200 healthy Iranian population, the frequency of all CYP2C19*1/*3, CYP2C19*2/*3 and CYP2C19*3/*3 genotypes was 0% (15). Moreover, in a study by Han et al. (2015) genetic polymorphisms showed notable impact on clopidogrel response in Japanese patients (16).

However, there is no data available on the frequencies of CYP2C19 polymorphisms in Iranian population. The aim of this study was therefore to identify the prevalence of the CYP2C19*2 and *3 alleles in Iranian population and to compare it with the studies conducted in other parts of the world. The results will be helpful in determining the right dose of clopidogrel. Patients who are under or intended to get clopidogrel as the antiplatelet therapy may benefit from the results of this study.

In four previous studies carried out on Iranian population CYP2C19 genotyping was detected using PCR-RFLP protocol (17-20).

Materials and Methods

Subjects and study design

A cross sectional study was performed in which the cases were selected from patients attending the Cardiology clinic in Boo-Ali Hospital or private clinics across Iran between July 2010 and 2011. The study participants included 154 ischemic heart disease patients who were under maintenance therapy of 75 mg clopidogrel as a daily dose for at least seven days. Patients were excluded if they had been treated with other antithrombotic drugs, such as heparin and glycoprotein inhibitors. Moreover, patients suffering from bleeding disorders or other coagulopathies were not included in the study. Among the study subjects, 75.3 % of cases were on concomitant dual antiplatelet therapy with aspirin and clopidogrel. According to the opinion of physicians about cases that were treated by clopidogrel, sub-optimal responses could be seen in cases that did not respond to the drug and showed adverse cardiovascular events. In this study, there were some patients who were considered as clinically resistant to clopidogrel. Cases who had used inhibitors and clopidogrel simultaneously, were excluded.

Table 1. Primer sequences, amplification conditions of PCR and Interpretation of digested fragments according to base pair.

	Allele		CYP2C19*2			CYP2C19*3	
Forward and reverse primer sequences	**Forward primer**		CCAGAGCTTGGCATATTGTATC			TCCAATCATTTAGCTTCACC	
	Reverse primer		GTAAACACACAACTAGTCAATG			ACTTCAGGGCTTGGTCAATA	
PCR amplification conditions	**Repeat**		1 cycle	45 cycle			1 cycle
	Time		7 minutes	30 sec	30 sec	20 sec	5 minutes
	Temperature		94	94	56	72	72

	Allele	PCR fragment (bp)	Normal fragment (bp)	Mutant fragment (bp)
Interpretation of digested fragments according to base pair	CYP2C19*2	322	110 + 212	322
	CYP2C19*3	266	170 + 96	260

Genotyping

Blood samples (5mL) were obtained from each patient. EDTA was added to each of these samples as anticoagulant. The cellular fraction was separated by centrifugation and DNA was extracted from the buffy-coat with a Fermentas kit. In next step, CYP2C19*2 and CYP2C19*3 genotyping was performed by PCR-restriction fragment length polymorphism (RFLP) method as described by Adithan et al. (21). Forward and reverse primer sequences for CYP2C19*2 and CYP2C19*3 PCR are presented in Table 1. PCR reactions were carried out in a 25μL reaction volume containing 100ng of DNA, 1μL of each primer, 2.5μL of 10x PCR buffer, 2.5μL MgCl2, 0.5 μL dNTPs 10 mM, and 1 unit of Taq DNA Polymerase.

The amplification conditions were performed according to Table 1. 10μL of PCR products were digested with 10 units of BamHI restriction endonuclease for CYP2C19*3 in 20μL reaction volume at 37 °C, overnight and SmaI restriction endonuclease for CYP2C19*2 in 20μL reaction volume at 30 °C, overnight. The digested PCR products were then separated on 6% polyacrylamide gel. The results were interpreted according to Table 1.

At the end, the PCR products of randomly selected homozygous and heterozygous mutant samples were sequenced for confirmation (14).

Ethics Statement

The study protocol was approved by the ethical committee of Boo-Ali Hospital and a written informed consent was signed by all participants.

Statistical analysis

The computer-based analysis program SPSS (Statistical Package for the Social Sciences, 12.0 for PC, SPSS, Inc., Chicago, Illinois) was used for statistical calculations in this study. Categorical variables were reported as counts (percentages), and continuous variables were reported as mean ± SD. We tested differences between the groups applying Chi-square test for categorical variables (or Fisher exact test when any expected cell count was <5 for a 2 × 2 table) and unpaired Student t-test or one-way analysis of variance for continuous variables. In addition, the Kolmogorov-Smirnov test was used to assess normal distribution. A P-value <0.05 was considered significant.

Results

Baseline characteristics of the study population

A total of 154 patients were enrolled in this study including 85 (55.2%) males and 69 (44.8%) females. The average age was 59.4±12.9 (SD) ranging from 18-81 years old. Furthermore, 116 (75.3%) cases were on aspirin therapy. In 2.6% of these cases the aspirin dose was higher than 100mg.

CYP2C19 status of the study population

The frequencies of wild type (*1/*1), mutated heterozygous (*2/*3, *1/*2, *1/*3) and mutated homozygous (*2/*2, *3/*3) were evaluated as 72.7% (112 cases), 23.4% (36 cases) and 3.9% (6 cases), respectively (Figure 2). Clinical resistance to clopidogrel was observed in 20 patients (83.3%) with wild genotype and four patients (16.7%) with mutated heterozygous cases (Figure 1).

Figure 1. Polyacrylamide gel electrophoresis after polymerase chain reaction–restriction (CYP2C19*2). Line1: Normal fragment (110, 212), Line 2: Mutant fragment (322, homozygous), Line 3: Mutant fragment (110, 212, 322 heterozygous).

According to the results, 20 of 24 patients (83.3%) who used clopidogrel had normal genotype and four (16.7%) were heterozygous for CYP2C19*2.
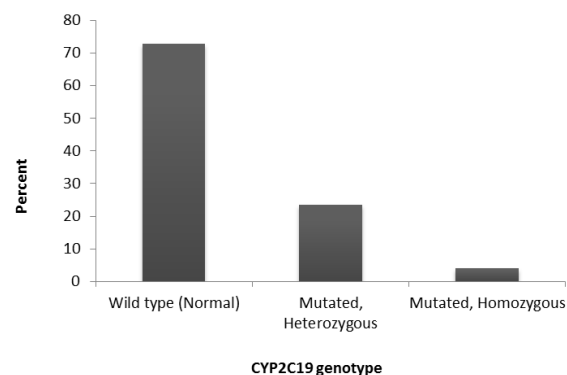

Figure 2. Relative frequency distribution of gene alterations in the study patients.

Among those who had wild genotype, 78.9% were male and 21.1% were female. Moreover 100% of women and 78.9% of men had normal genotype and were not resistant to clopidogrel while 21.1% of men who were heterozygous were resistant to clopidogrel. Moreover, 100% of heterozygous cases were male (Figure 3). Furthermore, the Chi-square test results indicated no significant association between gender and resistance to clopidogrel.
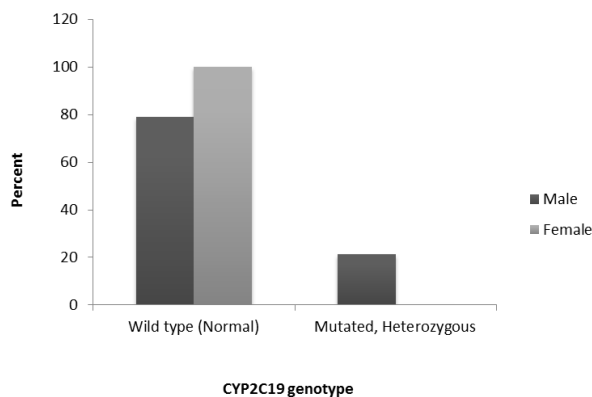

Figure 3. Frequency of clopidogrel resistance in male and female.

Discussion

The impact of CYP2C19 genetic variations on clopidogrel antiplatelet response has been documented in a number of studies (22, 23). The importance of genotyping CYP2C19 *2 and *3 has been highlighted by FDA. According to FDA recommendation antiplatelet therapy with clopidogrel must be done carefully in populations with a high prevalence of risk alleles (24).

Reduced CYP2C19 metabolism in intermediate and poor metabolizers decreases the maximum concentration of active metabolite by 30 to 50% resulting in lower platelet inhibition (25).

In March 2010, FDA announced that poor metabolizers of clopidogrel may not be fully protected from heart attack, stroke, and cardiovascular death. Molecular diagnostic tests can be used for determining the CYP2C19 genetic profile and identifying patients who are unable to convert passive clopidogrel to its actives forms (24).

In a recent meta-analysis and systematic review report, Snoep et al. concluded that among patients undergoing percutaneous intervention, one in every five people are likely to show non-responsiveness to clopidogrel therapy; these individuals are at an eightfold increased risk of developing an adverse clinical outcome (4).

In order to provide physicians with an estimation of the potential number of poor metabolizers among Iranian patients, we set out to study the prevalence of the CYP2C19 *2 and *3 risk alleles in Iranian population.

In the present study, nearly 75.3% of the patients were on dual antiplatelet therapy with clopidogrel and aspirin. The results showed the prevalence of CYP2C19 *2 and*3 alleles were 23.4% and 3.9%, respectively. The prevalence of these alleles in Caucasian population ranged from 0.11– 0.16 and 0.0–0.7, respectively (26). These frequencies were 0.11% and 0.002%, respectively in Egyptian population and 0.15% and 0.01%, respectively in Israeli Jewish population (11). In addition, the prevalence of CYP2C19 *2 and *3 in Lebanese population were 0.13% and 0.03 %, respectively (27). The results of the present demonstrate that the application of the FDA recommendations may be useful in Iran more than other countries. Physicians must identify patients who are at high risk for atherothrombotic events when undergoing clopidogrel therapy and consider other antiplatelet medications or alternative dosing strategies in poor metabolizers (27).

There were a number of limitations to this study. Firstly, the study was performed as a nonrandomized, observational and single center study. Secondly, the plasma levels of the clopidogrel active metabolite could not be measured and also the genetic association with the pharmacokinetic aspects of this medication could not be evaluated. Thirdly, the study had no aim to evaluate the association between clinical outcomes and genetic polymorphisms of CYP2C19. Further studies with these aims are required to fill the gaps in knowledge.

Conflict of Interest

The authors declare that they have no conflict of interest in this work.

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