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Allele and Genotype Frequencies of *CYP2C19* in Patients with Drug-Eluting Stents Following Percutaneous Coronary Intervention in Southwest of Iran

Habib Haybar [1, *], Mohammad Mehdi Ghadiri [1, 2], Pegah Ghandil [3], Ahmad Rouhizadeh [3], Ahmad Reza Asareh [1] and Ata A. Ghadiri [3, 4, **]

[1] Atherosclerosis Research Center, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran
[2] Department of Cardiology, School of Medicine, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran
[3] Cellular and Molecular Research Center, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran
[4] Department of Immunology, School of Medicine, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran

[*] *Corresponding author*: Atherosclerosis Research Center, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran. Email: takamoolsk@yahoo.com
[**] *Corresponding author*: Department of Immunology, School of Medicine, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran. Email: ataaghadiri@gmail.com

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Abstract

Background: Clopidogrel is a platelet inhibitor drug widely used in patients undergoing percutaneous coronary intervention (PCI) for the prevention of stent thrombosis. Genetic variation within *CYP2C19* gene causes variable clopidogrel response. The FDA has recommended *CYP2C19* genotyping in the patients taking clopidogrel, especially in the population with high prevalence rates of *CYP2C19* *2 and *3 alleles.
Objectives: The aim of this study was to determine the prevalence of *CYP2C19* gene polymorphisms in the population received Drug-Eluting Stents following PCI in the southwest of Iran.
Methods: This cross-sectional study was conducted on 102 patients undergoing PCI. Demographic characteristics and risk factors of patients were collected using a questionnaire and *CYP2C19* genotyping was carried out by PCR-RFLP. Then *CYP2C19* allele and genotype frequencies were determined and analyzed using χ^2 test.
Results: Data analysis showed that the frequencies of *CYP2C19**1, *CYP2C19**2, and *CYP2C19**3 allele were 79.4%, 15.2%, and 5.4%, respectively. The frequency of *CYP2C19**1/*1 genotype was 60.8%. Moreover, *CYP2C19**1/*2, *CYP2C19**1/*3, *CYP2C19**2/*3 heterozygote genotypes were shown in 28.4%, 8.8%, and 2.0% of the subjects, respectively. None of the patients had *CYP2C19**2/*2 or *CYP2C19**3/*3 genotypes.
Conclusions: The results of this study showed a high prevalence of *CYP2C19**2 polymorphism in the population lived in the southwest of Iran. The frequency of *CYP2C19**1/*2 genotype is compatible with the majority of the Iranian population and more similar to Caucasian populations.

Keywords: Clopidogrel, Percutaneous Coronary Intervention, *CYP2C19* Polymorphism

1. Background

Myocardial infarction (MI) is an event that occurs in several conditions as a result of several reasons like elevating inflammatory cytokines (1), and percutaneous coronary intervention (PCI) has been suggested to be a good treatment in reducing both short- and long-term death, non-fatal reinfarction, and stroke (2). On the other hand, Clopidogrel is one of the most common antiplatelet drugs used for the prevention of ischemic vascular atherosclerotic disease, acute coronary syndrome (ACS), as well as prevention of Stent thrombosis (3-5). Despite the benefits of using the clopidogrel, some patients experience recurrent ischemic events (6). Platelet reactivity assays showed inter-individual variability in the biological response to clopidogrel (7). Genetics, diabetes mellitus, obesity, smoking, and many drugs are the most important factors which may paly roles in different responses of patients to clopidogrel (8-10). Clopidogrel is a prodrug that is used orally and rapidly absorbed through the stomach. Approximately 85% of the absorbed clopidogrel is hydrolyzed and inactivated by plasma esterases and the reaming is converted to an active metabolite in a two sequential oxidative reaction hepatic cytochrome P450 cytochrome (CYP450) isoenzymes including CYP3A4, CYP3A5, and CYP2C19 (11). Since the enzymatic activity of CYP2C19 depends on the *CYP2C19* genotypes, genetic variation within *CYP2C19* gene causes variable clopidogrel response. There are more than 25

known variant alleles for encoding *CYP2C19* gene (12). The CYPC19*1 allele is the wild type form of the CYP2C19 that encodes the normal functional enzyme, while *CYP2C19**2 is the most reported loss-of-function allele in different populations with an allele frequency of 13% - 15% in Caucasians, 18% in African-American and 29-30% in Asian ethnicities (12, 13). Furthermore, *CYP2C19**3 is another major loss-of-function allele which influences the pharmacokinetic response to clopidogrel (11). Some studies showed a higher rate of major cardiovascular events after treatment with clopidogrel in the patients carrying these loss-of-function alleles (5, 14, 15). The importance of *CYP2C19* genotyping has been established, especially in populations with a high prevalence of *2 and *3 alleles by the United States Food and Drug Administration (FDA) (16, 17). Thus to determining the allele and genotype frequencies of *CYP2C19* gene in patients undergoing PCI, genotyping is recommended for application in the prognosis of the response to the treatment and prevention of the complications of stent restenosis (18-21).

2. Objectives

In this study, we determined the frequencies of *CYP2C19* polymorphisms in patients who received Drug-Eluting Stents (DES) following PCI who lived in the southwest of Iran.

3. Methods

3.1. Patient Selection

This cross-sectional study was conducted by 102 patients referred to PCI from the department of Cardiology of Imam Khomeini and Golestan University Hospitals in Ahvaz, from Khuzestan province of Iran. Then PCI was performed for all patients with drug-eluting stents. All the patients received aspirin 80 - 325 mg daily for one week before PCI and did not receive thienopyridine derivatives in one week prior to the enrollment.

3.2. Collecting Data

Standardized questionnaires were used to collect data about demographic characteristics, laboratory data, clinical and procedural information of the patients included age, sex, ethnicity, exposure to tobacco smoke, diabetes mellitus, hypertension (systolic blood presser $\geq$ 140 mmHg or diastolic blood pressure $\geq$ 90 mmHg), dyslipidemia (LDL-C $\geq$ 100 mg/dL), body mass index (BMI), white blood cell (WBC) count, platelet (PLT) count, diameter and length of stent, and LVEF < 45%.

3.3. Ethics Committee

All data and sample collection for this study were approved by the Ethics Committee of Ahvaz Jundishapur University of Medical Sciences (REC.ajums.ac.ir 1394.469). Informed consent was taken from each participant and all of them explicitly provided permission for collection of clinical data and genotyping analyses.

3.4. DNA Extract

Genomic DNA was extracted from EDTA-containing whole blood samples using phenol-chloroform method.

3.5. CYP2C19 Genotyping

Here, *CYP2C19* genotyping was carried out by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method. Two specific PCR reactions for CYP2C9*2 and CYP2C9*3 variant alleles were conducted in parallel for each specimen in a final volume of 10 μl using primers and conditions that previously described by Zendehdel et al. (22). Subsequently, CYP2C9*2 PCR product was digested with SmaI and CYP2C9*3 PCR product with BamHI overnight at 37°C. The digested products were visualized on a 2% agarose gel stained with ethidium bromide.

3.6. Statistical Analysis

Continuous variables are presented as mean $\pm$ SD. Categorical variables are reported as counts (percentage). The gene counting method was used to estimate alleles and genotypes frequencies. *CYP2C19* allele and genotype frequencies were analyzed using χ^2 test. Differences in allele and genotype frequencies between patients of the present study were measured by Fisher exact test. Data analysis was performed by SPSS (version 22.0) software (SPSS, Inc., Chicago IL, USA). A P-value < 0.05 was considered statistically significant.

4. Results

Demographic, clinical, laboratory, and procedural information of the patients are shown in Table 1. Overall, 102 patients (mean age = 65.2 $\pm$ 10.8 years) participated in this study. Genotype and allele frequencies of the *CYP2C19* polymorphisms are shown in Table 2. The allele frequency of *CYP2C19**1, *CYP2C19**2, and *CYP2C19**3 were 79.4%, 15.2%, and 5.4%, respectively. Also, *CYP2C19**1/*1 genotype was recorded in 60.8% of the patients. In addition, 28.4%, 8.8%, and 2.0% of the subjects showed *CYP2C19**1/*2, *CYP2C19**1/*3, *CYP2C19**2/*3 heterozygote genotypes, respectively. The *CYP2C19**2/*2 and *CYP2C19**3/*3 genotypes were observed in none of the patients.

Table 1. Demographic, Clinical, Laboratory, and Procedural Information of the Patients (N = 102)[a]

Demographic Characteristics	Values
Age, y	65.2 ± 10.8
Sex	
Male	58 (56.9)
Female	44 (43.1)
Race	
Persian	69 (67.6)
Arabian	33 (32.4)
BMI, kg/m^2	25.5±3.3
Clinical information	
Smoking	29 (28.4)
HTN (BP ≥ 140/90 mmHg)	27 (26.5)
Diabetes mellitus	47 (46.1)
Hyper lipidemia (LDL-C ≥ 100 mg/dL)	35 (34.3)
Laboratory data	
WBC (1000/mL)	6.9 ± 2.2
PLT (1000/mL)	308 ± 90
Hb, g/dL	12.4 ± 1.9
Procedural information	
LVEF < 45%	8 (7.8)
Length of drug-eluting stent, mm	20.4 ± 6.1
Diameter of drug-eluting stent, mm	3.5 ± 1.3

Abbreviayions: BMI, body mass index; Hb, hemoglobin; HTN, hypertension; LVEF, left ventricular ejection fraction; PLT, platelets; WBC, white blood cells
[a]Values are expressed as mean ± SD or No. (%).

Table 2. Genotype and Allele Frequencies of *CYP2C19* in the Present Study (N = 102)

Genotype	N	Frequency (%)	95% CI
***1/*1**	62	60.8	50.6 - 69.0
***1/*2**	29	28.4	18.6 - 39.6
***1/*3**	9	8.8	3.9 - 15.7
***2/*3**	2	2	0.0 - 5.3
***2/*2**	0	-	-
***3/*3**	0	-	-
***1 allele**	162/204	79.4	70.6 - 86.9
***2 allele**	31/204	15.2	20.2 - 41.2
***3 allele**	11/204	5.4	5.9 - 17.6

Abbreviation: CI, confidence interval for variant allele frequency

5. Discussion

In this study, the frequencies of *CYP2C19* polymorphisms were evaluated in Iranian (southwest) patients who underwent PCI and received DES. The results of this study show that the overall relative frequency of *CYP2C19**2 alleles is high.

Saber et al. (23) investigated *CYP2C19* allele and genotype frequencies on 691 individuals in a multiethnic Iranian population using experimental and computational approaches. The mean frequencies of *CYP2C19**2 and *CYP2C19**3 alleles were calculated as 0.125 [99.9% CI, 0.112 - 0.139] and 0.006 [99.9% CI, 0.004 - 0.009], respectively by a cumulative meta-analysis performed in the study.

Comparing the studies in different Iranian populations with other populations showed that *CYP2C19* allele frequencies in Iranian population are different from Asian ethnicities and are in compliance with African and Caucasian ethnicities (23).

Although based on the results of studies in different populations *CYP2C19* genotyping is recommended to use a proper dosage of clopidogrel, some new studies demonstrated contradictions in this regard (17, 18, 20). A recent study by Mahdieh et al. (17) determined *CYP2C19*, *CYP3A5*, *CYP3A4*, and *ABCB1* polymorphisms in 388 Iranian patients undergoing PCI treated with clopidogrel during a 6-month period of follow-up. The frequency of *CYP2C19**2 was 16.5%. None of the SNPs individually were significantly associated with outcome events. Results of their study showed that combinations of different alleles of genes are involved in pharmacokinetic variability and joint factors are important; so they concluded that genotyping and analyzing an individual variant may not be as straightforward in risk assessment and pharmacogenetics.

Nozari et al. (21) in a case-match study assessed the role of *CYP2C19**2 polymorphism in the occurrence of in-stent restenosis during a 1-year follow-up period in Iranian patients who underwent PCI. The results of this study showed no significant association with in-stent restenosis one year after PCI in patients with *CYP2C19**1*2 genotype. In another study, Namazi et al. (24) evaluated the impact of P2Y12, CYP3A5, CYP2C19, and environmental factors on the clopidogrel response variability in 112 Iranian patients after PCI. No significant associations between clopidogrel responsiveness and polymorphisms of *CYP2C19*, *CYP3A5*, and *P2Y12*, as well as environmental factors, were shown ($P > 0.05$).

5.1. Conclusions

The results of this study showed a high prevalence of *CYP2C19**2 polymorphism in the population of Khuzestan province located in the southwest of Iran. The frequency of *CYP2C19**1/*2 genotype is compatible with the majority of the Iranian population and more similar to Caucasian populations. Since there is controversy in clopidogrel treatment strategy for patients who carry at least one of the non-functional *CYP2C19* alleles, further studies with

larger sample numbers should be performed to determine the prevalence of non-functional alleles in various populations and to attain an agreement about efficient treatment strategy.

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Footnotes

Authors' Contribution: All authors contributed equally to this work.

Conflict of Interests: The authors declare no conflict of interest.

Ethical Approval: All procedures and the participants involved in this study were in accordance with the ethical standards of the Institutional and/or National Research Committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

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