

Cytochrome P-450 CYP2C19 genetic polymorphism and its relation with clopidogrel resistance

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Abstract

Objective: To find out the prevalence of CYP2C19*2 genetic polymorphism in ischaemic heart disease patients, and to determine its relation with clopidogrel resistance in different genotype groups.

Method: The cross-sectional study was conducted from August 2015 to December 2019 at the Army Medical College, National University of Medical Sciences, Rawalpindi, Pakistan, and comprised ischaemic heart disease patients of either gender who were on clopidogrel therapy. CYP2C19*2 genotyping of all the patients was carried out through polymerase chain reaction-restriction fragment length polymorphism. Platelet aggregation analysis was done using a light transmission aggregometer. Data was analysed using SPSS 23.

Results: Of the 390 patients, 232(59.5%) were males and 158(40.5%) were females. The overall age range was 16-82 years. Clinical indications of clopidogrel were angina 198(50.8%), myocardial infarction 146(37.4%) and acute coronary syndrome 46(11.8%). CYP2C19*2 genotyping showed that 196(50.24%) patients were homozygous wild type carriers (GG or *1/*1), 159(40.8%) were heterozygous carriers (GA or *1/*2), and 35(9%) were homozygous polymorphic allele carriers (AA or *2/*2). Platelet aggregation studies showed that there were 157(80.1%) clopidogrel responders and 39(19.9%) clopidogrel-resistant patients among GG carriers, 118(74.2%) clopidogrel responders and 41(25.8%) clopidogrel-resistant among GA carriers, and 18(51.4%) clopidogrel responders and 17(48.6%) clopidogrel-resistant among AA carriers ($p=0.001$). Intergroup mean platelet aggregation was significantly different ($p=0.025$). Allelic frequency of dominant allele *1 and polymorphic variant allele *2 was 0.706(70.6%) and 0.294(29.4), respectively.

Conclusion: Homozygous and heterozygous carriers of CYP2C19 allele *2 was found to have higher prevalence of clopidogrel resistance in the studied population.

Key Words: CYP2C19, Genetic polymorphism, Clopidogrel. (JPMA 73: 2388; 2023) DOI: 10.47391/JPMA.8025

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Introduction

Clopidogrel is commonly used along with aspirin as dual antiplatelet therapy for the management of different ischaemic conditions, including angina pectoris, myocardial infarction (MI), acute coronary syndrome (ACS), ischemic stroke and peripheral vascular diseases. It is also prescribed before and after percutaneous coronary intervention (PCI) and coronary artery bypass grafting (CABG). Clopidogrel targets adenosine diphosphate (ADP) receptors which are present on the surface of the platelets. ADP receptors are of two types, P2Y1 (Purinergic Receptor P2Y1) and P2Y12 (Purinergic Receptor P2Y12). In normal circumstances, the activation of P2Y1 and P2Y12 receptors by ADP causes platelet activation and

aggregation. Clopidogrel irreversibly inactivates ADP P2Y12 receptors, augmenting synthesis of cyclic adenosine monophosphate (cAMP), which decreases platelet activation and aggregation. Inactivation of either of P2Y1 or P2Y12 receptors is enough for platelet inhibition.¹

Clopidogrel is a pro-drug and it is activated inside human body by a two-step oxidation process first into inactive thiolactone metabolite, and then into active metabolites, by the action of variety of cytochrome P450 (CYP) enzymes functioning in the liver. These cytochrome enzymes include CYP2C19, CYP3A4/A5, CYP2B6, CYP1A2 and CYP2C9, of which CYP2C19 is the primary enzyme responsible for the activation of major chunk of clopidogrel in both the oxidation steps.^{2,3}

Worldwide, genetic polymorphisms in CYP2C19 gene have been found to be related to clopidogrel resistance in different populations. Altered CYP2C19 enzyme cannot activate clopidogrel efficiently. Different single nucleotide polymorphisms (SNPs) of CYP2C19 have been discovered ranging from wild type*1 to polymorphic *2,

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*3, *4, *5, *6, *7, *8 and *17, of which CYP2C19 allele *2 (rs4244285) has been extensively related to clopidogrel resistance. The presence of alleles *2, *3, *4, *5, *6, *7 and *8 has been associated with poor clopidogrel response, while allele *17 is associated with exaggerated clopidogrel action due to enhanced metabolism (ultra-rapid metabolisers). On the basis of CYP2C19*2 genetic polymorphism, patients can be classified as normal or normal metabolisers with both wild type alleles (*1/*1), intermediate metabolisers with one polymorphic allele (*1/*2), or poor metabolisers with both polymorphic alleles (*2/*2).^{1,4} Patients with loss of functional CYP2C19 alleles are at increased risk of major adverse cardiac events (MACE) due to stent thrombosis which can occur as early as within few days after stent implantation.⁵

CYP2C19 genetic polymorphism not only affects the biotransformation of clopidogrel, but also of other drugs, like omeprazole, lansoprazole, pantoprazole, citalopram, amitriptyline, voriconazole and cyclophosphamide, as they, too, are dependent upon CYP2C19 enzyme for optimum activation.⁶

Different techniques of platelet aggregation studies have been utilised to assess the functioning of platelets after drug administration and in various haematological disorders. Some of these techniques include light transmission aggregometry, platelet function assay-100, VerifyNow rapid platelet function assay, Thromboelastography and vasodilator-stimulated phospho-protein (VASP) phosphorylation assay.⁷

The current study was planned to find out the prevalence of CYP2C19*2 genetic polymorphism in ischaemic heart disease (IHD) patients, and to determine its relation with clopidogrel resistance in different CYP2C19*2 genotype groups.

Patients and Methods

The cross-sectional study was conducted from August 2015 to December 2019 at the Department of Pharmacology, Army Medical College, National University of Medical Sciences, Rawalpindi, Pakistan, in collaboration with the Institute of Biomedical and Genetic Engineering, Islamabad, Pakistan, after approval from the institutional ethics review committee.

The sample size was calculated using the World Health Organisation (WHO) calculator. There was no relevant study regarding prevalence of CYP2C19*2 SNP in Pakistani population, so anticipated frequency of CYP2C19*2 SNP was kept at 50% with 95% confidence level and 5% margin of error. The sample was raised from among patients in the outpatient department (OPD) and

wards. Those included were Pakistani IHD patients of either gender suffering from either angina pectoris, MI or ACS. All the patients were taking antiplatelet drug clopidogrel 75mg/day orally for at least 7 days.⁸ Those excluded were patients who were pregnant, or had hepatic and renal dysfunction, malignancy, known allergy to clopidogrel, or had infusion of eptifibatid or tirofiban within the last 48 hours, and patients taking omeprazole.

After written informed consent from all the subjects, random blood sampling was taken using 10ml disposable syringe; 4.5ml blood was stored in plastic tube containing 0.5ml sodium citrate (9:1) for platelet aggregation studies, and the remaining blood was stored in acid citrate dextrose (ACD) vacutainer for deoxyribonucleic acid (DNA) extraction.

DNA was extracted from whole blood using salting out method, and specific segments on the DNA enclosing gene were amplified through polymerase chain reaction (PCR), using forward and reverse primers.⁹ The sequences of forward and reverse primers used were 5'-CAG AGC TTG GCA TAT TGT ATC-3' and 5'-GTA AAC ACA CAA AAC TAG TCA ATG-3', respectively. The obtained amplicons were digested by restriction enzyme SmaI (*Serratia marcescens* 1), at 37°C into small fragments, depending upon the length of nucleotides base pairs (bps), which were later visualised through agarose gel electrophoresis using ultraviolet light and 100bp DNA ladder.¹⁰ The digested fragments of DNA were categorised according to the length of bps into wild type (GG) with two fragments of lengths 212bp and 109bp, variant heterozygotes (GA) with three fragments of lengths 321bp, 212bp, 109 b.p., and variant homozygotes (AA) with one fragment of 321bp length.

Platelet aggregation studies of all the patients were performed using light transmission aggregometer (LTA, Chrono-Log 490 Model, Chrono-Log Corporation, Havertown, Pennsylvania, USA) within 3 hours of sampling. Platelet-rich plasma (PRP) and platelet-poor plasma (PPP) were separated from sodium (Na) citrate blood samples through centrifugation. Light was allowed to pass through PRP and PPP in the LTA. ADP was used as an agonist to stimulate platelets in the PRP. With the help of a software (AGGRO/LINK Opti8, Chrono-Log Corporation, Havertown, Pennsylvania, USA), platelet aggregation was expressed in terms of percentage of light transmission across PRP, and the results were compared with baseline readings and the light transmitted across PPP, which was used as a reference.¹¹ Patients with <50% platelet aggregation were categorised as clopidogrel responders, and patients with

≥50% platelet aggregation were categorised as clopidogrel-resistant.¹¹

Data was analysed using SPSS 23. Data was expressed as frequencies and percentages, and as mean and standard deviation, as appropriate. Clopidogrel response among different CYP2C19 genotype categories and deviation of genotype frequencies from Hardy Weinberg equilibrium was tested using chi-square test. One-way analysis of variance (ANOVA) was used to find difference in mean platelet aggregation of patients among different CYP2C19*2 genotype groups, and post-hoc analysis was carried out to observe difference in mean platelet aggregation between the groups. $P < 0.05$ was considered significant.

Results

Of the 390 patients, 232(59.5%) were males and 158(40.5%) were females. The overall age range was 16-82 years. Clinical indications of clopidogrel were angina 198(50.8%), myocardial infarction 146(37.4%) and acute coronary syndrome 46(11.8%). Ethnicities of the patients

were also noted (Table). There were 196(50.2%) normal metabolisers GG or *1/*1 cases, 159(40.8%) intermediate metabolisers GA or *1/*1, and 35(9%) poor metabolisers AA or *2/*2.

Among different CYP2C19*2 genotype groups, there was no significant difference in family history of IHD, history of smoking, prevalence of hypertension, cardiac failure, invasive procedures and surgeries, clinical indications of clopidogrel, and bleeding events ($p > 0.05$).

Allelic frequency of dominant allele (G) was 0.706(70.6%), and of recessive allele (A) was 0.294(29.4%). There was no significant difference found between anticipated and expected gene frequencies ($p = 0.114$).

There were 157(80.1%) clopidogrel responders and 39(19.9%) clopidogrel-resistant patients among GG carriers, 118(74.2%) clopidogrel responders and 41(25.8%) clopidogrel-resistant among GA carriers, and 18(51.4%) clopidogrel responders and 17(48.6%) clopidogrel-resistant among AA carriers ($p = 0.001$) (Figure 1).

Table: Demographic profile and haematological indices of the patients in different CYP2C19*2 genotype groups.

	Ethnicity/ Category	/ Units	GG N = 196 n (%)	GA N = 159 n (%)	AA N = 35 n (%)	p value
Demographic Profile						
Ethnic Groups	Punjabi		105 (51.2)	79 (38.5)	21 (10.2)	0.907
	Pashtun		35 (49.3)	29 (40.8)	7 (9.9)	
	Sindhi		16 (53.3)	14 (46.7)	0 (0.0)	
	Kashmiri		28 (49.1)	25 (43.9)	4 (7.0)	
	Others		12 (44.4)	12 (44.4)	3 (11.1)	
Gender	Male		113 (57.6)	96 (60.3)	23 (65.7)	0.641
	Female		83 (42.3)	63 (39.6)	12 (34.2)	
Haematological Indices						
Haematocrit	%		39.9 ± 4.3	39.2 ± 4.2	39.6 ± 4.4	0.682
No of Platelets	K/uL		180.3 ± 52.8	178.1 ± 62.6	179.0 ± 54.0	0.956
Mean Platelet Volume	fL		8.5 ± 1.6	8.5 ± 1.4	8.4 ± 2.0	0.912
Platelet Distribution Width	%		10.7 ± 2.4	10.9 ± 2.6	10.5 ± 2.7	0.755
Platelet Large Cell Ratio	fL		18.0 ± 11.4	17.5 ± 8.9	17.4 ± 8.5	0.879
White Blood Cells	109/L		7.6 ± 1.9	7.7 ± 2.1	7.6 ± 1.6	0.878
Red Blood Cells	1012/L		4.6 ± 0.5	4.7 ± 0.6	4.6 ± 0.6	0.725
Haemoglobin	g/dl		13.4 ± 1.5	13.1 ± 1.5	13.2 ± 1.4	0.255

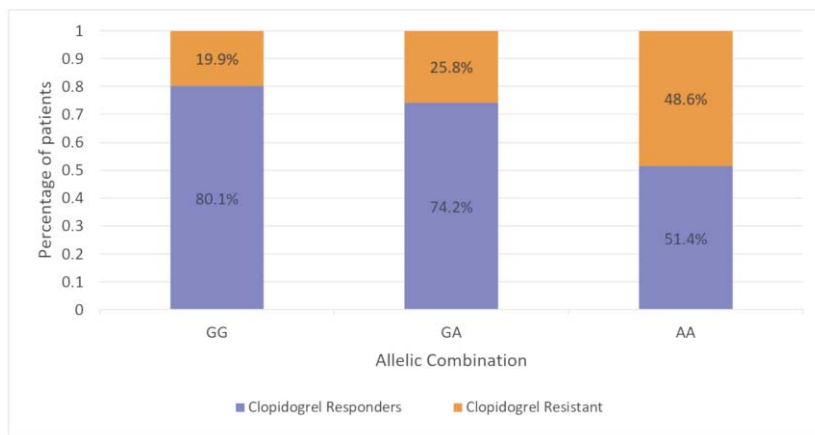


Figure-1: Clopidogrel responders and clopidogrel-resistant patients in different CYP2C19*2 genotype groups.



Figure-2: Mean platelet aggregation of different CYP2C19*2 allele categories.

There was significant difference in mean platelet aggregation of patients among different CYP2C19*2 genotype groups ($p=0.025$) (Figure 2). There was significant difference between mean platelet aggregation of normal and intermediate metabolisers ($p=0.049$), and normal and poor metabolizers ($p=0.020$), while there was no significant difference between intermediate and poor metabolisers ($p=0.241$).

Discussion

Genetic polymorphisms in hepatic cytochrome P450 enzyme can alter the activity of the cytochrome enzymes, mainly CYP2C19, involved in the biotransformation of pro-drug clopidogrel into active metabolite. This CYP2C19*2 genetic polymorphism study has demonstrated that almost 50% IHD patients, 40.8% with *1/*2 and 9% with *2/*2 polymorphic allelic combination, were intermediate or poor metabolisers of clopidogrel. Rest of the 50.2% patients having both wild type alleles *1*1 were normal metabolisers.

In a recent study carried out in Pakistan regarding CYP2C19 haplotypes and clopidogrel antiplatelet

response, the frequency of CYP2C19 allele 2 was 0.241 compared to 0.294 in the current study. The other found that out of 120 patients, 68 had *1/*1, 46 had *1/*2, and 6 had *2/*2 allelic combinations. No significant difference was found between frequencies of patients in different CYP2C19 genotype groups between both the sample populations, depicting uniform distribution of CYP2C19 alleles in both the sample populations.¹² However, the study did not find any association between presence of CYP2C19*2 polymorphic allele and clopidogrel resistance. In contrast, the current study showed that the percentage of clopidogrel responders was highest (80.1%) among normal metabolisers, followed by 74.2% in intermediate metabolisers, and 51.4% in poor metabolisers, while clopidogrel resistance was highest among poor metabolisers (48.6%), followed by intermediate (25.8%) and normal metabolisers (19.9%). Significant difference was found in clopidogrel response status among different genotype groups. Ahmed et al. found that H1 (Haplotype 1) and H2 (Haplotype 2) of CYP2C19 were extensively found in Pakistani population, with H1 mostly related to enhanced clopidogrel antiplatelet response.¹²

Vivek et al. found that 36% of south Indian patients were normal metabolisers, 40% were intermediate metabolisers and 24% were poor metabolisers of clopidogrel. In contrast, the current study demonstrated that normal metabolisers were significantly higher and poor metabolisers were significantly lower in Pakistani population compared to Indian population with no significant difference in intermediate metabolisers.¹³

Studies have confirmed that CYP2C19*2 polymorphism is a risk factor for clopidogrel resistance and normal metabolisers enjoy maximum benefits of clopidogrel therapy, while intermediate and poor metabolisers have compromised clopidogrel efficacy which can lead to suboptimal platelet inhibition and subsequent secondary ischaemic events, including stent thrombosis.¹⁴

In the current study, the frequency of CYP2C19 allele *2 associated with poor clopidogrel metabolism status was 29.4%, which is in accordance with another recent study done in Pakistan (29%), higher compared to Jordanian

(10%), European (15%), African (15%), Qatari (18%), Iranian (21%) and white (24%) populations, and lower compared to Chinese (30%) and Indian (35-38%) populations.¹⁵⁻¹⁷

CYP2C19*2 polymorphism (single nucleotide base change at position 681 G>A) occurs in exon 5 at the long arm of chromosome 10 (10q24.1-q24.3), which creates an aberrant splicing defect, resulting in the formation of truncated non-functional protein affecting conversion of clopidogrel into active metabolites.¹⁸ As a result, clopidogrel cannot inhibit platelets uniformly in all patients and can lead to suboptimal platelet inhibition in susceptible individuals.¹⁷

In the current study, significant difference was found between mean platelet aggregation of different genotype groups as platelet aggregation was highest among *2/*2 carriers, followed by *1/*2 carriers, and *1/*1 carriers ($p=0.025$). An earlier study also reported that clopidogrel intermediate and poor metabolisers had higher platelet aggregation compared to extensive metabolisers, and the presence of one or two CYP2C19*2 alleles is a risk factor for clopidogrel resistance.¹⁴

The current study has its limitations. There are many other SNPs of CYP2C19 related to clopidogrel resistance in different populations across the world, but due to financial constraints, the current study focussed on only one SNP. Many advanced methods of platelet aggregation studies are now available, but the study used light transmission aggregometer because it was the only method available.

Conclusion

The CYP2C19*2 SNP was present extensively in IHD patients, and the presence of CYP2C19*2 polymorphic allele was significantly related to clopidogrel resistance in such patients.

Disclaimer: The text is based on a PhD thesis.

Conflict of Interest: None.

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