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Research Article

Polymorphism in the P2Y12 Gene & Antiplatelet Response to Clopidogrel in the Pakistani Population

Manzoor Ahmed[1*], Muhammad Hanif Bangash[2], Mehwish Rafique[3]

[1]Queen Mary University London, United Kingdom
[2]Pakistan Institute of Nuclear Science and Technology Islamabad, Pakistan
[3]Shifa International Hospital Islamabad, Pakistan
Correspondence: a.manzoor@qmul.ac.uk

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Abstract

Clopidogrel, an antiplatelet drug, is frequently prescribed to patients with cardiovascular disease and those suffering from a stroke. Despite the drug being effective in most patients, some do not respond to the therapy and experience ischemic events early in the treatment, which might be due to poor platelet inhibition. The current study investigates the association between polymorphism in the P2Y12 adenosine diphosphate receptor (ADP) gene and platelet aggregation in response to clopidogrel in a Pakistani cohort. The study comprised 91 patients diagnosed with cardiovascular diseases and treated with clopidogrel. Antiplatelet response to clopidogrel was monitored by an aggregometer. The genotyping of the T744C genetic polymorphism of the P2Y12 receptor gene was carried out using the restriction fragment length polymorphism polymerase chain reaction method. Our results show that 53% of patients were homozygous for the P2Y12 wild-type genotype, 38% possessed one copy of the T allele, and 9% possessed both T alleles. Mean values of inhibition of platelet aggregation were different in all three groups. However, the differences in the means did not reach statistically significant levels. Therefore, we concluded that our study does not show an association between the T744C polymorphism of the P2Y12 ADP receptor gene and platelet inhibition by clopidogrel.

Keywords: Clopidogrel, platelet aggregation, antiplatelet, genetic polymorphism, adenosine diphosphate

Introduction

Clopidogrel, an antiplatelet drug targeting the thienopyridine P2Y12 receptor, is frequently employed in patients diagnosed with ischemic heart disease, ischemic stroke, and acute coronary syndrome (ACS)(Amsterdam et al. 2014, Kernan et al. 2014, Members et al. 2012). Clopidogrel functions as an irreversible antagonist of the receptor and is effective in the majority of patients. However, a significant patient population with ACS who are concomitantly treated with aspirin and clopidogrel faces the recurrence of an acute ischemic event early in the treatment (Parodi et al. 2011). This 'resistance' to the therapeutic effect of clopidogrel might be due to the poor platelet inhibition by the drug, which is likely to result in a poor therapeutic response to the antiplatelet therapy (Papathanasiou, Goudevenos, and Tselepis 2007).

Several studies investigating the clopidogrel 'resistance' report a prevalence of 15.9% to 49.5%, indicating a substantial population-based variation(Mallouk et al. 2012). This high variability among various populations suggests that environmental and genetic differences may play important roles in determining clopidogrel resistance. Genetic variation in the cytochrome

P4502*C19* gene has previously been associated with high adenosine diphosphate (ADP)-induced platelet aggregation in clopidogrel-treated patients (Wei et al. 2015, Jia et al. 2013). However, it is unlikely that *CYP2C19* genetic variation is the only responsible factor for the observed clopidogrel resistance. Various environmental factors may also lead to clopidogrel resistance, which may or may not depend on Clopidogrel's bioavailability (Berger et al. 2009, Cayla et al. 2016). The choice of blood preservative and the tests used to measure platelet aggregation influence the clopidogrel's antiplatelet response (Labarthe et al. 2005).

Clopidogrel is absorbed in intestinal cells as a prodrug and then transported to hepatocytes, where it is converted to an active metabolite under the action of different isoforms of CYP enzymes in two-step oxidation. CYP2C19 is the principal contributor in both these steps (Sangkuhl, Klein, and Altman 2010). Clopidogrel binds to ADP receptors and inhibits them from producing its pharmacological effect. It has been reported that polymorphisms in the ADP gene may also cause 'clopidogrel resistance.' The present study's principal objective is to determine the prevalence of resistance to clopidogrel therapy in Pakistani patients who are taking clopidogrel for the acute coronary syndrome (ACS) and to investigate its relationship with the polymorphism in the P2Y12 gene, especially with T744C variant.

Materials & Methods

The Institutional Review Board and Ethics Committee of Shifa Tameer-e-Millat University, Islamabad, Pakistan, sanctioned the study. All the methods and procedures were performed in accordance with the relevant guidelines and regulations. All individuals were mandated to provide informed consent to partake in the investigation. The study cohort comprised 120 acute coronary syndrome (ACS) patients taking 75 mg of clopidogrel at the time of inclusion in the study. Five milliliter of venous blood was taken in the EDTA tube and stored at 4^{o}C. Gen Jet Genomics DNA extraction Kit (ThermoScientific) was used to isolate the genomic DNA. A 1% agarose gel electrophoresis was used to quantify the genomic DNA. Genomic DNA was stored at -20^{o}C and was used later for further processing.

Platelet Aggregation

Whole blood aggregometry was used to investigate the antiplatelet activity, where ADP is a platelet aggregation-inducing agonist (Ahmed et al. 2013). Within 30-min to 5-h of placing the blood samples in clean test tubes, comprising of 3.2% sodium citrate anticoagulant (9:1), patient blood samples were tested at a temperature of 37 °C with 1200 rpm stirring speed. Following manufacturer guidelines, an equivalent amount (volume) of normal saline was used to dilute 500 μL of citrated blood. The response (platelet aggregation) was recorded after placing the electrode in the cuvette where platelet aggregation was induced by ADP (10 μM). For up to 6 min, recordings were obtained as a measure of electrical impedance in ohms. Percent mean platelet inhibition, after obtaining values from 3-4 individual experiments, was calculated.

Genotyping

We used polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) to genotype samples for T744C P2Y12 polymorphism, as previously described by Malek et al.(Malek et al. 2008). Genotyping of this variant was performed by amplification from 50 to 100 ng of genomic DNA, followed by digestion using th*e RsaI* restriction enzyme. The digestion gave rise to three profiles: wild TT homozygous (one fragment of 220 pb), TC heterozygote (two fragments of 220 and 196 bp), and mutated CC homozygous (one fragment of 196 bp). The digested product was separated on 3% agarose gel electrophoresis stained with Ethidium Bromide and visualized with UV rays.

Statistical Analysis

Version 26.0 of the IBM Statistical Package for Social Sciences SPSS was used for data-keeping and analysis. Mean + SD was used to represent continuous variables, whereas discrete variables were represented as percentages and frequencies. Fisher's Exact test was used to compare the mean inhibition of platelet aggregation in genotype groups. A p-value Less than 0.05 was regarded as statistically significant.

Results

The demographics of the patient population are given in table 1. The mean age of the patients was 60.176±10.95 years, the mean height was 165.33±6.88 cm, and the mean weight was 68.24±9.45 kg. Males were 59.3%, while females were 40.6% of the investigated participants. Sixty-seven percent of patients had a family history of coronary artery disease; 68% were hypertensive, 58% were diabetic, and 65% were smokers. CC genotype was found in 52.7% of patients, CT in 38.4% of patients, while the TT genotype was found in 8.7% of patients.

Table 1: Basic demographic, clinical, and genetic attributes of the investigated population.

	Mean	SD
Age	60.176	10.95
Hight	165.33	6.88
Weight	68.24	9.45
		%
Male	54	59.3
Female	37	40.6
Family History of CAD	61	67
Hypertensive	62	68
Diabetic	53	58
Smoker	59	65
CC	48	52.7
CT	35	38.4
TT	8	8.7

The mean inhibition of platelet aggregation in patients with the CC genotype was 59.45±16.99%; in the CT genotype, it was 63.20±17.9, while only 56±17.3% inhibition of platelet aggregation was observed in patients with the TT genotype. Although the mean inhibition of platelet aggregation in the CT group was higher than CC and TT groups, there was no statistically significant difference between the means of these groups.

Table 2: Mean values of inhibition of platelet aggregation along with standard deviation in different genotypes.

Genotype	Mean	N	SD
CC	59.45	48	16.99
CT	63.20	35	17.93
TT	56.00	8	17.31

Discussion

Many single nucleotide polymorphisms of the P2Y12 receptor gene were described to be in association with this inter-individual variability in ADP-induced platelet aggregation (Zoheir et al. 2013, Simon 2009, Sirotkina et al. 2009). T744C polymorphism has been associated with enhanced platelet aggregation, suggesting its potential effect on modulating Clopidogrel response (Fontana et al. 2003, Nordeen et al. 2013).

The present study investigated the relationship between clinical and demographical variables together with, more importantly, an important polymorphism within the P2Y12 gene and resistance to clopidogrel therapy in a Pakistani population. Previous studies throughout different regional and global populations exhibited a high variability when it comes to the prevalence of clopidogrel resistance, ranging from as low as approximately 5% to about 45% in German and Spanish populations, respectively (Müller et al. 2003, Angiolillo et al. 2005).

In the present study, we investigated whether the mutant allele T744C P2Y12 polymorphism has an association with the antiplatelet effect in ACS patients on clopidogrel. However, our study shows that there was no statistically significant correlation between the P2Y12 T744C genotypes and mean inhibition of platelet aggregation in patients on clopidogrel. Our results revealed that the CC genotype was the most prevalent among the patients, with a mean inhibition of platelet aggregation at 59.45% (16.99). A higher inhibition of platelet aggregation was observed in the CT genotype group at 63.20% (17.93). However, the TT genotype group displayed slightly less inhibition of platelet aggregation at 56.00% (17.31). But no statistically significant association was found among TT, TC, and CC genotypes. On the other hand, Cavallari et al. (Cavallari et al. 2007) found that nonsmokers carrying the minor haplotype H2 of the ADP receptor gene were highly associated with significant CAD (OR = 1.83, 95% CI = 1.17–2.87, and P = 0.007). However, similar findings were reported by Schettert et al.(Schettert et al. 2006), which did not provide evidence for a strong association between H1/H1 and H1/H2 haplotypes and an increased risk of cardiovascular events in a population with CAD.

Similar findings for CYP2C19 polymorphism and clopidogrel resistance have been reported previously. These findings are in line with previous reports (Hulot et al. 2006), where the correlation between clopidogrel resistance and the status of intermediate metabolizers failed to reach statistical significance. Bhatt et al. proposed that the presence of a loss-of-function allele could be linked to fewer bleeding complications but without a significant increase in the number of ischemic events (Bhatt et al. 2012). However, our findings are slightly different from several previous studies (Rath et al. 2015, Kim et al. 2008), some of which suggest that the *CYP2C19**2 allele is implicated in conferring resistance to clopidogrel therapy in multiple populations.

To the best of our knowledge, our study is the first in Pakistan to assess whether or not Clopidogrel response may be modulated by T744C P2Y12 polymorphism in a sample of Pakistan ACS patients. We also tried to determine the frequency of this polymorphism among Pakistani ACS patients. Further studies, including larger sample sizes and exploring interactions between this polymorphism and others, are still needed and may provide useful information to better understand the mechanism of Clopidogrel inter-individual resistance and also the risk of ACS occurrence in the option to improve the biomedical context.

Conflict of Interest

The authors declare that they have no competing interests.

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Study Approval

Yes. The study was approved by the Institutional Review Board & Ethics Committee of the Shifa Tameer-e-Millat University.

Consent Forms

Consent forms were signed by the patients and are available with the authors.

Authors Contribution

MA conceptualized the study and wrote the final manuscript, MA and MHB collected samples, carried out the experimental work, helped with the analysis and writing the first draft, MR performed the statistical analysis, and MA supervised the whole project.

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