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Prevalence of CYP2C19 and ITGB3 polymorphisms among Bangladeshi patients who underwent percutaneous coronary intervention

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Abstract

Introduction: Antithrombotic agents are the basic therapeutic option for patients with arterial thrombosis who underwent percutaneous coronary intervention (PCI). In Bangladesh, aspirin and clopidogrel are frequently prescribed as antithrombotics or platelet inhibitors. Studies reported the genetic polymorphisms of CYP2C19*2, CYP2C19*17, and ITGB3 cause an alteration of the pharmacodynamic and pharmacokinetic profile of aspirin and clopidogrel. Therefore, we aimed to assess the prevalence of CYP2C19*2, CYP2C19*17, and ITGB3 polymorphisms among Bangladeshi patients with cardiovascular disease (CVD) who underwent PCI.

Methods: Here we assessed a total of 1,000 CVD patients (male 782 and female 218) who underwent PCI and were treated with clopidogrel and/or aspirin. We performed genotyping of patients treated with clopidogrel and aspirin by polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP) and tetra-primer amplification refractory mutation system PCR (T-ARMS-PCR) methods. The PCR products of clopidogrel-treated patients were screened with agarose gel electrophoresis and then digested with SmaI and NsiI-HF for CYP2C19*2 and CYP2C19*17, respectively. We genotyped aspirin-treated patients with T-ARMS-PCR for missense rs5918 (PlA1/A1) polymorphism of the ITGB3 gene. Then we ran the digested PCR products on 2% agarose gel electrophoresis to detect the mentioned polymorphisms.

Results: Among the clopidogrel-treated patients, we observed 64.1% polymorphism (hetero + mutant) of CYP2C19*2 (loss-of-function allele) and 22.7% (hetero + mutant) of CYP2C19*17 (gain-of-function allele). On the other hand, among the aspirin-treated patients, polymorphisms of ITGB3 were 84.1% homozygous (PlA1/A1), 15.6% heterozygous (PlA1/A2), and 0.3% mutant homozygous.

Conclusion: In the present study, we observed a high prevalence of genetic polymorphisms of CYP2C19 and ITGB3 genes. Therefore, we recommend genotyping of CVD patients before prescribing clopidogrel or aspirin to prevent coagulation. Based on the genotyping study, the adjustment of doses or alternative generics might require to avoid therapeutic failure or toxicity in some cases.

Keywords

Aspirin, cardiovascular disease, clopidogrel, CYP2C19, epidemiology, public health, ITGB3, polymorphisms

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Introduction

Patients with the acute coronary syndrome (ACS) who underwent percutaneous coronary intervention (PCI) require adequate platelet inhibition to prevent recurrent ischemic events.[1,2] Oral antiplatelet drugs clopidogrel and aspirin are considered first-line therapy to prevent arterial thrombosis.[3] Physicians recommend these drugs for the management of ACS and to prevent thrombosis after stenting.[4] Also, they prescribe aspirin and clopidogrel combination for high-risk patients with drug-eluting stents.[2,5]

The hepatic cytochrome (CYP) P450 enzyme converts the prodrug clopidogrel into its active metabolite.[4] Several studies reported the altered pharmacokinetic and pharmacodynamic profile of clopidogrel due to CYP2C19 variants.[4–6] Variant allele accounting for the loss of function encompassing CYP2C19*2 to CYP2C19*8.[4] The CYP2C19*2 gene variant is a G681A mutation in exon five that encodes for a hidden link variant whereas CYP2C19*3, a G636A mutation in exon four, results in a premature stop codon.[5] The CYP2C19*17 allelic variant, a C806 T mutation in exon five, is responsible for increased catalytic activity. The active metabolite of clopidogrel shows higher antiplatelet actions due to the CYP2C19*17 variants. Therefore, an increased risk of bleeding is associated with clopidogrel due to CYP2C19*17 variants.[7] The gain-of-function CYP2C19*17T allele always occurs on a haplotype that also shelters the wild-type "CYP2C19*2 G." The impact of the gain-of-function *17 alleles may be due to the absence of the loss-of-function of CYP2C19*2 A allele.[8] Researchers conducted several studies concerning the association between CYP2C19 variants and clopidogrel therapy in patients with the coronary syndrome, myocardial infarction (MI), coronary artery disease, and patients who underwent PCI.[9–12] Patients with homozygous nonfunctional alleles of the CYP2C19*2 genes are called poor metabolizers of clopidogrel. On the other hand, patients with a gain of function of CYP2C19*17 alleles are called ultra-metabolizers of clopidogrel.[13] According to the studies in different ethnic population, 10%–25% poor metabolizers of clopidogrel were observed in Asian populations, whereas for the prevalence were 2%–3% in Caucasians and 4% in Africans, respectively.[4]

Another widely used drug for the prevention of ischemic cardiovascular and cerebrovascular events is aspirin. The long-term use of aspirin reduces the 25% risk of atherothrombotic stroke, MI, and death among high-risk cardiovascular disease (CVD) patients. Besides, it reduces the rate of bypass surgery, pulmonary embolism, arterial thromboembolic events, and deep vein thromboses in CVD patients.[14] Therefore, aspirin resistance and ITGB3 polymorphism are important factors that should consider while treating thrombotic plaques.[15] The polymorphic ITGB3 at position 1565 in exon two of the gene encoding glycoprotein IIIa leads to its diallelic polymorphism (PlA1/A2) that modulates platelet function. The prevalence of PlA2 ranges from 20% to 30% in the white population that results in enhanced thrombin formation and an impaired antithrombotic action of aspirin.[16] Many previous studies reported the genetic association with the hyporesponsiveness of antiplatelet therapy.[17–19]

The combined therapy of aspirin and clopidogrel deter platelets from adhering together to form clots. So, physicians record the incidents of bleeding to ensure the safety of this combination therapy. The need for clopidogrel as a part of the dual antiplatelet scheme represents standard care in clinical practice.[2,3,7] About 40 million patients across the world are being treated with clopidogrel to prevent atherothrombotic events.[8] However, the connection between post-PCI ischemic events and clopidogrel therapy is not well understood. About 33% of patients reported high on-treatment platelet reactivity (HTPR).[2] Regardless of the proven clinical benefits, response to clopidogrel is not optimal.[7] About 10%–15% of patients report periodic atherothrombotic events who regularly take clopidogrel.[6,8]

Therefore, the US Food and Drug Administration imposed a warning sign about the diminished efficacy of standard doses of clopidogrel in individuals with two reduced-functional CYP2C19 alleles. The authority also mentioned the importance of genetic tests of patients for necessary dose adjustment or alternative therapies.[20] Moreover, several studies showed that the higher loading or maintenance doses of clopidogrel in ACS or PCI patients do not reduce the death rate.[21,22] A randomized clinical trial demonstrated better effectiveness of prasugrel or ticagrelor over clopidogrel.[23] However, these drugs might not replace clopidogrel in many patients due to their long-term side effects, patient compliance, and cost.[22,23] On the other hand, the clinical pharmacogenetics implementation consortium (CPIC) guideline recommends an updated therapeutic approach. According to CPIC recommendation, physicians should emphasize the proper indication for CYP2C19 genotype-directed antiplatelet therapy for CVD patients who underwent PCI.[23] Therefore, the present study aimed to find the prevalence of these single-nucleotide polymorphisms (SNPs) among Bangladeshi CVD patients who underwent PCI.

Methods

Study population

In the present study, we assumed the confidence and error levels as 95% and 5%, respectively. According to this assumption, we require a minimum of 385 participants to achieve 80% statistical power.[24] This observational study recruited a total of 1,000 clopidogrel and/or aspirin-treated CVD patients (male 782 and female 218) who underwent PCI from the cardiology department of United Hospital, Dhaka, Bangladesh. We conducted this study between March 10, 2017, and November 30, 2018. The average age and body mass index (BMI) of patients were 59.26 ± 12.74 years and 28.53 ± 4.15 kg/m^2, respectively. We conducted this

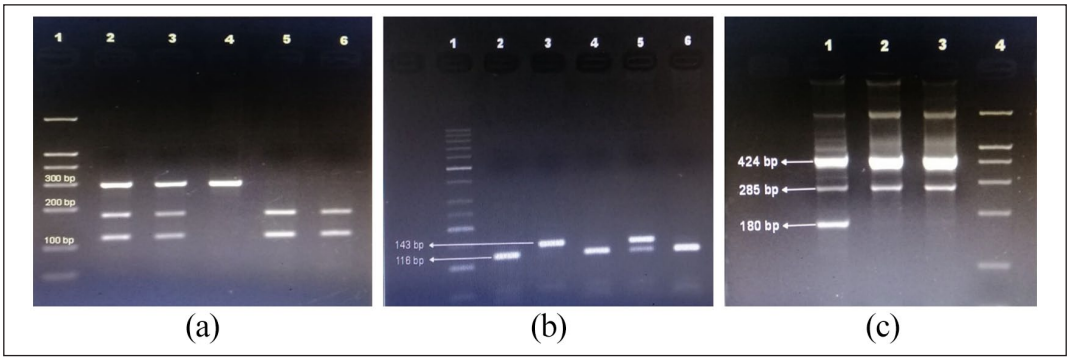

Figure 1. (a) PCR-RFLP based genotyping of CYP2C19*2 (lane 1 DNA ladder; lane 2,3 heterozygous; lane 4, mutant and lane 5, 6 homozygous); (b) PCR-RFLP based genotyping of CYP2C19*17 (lane 1 DNA Ladder; lane 2, 4, 6 homozygous; lane 3, mutant and lane 5, heterozygous); (c) T-ARMS-PCR based genotyping of the ITGB3 gene (lane 1, heterozygous; lane 2,3 normal, and lane 4 DNA ladder).

study following the Helsinki Declaration and its further amendments.[25] The study protocol was approved by the ethical review committee of the respective hospital. We had received written consent from all the patients in a prescribed form before they participated in this study.

DNA extraction

We extracted DNA from the collected blood sample following the prefixed protocol of the DNA extraction kit (Sacace blood DNA extraction kit).[26] We confirmed the purity of extracted DNA by measuring the ratio of absorbances for A260/280 (Eppendorf bio photometer with a single used cell).

Polymerase chain reaction

Total PCR reaction volume was 10 µL, containing 5 µL master mix, 0.15 µL forward primer (FP) and 0.15 µL reverse primer (RP), 2.7 µL NFW (nuclease-free water), and 2 µL DNA. The PCR mixtures were run in T100 thermocycler (Bio-rad) using the tetra-primer amplification refractory mutation system (T-ARMS-PCR) for the sample of aspirin-treated patients, and PCR-restriction fragment length polymorphism (RFLP) for the sample of clopidogrel-treated patients. We used Promega GoTaq Green Master Mix (Promega, USA) and Oligos (Bio-basic, Canada).

Genotyping

We completed genotyping of clopidogrel-treated patients by PCR-RFLP method and screened with 1.5% agarose gel electrophoresis (AGE). Later, digested with SmaI (For CYP2C19*2 polymorphism, overnight incubation at 25 °C) and NsiI-HF (For CYP2C19*17 polymorphism, incubated at 37 °C for 1 h). We ran the digested product on 2% AGE to detect the mentioned polymorphisms of CYP2C19*2 and CYP2C19*17. We genotyped aspirin-treated patients with T-ARMS-PCR for missense rs5918 (PlA1/A1) polymorphism of the ITGB3 gene.[27] Then, we analyzed the PCR product with 2% AGE. We performed sample analyses in the pharmacogenetics laboratory of Labaid Limited (Diagnostic), Dhaka, Bangladesh, according to the procedure described in our previous studies.[28]

Statistical analysis

We conducted all statistical analyses using a statistical package for social sciences (IBM SPSS, version 25.0) and Microsoft Excel 2016. We presented the data as frequencies and percentages. After data sorting, we performed data editing, coding, classification, and tabulation using Microsoft Excel 2016. Then we imported the excel file into IBM SPSS software for the application of descriptive statistics. A p value of less than 0.05 was considered statistically significant.

Results

We completed genotyping of CYP2C19 and ITGB3 genes to evaluate the therapeutic response of clopidogrel and aspirin. In clopidogrel-treated patients, digestion of CYP2C19*2 with SmaI resulted in products of 170 bp and 120 bp (homozygous, GG); 300 bp, 170 bp, and 120 bp (heterozygous, GA) and 300 bp (mutant, AA). Also, the digestion of CYP2C19*17 with NsiI-HF resulted in products of 116 bp and 27 bp (homozygous, CC); 143 bp, 116 bp, and 27 bp (heterozygous, CT) and 143 bp (mutant, TT) in the same patients. On the other hand, in aspirin-treated patients, two bands of homozygotes (PlA1/A1) resulting in 424 bp and 285 bp; 3 bands in heterozygotes (PIA1/A2) were in 424 bp, 285 bp, and 180 bp and two bands mutant (PIA2/A2) found in 424 bp and 180 bp after T-ARMS-PCR based genotyping of ITGB3 gene (Figure 1).

We noticed the prevalence of CYP2C19*2 polymorphisms (hetero + mutant) among clopidogrel-treated CVD patients who underwent PCI was 64.1%. But the prevalence of the same variants for CYP2C19*17 was only 22.7%. The

Table 1. Percentages of homozygous, heterozygous, and mutant homozygous people in response to drugs.

Therapeutic agents	Genetic polymorphisms	Number (*n*)	Percentage (%)
Clopidogrel			
	CYP2C19*2 (*n* = 1000)		
	Homozygous	359	35.9
	Heterozygous	472	47.2
	Mutant homozygous	169	16.9
	Hetero + mutant	641	64.1
	CYP2C19*17 (*n* = 1000)		
	Homozygous	773	77.3
	Heterozygous	204	20.4
	Mutant homozygous	23	2.3
	Hetero + mutant	227	22.7
Aspirin			
	ITGB3 (*n* = 1000)		
	Homozygous (PlA1/A1)	841	84.1
	Heterozygous (PlA1/A2)	156	15.6
	Mutant (PlA2/A2)	3	0.3
	Hetero + mutant	159	15.9

CYP: cytochrome; *n*: number.

percentage of homozygote in CYP2C19*2 was 35.9%, whereas the prevalence of the same variant was 77.3% for the CYP2C19*17 genes (Table 1). The polymorphism pattern is quite different for aspirin-treated patients compared with clopidogrel-treated patients. After assessing 1,000 patients for PlA1/A2 polymorphism of the ITGB3 gene, we observed 84.1% homozygous (PlA1/A1), 15.6% heterozygous (PlA1/A2), and only 0.3% mutant (PlA2/A2) (Table 1). Moreover, we presented the comparison of minor allele frequencies of studied polymorphisms of the CYP2C19 gene in different ethnic populations in Supplementary Table S.1.

Discussion

The advanced genomic technologies have made the treatment and management of cardiovascular patients easy for physicians.[29] Here we assessed the prevalence of polymorphisms potentially interfering with the efficacy of aspirin and clopidogrel for treating CVD patients who underwent PCI. The present study demonstrated 35.9% wild type, 47.2% hetero, and 16.9% mutant patients who carried CYP2C19*2 variant allele. Some other studies reported similar results among Malaysian, Chinese, Egyptian, and American healthy populations.[30–33] The combined prevalence of hetero and mutant was 64.1% in CYP2C19*2 variant allele among Bangladeshi CVD patients treated with clopidogrel as antithrombotic medication (Table 1). On the other hand, the percentage of CYP2C19*17 carriers was 22.7%. Both the results represent that a large number of CYP2C19 poor metabolizers and rapid metabolizers are regularly taking clopidogrel as an antithrombotic agent in Bangladesh. Therefore, physicians should consider this issue before prescribing clopidogrel or aspirin to CVD patients.

According to the previous report, clopidogrel non-responders might be carriers of CYP2C19*2/*2, CYP2C19*1/*2, and CYP2C19*1/*1.[30] Clopidogrel dose adjustment, adding a third antiplatelet, and switching to a novel generation P2Y12 inhibitor provide the optimum platelet inhibition.[34] Hulot et al. suggested that a doubling of the standard maintenance dose of clopidogrel (75 mg/day) is necessary for patients with the CYP2C19*2 loss-of-function polymorphism.[35] Physicians recommend a titrated clopidogrel dose and strict monitoring of potential bleeding for patients with CYP2C19*17 carriers.[36] According to CPIC guidelines, prasugrel provides better therapeutic responses over clopidogrel in low metabolizer genotype. Also, discontinuing or lowering clopidogrel doses in rapid metabolizer genotype decreases bleeding risk.[36] Another study among Chinese patients reported that personalized antiplatelet therapy based on the CYP2C19 genotype significantly reduces the incidence of major adverse cardiovascular events who underwent PCI.[35] However, some studies reported thromboembolic complications in CVD patients when adjusted clopidogrel doses were administered.[33]

New agents such as prasugrel, ticagrelor, and elinogrel do not involve CYP2C19 metabolism. Therefore, ticagrelor is more effective than clopidogrel in patients with ACS regardless of CYP2C19 and ABCB1 polymorphisms.[37] So, ticagrelor for managing arterial thrombosis can reduce the necessity of genetic testing. In patients with HTPR after PCI, prasugrel is more effective than clopidogrel in reducing platelet reactivity, particularly in CYP2C19*2 carriers.[38] The low-dose aspirin alters the activities of CYP2C19 but did not affect the activities of CYP1A2, CYP2D6, and CYP2E1. Therefore, physicians can adjust the doses of substrate drugs of CYP2C19 when they are administered in combination with

low-dose aspirin to ensure their efficacy.[39] The present study results support the therapeutic effectiveness of aspirin and clopidogrel in CVD patients with proper adjustment of doses after checking genetic polymorphisms. This finding is consistent with several past reports regarding the use of aspirin and clopidogrel in managing arterial thrombosis.[40–43] However, a study observed no significant association between polymorphisms in the platelet receptors and the therapeutic response of aspirin or clopidogrel in cardiac patients.[44] Also, the P2Y12 receptor performs a key role in platelet aggregation. Therefore, the presence of any variant alleles can alter the platelet activity in CVD patients who underwent PCI.[45,46]

The present study has some strengths and limitations. The main strong point is that this is the first genetic association study concerning CYP2C19 and ITGB3 polymorphisms among Bangladeshi CVD patients. Another strength is that we recruited 1000 patients, which therefore increased the statistical power. However, we selected known SNPs from a public or GWAS database instead of novel SNPs and the expressions. The lack of complete clinical and environmental data downgrade the outcomes of the present study.

Conclusions

We found a high prevalence of CYP2C19 and ITGB3 polymorphisms among Bangladeshi CVD patients who underwent PCI. Therefore, we suggest genotyping of CVD patients before prescribing aspirin and clopidogrel for optimum efficacy. Based on the genotyping report, the adjustment of doses of these drugs or alternative generics might require for avoiding therapeutic alterations. Moreover, we recommend further studies to analyze the metabolites produced in CVD patients with variant genetic composition and suggest alternative therapeutic options.

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Author contributions

Study conception and design: M.R.I., T.T.N., T.B., M.S.I., and Z.N.; Sample collection: T.T.N., N.A.M.M., S.N.I.R., and I.J.; Data analysis: M.R.I., T.T.N., N.A.M.M., and S.N.I.R.; Manuscript writing, editing, and reviewing: M.R.I., T.T.N.,. M.S.I., I.J., T.B., and Z.N. Supervision of the whole study: A.H. and Z.N. All authors approved the final version of the manuscript except A.H.

Declaration of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Ethical approval and consent to participate

The ethical review committee (ERC), Department of Cardiology, United Hospital, Dhaka, Bangladesh, approved this study protocol (ERC/UH/020031). Before the study, we obtained informed written consent from all subjects included in this study in a prescribed form. We conducted this study according to the Declaration of Helsinki.

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Informed consent

We obtained informed written consent from all subjects included in this study in a prescribed form. Also, a written consent was taken from legally authorized representative of the subjects whose decisional capacity was doubtful.

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Availability of data and materials

The data used in this study can be available from the corresponding author on a reasonable request.

Supplemental material

Supplemental material for this article is available online.

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