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RESEARCH ARTICLE

The clinical effects of *CYP2C19 *2* allele frequency on Palestinian patients receiving clopidogrel after percutaneous coronary intervention

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Abstract

Background *CYP2C19* loss-of-function polymorphic alleles (*2 and *3) have been documented to impair clopidogrel metabolism, and represent a risk factor for major adverse cardiac events. *CYP2C19* polymorphism exhibits marked ethnic heterogeneity. *Objective* To determine the prevalence of *CYP2C19 *2* and *3 alleles in a cohort of Palestinian patients managed with percutaneous coronary intervention and dual antiplatelet therapy, and to determine their role in causing major adverse cardiac events. *Setting* The blood samples were collected at the European Gaza Hospital, and the molecular techniques performed at the molecular genetics laboratory of the Islamic university of Gaza. *Method* The frequency of *CYP2C19 *2* and *3 alleles was determined in 110 patients managed with percutaneous coronary intervention and clopidogrel. Genotyping was performed by PCR–RFLP. Personal and clinical data was obtained from patient record and 6-month follow-up for major adverse cardiac events. *Main outcome measure* *CYP2C19* genotype, personal and clinical data and incidence of major adverse cardiac events. *Results* The frequency of *CYP2C19 *1*, *2 and *3 alleles was 82.3%, 15.5% and 2.3% respectively. Genotyping analysis showed that, 67.3% were homozygotes for *CYP2C19 *1*, 27.3% were *1/*2, 2.7% with *1/*3 genotype, 1.8% were *2/*3 and 0.9% were *2/*2. These frequencies were consistent with those of Caucasian populations. According to this study the poor metabolizers phenotype frequency was 2.7%, which is in the same range reported in Caucasians (2–5%) and lower than Oriental populations 13–23%. A strong significant relation was found between major adverse cardiac events and carrying the variant allele *CYP2C19 *2* ($P=0.001$). On the other hand, there was no significant relation between major adverse cardiac events and carrying the variant allele *CYP2C19 *3* ($P=0.324$). *Conclusion* The *CYP2C19 *2* allele is relatively common in our population, and its associated reduced metabolic activity deserves attention as it leads to an increased incidence of major adverse cardiac events in the follow-up of patients receiving clopidogrel.

Keywords Clopidogrel · Coronary artery disease · *CYP2C19* polymorphism · PCI · Percutaneous coronary intervention

Impacts on practice

- A genotype-guided treatment reduces major adverse cardiac events in patients who had a percutaneous coronary intervention by individualizing the anti-platelet therapy in a cost-effective manner.
- Percutaneous coronary intervention patients who are normal metabolizers can be safely maintained on clopidogrel.
- A different anti-platelet therapy should be used to reduce the risk of adverse cardiac events post percutaneous coronary intervention in patients identified as *CYP2C19* intermediate or poor metabolizers.

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Introduction

Cardiovascular diseases (CVDs) are the leading cause of death globally, with an estimated 17.7 million deaths in 2015, representing 31% of all global deaths [1]. More than 80% of CVD deaths are in low- and middle-income countries [2]. The leading cause of CVD-related deaths in 2013 was ischemic heart disease (IHD), followed by cerebrovascular disease [3]. Percutaneous coronary intervention (PCI) is a backbone in the management of acute coronary syndrome (ACS) [4, 5]. In ACS patients treated with PCI, dual antiplatelet therapy (DAPT; Aspirin and Clopidogrel) is associated with a significant reduction in ischemic risk (e.g., MI and stent thrombosis) [6–8]. Clopidogrel irreversibly inhibits the adenosine diphosphate receptor (ADP)-mediated activation of the platelets via the ADP receptor (P2RY12) [9, 10]. It is a prodrug requiring activation by two-steps oxidation catalyzed by hepatic cytochrome P450 (CYP) isozymes, mainly by *CYP2C19* [11–13].

Between 5 and 44% of patients treated with standard clopidogrel doses display resistance, i.e. show evidence of residual post treatment P2Y12 activity [14, 15]. Genetic polymorphisms in *CYP2C19* have been documented to impair clopidogrel metabolism, and represent a risk factor for major adverse cardiac events (MACE) [16–20]. The *CYP2C19 *1* wild-type form produces a full activity CYP2C19 enzyme, while the most common variant, *CYP2C19*2* allele (rs4244285; c.681G > A), produces a nonfunctional truncated protein [21, 22]. *CYP2C19 *3* (rs4986893; c.636G > A) is another relatively common allele which creates a premature stop codon [23, 24]. Both polymorphic variants are known as poor metabolizers (PM). The polymorphism associated with clopidogrel therapy exhibits marked ethnic heterogeneity [25]. *CYP2C19 *2* and *CYP2C19 *3* account for more than 99% of Oriental and approximately 87% of Caucasian PM alleles [26, 27].

In Gaza strip, 5044 deaths were reported in 2016, among which CDV-related deaths were leading (57.1% in 2016 and 44.5% in 2015) [28]. The number of cardiac catheterization procedure in 2016 was 5366 (27.8% more than 2015), about 32% of which were PCI [29]. All patients receive clopidogrel as a part of their post-PCI management protocol but unfortunately the *CYP2C19* PM polymorphic alleles are not evaluated.

Aim of the study

To determine the prevalence of *CYP2C19 *2* and *3* alleles in a cohort of Palestinian patients who were managed with PCI and DAPT, and to determine their relation to MACE as an important indicator of management success.

Ethics approval

All procedures performed in the study were in accordance with the ethical standards of the ethical committee of the Palestinian health research council (approval number: PHRC/HC/04/15) and the Palestinian Ministry of Health (approval number: 826), according to the World Medical Association Declaration of Helsinki. Informed consent was obtained from all individual participants included in the study.

Methods

Study population and sample collection

Between March and the end of May 2014 a total of 110 consecutive unrelated post-PCI patients were recruited from the cardiology department of the European Gaza Hospital (EGH). To be eligible, the patients must have had to be treated with DAPT. All patients enrolled in the study are living in Gaza strip a geopolitically isolated Palestinian area.

Approximately 2.5 ml of venous blood were collected in an EDTA tube from each participant and processed for genomic DNA extraction. Clinical and laboratory data were collected from the medical records of the patients. The patients were successfully contacted 6 months later to follow up for their general condition and for evidence of cardiovascular problems or complications that might have occurred following PCI.

DNA extraction and PCR/RFLP detection of *CYP2C19 *2, CYP2C19 *3*

Genomic DNA was extracted from peripheral-blood leucocytes using the Wizard Genomic DNA Extraction Kit (Promega, USA) according to the manufacturer instructions. Genotyping was performed according to a previously published work [30]. Two PCR reactions specific for *2 and *3 alleles were conducted, for each sample, in 25 µl-final volume. The reactions contained 0.2 pmol/µl of the specific sense and antisense primers, about 100 ng DNA and 1X GoTaq Green master mix (Promega, USA).

*CYP2C19 *2* amplification was done with the sense primer (5′-CAGAGCTTGGCATATTGTATC-3′, annealing 71-bp upstream from intron 4/exon 5 junction) and the antisense primers (5′-GTAAACACACAAAACTAGTCAATG-3′, annealing 73-bp downstream from the exon 5/intron 5 junction). The cycling profile consisted of an initial denaturation step at 94 °C for 3 min; followed by 37 cycles of 94 °C for 30 s, 52 °C for 30 s, and 72 °C for 40 s, and a 5-min final extension at 72 °C. The resulting 321-bp product of was

digested with *Sma*I (New England Biolabs) at 25 °C for 1 h followed by an inactivation step at 75 °C for 15 min. *Sma1* cuts the 321-bp PCR product containing the wild type allele into 212-bp and 109-bp fragments while it doesn't cut PCR products containing the mutant allele. A homozygous genotype yields one 321-bp band while a heterozygous genotype produces three bands (321-bp, 212-bp, and 109-bp).

*CYP2C19 *3* amplification, was done with the sense primer annealing 21-bp upstream to intron 3/exon 4 junction (5′AACATCAGGATTGTAAGCAC-3′) and the antisense primer annealing 88-bp downstream to exon 4/intron 4 junction (5′-TCAGGGCTTGGTCAATATAG-3′). The cycling profile was identical to that of *CYP2C19 *2*. The resulting 272-bp product was digested with *Bam*HI (New England Biolabs) at 37 °C for 3 h. followed by an inactivation step at 75 °C for 15 min. *BamH1* does not cut the PCR products containing the mutant allele but it cuts the PCR products containing the wild type allele into 175-bp and 96-bp fragments. A *CYP2C19 *3* homozygote yields one 271-bp band while a heterozygote produces three bands (271-bp, 175-bp, and 96-bp).

The digestion products were resolved by 2.5% agarose gel electrophoresis and identified by comparison to 100-bp DNA ladder.

Data analysis

The patient was considered as normal metabolizer (NM) in case of (**1/*1*) for both alleles. Heterozygotes for either allele (**1/*2* or **1/*3*) were considered intermediate metabolizers (IM). Finally, homozygotes (**2*/*2* or **3/*3*) and compound heterozygotes (**2/*3*) were considered poor metabolizers (PM).

At 6-months follow-up, major adverse cardiac events (MACE), including death, myocardial infarction (MI), acute coronary syndrome leading to hospitalization, in-stent restenosis/thrombosis and nonfatal stroke, were recorded. MACE data was collected from the patients and from their records at the hospital after evaluation by cardiologists during their follow up visits.

The IBM SPSS software version 22, was used for statistical analysis. All the statistical tests were two sided; a P value of < 0.05 was considered statistically significant and 95% CI were used to describe the strength of association.

Results

Genotyping results

Table 1 summarizes the frequency of *CYP2C19* genotypes in the study population (n = 110). More than half of the study population (67.3%) are homozygotes for the wild type allele (**1/*1*); 27.3% are heterozygotes to the *CYP2C19*2* allele (*1*/2**); 2.7% are heterozygotes for the *CYP2C19*3* allele (**1/*3*); 1.8% are compound heterozygotes (**2/*3*) and 0.9% homozygotes for (**2/*2*) mutant allele (Table 2). The **3/*3* homozygous genotype was not detected in the study population. By assuming random mating of population in Gaza strip and applying the Hardy–Weinberg equation for three alleles, the allelic frequency of the wild type allele **1* was 82.3%, while the frequency of the polymorphic allele **2* was 15.5% and that of the polymorphic allele **3* was 2.3%. The three alleles are in equilibrium and genotype frequencies are not significantly different from those expected by Hardy–Weinberg equation (P value = 0.325; Table 2). In the present study, the distribution of patients by *CYP2C19* phenotypes was: 67% NM, 30% IM and 3% PM (Table 2).

Table 1 Observed genotype distribution of *CYP2C19*

CYP2C19 genotype		Frequency	%
Allele **2* c.681G > A (rs4244285)	*GG* (**1/*1*)	77	70.0
	GA (**1/*2*)	32	29.1
	AA (**2/*2*)	1	0.9
	Total	110	100
Allele **3* c.636G > A (rs4986893)	*GG* (**1/*1*)	105	95.5
	GA (**1/*3*)	5	4.5
	AA (**3/*3*)	0	0.0
	Total	110	100

Table 2 Observed versus expected genotype distribution of *CYP2C19*

Phenotype frequency		Genotype frequency			P value
		Genotype	Observed	Expected by Hardy–Weinberg equation	
NM	74 (67%)	**1/*1*	74 (67.3%)	74.5 (67.7%)	0.325
IM	33 (30%)	**1/*2*	30 (27.3)	28.0 (25.5%)	
		**1/*3*	3 (2.7%)	4.1 (3.7%)	
PM	3 (3%)	**2/*2*	1 (0.9%)	2.6 (2.4%)	
		**2/*3*	2 (1.8%)	0.8 (1.0%)	
		3/*3*	0 (0%)	0.1 (0%)	

Major adverse cardiac events and *CYP2C19* polymorphism

Overall, 9 out of the 110 patients enrolled in the study (8.2%) experienced at least one MACE. Seven of them were IM (**1/*2* = 6, **1/*3* = 1), one case was PM (**2/*2* genotype) and one case was NM (**1/*1* genotype). The distribution of MACE by CYP2C19 phenotype (Table 3) is statistically significant ($\chi^2 = 14.042$, P value = 0.000). The distribution of cases with MACE by *CYP2C19*2* genotype

Table 3 Distribution of MACE by the *CYP2C19* phenotype

Phenotype	MACE		*P* value
	Yes	No	
NM	1	73	0.000
IM/PM[a]	8	28	
Total	9	101	

[a]IM (n = 1) and PM (n = 7) were combined together in one category (IM/PM) for statistical analysis

is presented in Table 4. Most of the cases (7/9) were either hetero- or homozygous for the polymorphic allele *2. This distribution was found statistically significant ($\chi^2 = 10.65$, *P* value = 0.001). In comparison, among the five cases carrying the (*1*/3**) genotype only one case had MACE (Table 4). This distribution was not statistically significant (*P* value = 0.324).

Major adverse cardiac events and other risk factors

As represented in Table 5, there was no statistically significant relationship between risk of MACE and age (*P* value = 0.155); gender (*P* value = 0.136); body mass index (*P* value = 0.103); hypertension (*P* value = 0.556), diabetes mellitus (*P* value = 0.417) and Family history of heart disease (*P* value = 0.062).

Discussion

Genetic polymorphisms in *CYP2C19*, an enzyme required for clopidogrel bioactivation have been shown to be associated with clopidogrel antiplatelet effectiveness, and represents a risk factor for recurrent ischemic cardiac events [31]. To the best of our knowledge, this is the first study in Palestine reporting the impact of *CYP2C19* loss-of-function alleles, *CYP2C19 *2* and *CYP2C19 *3*, in association with

Table 4 Distribution of MACE by the *CYP2C19 *2* allele genotype

Genotype	MACE		*P* value
	Yes	No	
(*1/*1)	2	75	0.001
(*1/*2) or (*2/*2)[a]	7	26	
Total	9	101	
(*1/*1)	8	97	0.324
(*1/*3)	1	4	
Total	9	101	

[a](*1/*2) and (*2/*2) were combined together in one category for statistical analysis

Table 5 Relationship between MACE and other clinical conditions

	MACE			*P* value
	Yes	No	Total	
Mean age ± SD (year)	54.6 ± 9	60.7 ± 11.3		0.155
BMI (Mean ± SD)	27 ± 2.6	29.5 ± 4.4		0.103
Gender (frequency)				
Male	8 (11%)	65 (89%)	73	0.136
Female	1 (2.7%)	36 (97.3%)	37	
Hypertension (frequency)				
Yes	7 (77.8%)	69 (68.3%)	76	0.556
No	2 (22.2%)	32 (31.7%)	34	
DM (frequency)				
Yes	7 (77.8%)	65 (64.4%)	72	0.417
No	2 (22.2%)	36 (35.6%)	38	
Family history of heart disease (frequency)				
No	8 (88.9%)	58 (57.4%)	65	0.062
Yes	1 (11.1%)	43 (42.6%)	44	

MACE in CVD patients managed with PCI and clopidogrel as part of DAPT. An understanding of the distribution of SNPs is crucial for the future application of pharmacogenomics to different population groups [32].

In this study, the frequencies of *CYP2C19 *1*, *2* and *3* alleles are 82.3%, 15.5% and 2.3% respectively, and the frequency of the NM, IM and PM phenotypes is 67%, 30% and 3% respectively. The distribution of *CYP2C19* variants in the Palestinian population is consistent with the relatively high worldwide frequency of *CYP2C19 *2* allele (22% on average, ranging from 11% in Americans to 36% in South Asian) [33]. This suggests that the mutation is old and has occurred before the separation of Caucasians, Oriental and Black populations [34]. The frequency of *CYP2C19 *2* allele was found consistent with other Middle Eastern populations, like Egyptian (12.6%) [35], Saudi (11.2%) [36], Lebanese (13%) [37] Jordanian (16%) [38], Israeli Jewish (15%) [39], and Tunisian (11.5%) [40]. *CYP2C19 *2* frequency in our population is slightly different from previous data in Gaza strip (9.6% pediatric hematological malignancy patients and 5.7% in healthy controls) [41]. It is also different from Palestinian population in the West bank and Jerusalem (9.5%) [42]. Frequency differences may reflect the cultural tendency of community to mate in a consanguineous manner. Furthermore, the Palestinian population of Gaza strip is isolated apart from other Palestinian and Arab populations due to political restrictions on movement in and out from Gaza strip.

The *CYP2C19 *3* allele, which was very low in our population, is specific to Asian and Oceanian ethnical groups (ranges from 0% in many different populations to 6% in East Asians with average of 1%) [43]. The low frequency, or sometimes absence of this allele in different Caucasians

populations confirms the Asian specificity of this mutation and suggests that this allele occurred quite recently, after the differentiation of Caucasian and Oriental groups [34, 44]. The *CYP2C19 *3* allele frequency in our study population is higher than those recorded in Egyptian (0.2%) [35] and Israeli populations (1%) [39], while it was not detected in Jordanian [38], Saudi [45] and Iranian populations [46, 47]. Our result is consentient with Lebanese (3%) [37] and with previous data in patients from Gaza strip (3%) [41]. The PM phenotype frequency in Palestinian patients is 2.7%, which is in consistence with the range reported in Caucasians (2–5%) and lower than in Oriental populations 13–23% [48, 49].

Despite the small number of patients with MACE, our findings show that there is a significant relation between MACE and carrying the variant allele *CYP2C19 *2* (*P* value = 0.001). Similar previous results reported that *CYP2C19 *2* carrier status is significantly associated with an increased risk of MACE, in-stent thrombosis and restenosis following PCI [19, 20, 50–53]. The *CYP2C19 *3* allele in our population is not significantly related to MACE (*P* = 0.324). It may be due to the small size of patients with restenosis or the lack of homozygous genotypes of the **3* allele. Overall, reduced metabolic activity of CYP2C19 as a result of carriage of allele *2 alone or together with allele *3 is significantly related to MACE. There is controversy over the role of high-dose clopidogrel in reducing MACE according to *CYP2C19*2* alleles in patients undergoing PCI. While some studies demonstrated no reduction in MACE upon high-dose clopidogrel therapy [54, 55], others concluded that high-maintenance-dose clopidogrel significantly reduces the risk of MACE [56, 57]. This reduction was suggested to be more meaningful in heterozygous carriers of *CYP2C19*2* allele but not in homozygotes [58]. In accordance with this suggestion, the Dutch Pharmacogenetics Working Group (DPWG) recommended choosing an alternative drug, or doubling the dose of clopidogrel to 150 mg daily dose, 600 mg loading dose for IM, and an alternative drug for PM [27].

Many other non-genetic factors may favor MACE. Such factors include diabetes mellitus (DM), hypertension, gender, age and BMI. Unlike other studies [59–62], our study results show no statistically significant relation between DM and MACE after PCI (*P* = 0.417). Similarly, out of the nine patients who developed MACE, 7 were hypertensive. However, this distribution was not statistically significant (*P* value = 0.556). On the contrary, hypertension was proposed by others to be an important predictor of MACE [62]. Our results advocate no relation between gender and age and MACE (*P* value = 0.136 and 0.115 respectively). Previous studies examining gender differences in patients undergoing coronary angioplasty have reported that women had a higher in-hospital mortality and were at increased risk for an adverse outcome in comparison with men [63–65]. However, this relationship was claimed by other researchers not to exist, and sex and age were not found to be predictors of MACE [62, 66, 67]. The mean BMI for MACE cases was 27 ± 2.6 kg/m^2, and for non-MACE cases was 29.5 ± 4.4 kg/m^2. The statistical analysis, shows no significance relation in this distribution (*P* value = 0.103). Also, out of the nine patients who developed MACE, 4 developed restenosis in the LAD and 5 in the RCA artery, and no restenosis occurs in the LCX and OM arteries. This distribution however was not statistically significant (*P* value = 0.26).

The main limitation of the study would be the low number of patients with MACE (n = 9). However, our results support several previous reports on the relation between *CYP2C19* polymorphism and incidence of MACE. Knowledge of the distribution of *CYP2C19* loss of function alleles (**2* and **3*) is crucial for the future application of a genotype-guided treatment, which could be more cost-effective and result in a reduction of clinical adverse outcomes.

Conclusion

The *CYP2C19 *2* polymorphic allele is relatively common in the Palestinian population of Gaza strip (frequency = 15.5%). Its associated reduced metabolic activity plays a major role in incidence of MACE in the follow-up of patients receiving clopidogrel.

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Conflict of interest The authors declare that they have no conflict of interest.

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