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Research Article

2C19*2 Polymorphism and Platelet Aggregation in Patients on Clopidogrel After Percutaneous Coronary Intervention

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Abstract

Background: Regardless of the clinical advantage accomplished with the utilization of clopidogrel in ACS or with coronary intervention, many patients keep on having thrombotic events.

Purpose: We aimed to find the relation between CYP2C19*2 gene polymorphism and clopidogrel poor response reflected by platelet aggregation.

Method: 30 patients scheduled for implantation of DES or BMS. Platelet aggregation was detected 4 times by LTA using ADP 5mol/l as agonist before 600mg loading of clopidogrel, after 24 hours, after 5 days and after 3 months. CYP2C19*2 genotype was detected by Conventional method of PCR amplification followed by restriction enzyme digestion for CYP2C19*2 gene.

Results: 27 patients had *1/*1 genotype and 3patients had *1/*2 genotype. Basal platelet aggregation did not vary significantly between genotype *1/*1 and genotype *1/*2. Platelet aggregation was significantly high in genotype *1/*2 after 24 hours from the loading dose of clopidogrel, after 5 days and after 3 months. Clopidogrel resistance in our definition was found in all patients with *1/*2 genotype and in 3 patients with *1/*1 genotype after 24 hours and after 5 days while in 1patient only with *1/*1 genotype after 3 months

Conclusions: CYP2C19*2 gene polymorphism may be associated with clopidogrel resistance which was reflected by platelet aggregation according to the definition that we used. In order to reach a more definitive conclusion, a standard definition of clopidogrel resistance is needed for future studies.

Keywords: *Polymorphism; Platelet; ClopidogrelT*

Introduction

Blood platelets have a great share in formation of thrombotic complications of the coronary arteries and perform a main role in the catastrophic events which occur after percutaneous coronary intervention as stent thrombosis [1].

Clopidogrel and aspirin together have been considered a main strategy in management patients with acute coronary lesions and in patients who are going to do percutaneous coronary intervention with BMS or DES [2].

Clopidogrel acts by inhibiting the platelet aggregation which is performed by ADP. Also it requires many activation stages which are done by CYP-450 isoenzymes resulting in performing its active form which blocks irreversibly the platelet P2Y12 receptor [3].

Regardless of the clinical advantage accomplished with the utilization of clopidogrel in acute coronary occlusion or with coronary intervention, many patients keep on having thrombotic occasions. This has been, to some extent, ascribed to the reality than a few persons may have less clopidogrel-actuated effects and continue with improved platelet reactivity, which is vital for the advancement of acute thrombotic events [4].

Clinical, hereditary, and cellular elements are included in the variation of clopidogrel effects. Especially, gene polymorphisms of enzymes required for clopidogrel activation which record for around 15% to 20% of the variety and are emphatically identified with poor anticipation in patients taking clopidogrel [5].

CYP2C19 is considered the main enzyme for activation of clopidogrel. So, polymorphisms of the CYP2C19 gene might be associated with reduced AntiPlatelet effects of this drug and might led to variable clinical effects among different patients [6].

The FDA pointed out this task by means of a boxed cautioning about the decreased effect of the medication in patients with debilitated capacity to change over the medication into its active structure due to hereditary polymorphism in CYP2C19, in light of this boxed cautioning, the American College of Cardiology Foundation/American Heart Association discharged a clinical master agreement report expressing "the proof base is deficient to prescribe either genetic testing or platelet testing right now." Since that announcement, extra studies have been distributed that question about the significance of platelet or genetic testing [7].

We perform this study in order to

- Find out whether the AntiPlatelet effect of clopidogrel in patients treated with percutaneous coronary intervention varies through follow-up.
- Evaluate the impact of CYP2C19*2 polymorphism on the AntiPlatelet effects of clopidogrel after implantation of DES or BMS.

Subjects and Methods

Study population

The present study is prospective Cohort study that done at Specialized Medical Hospital "Cardiology Department", Mansoura University Hospitals. The study included 30 patients with coronary lesions experiencing coronary angiography and is going to insert DES or BMS.

Exclusion criteria

Known contraindication to dual AntiPlatelet therapy, Glycoprotein IIb/IIIa inhibitors administration before coronary artery angiography, Significant bleeding, Major surgery within 4 weeks, Malignancy, stroke in the previous 3 months, Hematocrit< 34% and/or Creatinine clearance <25 ml/min.

Data acquisition

In cardiovascular Medicine department data was carefully collected. The patient data was written on the admission report, once patient admitted. The components of the report include patient history, cardiovascular risk factors, medication, physical examination and past medical history.

Venous blood tests were taken from every patient on their confirmation. Complete blood count, blood glucose, serum creatinine and INR of every participant were obtained.

Electrocardiographic analysis

Resting standard 12 drives surface ECG was accomplished for all patients. A standard alignment of 25 mm/s and 10 mm/mV was utilized for all ECGs. ECG were evaluated for rate and rhythm, presence or absence of significant ischemic changes (pathological Q wave, ST segment deviation and T wave inversion, presence or absence of significant arrhythmia (Premature atrial or ventricular ectopic, tachyarrhythmia or bradyarrhythmia).

Echocardiography

The participants were initially evaluated by echocardiography prior to PCI using General Electric Vivid 3 ultrasound system (GE Healthcare, USA). Echo were evaluated for systolic and diastolic functions, RSWMA, valvular lesions and pulmonary artery systolic pressure.

Coronary angiography and intervention

Coronary angiography was done to all of the participants at our department utilizing Siemens Angiocore Machine (Germany) by experienced doctors.

All participants will be loaded with Clopidogrel 600 mg at least 24 h before PCI then 75 mg/day preceded for 12 months for DES and 3 months for BMS. All patients will be treated with ASA 100 mg/day, freely to past or not incessant use. Effective PCI were accomplished for all occluded coronary lesions utilizing different types of coronary stents.

Platelet function assays

Platelet aggregation was assessed using ADP agonist. Blood samples for platelet aggregation will be drawn as the following: First sample before administration of the loading dose of clopidogrel (basal sample). Second sample was at time of catheterization before administration of heparin (after 24 hours from basal sample). Third sample was at day 5 after loading dose of clopidogrel. Fourth sample was after 3 months from date of the loading dose.

- **Sample:** Performed in a solitary specific research center at Mansoura University Hospitals. Venous blood was acquired into plastic tubes containing the anhydrous salt of trisodium citrate (3.2%) and blended at a proportion of 1 section citrate to 9 sections blood. Each sample was quickly handled and used in our study.
- **Estimation of platelet aggregation:** The standard turbidimetric aggregation detection strategy was performed firstly by **(**Born, 1962) [8]. Platelet rich plasma was set up by rolling of venous blood which is full of citric acid at 150 × g for 10 min then for 20 min. Platelet poor plasma was prepared by rolling of citrated venous blood at 2000 × g for 20 min. Aggregation was measured in a Chronolog Lumiaggregometer (Havertown, PA, USA) under consistent blending situations (1000 rpm) at 37^0C then done by adding (5 mol/L) ADP (Chronolog, Havertown, PA, USA) to PRP cuvette using PPP as a reference cuvette, and the change in light transmission (LTA) was recorded on a chart record. Platelet aggregation was expressed as the maximal percent change in light transmittance from baseline, with platelet-poor plasma used as a reference [8].
- **Definition of Drug Resistance:** Drug resistance was defined as an absolute difference between baseline aggregation and post treatment aggregation (Δ aggregation[%]) of 10% or less with ADP used as the agonist [8].

Genetic investigations

- **Methods:** 2 ml of blood samples was taken from each patient for genomic DNA extraction into tubes containing EDTA as an anticoagulant and stored at -20 °C. The extracted DNA used for detection of CYP2C19 gene polymorphisms by polymerase chain reaction(PCR) and restriction enzymes followed by agarose gel electrophoresis.
- **DNA extraction Principle:** G-spin TM total Genomic DNA Extraction Mini Kit provide fast and easy methods for purification of total DNA from blood samples for reliable PCR. The kit utilizes silica-based membrane technology in the form of a convenient spin column, eliminating the need for toxic phenol-chloroform extractions or time consuming [9].
- **Reagents:** Buffer BL (Buffer lysis), Buffer WA (Wash A), Buffer WB(Wash buffer), Buffer CE (elution buffer), Spincolumns, Ethanol (96 - 100%), RNase A (Lyophilized powder): It was prepared by dissolving RNase A in 0.3 ml of pure distilled water to each vial and Proteinase K (Lyophilized powder): It was prepared by dissolving proteinase K in 1.1 ml of pure distilled water to each vial.

Procedure of DNA extraction [10]

Cell lysis

- 200 µl of participant blood and 20 µl Proteinase K were added to 5 µl RNase A plus a 1.5 ml micro centrifuge tube. The tube was vortexed to mix thoroughly to obtain uniform suspension.
- 200 µl of BL buffer were added to the above mix then the tube was vortexed to mix thoroughly to obtain uniform suspension.
- Sample was incubated at 56°C for 10 minutes then centrifuged for 1 min at 13,000 rpm.

Binding of DNA

- The formed lysate was stacked to the genomic DNA purification column and embedded in a collection tube. The column was rolled for 60 sec at 13.000 rpm then it's inserted into a new 2 ml collection tube.

Washing of the column

- A first wash was done using 700 ul of WA buffer was added to the column and centrifuged for 1 min at 13,000 rpm. A second wash was done using 700 ul of WB buffer what's more, rolled for 60 sec at 13,000 rpm.

Evaluation of DNA

- **DNA quantification:** Using the DNA spectrophotometer GENWAY,(7300/7305) Genova, UK) [11].
- **DNA qualification:** Extracted DNA quality was assessed by agarose gel electrophoresis [12].
- **Agents of agarose gel electrophoresis**: Tris-Borate EDTA buffer, loading dye, Ethidium Bromide, (3%) agarose gel.
- **Genotyping of CYP2C19*2 gene polymorphisms:** Ordinary technique for PCR intensification took after by confinement chemical absorption for CYP2C19*2 gene was done according to the method of **Goldstein and Blaisdell** [13].
- **Primers:** The primers were selected according to the method of Goldstein and Blaisdell [13], 2 primers were purchased from Biolegio BV Company.

SNP	Product
CYP2C19*2	5′AATTACAACCAGAGCTTGGC′3(Forward) 5′TATCACTTTCCATAAAAGCAAG3′(Reverse)

Table a

PCR formation

For intensification of CYP2C19*2 gene the following mix was prepared: PCR formation/25 ul reaction.(Table b)

Material	Concentration	Amount	Final concentration/25 ul
PCR Master mix	2X	20 ul	
Primer CYP2C19*2-forward	10 pmol/ul	0.25 ul	20 pmol
Primer CYP2C19*2 reverse	10 pmol/ul	0.25 ul	20 pmol
DNA	10 ng/ul	5 ul	50 ng
Distilled water		5 ul	

Table b

Restriction endonuclease analysis for CYP2C19*2 gene polymorphism

0.5 ul (6 units) of the restriction endonuclease SmaI (purchased from New England BioLabsInc.) was added to 5 ul of amplified PCR product then 7.5 ul of sterile distilled water were added and mixed well. The reaction mixture was incubated at 37ºC for one hour. Inactivation of the enzyme was done by heating for 40 minutes at 65ºC in a heat block. The reaction mixture then subjected to 3% agarose gel electrophoresis (8 ul of restriction analysis mixture +2 ul loading dye). Electrophoresis was done for run about one hour at 70 volts. Products of 169bp fragment as one band represents CYP2C19*1/*1 genotype while products of 169 bp, 120 bp and 49 bp as three bands means mutated CYP2C19*1/*2 genotype (13).

Results

A total of 30 patients with known coronary artery disease that were admitted to cardiology department at specialized medical hospital Mansoura University Hospitals and underwent implantation of DES or BMS.

Demographics

Demographic data of the study population are summarized in (Table 1). The mean age among all patients was 60.00 (± 6.11) years, with their ages ranging from 48 to 70 years. The male/female distribution was 17 (52.7%) versus13 (43.3%).

In our study 12 patients (40%) were smokers, 25 patients (83.3%) had documented hypertension, 18 patients (60%) had documented DM, 12 patients (40%) were HCV +ve, 4 patients (13.3%) had history of old myocardial infarction, The patients serum creatinine ranged between 0.7 and 1.4 mg/dL (mean 1.05 ± 0.22 mg/dL), INR ranged between 0.8 and 1.5 (mean 1.06 ± 0.16), The patients hemoglobin ranged between 9.0 and 15.0 mg/dL (mean 11.37 ± 1.76 g/dL).

According to ECG and echocardiography 4 patients (13.3%) had normal ECG before PCI, 13 patients (43.3%) had nonspecific ECG changes, and 13 patients (43.3%) had significant ST changes, the patient's ejection fraction ranged between 40% and 72% (mean 61.60% ± 7.59%).

Items	Study patient (n = 30)	
	No	%
Sex		
Male	17	56.7
Female	13	43.3
Age/years		
Mean ± SD	60.00 ± 6.11	
Min - Max	48.00 - 70.00	
Smoking		
Non - smoker	18	60.0
Smoker	12	40.0
HTN	25	83.3
DM	18	60.0
HCV+ve	12	40.0
Creatinine		
Mean ± SD	1.05 ± 0.22	
Min - Max	0.70 - 1.40	
INR		
Mean ± SD	1.06 ± 0.16	
Min - Max	0.80 - 1.50	
HB		
Mean ± SD	11.37 ± 1.76	
Min - Max	9.00 - 15.00	
Hx of MI	4	13.3
Hx of CABG	0	0
ECG		
Normal	4	13.3
non specific change	13	43.3
sig.ST deviation	13	43.3
EF		
Mean ± SD	61.60 ± 7.59	
Min - Max	40.00 - 72.00	

Table 1: *Demographics and clinical characters of our patients.*

Legend: *SD: Standard Deviation; HTN: Hypertension; DM: Diabetes Mellitus; MI: Myocardial Infarction; CABG: Coronary Artery Bypass Graft; EF: Ejection Fraction.*

Coronary angiographic data

Coronary angiographic information of the studded patients is summarized in (Table 2). Among the 30 patients selected in our study: The most influenced artery was the LAD in 20 patients (66.7%) then the RCA in 11 patients (36.7%) and finally LCX in 8 patients (26.7%) and nobody had left main lesion. 21 patients (70%) experienced single vessel affection and 9 (30%) patients experienced multi vessel affection(Table 2, Figure 1,2)

	No. Pt.	%
Left anterior descending	20	66.7
Left circumflex	8	26.7
Right coronary artery	11	36.7
Left main	0	0
Single vessel disease	21	70
Multi-vessel disease	9	30

Table 2: *Coronary angiographic information among our cases.*

Legend: *LAD: Left Anterior Descending Artery; LCX, Left Circumflex Artery; RCA, Right Coronary Artery; LM, Left Main Artery.*

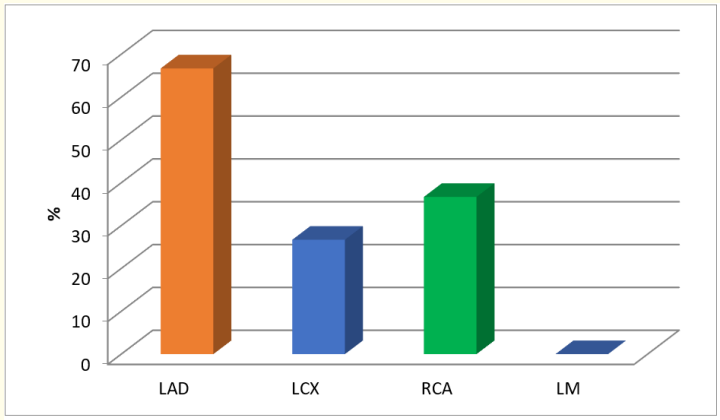

Figure 1: *Coronary angiographic information among our cases.*

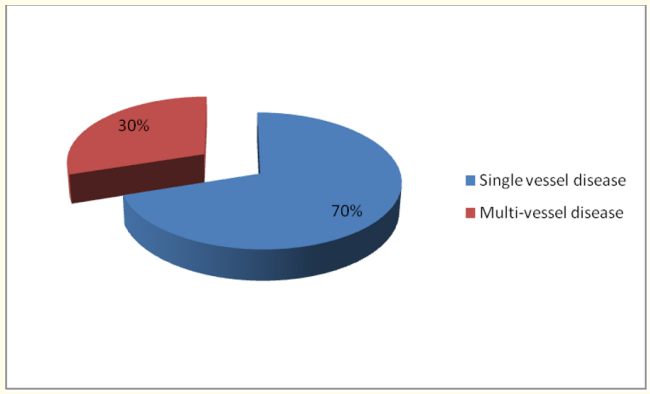

Figure 2: *Coronary angiographic information among considered cases as per the quantity of influenced vessels.*

In the 20 patients whom LAD was diseased, the proximal LAD was the most affected segment among 10 patients (33.3%), then mid segment among 8 patients (26.7%) and 2 patients (6.7%) had long lesion involve more than one segment, all of the stents which was used in LAD was DES (Table 3).

LAD	Study group (n = 30)	
	No	%
LAD		
Lesion	20	66.7
Segment		
Proximal	10	33.3
Mid	8	26.7
long lesion	2	6.7
Distal	0	0
type of stent		
DES	20	100
BMS	0	0

Table 3: *Coronary angiographic data among LAD affected patients.*

Legend: *LAD: Left Anterior Descending Artery; DES: Drug Eluting Stent; BMS: Bare Metal Stent.*

In the 8 patients whom LCX was diseased, the mid LCX was the most affected segment among 6 patients (20%), then proximal and distal segments each of them occur in 1 patient (3.3%), according to the type of stent used 4 stents was DES the other 4 stents was BMS (Table 4).

LCX	Study group (n = 30)	
	No	%
LCX		
Lesion	8	26.7
Segment		
Proximal	1	3.3
Mid	6	20.0
Distal	1	3.3
Long lesion	0	0
Type of stent		
BMS	4	50
DES	4	50

Table 4: *Coronary angiographic data among LCX affected patients.*

Legend: LCX: Left Circumfles Artery; DES: Drug Eluting Stent; BMS: Bare Metal Stent.

In the 11 patients whom RCA was diseased, the mid RCA was the most affected segment in 7 patients (23.3%), then proximal segment in 2 patients (6.7%), then the distal segment in 1 patient (3.3%) and 1 patient (3.3%) had long lesion involve more than one segment, 7 of the stents which was used in RCA was DES and 4 stents was BMS (Table 5).

Platelet aggregation

The patients Baseline Platelet aggregation to 5 mol/L ADP ranged between 46.7% and 80% (mean 62.27 ± 10.31%), while after 24 hours Platelet aggregation to 5 mol/L ADP ranged between 17.2% and 70% (mean 43.03 ± 14.04%), and after 5 days Platelet aggregation

RCA	Study group (n = 30)	
	No	%
RCA		
Lesion	11	36.7
Segment		
Proximal	2	6.7
Mid	7	23.3
Distal	1	3.3
Long lesion	1	3.3
Type of stent		
BMS	4	36.4
DES	7	63.6

Table 5: *Coronary angiographic data among RCA affected patients.*

Legend: RCA: Rightcoronary Artery; DES: Drug Eluting Stent; BMS: Bare Metal Stent.

to 5 mol/L ADP ranged between 15% and 68.6% (mean 39.16 ± 14.78%), and after 3 months Platelet aggregation to 5 mol/L ADP ranged between 13% and 65.2% (mean 36.30 ± 14.22%) (Table 6) (Figure 3).

Platelet aggregation	Study group (n = 30)			
	Basal	After 24h	After 5days	After3month
Mean *± SD*	62.27 ± 10.31	43.03 ± 14.04	39.16 ± 14.78	36.30 ± 14.22
Min-Max	46.70 - 80.00	17.20 - 70.00	15.00 - 68.60	13.00 - 65.20
Test of sig.	P1 = ≤0.001**, p2 = ≤0.001**, p3 = ≤0.001**, p4 = ≤0.001**, p5 = ≤0.001**, p6 = ≤0.001**			

Table 6: *Platelet aggregation [%] in response to 5 mol/L ADP.*

*(Paired t-test used) **: highly significance, p1: p value for contrasting amongst basal and after 24h, p2: p value for looking at amongst basal and following 5 days, p3: p value for looking at amongst basal and 3 month, p4: p value for looking at between following 24h and 5 days, p5: p value for looking at between following 24h and 3 month, p6: p value for looking at between 5 days and 3 month.*

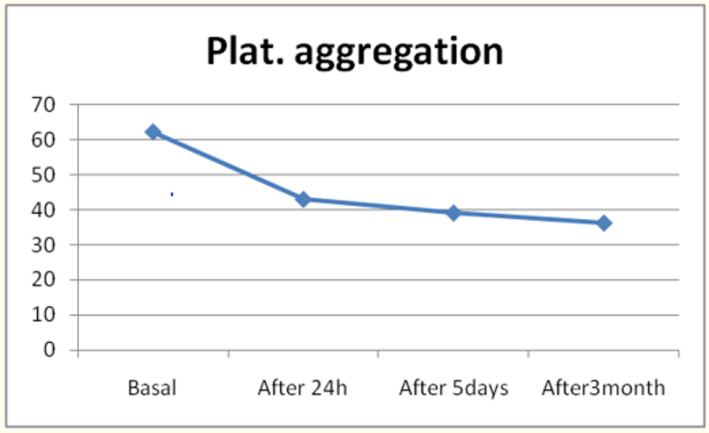

Figure 3: *Platelet aggregation [%] in response to 5 mol/L ADP.*

Drug resistance

At 24 hours in after stenting, 6 patients (20%) met the meaning of resistance, at 5 days subsequent to stenting, 6 patients (20%) met the meaning of resistance and at 3 months in the wake of stenting, 4 patients (13.3%) met the meaning of resistance (Table 7) (Figure 4)

Platelet aggregation	Resistant		Responder		p-value
	No	%	No	%	
After 24h	6	20.0	24	80.0	P1 = 1
After 5days	6	20.0	24	80.0	p2 = 0.5
After 3month	4	13.3	26	86.7	p3 = 0.5

Table 7: *Resistant and Responder patient after 24 hours, 5 days and 3 months.*

McNemar test used.

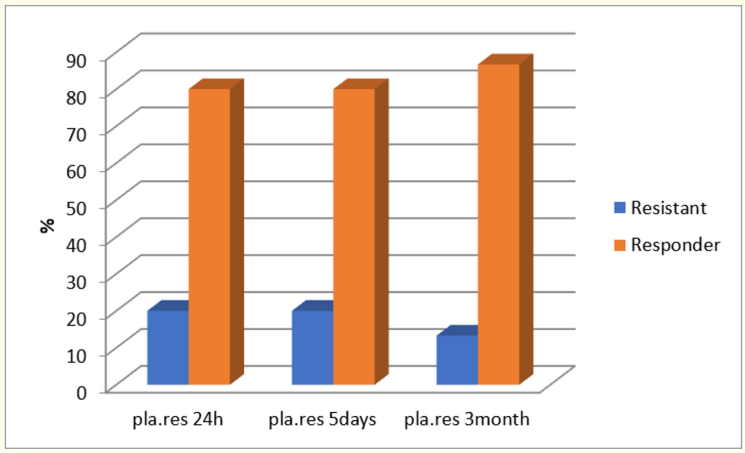

Figure 4: *Resistant and Responder patient after 24 hours, 5 days and 3 months.*

Molecular investigations

A series of procedures were performed in Medical Biochemistry Department, Faculty of Medicine, Mansoura University. These procedures included; DNA extraction from whole blood as in (Figure 5). DNA extraction was followed by PCR amplification of CYP2C19*2 gene as seen in (Figure 6). Conventional method of PCR amplification followed by restriction enzyme digestion for CYP2C19*2 gene (Figure 7).

The distribution of various genotypes of CYP2C19*2 among study population is shown in (Table 8) and (Figure 8). 27 (90%) patients had *1/*1 genotype while 3 patients (10%) had *1/*2 genotype of CYP2C19*2 gene.

Demographic characters of patients with CYP2C19*2 genotype *1/*1 is shown in Table 9. 27 patients (90%) was genotype *1/*1 with mean age among all the 27 patients was 59.62 ± 6.19 years, with their ages ranging from 48 to 70 years. The male/female distribution was 15 (55.6%) versus12 (44.4%). 10 patients were smokers (37%), 23 patients (85.2%) had documented hypertension, 16 patients (59.3%) had documented DM, 11 patients (40.7%) were HCV +ve.

Demographic characters of patients with CYP2C19*2 genotype *1/*2 is shown in Table 9. 3 patients (10%) was genotype *1/*2 with mean age among all the 3 patients was 63.33 ± 5.03years, with their ages ranging from 58 to 68 years. The male/female distribution was 2 (66.7%) versus1 (33.3%). 2 patients was smokers (66.7%), 2 patients (66.7%) had documented hypertension, 2 patients (66.7%) had documented DM, 1 patient (33.3%) was HCV +ve.

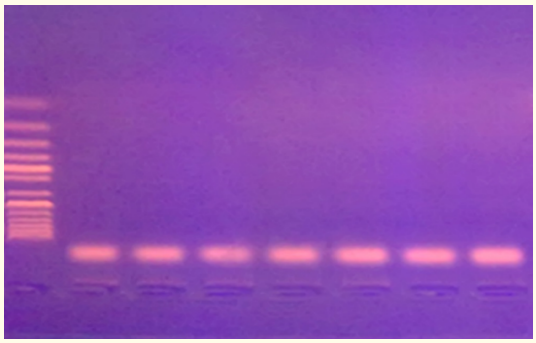

Figure 5: *Extracted genomic DNA gel electrophoresis: 1% agarose gel electrophoresis; lane 1: 1kb DNA ladder, Lanes 2 - 8: extracted genomic DNA.*

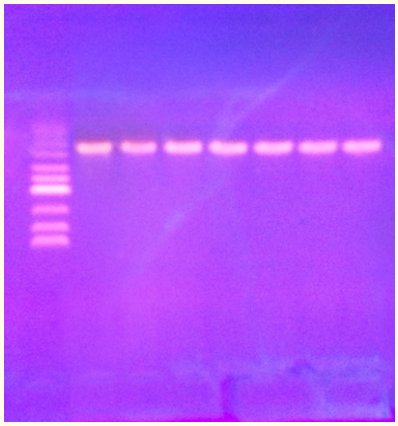

Figure 6: *PCR amplification for CYP2C19*2 geneson 3% agarose gel recolored by ethidium bromide. DNA was stacked in well 1 and the PCR-amplification results were stacked in wells 2 to 8.*

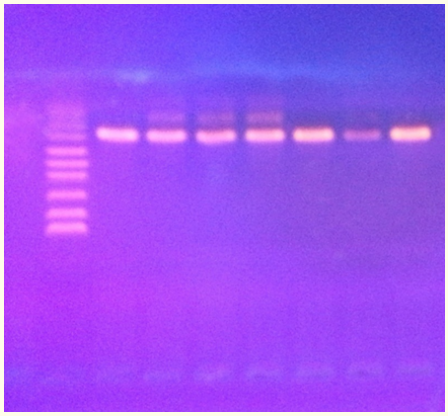

Figure 7: *PCR (SmaI absorption) fragmentaion designs on 3% agarose gel are recolored by ethidium bromide for CYP2C19*2, CYP2C19*2 *1/*1 genotypes (wells 2, 6, 7 and 8) and CYP2C19*2 *1/*2 genotypes (From 3 to 5 wells). DNA step was stacked in well 1 and the sizes of the PCR-fragments were 169 bp, 120 bp and 49 bp. 50 bp DNA marker was loaded inLane 1to show the sizes of thePCR-restriction fragments.*

Genotype	Study group (n = 30)	
	No	%
1*/1*	27	90.0
1*/2*	3	10.0

Table 8: The distribution of various genotypes of CYP2C19*2 among study population.

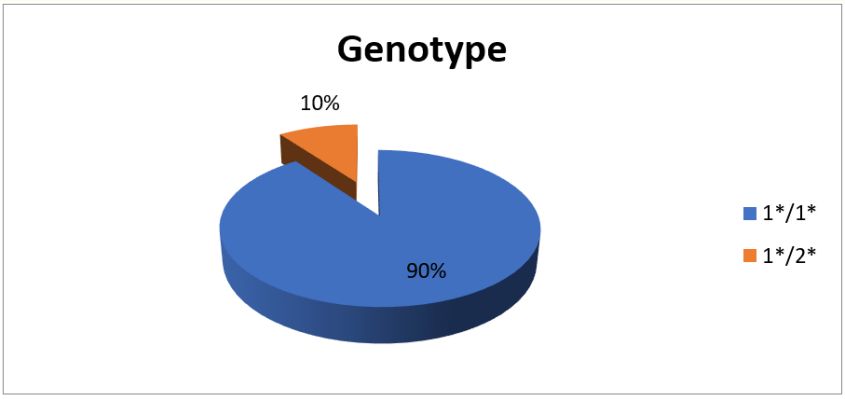

Figure 8: *The distribution of various genotypes of CYP2C19*2 among study population.*

Items	1*1* (n = 27)		1*/2* (n = 3)		Test of sig.	p-value
	No	%	No	%		
Sex						
Male	15	55.6	2	66.7	FET	1
Female	12	44.4	1	33.3		
Age/years						
Mean ± *SD*	59.62 ± 6.19		63.33 ± 5.03		t=0.99	0.329
Min-Max	48.00 - 70.00		58.00 - 68.00			
Smoking						
Non-smoker	17	63.0	1	33.3	FET	0.548
Smoker	10	37.0	2	66.7		
HTN	23	85.2	2	66.7	FET	0.433
DM	16	59.3	2	66.7	FET	1
HCV	11	40.7	1	33.3	FET	1

Table 9: *Demographic characters of patients with CYP2C19*2 genotype *1/*1 and genotype *1/*2.*

Legend*: FET: Fisher's Exact Test; t: Student t-test.*

Genotype and platelet aggregation

Table 10 show that there was no significant difference in the basal sample of platelet aggregation before administration of the loading dose of clopidogrel between patients of genotype *1/*1 and *1/*2. Basal platelet aggregation in patients of genotype *1/*1 ranged

between 46.7% and 80% with mean aggregation 62.37 ± 10.48% while Basal platelet aggregation in patients of genotype *1/*2 ranged between 52.5% and 73% with mean aggregation 61.33 ± 10.53%.

Plat. Aggregation	1*1* (n = 27)		1*/2* (n = 3)		Test of sig.	p-value
	Mean ± SD	Min-Max	Mean ± SD	Min-Max		
Plat. aggregation basal	62.37 ± 10.48	46.70 - 80.00	61.33 ± 10.53	52.50 - 73.00	t = 0.24	0.809
plat. agreg.24h	40.74 ± 12.82	17.20 - 70.00	63.60 ± 3.93	60.40 - 68.00	t = 2.45	0.014*
plat. agreg.5days	36.67 ± 13.34	15.00 - 68.60	61.53 ± 4.48	58.70 - 66.70	t = 2.45	0.014*
plat. agreg.3 month	33.84 ± 12.66	13.00 - 58.50	58.40 ± 5.88	55.00 - 65.20	t = 2.52	0.012*

Table 10: *Genotype and Platelet aggregation.*

Legend: t: Student t-test.

**Statistically significant at p ≤ 0.05.*

***: highly significance p ≤ 0.001.*

After 24 hours there was great alterations in platelet aggregation between patients of genotype *1/*1 and *1/*2. Platelet aggregation in patients of genotype *1/*1 ranged between 17.2% and 70% with mean aggregation 40.74 ± 12.82% while in patients of genotype *1/*2 ranged between 60.4% and 68% with mean aggregation 63.60 ± 3.9%.

After 5 days there was great alterations in platelet aggregation between patients of genotype *1/*1 and *1/*2. Platelet aggregation in patients of genotype *1/*1 ranged between 15% and 68.6% with mean aggregation 36.67 ± 13.34% while in patients of genotype *1/*2 ranged between 58.7% and 66.7% with mean aggregation 61.53 ± 4.48%.

After 3 months there was great alterations in platelet aggregation between patients of genotype *1/*1 and *1/*2. Platelet aggregation in patients of genotype *1/*1 ranged between 13% and 58.5% with mean aggregation 33.84 ± 12.66% while in patients of genotype *1/*2 ranged between 55% and 65.2% with mean aggregation 58.4 ± 5.88%.

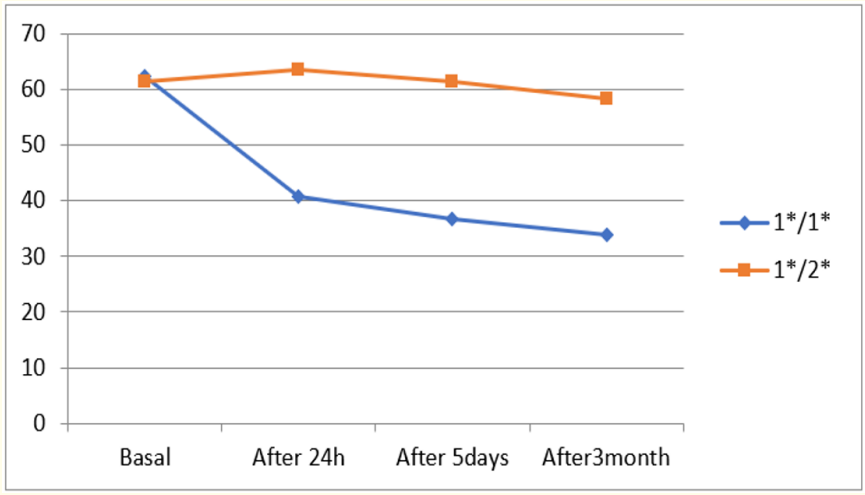

Figure 9: *Genotype and Platelet aggregation.*

Genotype and drug resistance

In our study we found that 3 patients with genotype *1/*1(11.1%) met the criteria of resistance after 24 hours and after 5 days from the basal sample, while 1 patient only with genotype *1/*1(3.7%) met the criteria of drug resistance after 3 months (Table 11).

On the other hand all the 3 patients with genotype *1/*2(100%) met the critria of drug resistance after 24hours, 5 days and 3 months (Table 11) (Figure 10).

Drug resistance	1*1* (n = 27)		1*/2* (n = 3)		p-value
	No	%	No	%	
After 24h	3	11.1	3	100.0	0.005*
After 5 days	3	11.1	3	100.0	0.005*
After 3 month	1	3.7	3	100.0	0.001*

Table 11: *Genotype and Drug resistance.*

Fisher's Exact Test used.

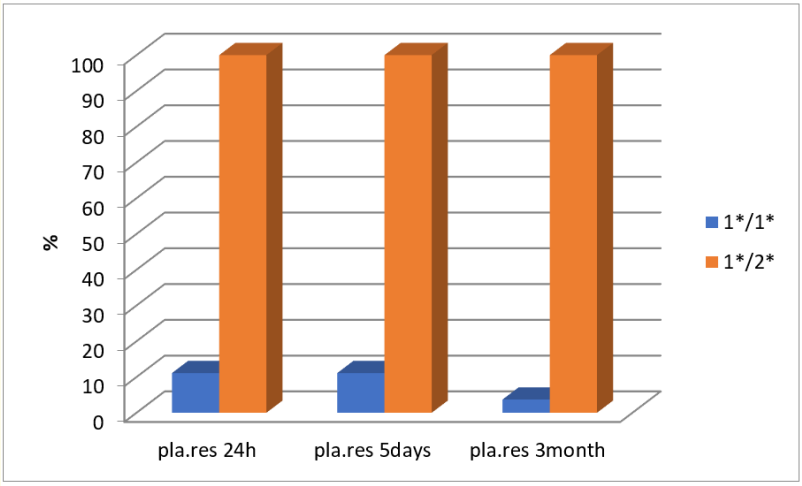

Figure 10: *Genotype and drug resistance.*

Discussion

Clopidogrel is a prodrug that requires activation into its dynamic metabolite before it focuses on the P2Y12 receptor on blood platelets. *In vivo* activation of the medication is a 2-stage process that is firmly connected to the cytochrome P450 enzymes. Diverse isoenzymes are in charge of clopidogrel activation and among them the isoenzyme CYP2C19 was found to assume a key part in this setting by adding to both clopidogrel activation steps [14].

There are no impacts of clopidogrel on any receptor other than P2Y12 to clarify the size of the clinical advantage. The majority of the set up clinical impacts are ascribed to diminished platelet responsiveness to ADP [15]. Along these lines, the patient with insufficient P2Y12 hindrance dictated by ex vivo testing sensibly has an expanded danger for thrombosis. Recurrent ischemic events and the variable AntiPlatelet clopidogrel effect are 2 powerful contentions against the utilization of clopidogrel [16].

The CYP2C19 polymorphism has a wide interethnic variations, stretching out from 25–30% among Caucasians to 35 - 45% among African-Americans and 50–60% in East Asians, whereby the CYP2C19*2 allele is the most frequent defective allele (75 - 80% in Caucasians and East Asians) [17].

The CYP2C19 isoenzyme is included in both stages of clopidogrel biotransformation and seems to have critical impact. The loss of function (LoF) alleles of CYP2C19*2 are most ordinarily marked as *1/*2 (GA) and *2/*2 (AA), while the no LoF alleles of CYP2C19*2 are normally named as *1/*1 (GG) by numerous studies [18].

Many studies have exhibited a relation between carriage of CYP2C19 loss-of-function alleles, particularly of the CYP2C19*2 loss-of-function allele, or of any two CYP2C19 loss-of-function alleles (*2,*3,*4, or *5) and a more rate of unfavorable cardiovascular occasions like stent thrombosis [19].

The meaning of clopidogrel resistance is essential. Most studies characterize clopidogrel resistance as indicated by ADP-hindered rate ≤ 10% [20,21]. ADP-induced maximal platelet aggregation (MPA) ≥50 [22,23], and vasodilator phosphoprotein (VASP) index ≥50% [24,25]; by the way, few studies define it as ADP inhibited percentage ≤20% [26], ADP-inhibited percentage ≤30% [27], or ADP-induced MPA ≥70% [28].

The present study is prospective Cohort study that performed at Specialized Medical Hospital "Cardiology Department", Mansoura University Hospitals. The study involved 30 patients with coronary artery disease undergoing coronary angiography and waiting for implantation of DES or BMS.

The study sample involved 30 patients who met the study inclusion criteria, with a diagnosis of coronary artery disease. The male/female ratio was (52.7%) versus (43.3%); and the mean age was 60.00 (± 6.11) years.

Platelet aggregation and drug resistance

The patients Baseline Platelet aggregation to 5 mol/L ADP ranged between 46.7% and 80% (mean 62.27 ± 10.31%), while after 24 hours Platelet aggregation to 5 mol/L ADP ranged between 17.2% and 70% (mean 43.03 ± 14.04%), and after 5 days Platelet aggregation to 5 mol/L ADP ranged between 15% and 68.6% (mean 39.16 ± 14.78%), and after 3 months Platelet aggregation to 5 mol/L ADP ranged between 13% and 65.2% (mean 36.30 ± 14.22%).

At 24 hours after PCI, 6 patients (20%) met the definition of resistance, at 5 days after PCI, 6 patients (20%) met the definition of resistance and at 3 months after PCI, 4 patients (13.3%) met the definition of resistance.

In another study Samples were obtained at baseline and at 2 hours, 24 hours, 5 days, and 30 days after coronary interference [29]. Baseline aggregation to 5 mol/L ADP was 62 ± 18%, Platelet aggregation was 58 ± 22% when detected after 2 hours, 37 ± 22% when detected after 24 hours, 32 ± 18% when detected after 5 days, and 31 ± 15% when detected after 30 days. At 2 hours after PCI, 63% of patients met the meaning of resistance and at 24 hours, resistance tumbled to 31%. No further changes were seen at 5 days as resistance was seen in 31%. At 30 days after PCI, the rate of resistance tumbled to 15%.

Also in another study Samples were obtained before clopidogrel administration (baseline) and second sample after 4 hours [30]. Baseline aggregation to 5 mol/L ADP was 65 ± 21%, 5% of patients met the definition of resistance after 4 hours with aggregation to 5 mol/L ADP was 67 ± 24% while 90% of patients were responders with aggregation to 5 mol/L ADP fall to 15 ± 16%.

In another study [31], Blood specimens were obtained from 110 patients for platelet assay before and 24 h after administration of clopidogrel, platelet aggregation rate at pre-and-post treatment was determined, patients with laboratory clopidogrel low responders (ΔA < 10%) and non clopidogrel low responders (ΔA > 10%) was 26 (23.6%) and 84 (76.4%).

In the study of (Campo., *et al.* 2011), Platelet function assay and clopidogrel low response definition was done by different method using Verify Now P2Y12 assay, the results are formed in P2Y12 reaction units (PRU). Clopidogrel low response was detected when a PRU value ≥ 235. Blood specimens were taken at baseline (just before PCI and 12 hours after loading dose of clopidogrel) and at 1 and 6 months after PCI. According to their specified definition of Clopidogrel poor response, 36%, 13%, and 13% patients are clopidogrel low responders basally, one month, and 6 months, respectively [32].

CYP2C19*2 genotyping

The distribution of various genotypes of CYP2C19*2 among our study population were:27 (90%) patients had *1/*1 genotype while 3 patients (10%) had *1/*2 genotype of CYP2C19*2 gene while no patient had *2/*2 genotype.

The distribution of various genotypes of CYP2C19*2 in other studies were: 70% were CYP2C19 (*1/*1), 28.6% were CYP2C19 (*1/*2) and 2.1% were (*2/*2) [33], while in another study 69% were CYP2C19 (*1/*1), 29% were CYP2C19 (*1/*2) and 2% were (*2/*2) [34] and also in another study included 110 patients, 59 patients (53.6%) were CYP2C19 (*1/*1), 43 were (39.1%) CYP2C19 (*1/*2), and 8 were (7.3%) (*2/*2) [31].

In our study There were no significant alterations between patients with genotype *1/*1and patients with genotype *1/*2 in baseline demographic and clinical characteristics.

This finding is in agreement with (Yamamoto., *et al*. 2011) who found that there were no great alterations in patient characters among different genotypes [35].

Also (Trenk., *et al*. 2008) found that there were no noteworthy contrasts between patients with the allelic variation CYP2C19*2 aside from a marginally higher extent of smokers and a somewhat higher extent of patients with B2 or C coronary lesions as indicated by the American Heart Association/American College of Cardiology definitions in the group having the *2 allelic variation of CYP2C19 [33].

In the present study there was no significant difference in the basal sample of platelet aggregation before administration of the loading dose of clopidogrel between patients of genotype *1/*1 and *1/*2, However there was significant difference in platelet aggregation between patients of genotype *1/*1 and *1/*2 after 24 hours, 5days and 3months from administration of the loading dose of clopidogrel.

According to our definition of clopidogrel resistance, All patients with genotype *1/*2 show clopidogrel resistance after 24 hours, 5days and 3months from taking the first dose of clopidogrel while 3patients only with genotype *1/*1 show clopidogrel resistance after 24 hours and 5days from taking the first dose of clopidogrel and after 3months from taking the first dose of clopidogrel only one only with genotype *1/*1 show clopidogrel resistance.

This result is in agreement with (Hulot., *et al*. 2006) who reported that the reaction to clopidogrel was unequivocally impacted by the CYP2C19 genotypic status. Twenty of the 28 subjects were CYP2C19 (*1/*1), and the other 8 subjects were CYP2C19 (*1/*2). At standard, platelet accumulation within the sight of ADP did not vary altogether as indicated by the CYP2C19 genotype. After treatment with clopidogrel 75 mg once every day, platelet aggregation in light of ADP continuously diminished in *1/*1 subjects, while it didn't change in *1/*2 subjects [36].

Also this finding is in harmony with previously published meta-analysis of 8 studies which was conducted to evaluate the association between CYP2C19*2 gene polymorphism and clopidogrel resistance reflected by platelet function assays and suggested that CYP2C19*2 gene polymorphism may be associated with clopidogrel resistance which was reflected by platelet function assays. This finding may potentially be used in considering individual AntiPlatelet therapy with clopidogrel. In order to reach a more definitive conclusion, a standard definition of clopidogrel resistance is needed for future studies [18].

However, (Nasyuhana Sani., *et al*. 2013)**2013** studied the impact of The CYP2C19∗1/∗2 Genotype on clopidogrel effect in normal Malaysian persons and found that CYP2C19*2 allele was significantly connected with hindered clopidogrel effectiveness. This is not just found in persons with two LoF CYP2C19∗2 alleles (*2/*2), additionally in persons with one LoF CYP2C19∗2 (*1/*2) and CYP2C19 (∗1/∗1) alleles. This perception suggests that a diminished AntiPlatelet impact of clopidogrel happens regardless of CYP2C19*2 genotype status [37].

And in another Malaysian study. There was no huge alteration between the clopidogrel effect in poor metabolizres, intermediate metabolizers and extensive metabolizres which may be due to significant inter-ethnic difference in the distribution of CYP2C19 polymorphism [38].

Conclusion

In conclusion, our study suggests that CYP2C19*2 gene polymorphism may be associated with clopidogrel resistance which was reflected by platelet aggregation according to the definition that we used. In order to reach a more definitive conclusion, a standard definition of clopidogrel resistance is needed for future studies.

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