Role of Platelet Reactivity Index (PRI) in Antiplatelet Therapy and its Association with Vasodilator Stimulated Phosphoprotein (VASP) for Iraqi Patient Undergoing PCI.

Bassam A. Alzubaidi[1], Abdulkareem H. Abd[2], Hayder Abdulhafedh.Kurji[3]
[1,2] Dept. of Pharmacology, College of Medicine, Al-NahrainUniversity, Baghdad, Iraq.
[3] Bilad Alrafidain University College/Iraq
bassam_ph1991@yahoo.com

ABSTRACT

Background: VASP, an actin-regulatory protein family member, controls platelet adhesion .Ticagrelor isthe first member of a brand-new agent of chemicals, thecyclopentyltriazolopyrimidines, is Ticagrelor. ADP's prothrombotic effects can be prevented by Ticagrelor's ability to block the platelet P2Y12 receptor, just as the thienopyridines. Dissimilar the thienopyridines, which are irreversible inhibitors , reversibly binding to the P2Y12 receptor, Ticagrelor almost entirely blocks ADP-stimulated platelet aggregation, as demonstrated in vitro. Because it does not require metabolic activation, Ticagrelor is an alternative to the thienopyridines. All patients receiving Ticagrelor achieve adequate antiplateleteffects. **Objective:** Inthis study, we examined the roleof platelet reactivity index (PRI) in antiplatelet treatment and its association with vasodilator stimulated phosphoprotein (VASP) for Iraqi patient undergoing percutaneouscoronary intervention (PCI). **Methods:** In this study, patients who were eligible for PCI were included. At least two weeks after a 90-mg dose of ticagrelor was administered following PCI, blood samples were collected from patients. Vasodilator-stimulated phosphoprotein phosphorylation assay (VASP-P) was used to detect the inhibition percentage of platelet adhesion. Consistently high or low levels of platelet activationon therapy can be identified by the evaluation ofplatelet reactive index (PRI) using the VASPELISA assay.

Results: The body mass index (BMI) was significant higher in patients with coronary heartdisease contrasted to thecontrol group ($P < 0.001$). There was a significant difference inthe platelets count in patientswith coronary heart disease in comparison tothe control group ($p<0.05$). The study groups were categorized according to the PRI-VASP % and it was found that (26.46 ± 0.75) of the patients while on (80.04 ± 0.98) of the control. The PRI-VASP % in patients was a strong significantly increased in comparison to PRI-VASP % in control group (26.46 ± 0.75versus 80.04 ± 0.98, $P<0.001$) respectively. According to the findings, the subjects were grouped into the mean of serum VASP, and it was found that 139.56 ± 13.66 ng/L of the patients while on 238.85 ± 20.74 ng/L of the control. The mean of VASP level in patients was a strong significantly decreased in comparison to mean of serum VASP level mean in control group (24.97 ± 0.33 ng/Lversus 22.55 ± 0.28 ng/L $P<0.001$) respectively. **Conclusion:** Ticagrelor medication protects all patients, according to present findings in this study in Iraqi population. Also, there was no association between VASP phosphorylation and VASP.

Keywords: Vasodilatorstimulated phosphoprotein, Platelet reactivity, PCI

1. Introduction

In the world, coronary artery disease (CAD) isthe most frequentdisease. This is the most lifethreatening phase ofcoronary artery disease (CAD). When it comes to ACS patients, the disease's prevalence and management vary greatly from country to country [1].

VASP, an actin-regulatory protein family member, controls platelet adhesion. Phosphorylation of VASP is another tool used to evaluate platelet reactivity in patients taking antiplatelet medications called P2Y12 receptor antagonists. As a result, the contribution of VASP in the formation of thrombus and the antiplatelet impact of P2Y12 receptor antagonists is unclear [2]. The cyclic nucleotide-dependent kinases PKG and PKA regulate the human Vasodilator Stimulated Phosphoprotein (VASP) gene, which encodes a protein known as vasodilator-stimulated phosphoprotein [3].

Glycoprotein-stimulated protein phosphorylation (GSP) has been shown to inhibit agonist-stimulated signaling of calcium, fibrinogen binding, aggregation and adhesion in platelets, platelets' phosphorylation of the cytoskeletal and focal adhesion protein VASP is also stimulated [4].

Men andwomen worldwide die as a result ofcoronary artery atherosclerosis. An abnormality in the walls of the heart's arteries caused by atherosclerotic plaques is the most prevalent symptom of heart disease. Endothelial dysfunction, accumulation of lipids, calcium, vascular inflammation, cellular debris, and cholesterol, in the intima of the vessel wall characterize atherosclerosis, adisease ofthe large and mediumsized muscle arteries. As a result of this Atherosclerotic buildup there is vascular remodeling, Plaque development acute and chronic luminal blockage, and irregularities in blood flow with reduced oxygen supply into the heart [5].

Ten to fifteen years of full growth of atherosclerotic plaques (or atheromas) [6]. People often begin to predispose symptoms of early atherosclerosis in their heart arteries and aorta bythe age of 20 [7]. The Glagov

doi.org/10.31838/hiv22.02.33 Received: 12.01.22, Revised: 13.05.22, Accepted: 13.08.22

phenomenon (positive remodeling) is an outward remodeling [8] a situation inwhich the artery wallbulges outward but the lumen residues intact although these plaques continue to grow, they are unlikely to induce angina since they are not hemodynamically significant for a lengthy period of time. Plaque doesnot started to intrude into the lumen before it takes up 40% of the crosssectional area of the vessel.

2. Materials and Methods

Study design

In a study group of Iraqi patients, this is a crosssectional study that was conducted prospectively. Between 10 February and 1 July 2021, the research was conducted. Both Baaquba teaching hospital's Specialized Ward for Cardiac Surgery and ASCO lab (Advanced Scientific Learning Center) private center in Baghdad were used for the lab work, which was done in two laboratories. 80 participants (male and female) between the ages of 35 and 65 were included in this study. From the Baaquba teaching hospital Specialized ward for Cardiac Surgery in Diyala were recruited patients with CADs, including unstable angina, acute MI and acute coronary syndrome, those undergoing PCI, who were already receiving antiplatelet therapy at least two weeks after PCI (ticagrelor plus aspirin).

All Iraqi patients of Arabic origin (35-65 y.) Participants comprised those with unstable angina, acute MI, or who had undergone PCI and were taking ticagrelor and aspirin for at least two weeks after PCI. Patients with hepatic and renal failure as well as those patients received macrolide , INH, ketoconazole.

The InstituteReview Board of theMedical College /Al-Nahrain University granted ethical approval for this investigation. To ensure compliance with the Declaration of Helsinki, all patients enrolled in this study were made aware of the test that would be performed and submitted written informed consent.

Data Collection

Clinical researchers at Ba'aquba Teaching Hospital, comprising nurses and residents, obtained all of the information. Trained technicians input the Case ReportForms into thedatabase twice. Two auditors took a 5% to 10% random sample of medical records. Medical records were used to verify the accuracy of the provided data. A supervisor determined the variable when therewas a disagreement over its final value. All patients who were discharged from the study alivewere required to return for an outpatient follow-up visit one month after surgery as part of the present study protocol. Medicinal records inthe outpatient clinic were reexamined for those who had reported any adverseeffects over the onemonth period. If a patient had been treated elsewhere for a serious medical condition, they were expected to mail a copy of their records to the clinic. Overall, there was a 100% success rate.

Vasodilatorstimulated phospho-protein phosphorylationassay

An skilled technician utilized platelet VASPkits (Biocytex Co Ltd, Marseille, France) per the instructions in the manual to do the VASP phosphorylation test following blood collection. ADP or prostaglandin E1 were used to mix blood samples invitro. After a 16C2FITC antibody incubation, a goat antimouse fluorescence staining in isothiocyanate polyclonal reagent was used to identify each blood sample. A vasodilator-stimulated phosphoprotein phosphorylation on platelet serine239 was recognized using an enzyme-linked immunosorbent test in fresh whole blood. The optical density at 450 nm was used to compute PRI when prostaglandin E1 or ADP were introduced to the samples.

Statistical analysis

The mean ± SEof mean were calculatedby using the IBM SPSS version 28.0. WinPepi version 11.65 calculates the odd ratio, 95 percent confidence interval, and Fisher's exact test when it comes to genotyping and allele frequencies [9]. Students' T-tests and ANOVA tables were used for calculating the likelihood ratio. Such. A Hardy-Weinberg calculator on the online can be used to estimate allele frequencies [10].

3. RESULTS

From 10 February 2021 to 1 July 2022, 100 participants, 50 of them were CADs patients and the other 50 were healthy individuals (controls).The age mean of the patients was (56.78 ± 1.02) years, while the mean age of healthy persons involved in this study was (47.93 ± 1.89) years . The mean of body mass index (BMI) for patients was (26.09 ± 2.33 Kg/m²) versus (23.55 ± 0.28 Kg/m²) to control. Including the underlying diseases, the patient group had 40 (80.0 percent) hypertension, 30 (60.0 percent) diabetes, 9 (18 percent) dyslipidemia, and 10 (20.0 percent) had no other disease, while the control group had 20 (40.0 percent) hypertension, 10 (20.0 percent) diabetes, 5 (10 percent) dyslipidemia, and 10 (20.0 percent) had no other disease as presented in (Table 1).

According to the findings, the subjects were grouped into the mean of serum VASP and it was found that 139.56 ± 13.66 ng/L of the patients while on 238.85 ± 20.74 ng/L of the control. The mean of VASP level in patients was a strong significantly decreased in comparison to mean of serum VASP level mean in control group (24.97 ± 0.33 ng/Lversus 22.55 ± 0.28 ng/L P<0.001) respectively as presented in (Table 1).

The study groups were categorized according to the PRI-VASP % and it was found that (26.46 ± 0.75) of the patients while on (80.04 ± 0.98) of the control. The PRI-VASP % in patients was a strong significantly increased in comparison to PRI-VASP % in control group (26.46 ± 0.75versus 80.04 ± 0.98, P<0.001) respectively as presented in (Table 1).

TABLE 1. Demographics and baseline characteristics

Baseline characteristics	AT(n=50)	control(n=50)	P value
Age(y)(mean ± SE)	56.78 ± 1.02	47.93 ± 1.89	< 0.001

Gender			
Male	36 (72%)	24 (48%)	
Female	14 (28%)	26 (52%)	
BMI(Kg/m2)	25.97 ± 0.33	23.55 ± 0.28	< 0.001
Hypertension	40 (80.0%)	20 (40.0%)	
Dyslipidemia	9 (18.0%)	5 (10.0%)	
Diabetes mellitus	30 (60.0%)	10 (20.0%)	
Platelets count (*103/uL)	230.60 ± 5.78	213.16 ± 5.69	0.034
Serum Albumin (g/l)	35.94 ± 0.36	42.14 ± 0.73	< 0.001
Alkaline phosphatase (U/L)	79.46 ± 3.22	69.08 ± 2.22	< 0.05
Creatinine (mg/dl)	0.87 ± 0.02	0.86 ± 0.03	0.951
Urea (mg/dl)	41.21 ± 0.99	36.94 ± 0.80	0.001
Serum VASP.(ng/l)	139.56 ± 13.66	238.85 ± 20.74	< 0.001
PRI-VASP %	26.46 ± 0.75(19-38)	80.04 ± 0.98(70-90)	< 0.001
Statins	40 (80.0%)	20 (40.0%)	
B-blocker	50 (100%)	15 (30%)	
Values are mean±SE or n(%).n, sample size. AT, Aspirin+ticagrelor. BMI, bodymass index. SE, standard error.VASP, vasodilator phospho-protein. PRI, platelet reactivity-index.			

Clinical Outcomes

Antiplatelet therapy patients in the AT group did not experience any life-threatening bleeding during the course of the research, and mild hemorrhage was no statistical differences between thetwo groups. Chest drainage volumeswere also found to be similar among patients. Within thefirst 30 days following surgery, no one died, no one suffered a MI, and noone required additional revascularization procedures were needed.

4. Discussion

VASP is abundant in platelets and is phosphorylated in response to drugs that boost intracellular cAMP and cGMP levels, such as vasodilators and platelet inhibitors. VASP's mechanism of platelet inhibition is unclear, however phosphorylation appears to be a key factor [3].

In this circumstance, P2Y12 receptor inhibition can be reflected by the level of VASP phosphorylation. PRI-VASP% value was estimated in 100 participants (50 patients with cardiovascular diseases using antiplatelet therapy and 50 healthy person as controls) have been randomly selected from our samples werean ELISAkit was used to perform the tests. In these results indicated that there was a strong significant decrease of PRI-VASP% value in the patients with cardiovascular diseases using antiplatelet (ticagrelor) therapy compared with a value of controls group.

The PRI-VASP % seen in this investigation in patients with CAD receiving antiplatelet medication is agreement with other studies reported by Xu and his coworkers [11], they were found a strong significant decrease of PRI-VASP% value in patients with cardiovascular diseases using antiplatelet (ticagrelor) therapy when compared to PRI-VASP% value in controls group. In keeping with our findings, Ding et al discovered that the of PRI-VASP% value in Chinese patients was much lower than in the control group [12]. On day 45, Kubica and his coworkers [13] observed that the PRI percentage value at 90mg MD of ticagrelor was 14.1.

VASP serum levels was estimated in 100 participants (50 patients with CAD using antiplatelet therapy and 50 healthy person as controls) have been randomly selected from our samples were measuredby using ELISA kit .

VASP levels in the serum of individuals with CAD were found to be significantly lower, according to our findings using antiplatelet therapy compared with the levels of this protein in the serum of controls group.

These findings could be simply CADs are inflammatory processes [14]. When it comes to inflammation, proteases areversatile enzymes, chiefly extracellularmatrix degrading enzymes, thatplay a role in a wide range ofphysiological processes. Dysregulation and over-expression of these genes during inflammation can have devastating effects, encouraging the development of vascular disorders as a result of their involvement [15]. An enzyme called a protease is responsible for the breakdown of proteinsinto smaller polypeptides or single amino acids, which is called proteolysis [16]. As a result, VASP is reduced in the patient group contrasted with control group.

This parameter is inactive form and included to support my thesis via as an extra parameter and could give me an idea that it will not be useful in the future. It's worth noting may that no one has ever measured the level of serum VASP in patients with CADs , this may be no clear knowledge about its protein kinetics .

From our data there was no association between VASPphosphorylation and VASP (r = 0.027). These findings may be supported by Fedor and his co-workers [17] when they said that the inhibition of the P2Y12 receptor is correlated with VASP phosphorylation, whereas the active form of P2Y12 is correlated with VASP non-phosphorylation. It is noteworthy an adequate response to target treatment is defined as a PRI value below 50%, which indicates a lower risk of post-PCI stent thrombosis ,myocardial infarction , and cardiac death [17].

This study concluded that tailoring ticagrelor to platelet reactivity as evaluated by PRI is safe and can enhance significantly clinical outcomes in patients taking the drug after PCI. Ticagrelor medication protects all patients, according to present findings in this study. The outcome is a PRI value in the range of 0-100 percent, which represents platelet reactivity to ADP.

From our data there was no association between VASPphosphorylation and VASP.

5. Study Limitation

The study may have significant limitations, involving a small sample population and the analysis of SNPs. Antiplatelet therapy guidelines at the time of this study recommended a loading doseof 180 mg of ticagrelor, therefore no comparison ofthe 90 mg and 180 mg loading doses of ticagrelor could be made. Also more one method of PRI computing can be used.

6. Acknowledgments

I'd want to show my thanks to all of the doctors, nurses, and other hospital employees that assisted with the recruitment of study participants.

7. Conflict of interest

There aren't any potential conflicts.

8. Funding

This article was written without the assistance of any funding agency on the part of the author.

9. List of abbreviation

CAD = Coronaryartery disease, ACS = Acute coronary syndromes, ADP = adenosinediphosphate, AT =aspirin+ticagrelor, PRI =platelet reactivity index, VASP-P = vasodilatorstimulated phosphoprotein phosphorylation , VASP = vasodilatorstimulated phosphoprotein, PCI =Percutaneouscoronary intervention. ,ELISA = enzymelinked immunosorbentassay.

References

1. Hamid MB. Clinical Characteristics and Outcomes of Acute Coronary Syndromes in a Group of Iraqi Patients. Iraqi Journal of Medical Sciences. 2016;14(4). Available from: https://www.iraqijms.net/upload/pdf/iraqijms5888e4c5601a6.pdf

2. Ito Y, Ohno K, Morikawa Y, Tomizawa A, Mizuno M, Sugidachi A. Vasodilator-stimulated phosphoprotein (VASP) is not a major mediator of platelet aggregation, thrombogenesis, haemostasis, and antiplatelet effect of prasugrel in rats. Scientific reports. 2018;8(1):1-11. https://doi.org/10.1038/s41598-018-28181-8

3. Harbeck B, Hüttelmaier S, Schlüter K, Jockusch BM, Illenberger S. Phosphorylation of the vasodilator-stimulated phosphoprotein regulates its interaction with actin. Journal of Biological Chemistry. 2000;275(40):30817-25. https://doi.org/10.1074/jbc.M005066200

4. Schwarz UR, Walter U, Eigenthaler M. Taming platelets with cyclic nucleotides. Biochemical pharmacology. 2001;62(9):1153-61. https://doi.org/10.1016/S0006-2952(01)00760-2

5. Tardif J-C. Coronary artery disease in 2010. European heart journal supplements. 2010;12(suppl_C):C2-C10. https://doi.org/10.1093/eurheartj/suq014

6. Stary HC, Chandler AB, Dinsmore RE, Fuster V, Glagov S, Insull Jr W, Rosenfeld ME, Schwartz CJ, Wagner WD, Wissler RW. A definition of advanced types of atherosclerotic lesions and a histological classification of atherosclerosis: a report from the Committee on Vascular Lesions of the Council on Arteriosclerosis, American Heart Association. Circulation. 1995;92(5):1355-74. https://doi.org/10.1161/01.CIR.92.5.1355

7. Samady H, Eshtehardi P, McDaniel MC, Suo J, Dhawan SS, Maynard C, Timmins LH, Quyyumi AA, Giddens DP. Coronary artery wall shear stress is associated with progression and transformation of atherosclerotic plaque and arterial remodeling in patients with coronary artery disease. Circulation. 2011;124(7):779-88. https://doi.org/10.1161/CIRCULATIONAHA.111.021824

8. Korshunov VA, Schwartz SM, Berk BC. Vascular remodeling: hemodynamic and biochemical mechanisms underlying Glagov's phenomenon. Arteriosclerosis, thrombosis, and vascular biology. 2007;27(8):1722-8. https://doi.org/10.1161/ATVBAHA.106.129254

9. Abramson JH. WINPEPI updated: computer programs for epidemiologists, and their teaching potential. Epidemiologic Perspectives & Innovations. 2011;8(1):1-9. https://doi.org/10.1186/1742-5573-8-1

10. McMurran SL. The Hardy-Weinberg Principle. Primus. 2010;20(6):529-49. https://doi.org/10.1080/10511970.2010.489544

11. Xu F, Feng W, Zhou Z, Zhang Y, Diao X, Hu S, Zheng Z. Antiplatelet effects of ticagrelor versus clopidogrel after coronary artery bypass graft surgery: A single-center randomized controlled trial. The Journal of Thoracic and Cardiovascular Surgery. 2019;158(2):430-7. e4. https://doi.org/10.1016/j.jtcvs.2018.10.032

12. Ding P, Wei Y, Chen N, Liu H. Validation of a New ELISA-Based Vasodilator-Associated Stimulated Phosphoprotein Phosphorylation Assay to Assess Platelet Reactivity Index in a Chinese Population. Clinical and Applied Thrombosis/Hemostasis. 2018;24(3):452-61. https://doi.org/10.1177%2F1076029616689300

13. Kubica J, Adamski P, Buszko K, Barańska M, Sikora J, Marszałł MP, Sobczak P, Sikora A, Kuliczkowski W, Fabiszak T. Platelet inhibition with standard vs. lower maintenance dose of ticagrelor early after myocardial infarction (ELECTRA): a randomized, open-label, active-controlled pharmacodynamic and pharmacokinetic study. European Heart Journal-Cardiovascular Pharmacotherapy. 2019;5(3):139-48. https://doi.org/10.1093/ehjcvp/pvz004

14. Sayin M, Cetiner M, Karabag T, Dogan S, Aydin M, Yavuz N. Op-142 the Relationship Between the Gensini Score and Complete Blood Count Parameters in Coronary Artery Disease. International Journal of Cardiology. 2012(155):S32. http://doi.org/10.1016/S0167-5273(12)70083-9

15. Mancek-Keber M. Inflammation-mediating proteases: structure, function in (patho) physiology and inhibition. Protein and peptide letters. 2014;21(12):1209-29. Available from: https://www.ingentaconnect.com/content/ben/ppl/2014/00000021/00000012/art00003

16. López-Otín C, Bond JS. Proteases: multifunctional enzymes in life and disease. Journal of Biological Chemistry. 2008;283(45):30433-7. https://doi.org/10.1074/jbc.R800035200

17. Fedor M, Simonova R, Fedorova J, Skornova I, Duraj L, Samos M, Stasko J, Kovar F, Mokan M, Kubisz P. Role of VASP Phosphorylation Assay in Monitoring the Antiplatelet Therapy. Acta Medica Martiniana. 2013;13(1):21-6. https://doi.org/10.2478/acm-2013-0008