Platelet activity with hemoglobin level in patients with hemodialysis

Prospective study

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Abstract

Background: VerifyNow (VN; Accumetrics, San Diego, CA) P2Y12 reaction unit (PRU) has an inverse relation with hemoglobin level (Hb). Chronic kidney disease (CKD) is associated with low response to clopidogrel and low Hb. Our aim is to investigate the relation between PRU and Hb, and to assess whether Hb directly affects PRU or not in patients with CKD undergoing hemodialysis (HD).

Methods: We analyzed the relation between PRU and Hb in 43 HD patients and compared it with a control group of 127 patients with normal renal function. Both groups underwent percutaneous coronary intervention for stable coronary artery disease. We also compared PRU between the 2 groups considering Hb as a confounding factor.

Results: In the control group, Hb and PRU showed a significant inverse correlation (correlation coefficient $r = -0.340; P < .001$), but not in the HD group (correlation coefficient $r = -0.099; P = .53$). PRU was higher in the HD group than the control group after adjusting for the influence of Hb (299.2 [95% confidence interval: 278.4–316.7] vs 248.7 [95% confidence interval: 227.7–269.0]; $P < .001$), even after propensity score matching (299.2 [95% confidence interval: 278.4–316.7] vs 241.7 [95% confidence interval: 221.8–262.2]; $P < .001$).

Conclusions: PRU was higher regardless of lower Hb in HD patients than normal renal function patients. Therefore, Hb was not a crucial factor to decide PRU in CKD on HD patients in this study.

Abbreviations: ACEI = angiotensin-converting enzyme inhibitor, ADP = adenosine diphosphate, ARB = angiotensin receptor blocker, BMI = body mass index, CAD = coronary artery disease, CKD = chronic kidney disease, Cr = creatinine, DM = diabetes mellitus, EPO = erythropoietin, Hb = hemoglobin, HD = hemodialysis, HDL = high-density lipoprotein, LDL = low-density lipoprotein, LOF = loss of function, LTA = light transmission aggregometry, PLT = platelets, PRU = P2Y12 reaction unit, PS = propensity score, TG = triglycerides.

Keywords: clopidogrel, hemodialysis, hemoglobin, platelet reactivity, P2Y12 reaction unit

1. Introduction

Clopidogrel has been widely prescribed to prevent major adverse cardiac events in coronary artery disease (CAD). But the response to clopidogrel has significant interindividual variation.\cite{1,2} According to a meta-analysis, impaired platelet responsiveness to clopidogrel is associated with adverse clinical outcomes.\cite{3}

Clopidogrel on-treatment platelet reactivity is affected by many factors such as genetic variations (CYP2C19), diabetes, female sex, smoking, and body mass index (BMI).\cite{4} The VerifyNow (VN; Accumetrics, San Diego, CA) P2Y12 reaction unit (PRU) is an assay that measures the agglutination of fibrinogen-coated beads to stimulated platelets in citrated whole blood.\cite{5} PRU has been used as a measure of residual platelet reactivity in patients taking P2Y12 receptor antagonists in practice. It has been reported that lower hemoglobin (Hb) or hematocrit level is associated with a higher VerifyNow PRU.\cite{6} Usually, chronic kidney disease (CKD) patients are in a state of chronic anemia. There are several mechanisms; erythropoietin (EPO) deficiency, shortened red cell survival, retained inhibitors or toxic metabolites that inhibit erythropoiesis, and blood loss resulting from the qualitative platelet defect present in uremia.\cite{9} In former study, we found that platelet responsiveness to clopidogrel is lower in patients with CKD than in patients with normal renal function.\cite{10,11} However, there are no literature yet to confirm that Hb affects PRU in CKD on hemodialysis (HD) patients.
The aim of this study was to analyze the relation between PRU and Hb level, and to assess whether Hb level directly affects PRU or not in patients with CKD undergoing HD.

2. Materials and methods

2.1. Patients

This PIANO (Effect of Platelet Inhibition According to Clopidogrel Dose in Patients with Chronic Kidney Disease)-9 CKD study is a single-center, nonrandomized, prospective analysis. Forty-three consecutive CKD patients undergoing chronic HD were enrolled as HD group, and 127 patients with normal renal function (estimated glomerular filtration rate >60 mL/min/1.73 m² and no proteinuria) were enrolled as the control group, from September 2009 to June 2011. Both groups underwent percutaneous coronary intervention (PCI) for stable CAD. Patients were screened for platelet reactivity if they took low-dose aspirin (100mg/day) and clopidogrel (75 mg once daily maintenance dose) for at least 14 days as part of the standard treatment regimen. We also analyzed the relation between PRU and Hb level in the HD group and control group. We also analyzed the relation between Hb level and PRU value and Hb level in the HD group and control group. The exclusion criteria were as follows: known allergy to aspirin or clopidogrel; concomitant use of other anti-thrombotic drugs (oral anticoagulants and dipyridamole); thrombocytopenia (platelet count <100,000/mL); liver disease (bilirubin >2mg/dL); active bleeding or bleeding diathesis; concomitant use of a cytochrome P450 inhibitor or nonsteroidal anti-inflammatory drug; or recent treatment (<30 days) with a glycoprotein Ib/IIa antagonist.

The study protocol was approved by the Institutional Ethics Committee, and all patients were exempted from informed consent for participation.

2.2. Platelet function measurements

Just before the HD sessions in HD patients, antecubital vein was used for each blood sample collection. The first 2mL of blood was discarded to avoid spontaneous platelet activation, using multiple syringe sampling technique. Within 1 hour after blood draw the samples were centered.

For the HD group, 2 platelet function assays were performed. The first was LTA, and the second was the VerifyNow P2Y12 assay. The LTA of platelet-rich plasma was assessed by a turbidimetric method using a two-channel aggregometer (Chrono-Log Model 490; Chrono-Log Corp., Havertown, PA) after stimulation with 5μmol/L adenosine diphosphate (ADP); aggregation percentages were recorded 7 minutes later. The VerifyNow P2Y12 assay estimates platelet-induced aggregation by recording increases in light transmission; data are reported as both PRU and percentage inhibition. For the control group, the VerifyNow P2Y12 assay test was performed.

2.3. Statistical analysis

All statistical analyses were performed using SPSS for Windows version 20.0 (SPSS Inc, Chicago, IL) and R version 3.2.3. A two-sided P < .05 was considered significant. Continuous variables, presented as means ± standard deviations, were compared using unpaired Student t test. Categorical variables, presented as frequencies and percentages, were compared using the Chi-squared test or Fisher exact test as appropriate. For correlation analysis, Pearson correlation coefficient r was used. We used a propensity score (PS)-matched analysis to control confounding bias. Many studies reported that platelet reactivity on clopidogrel is independently affected by diabetes, sex, BMI, smoking, CYP2C19 loss of function allele, and Hb level. So these variables were included on the PS. The patients were matched using pair matching (1:1 matching) and optimal matching method.

3. Results

3.1. Demographics and baseline characteristics

Baseline characteristics, risk factors of CAD, and concomitant medications of the study patients are shown in Table 1. In comparison to the control group, the HD group was younger, leaner, and showed lower Hb and cholesterol levels despite less statin use. Otherwise, there were no significant differences between the 2 groups.

3.2. Association between PRU and Hb

For all patients, there was a significant inverse correlation between PRU and Hb level (r = −0.468; P < .001) (Fig. 1A). Unstandardized regression coefficient calculated by univariate linear regression analysis was −18.2, which means that the PRU decreased by 18.2 units for every 1mg/dL increase in Hb level. In the control group, there was also a significant inverse correlation between PRU and Hb level (r = −0.340; P < .001) (Fig. 1B). But in the HD group, PRU and Hb level showed no correlation (r = −0.099; P = .53) (Fig. 1C), and platelet aggregation value by LTA did not shown correlation (r=0.003; P = .98) (Fig. 1D) with Hb level.

3.3. Hb-matched and PS-matched PRU

The HD group showed higher PRU (299.2 ± 67.3 vs 228.2 ± 69.0; P < .001) (Fig. 2A) and lower Hb level (11.2 ± 1.0 vs 13.7 ± 1.7; P < .001) (Fig. 2B) than the control group. Considering the significant relation between PRU and Hb, we compared the Hb-matched PRU between the 2 groups and found it to also be higher in the HD group than control group (299.2 [95% confidence interval: 278.4–316.7] vs 248.7 [95% confidence interval: 227.7–269.0]; P < .001) (Fig. 2C). PS-matched analysis considering diabetes, sex, BMI, smoking, CYP2C19 loss of function allele, and Hb showed higher PRU in the HD group than control group (299.2 [95% confidence interval: 278.4–316.7] vs 241.7 [95% confidence interval: 221.8–262.2]; P < .001) (Fig. 2D).

Table 1 also shows PS-matched characteristics.

4. Discussion

4.1. PRU and Hb in HD patients

We demonstrated a significant inverse relation between PRU and Hb level in patients with normal renal function, which means that PRU increased as Hb level decreased. This finding coincides with results from previous studies. We questioned that the inverse relation between PRU and Hb was similarly shown in...
Finally, our results implicated that not only the Hb level but other parameters were implicated in platelet activity measurement. Measurements included diabetes, sex, BMI, smoking, CYP2C19 loss of function allele, and hemoglobin.

Table 1
Baseline demographics and clinical characteristics of the study population before and after propensity score matching.

| Total population | Hemodialysis (N = 43) | P | Control (N = 127) | Propensity-matched population |
|------------------|-----------------------|---|------------------|-------------------------------|
| **Age (yrs)**    | 65.0 ± 10.6           | <.001 | 54.2 ± 14.4       | 69.5 ± 9.5                    |
| **Male**         | 79 (62.2%)            | .17 | 21 (48.8%)       | 14 (32.6%)                    |
| **BMI (kg/m²)**  | 25.0 ± 3.3            | .01 | 23.4 ± 3.9       | 24.1 ± 3.5                    |
| **DM**           | 41 (32.3%)            | .22 | 9 (20.9%)        | 10 (23.3%)                    |
| **Hypertension** | 89 (70.1%)            | .68 | 28 (65.1%)       | 36 (83.7%)                    |
| **Dyslipidemia** | 35 (27.6%)            | .96 | 11 (25.6%)       | 14 (32.6%)                    |
| **Smoking**      | 30 (23.6%)            | .88 | 9 (20.9%)        | 2 (4.7%)                      |
| **CYP2C19 LOF allele** | 61 (83.8%) | .46 | 24 (55.8%) | 27 (62.8%) |
| Laboratory findings |                     |     |                  |                               |
| **Hb (g/dL)**    | 13.7 ± 1.7            | <.001 | 11.2 ± 1.0       | 12.0 ± 1.2                    |
| **PLT (10³/mm³)**| 240.9 ± 67.0          | .02 | 211.7 ± 68.8     | 251.0 ± 80.3                  |
| **Cr (mg/dL)**   | 0.8 ± 0.2             | <.001 | 10.5 ± 4.0       | 8.0 ± 0.3                     |
| **Total-C (mg/dL)** | 187.7 ± 42.2        | <.001 | 145.7 ± 30.0     | 184.1 ± 47.7                  |
| **LDL-C (mg/dL)**| 116.1 ± 35.1          | <.001 | 85.2 ± 27.2      | 113.8 ± 39.0                  |
| **HDL-C (mg/dL)**| 48.4 ± 19.1           | <.001 | 49.2 ± 12.2      | 47.9 ± 14.9                   |
| **TG (mg/dL)**   | 160.1 ± 123.9         | <.001 | 98.6 ± 48.2      | 139.1 ± 72.8                  |
| Medications      |                       |     |                  |                               |
| **Beta blocker** | 62 (48.6%)            | .38 | 17 (39.5%)       | 22 (51.2%)                    |
| **ACEI or ARB**  | 70 (55.1%)            | >.001 | 24 (55.8%)       | 22 (51.2%)                    |
| **CCB**          | 43 (33.9%)            | .30 | 19 (44.2%)       | 16 (37.2%)                    |
| **Diuretics**    | 12 (9.4%)             | .18 | 8 (18.6%)        | 5 (11.6%)                     |
| **Statin**       | 117 (92.1%)           | <.001 | 7 (16.3%)        | 39 (90.7%)                    |

Values are shown as mean ± standard deviation. 
ACEI = angiotensin-converting enzyme inhibitor, ARB = angiotensin receptor blocker, BMI = body mass index, C = cholesterol, CCB = calcium channel blockers, Cr = creatinine, DM = diabetes mellitus, Hb = hemoglobin, HDL = high-density lipoprotein, LDI = low-density lipoprotein, LOF = loss of function, PLT = platelets, TG = triglycerides.

Table notes:
1 Variables included in the propensity score matching were diabetes, sex, BMI, smoking, CYP2C19 loss of function allele, and hemoglobin.

4.2. Platelet activity in HD patients

Our data supported several previous studies that CKD patients had significantly higher PRU than patients with normal renal function. Guo et al. suggested that renal impairment is associated with higher PRU and elevated major adverse cardiovascular events risks. Breet et al. presented that CKD patients with higher PRU were associated high risk of cardiovascular events. Despite this significant implication of higher PRU in CKD patients, the mechanism or cause of the higher PRU has not been elucidated so far. We assumed that the lower Hb level could affect higher PRU in CKD patients, so we compared PRU after Hb level adjustment by PS-matching between 2 different renal function groups to offset the effect of lower Hb in CKD on HD patients. In addition, we also adjusted for other confounding factors such as sex, BMI, smoking, diabetes, and CYP2C19 loss of function allele as well as Hb, which could affect the PRU result. Nevertheless, PRU was still higher in CKD on HD patients than normal renal function. Finally, our results implicated that not only the Hb level but other confounding factors mentioned above were not crucial factors for high PRU in CKD on HD patients.

4.3. Concept of enhanced platelet activity in HD patients

The CKD increases the risk of adverse cardiovascular outcomes after PCI. The investigation about the reasons of enhanced platelet activity in HD patients could contribute to improve post-PCI cardiovascular outcomes. We already know that platelet responsiveness to clopidogrel is lower in patients with CKD than in patients with normal renal function. Morel et al. summarize the putative mechanisms involved in impaired P2Y12 inhibition by thienopyridines in CKD as complex metabolism including hepatic conversion and intestinal absorption of clopidogrel that can be altered in uremia and accumulation of denucleotide polyphosphates that act as partial agonists of the P2Y12 receptor, enhancing platelet turnover. Moreover, Stohlawetz et al. reported EPO that was routinely injected in CKD patients markedly enhances endothelial activation and platelet reactivity.

4.4. Emerging biomarkers for platelet activity

Recently, microRNAs, small noncoding RNAs that regulate gene expression, have been emerged as potential biomarkers for cardiovascular disease. MicroRNAs also play a key role in atherosclerosis and platelet activation acting on not only intracellularly, but also extracellularly. The blood level of specific circulating microRNAs correlate with platelet reactivity. Further studies considering the role of microRNAs
as platelet activity marker can elucidate the enhanced platelet activity in CKD patients.

4.5. Study strength and limitation

This was the first study to assess the relation between PRU and Hb level in patients with CKD undergoing HD. Previous articles excluded renal insufficiency, did not mention about renal function, or enrolled only a small proportion (17%) of CKD patients. Furthermore, we newly found that PRU showed still higher level in CKD patients in spite of the PS matching with Hb and other confounding factors.

On the other hand, there are several limitations in our study. First, this study consisted of small number of patients in a single center with nonrandomized nature. Second, our study group contains only HD patients. Third, we could not confirm the key mechanism that affects PRU in HD patients. Further studies are

Figure 1. Relation among VerifyNow P2Y12 reaction unit (PRU), light transmission aggregometry (LTA) value, and hemoglobin (Hb) level. (A) Relation between PRU and Hb level in total patients. (B) Relation between PRU and Hb in the normal renal function group. (C) Relation between PRU and Hb in the hemodialysis group. (D) LTA value after stimulation with 5 μmol/L ADP. ADP = adenosine diphosphate, Hb = hemoglobin, LTA = light transmission aggregometry, PRU = P2Y12 reaction unit.
necessary to reveal the cause of enhanced platelet reactivity in CKD on HD.

5. Conclusion

In conclusion, PRU showed higher level regardless of lower Hb in CKD on HD patients than normal renal function patients. Therefore, Hb was not crucial factor to decide PRU in CKD on HD patients in this study.

Author contributions

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