Platelet Aggregation Tests Are Affected in Pseudothrombocytopenia

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DOI: 10.1309/LM9UXAORTFONZ6U5

Pseudothrombocytopenia (PTCP) is characterized by a low platelet count (platelet count below $150 \times 10^3/\mu L$) associated with in vitro platelet agglutination resulting from the presence of antiplatelet antibodies such as IgG, M, and A reacting with platelets in blood drawn into ethylenediaminetetraacetic acid (EDTA). The mechanism of this reaction appears to involve antiplatelet antibodies against glycoprotein IIb. Low platelet counts are detected in automated blood counts in EDTA tubes, whereas platelets form clusters in a peripheral blood smear.\(^1\)\(^-\)\(^3\) The frequency of PTCP is reported in the rate of 0.09% to 0.21% in both healthy subjects and patients with various diseases.\(^4\)\(^-\)\(^6\) Pseudothrombocytopenia incidence is 17% in patients with thrombocytopenia.\(^5\) Although there is no evidence of purpura or bleeding in patients with PTCP, platelet function abnormalities have been rarely reported in a few studies based on the detection of the antibody.\(^1\)\(^-\)\(^7\) To our best knowledge, there is no clinical study in which platelet aggregation with aggregometry is evaluated in PTCP. So we aimed to investigate platelet functions with aggregometry using adenosine diphosphate (ADP), collagen, epinephrine, and ristocetin.

Materials and Methods

Fifteen patients with PTCP (9 female with a mean age 48 ± 4 years) and 19 healthy persons (13 female with a mean age 55 ± 2 years) were enrolled in this study. Inclusion criteria of the patients with PTCP were low platelet count ($<150 \times 109/\mu L$) in routine EDTA-anticoagulated blood and the appearance of sufficient platelet clumping on peripheral smear. Average in 10 high power field on a blood film microscopically and multiplying by 15000 gives a platelet count reason-

Abstract

Objective: The aim of the study is to evaluate the platelet functions with platelet aggregation tests in patients with pseudothrombocytopenia (PTCP).

Methods: Fifteen (9 female and 6 male) patients with a mean age of 48 ± 4 years with PTCP (platelet count below $150 \times 10^3/\mu L$) and 19 (13 female and 6 male) healthy controls with a mean age of 55 ± 2 years were enrolled in this study. In both groups, whole blood counts, peripheral smear, and platelet aggregation tests including ristocetin, epinephrine, collagen, and ADP were investigated.

Results: Adenosine diphosphate (ADP) 63 ± 6% and epinephrine-induced 61 ± 5% platelet aggregations in the patients with PTCP were significantly lower in those of healthy controls 93 ± 3.5% and 80 ± 4%, respectively, and P=0.001 and P=0.006, respectively.

Conclusion: Platelet aggregation tests may decrease in PTCP, but its importance is unclear. Therefore, this condition should be investigated with larger series and molecular studies.

Keywords: pseudothrombocytopenia, platelet aggregation, epinephrine, ADP

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Abbreviations

PTCP, pseudothrombocytopenia; ADP, adenosine diphosphate; EDTA, ethylenediaminetetraacetic acid; PRP, platelet-rich plasma; PPP, platelet-poor plasma; MPV, mean platelet volume; PDW, platelet distribution width
the aggregation rate (seconds).\textsuperscript{5,10} All platelet aggregation tests were completed once. Informed consent was obtained from all participants. The study protocol was also approved by the local ethical committee.

**Statistical Analysis**

All results were given as mean ± standard error. The comparison of these results was performed with the Mann Whitney U test. The statistical analysis was carried out by using Statistical Package for Social Sciences (SPSS), version 13.0 (Chicago, IL). Significance was defined as $P<0.05$.

**Results**

Table 1 lists the patients’ demographic features and the results of platelet parameters and platelet aggregation tests in both groups. Platelet parameters including platelet count ($P=0.001$), mean platelet volume (MPV), platelet distribution width (PDW), and platelet-crit in the patients with PTCP were statistically different than the controls ($P<0.05$ for other parameters).

Adenosine diphosphate (63 ± 6%) and epinephrine-induced (61 ± 5%) platelet aggregations in the patients with PTCP were significantly lower than those of the healthy controls (93 ± 3.5% and 80 ± 4%), respectively. Significant values were $P=0.001$ for ADP and $P=0.006$ for epinephrine. However, collagen (75 ± 6%) and ristocetin-induced (71 ± 3%) platelet aggregations were not statistically different than the controls ($P>0.05$ for both parameters).

Platelet satellitism was not detected in any of the patients with PTCP on peripheral smear.

**Discussion**

In this study, we observed a significant decrease in ADP and epinephrine-induced platelet aggregation in the patients with PTCP. Platelets play a vital role in the normal hemostasis, and derangements of their function can lead to hemorrhage or thrombosis. While we have made progress in elucidating the molecular mechanisms leading to platelet adhesion, aggregation, shape change, and secretion, clinically useful tests of platelet function have lagged behind.\textsuperscript{11} Although their importance is not clear, anti-platelet antibodies can be detected in patients with autoimmune diseases, infections, cancer, healthy individuals, and pregnant women. Most of these antibodies are IgM type.\textsuperscript{12,13}

Vicari and colleagues\textsuperscript{14} found the frequency of PTCP at the rate of 0.13% in 33,623 blood samples with EDTA obtained from healthy blood donors. It was commented that EDTA-dependent PTCP is a rare but misleading phenomenon. The recognition of this situation is important in order to avoid expensive and invasive procedures.

Complete blood counts have a 90% sensitivity and 100% specificity to determine platelet counts.\textsuperscript{15} When PTCP is accepted as platelet counts less than 100 × 10^3 μL, the frequency of PTCP is 0.2% in platelet donors.\textsuperscript{16} In most studies, platelet counts less than 150 × 10^3 μL have been accepted for PTCP.\textsuperscript{1,4}

Moreover, we found small statistical differences for MPV, platelet-crit, and PDW in patients with PTCP. These parameters were lower when compared with the controls. No effect was seen on cell morphology or staining characteristics. It may be related to EDTA, but there is no consensus on this condition. In 1 study, MPV and PDW values of healthy controls in EDTA solution were higher than those in sodium citrate solution. The authors explained that shear stress was responsible for the platelet activation;\textsuperscript{17} however these values of PTCP patients were lower in another study.\textsuperscript{15} Interestingly, it was found that MPV and PDW values in EDTA solution were lower in cats.\textsuperscript{18}

It was found that the expression of GpIIb/IIIa in the presence of EDTA was significantly reduced in individuals with PTCP, compared to controls in 1 study. CD62, CD63, and thrombospondin-antigen were upregulated in the presence of EDTA. They concluded that these alterations in the expression of platelet antigens could also be induced on platelets from normal donors by incubation with sera of PTCP subjects and EDTA.\textsuperscript{19}

Adenosine diphosphate is a weak agonist compared to collagen or thrombin. In citrated PRP, low concentrations of ADP cause only a primary, incomplete, and reversible phase of aggregation, but at concentrations more than 1–3 μM primary aggregation of human platelets does not reverse and is followed by a secondary and irreversible phase.\textsuperscript{20} A severely impaired aggregation response to ADP and impaired aggregation in response to collagen/thrombin may indicate a very rare abnormality of the P2Y12 ADP receptor.\textsuperscript{21} Epinephrine (5–10 μM), another weak agonist, aggregates platelets in citrated PRP without an initial change in platelet shape, but epinephrine is the least consistent agonist.\textsuperscript{22} We did not perform platelet aggregation tests with higher concentrations of ADP and epinephrine (>3 μM). At these concentrations, these tests might return to normal.

Platelet aggregometry can evaluate platelet functions, but it has some limitations since standardization of this procedure is difficult.\textsuperscript{23} Reproducibility of these tests is important to avoid the erroneous results.\textsuperscript{24} However, these tests were not repeated in our study. In our study, ADP 63 ± 6% and epinephrine-induced 61 ± 5% platelet aggregations in the patients with PTCP were significantly lower in those of healthy controls 93 ± 3.5% and 80 ± 4%, respectively ($P=0.001$ and $P=0.006$).

Platelet aggregation is a complex phenomenon. Divalent cations, such as calcium and magnesium, are required for
platelet aggregation. They alter the specificity of the integrin αIIb for its ligands. The molecular basis of integrin signaling, which occurs in platelet αIIb, is an integral part of thrombus formation. Gp IIb (αIIb) and Gp IIIa (β3) were identified as the abnormal proteins present in the patients with Glanzmann thrombasthenia. The signaling pathways of Gp αIIb β3 are complex and have been extensively studied; however the terminal effector molecules affecting activation remain unknown. Not only does the αIIb β3 receptor have some important roles with respect to platelet functions, but it also affects the coagulation pathways and inflammatory process.25-28

Platelet aggregation tests may be affected in some hereditary and acquired disorders such as Bernard-Soulier, Glanzmann thrombasthenia, Hermansky-Pudlak syndrome, α granule abnormalities, uremia, paraproteinemia, myelodysplastic syndrome, and myeloproliferative disorders.29 Since the level and/or activity of each of the receptors for the agonists can vary among normal subjects, it is recommended that the dose-response curve to each agonist be obtained from the patient under study and compared to a reference range obtained from multiple normal subjects.30 Girtovitis and colleagues observed a decrease in ADP- and epinephrine-induced platelet aggregation especially in patients with myelodysplastic syndrome. The Quebec platelet disorders, characterized by mild thrombocytopenia and defective epinephrine-induced platelet aggregation, are seen with a decrease in the amount of multiple α-granule proteins.32 Moreover ADP may play a crucial role in hemostasis and thrombosis, and its receptors are potential targets for antithrombotic drugs such as clopidogrel and ticlopidine.33 Our study’s limitation was selective hereditary deficiencies of platelet aggregation. In conclusion, platelet aggregation tests may decrease in PTCP, but its importance is not clear. As a result, this condition should be investigated with larger series and molecular studies.1M