Platelet function alterations in dengue are associated with plasma leakage

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Summary

Severe dengue is characterised by thrombocytopenia, plasma leakage and bleeding. Platelets are important for preservation of endothelial integrity. We hypothesised that platelet activation with secondary platelet dysfunction contribute to plasma leakage. In adult Indonesian patients with acute dengue, we measured platelet activation status and the response to the platelet agonist TRAP using flow cytometer-based assays. Patients were monitored daily for plasma leakage by ultrasonography. Acute dengue was associated with platelet activation with an increased expression of the activated fibrinogen receptor ($\alpha_{IIb}\beta_3$), the lysosomal marker CD63 and the alpha-granule marker CD62P (P-selectin). Upon maximal platelet activation by TRAP, platelet function defects were observed with a significantly reduced maximal activated $\alpha_{IIb}\beta_3$ and CD63 expression and reduced platelet-monocyte and platelet-neutrophil complexes. Patients in the lowest tertile of activated $\alpha_{IIb}\beta_3$ and CD63 expression had an odds ratio for plasma leakage of 5.2 (95% confidence interval [CI] 1.3–22.7) and 3.9 (95% CI 1.1–13.7), respectively, compared to the highest tertile. Platelet-derived serotonin has previously been related to plasma leakage and we found increased intra-platelet serotonin concentrations in our patients. In conclusion, platelet activation with platelet function alterations can be found in patients with acute dengue and this may contribute to dengue-associated plasma leakage.

Keywords

Dengue virus infection, platelet function, flow cytometry, plasma leakage, serotonin

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Introduction

Dengue has become the most important arthropod-borne viral infection in the world (1). Severe dengue is characterised by thrombocytopenia, vascular leakage and haemorrhage. The pathogenic mechanisms underlying these complications, which typically occur during or shortly after defervescence and are not associated with morphological endothelial damage, are still incompletely understood (2–4). In recent years, the role of platelets in regulating endothelial integrity and dengue-associated plasma leakage has gained increased interest (5–9).

Sufficient numbers of functional platelets are required for preservation of endothelial integrity during inflammation (10). The observation that petechiae and bleeding complications in dengue patients frequently occur with platelet counts well above the threshold for spontaneous bleeding, suggests that dengue is not only associated with thrombocytopenia, but also with functional platelet defects. Studies using platelet aggregometry indeed showed reduced aggregation in dengue patients (11–13), but because the reliability of aggregometry is compromised in conditions with thrombocytopenia, these findings should be interpreted with caution. We have developed a novel flow cytometer-based platelet function test using anti-coagulated unprocessed blood that is well suited for use in thrombocytopenia (14). In this test, expression of the alpha-granule marker CD62 (P-selectin), the lysosomal marker CD63 and binding of the monoclonal antibody PAC-1 to the activated $\alpha_{IIB}\beta_3$ (the GPIIbIIIa complex, CD41/ CD61) receptors are used as markers for platelet activation (15–17). Reduced expression of these markers upon addition of a platelet agonist suggests the presence of thrombocytopathy (18). Activated platelets form complexes with monocytes and neutrophils by binding of platelet CD62P to its counter-ligand on leukocytes, P-selectin glycoprotein ligand-1 (PSGL-1) (19). These complexes can also be