Flow Cytometric Quantitation of Red Blood Cell Vesicles in Thalassemia

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Abstract

**Background:** Thalassemia is a hereditary hemolytic anemia caused by mutations in the globin gene complex. Circulatory disturbances including arterial and venous thrombosis have also been noted in these patients. Aggregability of abnormal RDC and the high level of membrane-derived microparticles stemming from activated platelets and other blood cells are thought to be responsible for the associated thrombotic risk. Destruction of RBC is also thought to be an important pathophysiological consequence, particularly through the formation of circulating vesicles. To our knowledge, there has been no attempt to quantitatively evaluate the number of RBC vesicles in thalassemia. This prompted us to study the level of RBC vesicles in the peripheral blood of thalassemia patients using quantitative flow cytometry.

**Methods:** Whole blood from each subject was doubly stained for RBC and platelet or annexin V markers, together with the known density TruCount™ beads. RBC vesicles were gated according to their forward/side scatter and RBC marker. Percentage of RBC vesicles and their absolute number were analyzed by flow cytometry.

**Results:** Our data indicated that RBC vesicles were annexin V-positive. The number of annexin V-positive events was higher than their intact RBCs. RBC vesicles were present in both normal and thalassemic blood samples, but the numbers of RBC vesicles were significantly higher in thalassemia. Both the percentage and the absolute number of RBC vesicles were especially marked in splenectomized subjects with \(\beta\)-thalassemia/Hemoglobin E. When clinical and hematological indices were compared with RBC vesicles, there was an inverse relationship between the degree of severity in thalassemia patients and the number of RBC vesicles.

**Conclusion:** Flow cytometric quantitation of RBC vesicles is simple, reliable and may offer new insights in to study of the relationship between defective hemoglobin synthesis, RBC perturbation and pathophysiological complications in thalassemia.

**Keywords:** Annexin V; Coagulation; Flow cytometry; Glycophorin A; Phosphatidylserine; Red blood cell; Thalassemia; Vesicle

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