Elevated levels of circulating procoagulant microparticles in patients with β-thalassemia intermedia

Patients with β-thalassemia intermedia (βTI) are completely asymptomatic until adult life, experiencing only mild anemia, maintaining hemoglobin levels between 7 and 10 g/dL, and require only occasional blood transfusions, if any. MPs are shed submicrometric plasma membrane fragments (~0.1-1 μm) harboring negatively-charged procoagulant phosphatidylserine (PS) in their extracellular membrane leaflet. They mainly derive from apoptotic or activated cells, and generally present a procoagulant potential.[1] Increased levels of circulating MPs were described in many disorders with major vascular and thrombotic symptoms.[2]

We determined the level of circulating procoagulant MPs and their cellular origin in patients with βTI and in healthy volunteers. We prospectively included 24 patients (13 men and 11 women, mean age±SD, 29.9±8.1 years). This study was approved by the Institutional Research Board of the American University of Beirut. Among the βTI patients, 17 had splenectomy and 9 had osteoporosis. None of the patients received transfusion, and none had acute events except 2 who experienced thrombosis at least three years prior to the study. Four patients were treated daily with 100 mg of aspirin and 7 occasionally with standard doses of desferal (30-40 mg/kg/d). Hematocrit values were 28.3±5.5 (mean±SD, n=22), ranging between 21 and 38%. Ferritin concentration was 1,048±520 ng/mL, (mean±SD, n=14), ranging between 300 and 2,200 ng/mL.

Control subjects were healthy volunteers with no medication (13 men and 3 women, mean age±SD, 28.4±3.2 years). Blood was withdrawn once from patients or healthy volunteers and platelet-free plasma fractions were prepared within the first hour of blood collection. Circulating procoagulant MPs were determined using a combined assay with solid-phase annexin V-based capture and prothrombinase activity measurement as previously described.[3] The cell origin of MPs was further assessed after capture onto insolubilized specific antibodies followed by measurement of prothrombinase activity. Antibodies to CD105 (R&D Systems) were used to identify MPs originating from endothelial cells, to glycoprotein (GP)Ib (a kind gift from Dr. F. Lanza, INSERM U.311, Strasbourg, France) for platelets, to CD11a (Leinco Technologies, Inc.), for leukocytes, and to glycophorin (R&D Systems) for red blood cells. Levels of MPs of patients detected by annexin V or of the different cell origins were compared with those of healthy volunteers. Statistical analysis was carried out using a statistical package for social sciences (SPSS). The level of circulating procoagulant MPs detected through binding to annexin V was elevated in βTI patients compared with healthy subjects (6.9±3.5 versus 4.7±2.9 nmol/L phosphatidylserine equivalent, mean±SD, p=0.04, independent sample t-test respectively) (Figure 1). The distribution of MP levels according to their cellular origins is also shown in this figure. Table 1 summarizes the values of circulating MPs according to their cellular origin and shows that patients have MP levels with highest statistical significance when of RBC, endothelial and leukocytic origins. A modest correlation between the MPs captured onto anti-glycophorin and anti-CD105 antibodies was observed in patients, but not in healthy volunteers (r=0.44, p<0.05, Pearson correlation). No other significant correlation was observed between the MPs of different cell origins that could characterize only the βTI patients. Platelet MPs were significantly higher in splenectomized patients versus non-splenectomized (1.6±0.5 nmol/L phosphatidylserine equivalent, n=7, *vs.* 3.9±2.6, n=17 respectively p<0.001). Increase in annexin-V labeled whole RBC was previously reported in patients with β-thalassemia major (βTM) and βTI.[4] It is well documented that patients with β-thalassemia have a hypercoagulable state, attributable in part to a decrease in the natural endothelial anticoagulant system, involving thrombomodulin, and proteins C and S.[5] Activation of platelets and endothelium was demonstrated in *ex vivo* models of βTM and more recently βTI with an increase in urinary thromboxane metabolites and prostacyclin derivatives.[5]

Endothelial activation characterizes those β-thalassemia patients who present a high predisposition to thrombosis and cardiovascular disorders.[6] Our results differ from the recent report from Pattanapanyasat *et al.*, who described an increase in MPs of platelet but not endothelial origin in a population of β-thalassemia/hemoglobin E (HbE), a form

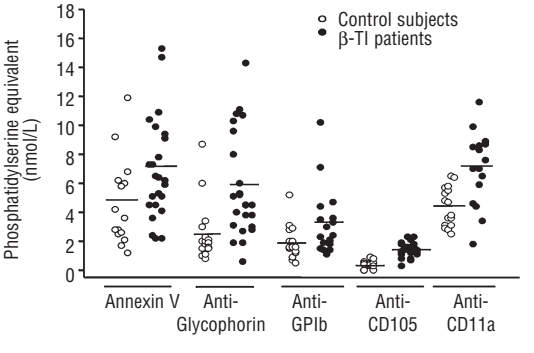

Figure 1. Individual values of circulating procoagulant microparticles in peripheral blood of 24 β-thalassemia intermedia patients, and 16 control subjects. Insolubilized annexin V or cell-specific antibodies were used to capture MPs, the latter testifying to their cellular origin. Each individual value is expressed as nmol/L phosphatidylserine equivalent. Corresponding mean ± SD values are reported in Table 1. Horizontal lines represent the mean.

Table 1. Antigenic characterization of circulating procoagulant microparticles in patients with β-thalassemia intermedia or in healthy subjects.

Specific antibodies	*Healthy subjects (n=16)*	*β-thalassemia (n=24)*	*p value*
Glycophorin	2.54±2.05	5.73±3.60	=0.003
GPIb	1.91±1.15	3.22±2.35	=0.04
CD105	0.41±0.30	1.41±0.49	<0.001
CD11a	4.38±1.36	6.97±2.41	=0.001

Values are expressed in nmol/L phosphatidylserine equivalent (mean ± SD).

characteristic of Southern Asia.[7] The 2 subtypes of anemia, although sharing common phenotype, certainly vary in feature and progression. The combination of HbE with β-thalassemia spans thalassemia phenotypes from a condition indistinguishable from thalassemia major to a mild form of thalassemia intermedia.[8] Endothelial MPs were recently described in different pathologies including myocardial infarction, ischemia, diabetes, or end stage renal failure.[9-12] These observations suggest endothelium activation or injury in patients with βTI, and further characterization of MPs is needed for more accurate assessment of the endothelial status. In another respect, it would have been valuable to know whether tissue factor (TF) is harbored by MPs, but the limited number of copies of TF molecules per MP impedes reliable determination by flow cytometry or TF activity measurement. In conclusion, this study shows that the levels of procoagulant MPs of RBC, leukocytic and endothelial origins are high in patients with β-thalassemia intermedia, but their possible deleterious role in endothelial dysfunction remains to be further investigated.

Aida Habib,[1] Corinne Kunzelmann,[2] Wael Shamseddeen,[3] Fatiha Zobairi,[2] Jean-Marie Freyssinet,[2] and Ali Taher[3]

[1]Department of Biochemistry, [3]Internal Medicine, American University of Beirut, Lebanon; The Chronic Care Center, Hazmieh, Lebanon; [2]INSERM, U770, Université Paris-Sud, Le Kremlin-Bicêtre, France; Université Louis Pasteur, Faculté de Médecine, Institut d'Hématologie et d'Immunologie, Strasbourg, France

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Correspondence: Aida Habib, Department of Biochemistry, American University of Beirut, PO Box 11-236 Beirut, Lebanon. Phone: international +961.135000 ext. 4882. Fax: international +961.1370814. E-mail: ah31@aub.edu.lb

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