CONCISE REPORT

Fragment D-Dimer Levels: An Objective Marker of Vaso-occlusive Crisis and Other Complications of Sickle Cell Disease

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Although abnormalities in coagulation tests have been reported during vaso-occlusive crises in patients with sickle cell disease, objective, readily performed laboratory tests that document the occurrence of this complication have not been available. We examined the relationship between fibrin D-dimer levels and the occurrence of complications in patients with sickle cell disease, using a commercially available latex bead agglutination assay. The patients were either asymptomatic, hospitalized for vaso-occlusive crisis, or had other complications of sickle cell disease including leg ulcers, chronic cholecystitis, aseptic necrosis, joint pain and infection. Fifty-seven percent of 187 samples from 96 patients had elevated levels of fibrin D-dimer. Ninety percent of 75 samples from asymptomatic patients were negative for fibrin D-dimer (<1 μg/ml) but 97% of 29 samples from patients with vaso-occlusive crisis and 85% of 83 samples from patients with other complications of sickle cell disease were positive. In serial studies, worsening or amelioration in clinical complications were reflected in increasing or decreasing levels of fibrin D-dimer, respectively. The molecular species of fibrin identified by the latex agglutination test was shown to be fragment D-dimer by successive immunoprecipitation and protein blot analysis. We conclude that the complications of sickle cell disease, including vaso-occlusive crisis, result in the production of fibrin D-dimer, and its detection may be used as a marker for the presence of the complication.

MATERIALS AND METHODS

Patient samples. EDTA plasma was obtained from blood samples drawn for routine blood count or hemoglobin electrophoresis. Ninety-six patients (68 adults and 28 children) are reported in the present study. Of these, 79 are homozygous for hemoglobin S (Hb SS), ten have Hb SC disease, five have Hb S β" thalassemia and one each has Hb SD disease and Hb S β' thalassemia. All of these patients have some history of one or more of the complications of sickle cell disease, including vaso-occlusive crisis, leg ulcers, infections (osteomyelitis, pneumonia, viral illness), chronic cholecystitis, aseptic necrosis and bone marrow infarction.

Measurement of fibrin D-dimer levels. To measure the levels of fibrin D-dimer in EDTA plasma samples we used the Dimertest latex agglutination assay (American Diagnostica, Greenwich, Conn) according to the manufacturer’s recommendations. The minimum amount of fibrin D-dimer detected by the assay is 1 μg/ml. All assays were performed by the same person, who was unaware of the clinical status of the patient. Statistical analysis of the relationship between disease activity and Dimertest results was performed using the G-test of independence.

Assessment of patient clinical status. The patient’s clinical status at the time the fibrin D-dimer assays were performed was assessed by history and physical examination. The evaluation was made without knowledge of the assay results. Patients were asymptomatic, were hospitalized in vaso-occlusive crisis, or were having

THE ROLE OF blood coagulation in the pathogenesis of sickle cell disease is poorly understood. It is, however, known that patients in vaso-occlusive crisis have evidence of intravascular coagulation since fibrinopeptide A levels are elevated in sickle cell crisis. In addition, the activation of coagulation factors may have a profound effect on blood viscosity in sickle cell patients. Sickled erythrocytes adhere to vascular endothelial cells and this adherence is enhanced by autologous plasma proteins. Therefore, the occlusion of the microvasculature by sickled erythrocytes may be enhanced by interactions with coagulation factors. Sickled erythrocytes or purified spicules from such erythrocytes accelerate blood clotting in vitro. Whether the activation of the coagulation pathway is contributory to vaso-occlusive crisis is unknown. In addition to episodes of painful crisis, many adult patients with sickle cell disease suffer from more chronic complications of the disease, including leg ulcers, aseptic necrosis of bone, and chronic cholecystitis.

To determine if laboratory measurements of fibrinolytic activity are related to the clinical status of the patient and might therefore provide an objective measurement of disease activity, we used a newly available, simple, and rapid latex bead agglutination assay (Dimertest) which is specific for the fragment D-dimer domain in Factor XIIIa-crosslinked fibrin. The monoclonal antibody used in the Dimertest, DD-3B6, measures both fragment D-dimer and fragment D-dimer/fragment E complex in plasma. Fragment D-dimer/fragment E complex is a terminal breakdown product of crosslinked fibrin; therefore its presence in plasma is evidence of fibrinolysis. For the purpose of discussion, fibrin D-dimer will refer to the fragment D-dimer domain in all molecular species of crosslinked fibrin and fibrin degradation products. Fibrin D-dimer levels are elevated in fibrinolytic states including disseminated intravascular coagulation, pulmonary embolism, and deep venous thrombosis. We have used this assay to show that there is a strong relationship between fibrin D-dimer levels and disease activity in patients with sickle cell disease.
other complications of sickle cell disease. Vaso-occlusive crisis was defined as multiple sites of skeletal or other pain lasting at least 12 hours. The other complications of sickle cell disease in these patients included leg ulcers, aseptic necrosis of bone, joint pain, stroke, cholecystitis and infection (pneumonia, osteomyelitis, viral infection).

**Biochemical analysis of fragment D-dimer.** In order to determine which molecular species of fibrin were recognized by the Dimertest, we analyzed fibrin-related antigens present in EDTA serum. EDTA plasma samples which contained measurable fibrin D-dimer were clotted with thrombin (gift of Dr John W. Fenton II, New York State Department of Health, Albany, NY) and the clot was removed. Fibrinogen-related antigens were immunoprecipitated from serum using rabbit antihuman fibrinogen and Cowan I strain *Staphylococcus aureus* cells (both from Calbiochem-Behring, San Diego) under conditions previously determined to remove all fibrinogen-related antigens. As a control, immunoprecipitations were performed using nonimmune rabbit serum. The precipitates were subjected to sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) under nonreducing conditions and transferred onto nitrocellulose paper by the method of Towbin et al. The blot was probed first with monoclonal antibody DD-3B6 obtained from the enzyme-linked immunosorbent assay (ELISA) form of the Dimertest, and second with 125I-labeled F(ab')2 sheep antimouse immunoglobulin (Amersham, Arlington Heights, Ill). Samples of purified fragment D and fragment D-dimer were electrophoresed in the same gels to serve as internal standards. The blot was then dried and autoradiographed.

**RESULTS**

Of the 187 samples of EDTA plasma from 96 sickle cell patients tested, 57% had elevated levels of fibrin D-dimer. Normal plasma samples from healthy donors were always negative. When the fibrin D-dimer levels were related to the clinical status of the patients (Fig 1), there was a significant association between disease activity and the presence of fibrin D-dimer (G = 132.068, P < 0.001). Of 75 samples from 38 asymptomatic patients, only 10% contained measurable levels of fibrin D-dimer. Samples from 14 patients in vaso-occlusive crisis were Dimertest-positive 97% of the time. Samples from six of the patients were taken when the patients were asymptomatic; all of these were Dimertest negative. Among all samples from patients in vaso-occlusive crisis, only one was negative and this patient’s diagnosis was not supported by physical findings. In the course of the same hospital admission, this patient experienced chest pain accompanied by a fall in Po2. A plasma sample drawn 24 hours later contained measurable fibrin D-dimer.

Eighty-six percent of samples from symptomatic patients not hospitalized for management of vaso-occlusive crisis had elevated levels of fibrin D-dimer. Serial samples were obtained from ten patients in this group who experienced changes in their clinical status other than hospitalization for vaso-occlusive crisis. In all cases, those who developed complications developed measurable levels of fibrin D-dimer while those whose complications resolved became negative in this assay.

There were 14 of the 96 patients (15%) in whom fibrin D-dimer levels did not correlate with clinical symptoms. In eight asymptomatic patients (three adults and five children), fibrin D-dimer levels were elevated while in six symptomatic patients fibrin D-dimer levels were normal. In the latter group, two patients had leg ulcers and four had subjective complaints of pain.

The amount of fibrin D-dimer measured by the agglutination assay was the same in EDTA plasma or serum prepared...
from thrombin-treated EDTA plasma. Immunoprecipitation/protein blot analysis of the fibrinogen-related antigens in these sera demonstrated that the monoclonal antibody DD-3B6 recognizes primarily fragment D-dimer in addition to some fragment D1 and higher molecular weight fibrin fragments in the Dimertest-positive samples from patients with either vaso-occlusive crisis or other complications of sickle cell disease (Fig 2).

DISCUSSION

There are no practical and readily available laboratory measurements currently in use which correlate with disease state in sickle cell disease. However, there are reports of abnormal coagulation activity in patients with sickle cell disease. Richardson et al.2 found elevated levels of factor VIII:C, factor VIIIIR:Ag, and antithrombin III in the plasma of eight patients in steady state which rose higher when the patients were in vaso-occlusive crisis. The onset of vaso-occlusive crisis is also accompanied by an increase in plasma high-molecular-weight fibrinogen complexes (HMWFC).18 Fibrinopeptide A (FPA) levels have been used to measure coagulation activity in patients in vaso-occlusive crisis. Leichtman and Brewer1 showed that the FPA level in pain-free patients is generally within normal range but increases with the onset of painful crisis. However, the measurement of both HMWFC and FPA is technically cumbersome and the latter assay is highly susceptible to false positive results due to traumatic venipuncture. Only one of these studies19 measured coagulation factors in patients with the more chronic complications of sickle cell disease. The single patient with leg ulcers reported showed normal levels of HMWFC.

The measurement of fibrin D-dimer with the latex bead agglutination assay showed significant correlation with disease activity in sickle cell disease. Asymptomatic patients were fibrin D-dimer negative, while patients in vaso-occlusive crisis or patients with other complications of sickle cell disease were positive. Fibrin D-dimer levels may be elevated in conditions other than sickle cell disease. We have found that 28% of 150 random samples from hospitalized patients had measurable levels of fibrin D-dimer.11 Most of these patients either had diseases associated with an increase in fibrinolytic activity or had recently undergone surgery.

Protein blot analysis of the species of fibrin recognized by the monoclonal antibody in plasma from sickle cell patients demonstrated that while the fragment D-dimer was the predominant species, presumably representing circulating fragment D-dimer/fragment E complexes, some fragment D1 and higher molecular weight forms of fibrin were detected in the blot. The latter may represent fragment DY or fragment Y dimers. Although DD-3B6 recognized fragment D in protein blot analysis, this fragment in fluid phase does not cause agglutination in the Dimertest assay.

The data reported here give no indication of the role abnormal coagulation activity plays in sickle cell disease. It is unclear whether abnormal fibrinolytic activity precipitates the onset of vaso-occlusive events, or is a result of vascular occlusion or vascular injury. However, the close correlation of fibrin D-dimer levels and the patient’s clinical condition may provide useful information in the management of patients with sickle cell disease and in the evaluation of therapeutic regimens.

ACKNOWLEDGMENT

We thank Martha Sutschenko and Mary Whorton for their help in sample collection.

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