D-D dimer levels in patients with sickle cell disease during bone pain crises and in the steady state

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Objective: To determine the presence of ongoing thrombosis by measuring the D-D dimer levels in bone pain crises (BPCs) and in the steady state of patients with sickle cell disease, comparing these levels with those in individuals with normal hemoglobin (HbAA) in southwest Nigeria.

Study design, patients, and methods: The study design involved 38 patients with homozygous sickle cell anemia (HbSS) and 78 adults with the HbAA phenotype, seen at the Hematology Day Care and Accident and Emergency units of the University College Hospital, Ibadan, Nigeria. The TintElize kit was used to quantitatively determine human D-D dimer levels in the plasma with enzyme immunoassay.

Results: The mean D-D dimer level measured of the 78 individuals with HbAA was 73.59 ng/mL. The mean D-D dimer level of the patients with HbSS during BPCs was 4002.40 ng/mL, while the mean level in the same patients in the steady state measured 6 weeks after their BPCs, with no other painful crisis episode before the sample was collected, was 1320.00 ng/mL.

Conclusion: This study demonstrated a significant increase in the D-D dimer levels of patients with HbSS in the steady state, when compared with those of individuals with HbAA of the same age and sex distribution. There was also an approximate threefold increase in the D-D dimer levels in the same patients with HbSS during BPCs. This confirms the activation of coagulation and fibrinolytic systems in patients with HbSS in the steady state, which is further escalated during BPCs. A multicenter study on the use of anticoagulants in BPCs in patients with sickle cell disease is required.

Keywords: anticoagulant, dimer, sickle cell disease, BPC, Nigeria, chronic hemolytic anemia

Introduction

Patients with homozygous sickle cell anemia (HbSS) are heterogeneous with respect to clinical, rheological, and hematological parameters, and attempts to demonstrate a correlation among these variables have produced conflicting results due to the difficulty in defining clinical severity in these patients. In the past, microvascular occlusion by the sludge of sickle red blood cells (RBCs) was thought to be the only mechanism responsible for the painful crises and subsequent damage to various end organs. However, there is increasing evidence that sickle cell disease (SCD) – as well as other chronic hemolytic anemias, such as β thalassemia, paroxysmal nocturnal hemoglobinuria, autoimmune hemolytic anemia, and unstable hemoglobinopathies – is characterized by a hypercoagulable state.^{1,2} Activation of the coagulation and fibrinolytic systems appears to play a role in the complex physiologic interactions of SCD.³ SCD is characterized by RBC membrane abnormalities, with abnormal exposure of phosphatidylserine,¹

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leading to sticky red cells and sludging. Hemostatic disorders in patients with SCD are typified by a hypercoagulable state, and plasma D-D dimer levels have been shown to correlate with the frequency and severity of painful vasoocclusive crises (VOCs) in SCD⁴; however, the role of coagulation mechanisms in the genesis of VOCs is still inconclusive. First, science must further elucidate the hemostatic status of patients with SCD.

Study design and patients

This was a prospective cross-sectional, hospital-based study. Patients with homozygous HbSS disease in bone pain crises (BPCs) presenting at the Hematology Day Care and Accident and Emergency Units, University College Hospital, Ibadan, Nigeria, during the period of the study were included. Ethical approval for this study was obtained from the Institutional Review Board, College of Medicine, University of Ibadan, Ibadan, Nigeria.

Patients with HbSS as well as other medical conditions not directly attributable to the disease (eg, renal/liver failure), other types of hemoglobinopathies and patients with HbSS presenting with other types of VOC (eg, abdominal crises, priapism, and acute chest syndrome) were excluded from the study. A total of 38 patients constituted the study population group while 78 HbAA individuals in the same age range served as the control subjects. Blood samples were taken from both groups on presentation at the hospital. Six weeks after the BPC, blood samples were taken again from the patient group which served as the auto-control.

Methods

Citrated plasma samples (3.2% citrate) from the patients and control subjects were stored frozen at -30°C . The D-D dimer levels were estimated every 2 months from the plasma samples using the TintElize[®] enzyme immunoassay (Biopool, Umea, Sweden). This immunoassay uses the double antibody principle. The plasma sample was added to a microtest well, which was coated with MA-8D3, a monoclonal antibody against D-D dimer. After an incubation period of 30 minutes, which is sufficient to allow $>85\%$ of the D-D dimer to bind to the coated antibodies, a horseradish peroxidase-labeled Fab fragment of anti-D-D dimer immunoglobulin G was added. The horseradish peroxidase-labeled anti-D-D dimer was allowed to react with the adsorbed D-D dimer. Then the well was emptied and washed to remove unbound conjugate. Following this, peroxidase substrate ($\text{OPD}/\text{H}_2\text{O}_2$) was added. The quantity of yellow that developed was measured with the enzyme-linked immunosorbent assay reader. The yellow

intensity is directly proportional to the amount of D-D dimer present in the sample.

Results

A total of 38 patients with HbSS, age range 17–41 years old, were enrolled in this study: 18 (47%) men and 20 (53%) women. Only 11 (28.9%) of the patients had BPCs more than 6 weeks before the BPC episode they had at the time of the study. Thirty-four (89.5%) of the patients had moderately severe BPCs (pain grade of 5–9). The BPC in 31 (81.6%) of the patients started within 4 days of the study, while three (7.9%) of the patients presented 10–14 days after onset of the crises.

The mean D-D dimer level measured of the 78 individuals with HbAA (Table 1) was 73.59 ng/mL (range = 10–430 ng/mL). D-D dimer values <500 ng/mL were considered normal according to the D-D dimer levels determined with the TintElize enzyme immunoassay.

Table 2 shows the D-D dimer levels and the frequency distribution of the patients with HbSS during BPCs and the steady state 6 weeks after the BPC episode. The mean value of the D-D dimer level measured during BPC was 4002.40 ng/mL (range = 230–12,480 ng/mL), while that measured in the same patients with HbSS in the steady state 6 weeks after the BPC episode with no other episode of painful crises before the sample was collected was 1320.00 ng/mL (range = 140–7610 ng/mL). Eighteen (47.4%) of the patients with HbSS during BPCs had D-D dimer levels >3000 ng/mL, while 36 (94.7%) of the patients with HbSS 6 weeks after a major BPC had levels <3000 ng/mL.

Four of the patients with HbSS presented to the hospital again with bone pain before the end of the sixth week. Their D-D dimer levels were estimated for the second episode and the repeat sampling 6 weeks with no further BPC episode in between. The patients' D-D dimer levels are shown in Figure 1.

Table 1 D-D dimer levels of individuals with HbAA

D-D dimer levels (ng/mL)	Number	Percentage
0–49	7	9.0
50–99	19	24.4
100–149	9	11.5
150–199	1	1.3
200–249	10	12.8
250–299	11	14.1
300–349	12	15.4
350–399	6	7.6
400–449	3	3.9
Total	78	100.0

Table 2 D-D dimer levels of patients with HbSS

D-D dimer levels (ng/mL)	Number (percentage) during BPCs	Number (percentage) in the steady state
0–499	2 (5.3)	8 (21.1)
500–999	5 (13.2)	12 (31.6)
1000–2999	13 (34.2)	16 (42.1)
3000–4999	7 (18.4)	1 (2.6)
5000–6999	4 (10.5)	0 (0.0)
7000–8999	4 (10.5)	1 (2.6)
9000–10,999	1 (2.6)	0 (0.0)
11,000–12,999	2 (5.3)	0 (0.0)
Total	38 (100.0)	38 (100)

Note: $P < 0.001$.

Abbreviation: BPCs, bone pain crises.

Discussion

SCD presents clinically with varying severity in different population groups. In this study, there was a significant increase in the D-D dimer levels in patients with HbSS (mean value = 1320 ng/mL) in the steady state compared with the D-D dimer levels of individuals with HbAA (mean value = 73.6 ng/mL) in the same age group and sex distribution. During BPCs in the patients with HbSS, the D-D dimer levels increased by about threefold with a mean value of 4002.40 ng/mL ($P < 0.001$). This confirms that activation of the coagulation and fibrinolytic systems in the steady state is further escalated during BPCs in individuals with HbSS.

Normally, phosphatidylserine is found in the inner monolayer of the cell membrane, whereas choline-containing phospholipids, such as phosphatidylcholine and sphingomyelin,

are located in the outer monolayer in the plasma membrane.⁵ Abnormal phosphatidylserine exposure functions as a recognition signal for cell removal during apoptosis of nucleated cells⁶ and a docking site for enzymatic complexes involved in coagulation and anticoagulation pathways.³ External exposure of phosphatidylserine alters the adhesive properties of RBCs⁷ and appears to be involved in the hemostatic changes observed in hemolytic anemias, particularly SCD.^{8–11} The number of phosphatidylserine-positive RBCs has been reported to be significantly correlated with plasma markers of thrombin generation, such as prothrombin fragment 1+2, D-D dimer, and plasmin–antiplasmin complexes in SCD, suggesting a significant role for RBCs in coagulation activation.^{8,9} Tissue factor, the principal initiator of coagulation, is abnormally expressed in circulating endothelial cells in patients with SCD.¹² Microparticles released during hemolysis may be tissue factor–positive.¹³ Several potential mechanisms for increased tissue factor expression have been described in SCD, including ischemia-reperfusion injury,¹⁴ increased levels of soluble CD40 ligand,¹⁵ and increased heme levels.¹⁶

These observations have been most frequently noted during episodes of painful vasoocclusion, but some abnormalities have also been detected in patients with SCD during the steady state. For this reason, it has perhaps been difficult to correlate such findings with the actual severity of SCD and it has been even more difficult to define the specific role played by disordered hemostasis in clinical manifestations that are referred to as “sickle cell crises.”

Figure 1 D-D dimer levels of four patients with bone pains at 6 weeks of a repeat bone pain crisis.

Note: P value = 0.033.

What role do the severity of the pain and the frequency of BPC have to play in determining the D-D dimer level at a given time? The precise level of D-D dimer circulating in the blood at a given time depends on the time elapsed since the thrombotic event, the initial size of the clot, and the rate of fibrinolysis. It is possible that the marked increase in D-D dimer levels observed in this study was due to multiple sites with varying severity of thrombi formation during BPCs in patients with HbSS. D-D dimer has a half-life of approximately 6 hours in the circulation of individuals with normal renal function. Patients with stabilized clots and who are not undergoing active fibrin deposition and plasmin activation may not have detectable D-D dimer elevations. Determining D-D dimer reflects activation of prothrombin plasminogen and of factor XIII. Thus, a direct test for circulating thrombin activity might give a clearer answer to the question of how severe the real state of coagulation activation is.¹⁷

The numerous complications of SCD that vary widely among patients are vexing for those experiencing them and their physicians. The frequency of VOCs, although of importance, cannot solely serve as a parameter of treatment efficacy; additional objective parameters are needed to effectively study and manage VOCs in SCD.¹⁸ Objective laboratory parameters that accurately reflect the vasoocclusive process in patients with SCD are needed to aid the clinician in daily assessment and management of this unpredictable disease. No single mechanism explains the vasoocclusion seen in SCD and the complexity of the process of vasoocclusion provides many possibilities for therapeutic intervention.¹⁹ The routine use of antithrombotic agents in patients with HbSS during a BPC is generally not favored. However, low-dose heparin therapy (ie, in prophylactic doses) during intractable BPC and acute chest syndrome in patients with HbSS have been documented to have beneficial effect.

Conclusion

This study demonstrated a significant increase in the D-D dimer levels of patients with HbSS in the steady state, when compared with those of individuals with HbAA of the same age and sex distribution. There was also an approximately threefold increase in the D-D dimer levels in the same patients with HbSS during BPCs. This confirms the activation of coagulation and fibrinolytic systems in patients with HbSS in the steady state, which is further escalated during BPCs. Finally, well-controlled clinical studies of therapeutic and prophylactic doses of anticoagulant use in BPCs and in

other forms of vasoocclusion employing appropriate clinical endpoints with the aim of reducing the severity and duration of SCD crises are required.

Disclosure

The authors declare no conflicts of interest in this work.

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