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Coagulation Changes in Individuals With Sickle Cell Trait

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Sickle cell disorders, such as Hb SS and Hb SC, are associated with a hypercoagulable state that may contribute to the vaso-occlusive episodes observed in the disorders. To what extent increased coagulation activity occurs in individuals with sickle cell trait has had limited study. Because such information may help clarify clinical and pathologic findings that may occur in these individuals and may be useful in clarifying the hypercoagulable state in sickle cell disease, we have examined individuals with Hb AS to determine the extent that increased coagulation activity does occur. We measured d-dimers, thrombin–antithrombin (TAT) complexes, prothrombin fragment 1.2 (F1.2), absolute blood monocyte levels, proteins C and S, and isotypes of antiphospholipid antibodies in individuals with Hb AS and in matched controls (Hb AA). Results showed that d-dimers, TAT, and F1.2 were increased significantly above normal levels. Absolute blood monocyte levels were increased. The d-dimers, TAT, F1.2, and monocyte counts showed significant increasing trends through groups of increasing severity (Hb AA, Hb AS, Hb SC, and Hb SS). Our study shows that individuals with Hb AS have increased coagulation activity, with d-dimers, TAT, and F1.2 being consistent indicators. The measures of coagulation activity in Hb AS are lower than in patients with Hb SC and Hb SS disease. These results extend our previous observation that the degree of coagulation activation parallels the degree of disease severity among sickle cell genotypes. The findings suggest that monocytosis, with the possible expression of monocyte-derived tissue factor, and the associated hypercoagulable state are driven by disease severity.
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Key words: sickle cell trait; coagulation; coagulation activity; hypercoagulability

INTRODUCTION

Although sickle cell trait (Hb AS) is a benign disorder, there is an increased risk for several abnormalities, including splenic infarction at high altitudes, hyposthenuria, hematuria, bacteriuria in women, bacteriuria and pyelonephritis in pregnancy, sudden death following exertion, pulmonary embolism, and glaucoma due to anterior chamber bleeding [1]. Previous studies have shown that a hypercoagulable state is present in patients with Hb SS and Hb SC [2,3] and in sickle cell trait [2] and that the changes in coagulation activity in Hb SS and Hb SC disease reflect the degree of disease severity of the hemoglobin disorder [3]. To further examine the extent to which increased coagulation activity occurs in individuals with sickle cell trait and to what degree the changes are comparable to the coagulation abnormalities observed in patients with Hb SS and Hb SC disease, we have made coagulation measurements in individuals with the trait and have compared results to controls, matched for age, gender, and race, and to previously described groups of patients with Hb SS and Hb SC disease [3].

METHODS

Twenty-three African-Americans with sickle cell trait (Hb AS) and 23 controls (Hb AA), who were

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matched for age, gender, and race, were examined. The individuals were 20–73 years of age and included 19 women and 4 men. The controls and patients were randomly selected and consisted of laboratory and non-laboratory personnel. Technicians performing the laboratory assays were blinded as to the origin of all samples. The study was approved by the Institutional Review Board of Mount Sinai Hospital, and informed consent was obtained from the participants.

Assays of Coagulation Activation

Measures of coagulation activation included d-dimers, thrombin–antithrombin complexes (TAT), and prothrombin fragment 1.2 (F1.2). The d-dimers were assayed by ELISA (American Bioproducts Co.; Diagnostica Stago, Asnieres-sur-Seine, France), TAT was determined by ELISA (Dade Behring, Chicago, IL), and F1.2 was assayed by ELISA (Dade Behring), as previously described [3].

Antiphospholipid Antibody Assay

Antiphospholipid antibodies (phosphatidylserine [PS], cardiolipin [CL], phosphatidylethanolamine [PE], phosphatidylglycerol [PG], and phosphatidylinositol [PI]) were measured by an ELISA as previously described [4]. Cut-off values were determined by using sera from 46 non-pregnant controls between the ages of 20 and 50 years. An absorbance equal to or greater than 2 standard deviations above the mean for that phospholipid and antibody isotype was considered positive. In some instances multiples of the mean have been used with essentially the same results. β_2-GPI was a necessary co-factor and was present in the fetal calf serum used as the blocker.

Proteins C and S Assays

Proteins C (activity, antigen) and S (activity, total, free antigen) were measured. The plasma was tested immediately for activity measurements and the remainder was quick frozen at −80°C until the time of the antigenic determinations. Appropriate standards and controls were performed with all assays.

Protein C activity was determined by chromogenic analysis using S2366 (Diapharm Chromogenic, Mölndal, Sweden) as the chromophore and Protac (American Diagnostica, Greenwich, CT) as the protein C activator. Protein C antigen was determined by electroimmunodiffusion (EID) (Laurell rocket electrophoresis). Agarose antibody plates were obtained from Helena Laboratories (Beaumont, TX) [5].

Protein S activity was measured by a clot-based analysis of dilute plasma with buffer (1:5) and preincubated with added activators and protein S deficient plasma (Bioclot Protein S kits, Biopool, Ventura, CA) [6]. Protein S antigen (total, free) was analyzed by EID on antibody-coated agarose plates (Assera-plate PS antigen kindly supplied by Diagnostica Stago). Total protein S antigen was determined by applying native plasma to the plate. Free protein S (unbound to C_{4b}-BP of complement) was also determined by EID after separating the free from the bound protein S. This was accomplished by cold precipitation of the bound protein S after mixing plasma (13:1) with 25% PEG (polyethylene glycol), centrifuging and separating the supernatant as free protein S.

Absolute blood monocyte counts were calculated from the percentage of monocytes present in total leukocyte counts. Urine osmolality was determined by freezing point depression (Advanced Instruments, Norwood, MA) on overnight fasting, first morning specimens.

Statistical Methods

The distributions of clotting variables (d-dimers, TAT, F1.2) and monocyte counts within disease groups tended to be skewed with large outliers to the right, and most showed evidence of extreme departures from normality according to the Shapiro–Wilk test. Due to the evidence of non-normality, together with the relatively small sample sizes on each group, we used non-parametric methods to compare medians rather than means. Correlations are based on rankings rather than original values.

The distribution of the measures of coagulation activity were described graphically and summarized with median values and inter-quartile ranges (IQR). Median values of each measure (d-dimers, TAT, F1.2) were each compared between the individuals with Hb AS and their matched controls using sign tests. Median values were also compared between the individuals with Hb AS and patients with Hb SC disease, between the individuals with Hb AS and patients with Hb SS disease and between patients with Hb SC and patients with Hb SS [3] using rank sum tests. Spearman correlation coefficients were used to examine the association between each of the coagulation measures and urine osmolality.

Correlations and partial correlations were used to analyze associations between d-dimers, monocyte counts, and disease severity. Disease severity was analyzed as a score representing the degree of increasing clinical severity in the various groups: 0 = Hb AA, 1 = Hb AS, 2.5 = Hb SC, 3 = Hb SS. Thus the variable, disease severity, would reflect the effect of an

average trend or the effect of a unit increase in the assigned score. Correlations were computed between d-dimers and monocyte counts, both unadjusted and adjusted for group scores; between group scores and monocyte counts, both unadjusted and adjusted for d-dimers; and, between group scores and d-dimers, both unadjusted and adjusted for monocyte counts [7].

RESULTS

Clinical data are shown in Table I. All participants in the study were healthy with no evidence of disease. Two individuals (#13, #22) had a past history of gross hematuria on one occasion. None of the individuals with sickle cell trait had present or past history of pulmonary embolism or leg ulcers. Ten individuals (#14–#23) had moderately decreased hemoglobin levels (10.4–11.8 g/dl). The individuals with decreased hemoglobin levels had no history of pregnancy for at least 2 years, no clinical or laboratory evidence of iron deficiency, and no reported use of oral contraceptives during the period of the study. The decrease in hemoglobin levels in this group may be related to several factors such as co-existent α-thalassemia [8], aging, race, or gender [9].

Median d-dimer, TAT, and F1.2 levels were significantly increased in the individuals with sickle cell trait (Table II). There was no correlation between d-dimer, TAT, and F1.2 and the hemoglobin concentration, whether normal or decreased. Similarly, the levels of Hb S and urine osmolality did not correlate with the levels of coagulation activity. The d-dimer, TAT, and F1.2 levels in individuals with Hb AS were less than those previously described in patients with Hb SC and Hb SS disease (Table II, Fig. 1) [3].

TABLE I. Clinical Data on Individuals With Sickle Cell Trait

	Age	Gender	Hb (g/dl)	HbS (%)	MCV (fl)	Urine osmolality (mOsm/kg H_2O)
1.	30	F	13.5	42	88	546
2.	34	F	13.9	42	89.5	592
3.	31	F	13.3	37	79.4	427
4.	46	F	13.9	42	93.4	247
5.	46	F	13.3	41	93.9	238
6.	28	M	15.1	42	94.4	801
7.	20	M	14.7	43	91	778
8.	72	M	14.5	40	87.4	469
9.	27	F	12.8	26	72	787
10.	43	F	12.4	41	87.5	462
11.	56	F	13.2	40	85	467
12.	50	M	12.9	41	87.5	439
13.	58	F	13.5	40	89.5	457
14.	43	F	10.8	41	92	412
15.	37	F	11.1	42	82.7	388
16.	28	F	11.4	43	93	ND[a]
17.	50	F	11.7	40	94.6	817
18.	47	F	10.8	38	88.6	338
19.	72	F	11.8	40	79	466
20.	25	F	11.7	39	80.9	472
21.	73	F	11.4	42	83	432
22.	37	F	10.4	31	81	472
23.	30	F	10.5	39	81	674

[a]ND, not done.

Absolute blood monocyte counts were lowest in the controls and were progressively higher in individuals with Hb AS and in patients with Hb SC and Hb SS (controls, 398 [274] $\times 10^9$ cells/l; median [IQR]; Hb AS, 476 [301]; Hb SC, 613 [691]; Hb SS, 949 [867]). Pairwise comparisons between groups were not significant at $P < 0.05$. The plots in Fig. 2 show the group median values for monocyte counts plotted against the group median values for d-dimers and reflect a near-linear relationship across the group

TABLE II. Measures of Coagulation Activation in Individuals With Sickle Cell Trait, Patients With Hb SC Disease, Patients With Hb SS Disease, and in Controls[a]

	Sickle trait	Control (sickle trait)	Hb SC	Hb SS	Control (Hb SC, HbSS)
D-dimer (μg/ml)	0.46 (0.27) [n = 23]	0.30 (0.25) [n = 23]	0.81 (0.70) [n = 16]	1.26 (1.63) [n = 18]	0.2 (0.2) [n = 17]
P	0.0005*, 0.0013**; 0.0002***		0.0792[b]		
TAT (ng/ml)	2.8 (2.5) [n = 23]	1.5 (1.0) [n = 23]	4.1 (3.7) [n = 15]	6.8 (8.1) [n = 18]	2.4 (2.35) [n = 18]
P	0.0072*, 0.0379**; 0.0003***		0.0203[b]		
F1.2 (nm)	0.6 (0.5) [n = 23]	0.5 (0.5) [n = 23]	2.3 (1.8) [n = 15]	2.6 (1.3) [n = 18]	0.76 (0.76) [n = 17]
P	0.0266*, 0.0004**; 0.0002***		NS[b]		

[a]Values are expressed as median (inter-quartile range); values for Hb SC and Hb SS were obtained from Ref. 3; NS, not significant at < 0.05 level; n = number of subjects.
[b]Hb SC v Hb SS.
*Sickle trait v control.
**Sickle trait v Hb SC.
***Sickle trait v Hb SS.

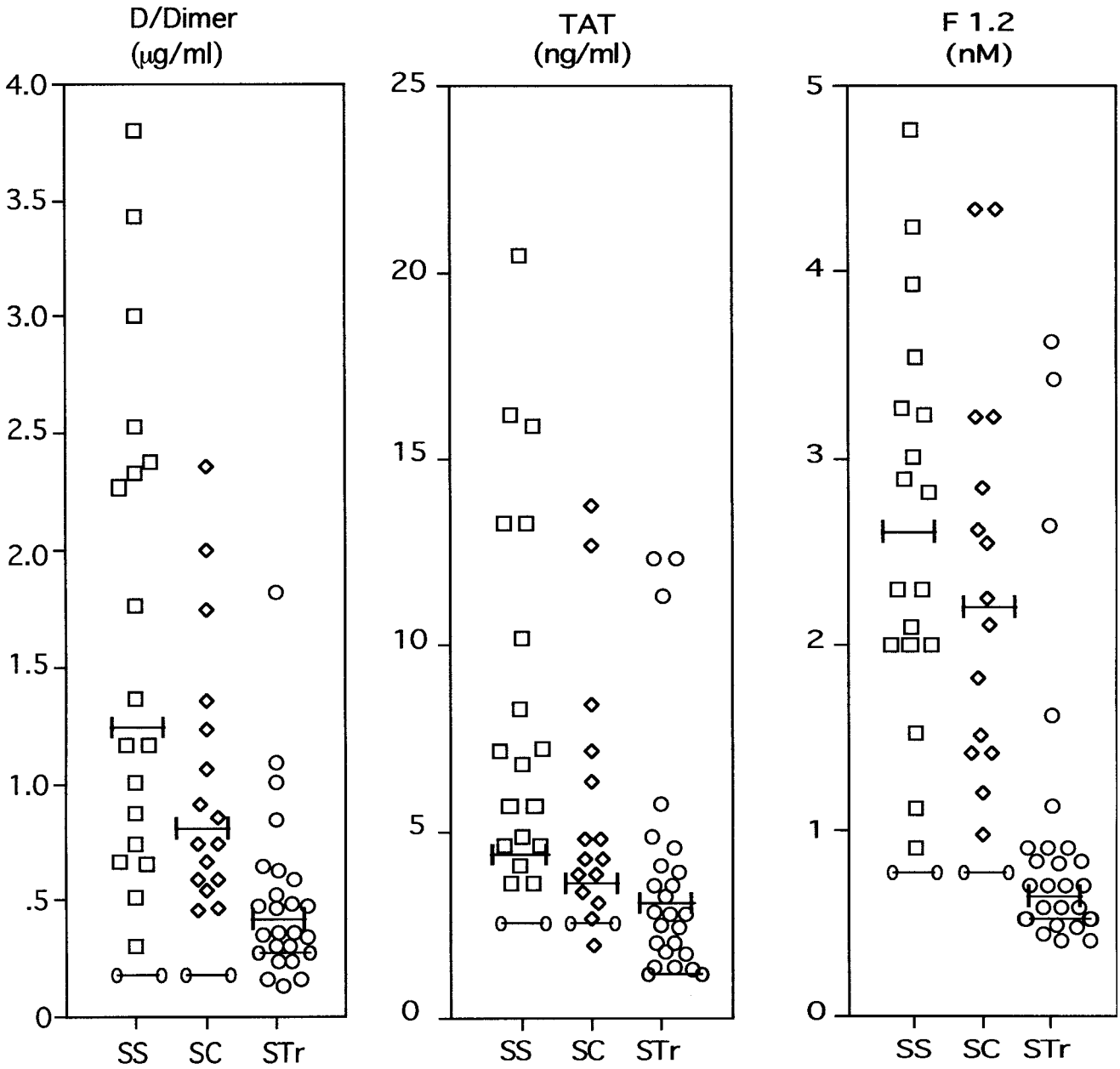

Fig. 1. Levels of d-dimers, TAT, and F1.2 in patients with Hb SS disease, Hb SC disease, in individuals with sickle cell trait and in controls. (I—I) Median values for Hb SS, Hb SC, and Hb AS. (O—O) Median values for controls.

means, with the groups ordered in terms of increasing disease severity. Plots of TAT and F1.2 did not show a similar trend.

Increasing trends in d-dimers and monocyte counts computed from all disease groups combined showed an apparent association between d-dimers and monocyte counts without adjusting for group ($r = 0.27$, $P = 0.04$, $n = 57$). The correlation coefficient for the trend in d-dimers by disease severity score (0 = Hb AA, 1 = Hb AS, 2.5 = Hb SC, 3 = Hb SS), adjusting for monocyte counts, was $r = 0.78$ ($P < 0.0001$, $n = 57$). The correlation coefficient for the trend in monocyte counts by disease severity score, adjusting for d-dimers, was $r = 0.33$ ($P = 0.013$, $n = 57$). The association between d-dimers and monocyte counts was not significant after adjusting for group.

Antiphospholipid antibodies, protein C (activity, antigen), and protein S (activity, total, free antigen) in individuals with Hb AS were in the normal range (results not shown).

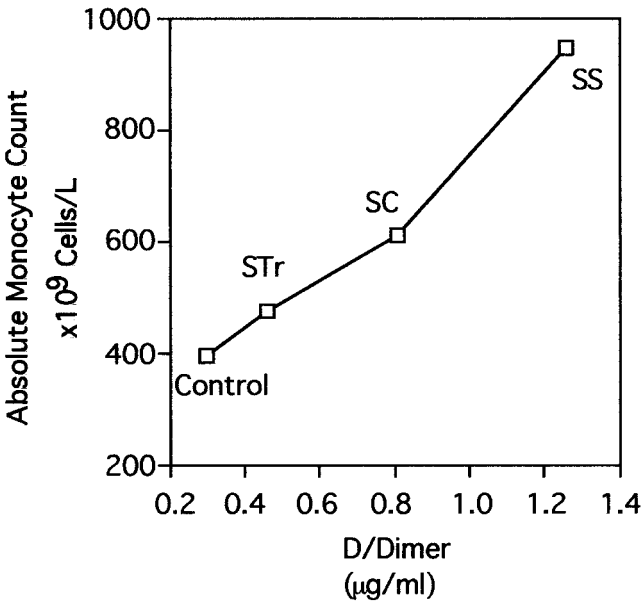

Fig. 2. Comparison of median absolute monocyte counts to median d-dimer levels in patients with Hb SS disease, Hb SC disease, in individuals with sickle cell trait, and in controls.

DISCUSSION

The study shows that the coagulation pathway in individuals with sickle cell trait is activated, as indicated by the significantly increased levels of d-dimers, TAT, and F1.2. The cause of the increased coagulation activity includes several possibilities. Loss of phospholipid asymmetry in the membranes of sickle red blood cells [2,10–12] and in the membranes of the vesicles shed from the cells [13–15], with exposure of phosphatidylserine (PS) on the membrane surface, would contribute to hypercoagulability. Although the loss of asymmetry, which is associated with deoxygenation of sickle red cells and production of membrane-shed vesicles, would be less likely to occur in Hb AS red cells than in Hb SS or Hb SC red cells, loss of asymmetry can occur because deoxygenation of red cells is observed in individuals with Hb AS [16]. Increased absolute blood monocyte levels, which are associated with increased expression of monocyte-derived tissue factor in both Hb SS and Hb SC disease [17], are also increased in individuals with Hb AS and may contribute to the increase in coagulation activity [18]. Consequently, both the loss of phospholipid asymmetry in sickle red cells and the sickle red cell-shed vesicles and the presence of monocytosis, with possible increased expression of monocyte-derived tissue factor, may contribute to the increase in coagulation activity in individuals with Hb AS.

It is of interest that the levels of d-dimers and blood monocytes show a near-linear trend across the genotypes as disease severity increases from controls through Hb AS, Hb SC disease, and Hb SS disease. The partial correlation between disease severity groups and monocyte counts, adjusting for d-dimers, is significant, whereas the partial correlation between d-dimers and monocyte counts, adjusting for disease severity groups, is not significant. The finding suggests that membership in a genotype has a stronger independent relationship with monocyte counts than do d-dimer levels and implies that the groups are driving the relationship between absolute monocyte levels and likely tissue factor expression and coagulation activity.

The measures of coagulation activity appear to reflect the levels of disease severity observed in the groups, i.e., in Hb SS disease, Hb SC disease, and Hb AS. Levels of d-dimers, TAT, and F1.2 decline in parallel with the decreasing degrees of disease severity observed in the genotypes with TAT and d-dimer levels being the most consistent measures of increased coagulation activity. The extent to which the differences in coagulation activity contribute to the differences in clinical and pathologic findings in Hb SS disease, Hb SC disease, and sickle cell trait is not clear. The findings of a hypercoagulable state in the individuals with Hb AS extend the results obtained in our previous study [3] in which the degree of hypercoagulability reflected the difference in disease severity between Hb SS and Hb SC disease. The progressive decline in absolute monocyte counts with the decreasing levels of group disease severity would be compatible with the findings of a similar decline in levels of coagulation activity associated with decreasing group disease severity.

The findings in our study differ from those observed in a previous study [2] in that d-dimers, which were significantly altered in the present study, were not measured in the earlier study and the relationships between group severity and coagulation activity, although parallel in the previous study, did not generally reach statistical significance.

Evaluation of the hypercoagulable state and its relationship to the clinical and pathologic findings in Hb AS have not been defined. Measures of coagulation activity during complications, such as hematuria, however, may be important. Although the total number of patients who have been examined in the present study are relatively small, we consider that the findings are relevant and may contribute to concepts of pathogenesis and management. The findings may also contribute to an understanding of the increased risk of unexplained sudden death in African-American recruits in the Armed Forces with Hb AS or to the more recently described increased risk of pre-eclampsia observed in African-American women with Hb AS [19].

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