CHANGES OF HEMOSTATIC FACTORS IN PATIENTS WITH HEMOGLOBINOPATHIES*

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In this study, protein C (PC), protein S (PS), heparin cofactor II (HCFII), prothrombin fragment 1+2 (PF1,2), thrombin-antithrombin III complex (TAT), von Willebrand factor (vWF) and thrombomodulin (TM) were investigated in 13 patients with beta thalassemia intermedia (TI) not requiring transfusion, six patients with sickle cell disease (SCD), and seven patients with HbS-beta thalassemia (S-BT) who were not in crisis. These hemostatic parameters were also studied in 12 healthy children assigned as a control group.

Protein C and Protein S (PC-PS) were found to be decreased in TI patients and normal in S-BT patients. PC was decreased in SCD patients. In the patients with TI and SCD, the mean PF1,2 level was elevated, whereas the TAT level was not statistically different from that of the control group. These results suggested that in patients with hemoglobinopathies: a) decreased natural anticoagulants and b) enhanced procoagulant activation have been encountered. Other unexpected and interesting results of this study are the decreased vWF and elevated HCFII levels in all three patient groups. *Key words: thalassemia, sickle cell disease, coagulation inhibitors, activation in coagulation.*

In thalassemic patients, many ischemic strokes, peripheral arterial or venous thrombosis, pulmonary emboli and renal infarcts have been reported[1,2]. These observations indicate that thrombosis is the one of the important life threatening complications in hemoglobinopathies. Thrombosis is observed in these patients especially after splenectomy[2]. Recently, several studies concerning hemostatic disturbance and their influences on the development of thrombosis have been reported in thalassemic patients[3,4]. In sickle cell disease (SCD), it has been generally suggested that the sickling phenomenon and its interaction with the vascular endothelium provoke sickle cell crisis; however, the role of hemostatic changes in the pathogenesis of vaso-occlusion has been encountered only

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recently[5,6]. At present, although studies have yielded controversial results, it is thought that vascular occlusion in sickle cell patients is a multifactorial event rather than merely a sickling phenomenon[5,6]. Hemoglobinopathies are frequent in Turkey and in other Mediterranean countries[7]. It has therefore been possible in this study to investigate several parameters of different hemostatic compartments in quite a high number of patients with hemoglobinopathies, in an effort to shed light on the mechanisms leading to thrombophilia in this group of disorders.

Material and Methods

The subjects of this study were 26 patients (mean age 14.5 ± 1.9 years) whose cases were followed at Hacettepe University Children's Hospital between 1996-1997 [13 patients with thalassemia intermedia (TI) not requiring blood transfusion, 7 patients with HbS-thalassemia syndrome (S-BT) and 6 patients with SCD]. No S-BT or SCD patients were in crisis. These hemostatic parameters were also studied in 12 healthy children assigned as a control group (mean age 8.4 ± 3.1 years). Following separation from the whole blood, plasma samples were stored at -70 °C until the study was performed. The plasma antigenic levels of protein C (PC), total protein S (PS), von Willebrand factor (vWF), thrombomodulin (TM) (asserochrome protein C, protein S, von Willebrand factor, thrombomodulin, Diagnostica Stago, Paris, France), thrombin-antithrombin III complex (TAT) and prothrombin fragment 1-2 (PF1,2) (Anzygnost TAT, PF1,2 micro, Behring, France) were measured by enzyme-linked immunoabsorbent assay (ELISA). The heparin cofactor II (HCFII) level (stachrome HCFII; Diagnostica Stago, Paris, France) was measured by colorimetric assay. Statistical analyses were performed using the Mann-Whitney U, Wilcoxon and Pearson tests.

Results

The results of all three groups are presented in Table I.

TI Group: Plasma PC, PS and vWF levels were found to be decreased, while PF1,2 and HCFII levels were increased, as compared to those of the control group ($p < 0.01$ for all five tests); TAT and TM levels were not different from the control levels. A positive correlation was found between the PF1,2 and TAT levels (r: 0.85, $p < 0.01$).

S-BT Group: vWF level was decreased, while PF1,2 and TAT levels were increased, as compared to those of the control group ($p< 0.01$, < 0.01, < 0.01 respectively). The mean plasma levels of PC, PS, HCFII and TM were normal.

SCD Group: PC and vWF levels were decreased (p:0.01 and < 0.01, respectively) and the HCFII level was increased ($p < 0.01$); TAT and TM levels in this group were normal.

Table I: Results of the Plasma Levels of Hemostatic Parameters in Three Groups Compared to the Control Group

Hemostatic Parameters		β-Thalassemia Intermedia (n:13) (1)*	S-β Thalassemia Group (n:7) (2)*	Sickle Cell Disease Group (n:6) (3)*	Control Group (n:12) (4)*	P**
PC	(mg/L)	69.3 ± 3.9	97.4 ± 11.5	81.5 ± 8.2	113.5 ± 8.8	1-4:<0.001 3-4:<0.01
PS	(mg/L)	48.3 ± 2.6	60.7 ± 6.7		100.1 ± 6.4	1-4:<0.001
PF1,2	(µg/L)	5.8 ± 2.4	6.6 ± 2.1	40.8 ± 10.1	1.6 ± 0.1	1-4:<0.01 2-4:<0.01
TAT	(µg/L)	80.8 ± 25.9	133.2 ± 32.4	61.0 ± 5	40.8 ± 10.1	2-4:<0.01 3-4:<0.01
HCFII	(U/ml)	88.6 ± 6.5	88.7 ± 2.2	105.0 ± 6.1	61.0 ± 5	1-4:<0.001 3-4:<0.01
vWF	(%)	52.6 ± 8.2	67.8 ± 12.9	17.9 ± 2.5	105.0 ± 6.1	1-4:<0.001 2-4:<0.01 3-4:<0.01
TM	(ng/ml)	18.6 ± 2.8	19.4 ± 2.3		17.9 ± 2.5	

* The groups are numbered as 1, 2, 3 and 4 for statistical analysis.
** Only significant "p" values are showed in the table.
PC: protein C, PS: protein S, PF1,2: prothrombin fragment 1+2, TAT: thrombin-antithrombin III complex, HCFII: heparin cofactor II, vWF: von Willebrand Factor, TM: thrombomodulin.

Discussion

Hemoglobinopathies complicated with hemostatic disorders showing bleeding or thrombosis have been reported in several studies[1,2,4]. However, in the majority of studies only one component of the hemostatic system is evaluated. In this study, the three different components of hemostasis have been studied.

Protein C PC and PS, important natural anticoagulants, inhibit active FV and FVIII. HCFII, together with dermatan sulfate, inhibits thrombin as well. PFI,2 is a secondary product during the conversion of prothrombin to thrombin. TAT is a stable substrate which is required for the inactivation of thrombin. PF1,2 and TAT have been accepted as sensitive indicators of activation in coagulation, while vWF and TM are the most important indicators of vascular injury, in addition to functioning in hemostasis[8].

In this study, PC and PS levels were found to be decreased in the TI group. In the SCD group, PC was also found to be decreased (PS was not studied in this group). These findings are in accordance with the results of previous studies which interpreted these levels as due to either impaired protein production in the liver or as secondary to increased thrombin generation[3,8]. In the S-BT group, an increase in PF1,2 indicated that the coagulation system was activated, whereas normal PC and PS levels suggested that the production of these proteins by the liver was not diminished in this group.

Phospholipids, leaking from the erythrocyte membranes following hemolysis, have been shown to activate coagulation cascade probably causing an elevation in PF1,2 and TAT levels[6,9]. It was therefore suggested that the activation of coagulation cascade seen in TI and SCD is probably seconday to hemolysis, which enhances thrombotic risks in these patients. The elevation in the HCFII level found in this study but not in previous publications needs to be evaluated in further studies[10,11].

von Willebrand Factor levels were found to be decreased in all three groups ($p < 0.01$ in all three groups). Acquired vWF deficiency was described in some conditions, such as hematological malignancies, and in patients to whom some drugs were administered. The low vWF levels could be explained by the presence of an antibody to the vWF, proteolysis of vWF directly, and/or absorption of vWF by malignant cells[12]. However, we could not interpret the statistically significant low vWF levels found in all three patient groups ($p < 0.01$) by these mechanisms.

There was no decrease in the TM levels in any of the patient groups, indicating that endothelial injury does not seem to contribute to thrombotic complications in these patients. However, measurement of this parameter in S-BT and SCD during crisis could be useful in the evaluation of endothelial injury that may occur in vaso-occlusive crisis in these patients.

In conclusion, our study indicated that some natural inhibitors such as PC and PS are decreased in hemoglobinopathies. These low levels may be secondary to increased thrombin generation or due to the impaired hepatic production of these proteins. Elevated levels of PF1,2 and TAT indicated that a subclinical thrombotic process occurs in hemolytic anemia. However, since thrombosis a multifactorial process, thrombotic tendency in patients with hemoglobinopathies may be enhanced by other factors.

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