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ORIGINAL ARTICLE

Increased Level of Factor VIII and Physiological Inhibitors of Coagulation in Patients with Sickle Cell Disease

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Abstract Sickle cell disease (SCD) is a hemoglobinopathy characterized by hemolysis, oxidative stress, and vaso-occlusive crises. Thromboembolism also remains a serious complication and probably underestimated in the SCD. Our objective was to seek the existence of hemostasis abnormalities that predispose to thrombosis such as elevation of FVIII and Physiological inhibitors of coagulation deficiency. We studied 81 patients with SCD, including 32 homozygous S/S, 20 double heterozygous S/β thalassemia and 29 heterozygous S/A. Controls AA were in number 60. For each patient and control we assayed the physiological coagulation inhibitors (Protein C, Protein S and Antithrombin) and the clotting FVIII. We found a significant increase in FVIII in all phenotypes of SCD compared to controls. Also, a significant decrease in levels of protein C and S was observed in patients with sickle cell homozygous or double heterozygous S β Thalassemia compared to controls. As against, for antithrombin no difference was observed between patients and controls. These hemostasis abnormalities therefore reflect the existence of a pro thrombotic state in sickle cell disease that can explain the increase of incidence of thrombosis in this pathology. Factor VIII clotting consistently high in SCD may well be a prime therapeutic target in the treatment of thrombotic manifestations of this disease.

Keywords Protein C · Protein S · Antithrombin · Factor VIII · Sickle cell disease

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Introduction

Sickle cell disease is a constitutional hemoglobinopathy due to a mutation GAG to GTG leading to the substitution of a Glutamine by a Valine on the sixth Amino Acid of the β globin chain.

This structural modification of hemoglobin promotes polymerization of the deoxygenated form, which indicates the distortion of red blood cell, substantially at low pressure oxygen [1].

Clinically there are essentially hemolytic anemia, oxidative stress and vaso occlusive crises (VOC) [2, 3]. thromboembolism also remains a serious complication and probably underestimated in the SCD [4].

In Algeria the SCD is a relatively common disorder with a prevalence of 4.9/100,000 population for major syndromes and 1.2 % for sickle cell trait. It represents a real problem of health since 38.5 % of patients have over 3 VOC per year and 43 % are hospitalized more than 10 days per year [5].

Given a higher incidence of thromboembolism in sickle cell disease are there haemostatic abnormalities that predispose?

In this regard a case–control study of 81 patients was performed in the Hemobiology Department of the University Hospital Center of Oran and allowed for the existence of an association between SCD and haemostatic abnormalities seeking the existence of a thrombotic process.

Patients and Methods

Study Design and Population

153 patients were contacted from the local registry of SCD. Exclusion criteria were as follows: blood transfusion in the

last 3 months, VOC in progress and any treatment or condition that might interfere with the assessment of thrombophilia as: pregnancy and postpartum, use of oral contraceptives, recent surgery and prolonged bed rest. Also, patients who had liver failure with increased Prothrombin Time (PT) and/or clinical signs of cirrhosis and those who had kidney failure or cancer were excluded. Knowing the effect of potential bias of inflammation on FVIII clotting and protein S and for a better assay specificity, we excluded patients with a C reactive Protein (CRP) upper than 15 mg/l. Samples were taken at 3 months distance from the thrombotic event.

In history, in case of occurrence of vasoocclusive crises patients received treatment based on analgesics and hydratation except in one patient who received treatment with hydroxyurea because of her stroke antecedents. Anemia was treated by red blood cells transfusion.

We tried to increase the power of our study using a case–control ratio of each phenotype close 2. Thus, 60 controls were selected from families sharing similar cases of constitutional abnormalities of hemostasis risks. The controls are all with AA phenotype in hemoglobin electrophoresis.

Patients and controls were matched for age, sex, blood group and ethnic origin from sub-Saharan Africa or no.

Assaying of the Physiological Coagulation Inhibitors and FVIIIc

After informed consent samples were collected in tubes containing citrate (0.109 M). Plasmas obtained by centrifugation (2500 rpm for 15 min) of whole blood were processed the same day within a period not exceeding 2 h.

We assayed the activity of protein C (PC), protein S (PS) and clotting Factor VIII (FVIII: c) by chronometric method and we determined Antithrombin (AT) activity by colorimetric method using reagents Stago and STA Compact® (Stago®, Diagnostica Stago, France).

For the interpretation of the results we used the normal values specified by the manufacturer; PC [70–130 %], PS [55–145], AT [80–120 %], FVIII:c [60–150 %].

Statistical Analysis

The mean and standard deviation were calculated for each group. T-Student test was used to compare the results observed in each group SS, Sβ thalassemia and AS with the group of controls AA. The statistical relationship between haemostatic abnormalities predisposing to thrombosis and phenotypes of SCD was determined by the χ_2 test. Difference between patients and controls was considered significant when $P < 0.05$ and non-significant in the other cases.

Results

Among 153 patients enrolled in the local registry of SCD, 127 were present and examined for eligibility. 93 were deemed eligible after having eliminated 34 including 4 for contraceptive use, 20 for vaso-occlusive crisis and 10 for transfusion. The upper 15 mg/l CRP management allowed us to eliminate 12 patients to finally have 81 patients included in the study.

The median age of patients was 28 years with interquartile range from 15 to 35 years and sex ratio M/F was 1.25. 23 % of patients were from sub-Saharan africa and 43 % had blood group "O". Among patients 32 had S/S phenotype, 20 S/β Thalassemia (16 S/β° and 4 S/β+) phenotype and 29 S/A phenotype. The median age of controls was 26 years with interquartile range from 13 to 35 years and sex ratio M/F was 0.82. 22 % of patients were from sub-Saharan Africa and 42 % had blood group "O". The mean hemoglobin was 8.7 (±0.7) g/dl in patients with SS phenotype, 9.2 (±1.1) in patients phenotype S/β thalassemia, 10.3 (±0.5) in those with S/A phenotype and 12.4 (±1.4) in controls.

We found that 6 patients (7.5 % of patients) had a history of venous thromboembolism.

Table 1 reports the results of the 4 groups. We found a significant increase in $FVIII_C$ in patients with phenotypes SS, Sβ Thalassemia and SA compared to controls AA.

Also, a significant decrease in levels of protein C and S was observed in patients with sickle cell homozygous or double heterozygous S β Thalassemia compared to controls.

As against, for antithrombin no difference was observed between patients and controls.

Unlike SS and Sβ thalassemia, sickle cell trait was associated with an increased rate of coagulation inhibitors: antithrombin and protein C.

The elevation of FVIII was allele dependent since we observed that the presence of allele Hb S caused a 30 % FVIII increase compared to the controls and 97 % when two alleles HbS were present.

The threshold values recommended by the supplier of reagent has a bio clinical interpretation of the results as summarized in Table 2.

It was a statistical relationship between PC and PS deficiency with S/S and S/β thalassemia phenotypes but not with sickle cell trait when AT deficiency was associated only with S/S phenotype. Clotting FVIII increase was associated with the presence of the S allele (Table 2).

The only patient treated with hydroxy urea showed no abnormalities of hemostasis predisposing to thrombosis in parallel with an absence of VOC since the introduction of this treatment.

Table 1 Rates of FVIII:c and inhibitors of coagulation according to the profile of the hemoglobin electrophoresis

	S/S			S/β thalassemia			S/A			A/A	
	Mean	SD	*P*	Mean	SD	*P*	Mean	SD	*P*	Mean	SD
Protein C	80	25	0.04	78	13	0.01	118	37	0.01	101	17
Protein S	54	7	0.000	57	9	0.000	82	13	0.003	86	10
Antithrombin	93	16	0.13	100	9	0.47	108	15	0.02	102	11
$FVIII_C$	203	52	0.000	161	40	0.000	134	40	0.000	103	19

SD standard deviation

Table 2 Relationship between hemostasis abnormalities predisposing to thrombosis and SCD phenotypes

	Patients N (%)	Controls N (%)	*P*
S/S phenotype			
PC deficiency	15 (46.87)	1 (1.66)	0.000
PS deficiency	17 (53.12)	1 (1.66)	0.000
AT deficiency	9 (28.12)	0 (0.00)	0.000
FVIIIc increase	31 (96.87)	4 (6.66)	0.000
S/β thalassemia phenotype			
PC deficiency	7 (35)	1 (1.66)	0.000
PS deficiency	7 (35)	1 (1.66)	0.000
AT deficiency	0 (0.00)	0 (0.00)	*
FVIIIc increase	8 (40)	4 (6.66)	0.000
S/A phenotype			
PC deficiency	2 (6.89)	1 (1.66)	0.200
PS deficiency	1 (3.44)	1 (1.66)	0.595
AT deficiency	0 (0.00)	0 (0.00)	*
FVIIIc increase	9 (31.03)	4 (6.66)	0.002

PC protein C, *PS* protein S, *AT* antithrombin

* No statistics are computed because AT is a constant

Discussion

Deficits in physiological coagulation inhibitors may be from constitutional or acquired origin.

However, the presence of associated deficits in 2–3 coagulation inhibitors directs us to an acquired deficit and we spotted to make family surveys.

For all cases of deficits in physiological coagulation inhibitors that we found we realized family surveys and the result was that no case of inhibitors deficiency was found in the parents.

This leads us to conclude that the deficits observed in physiological coagulation inhibitors are acquired and unconstitutional.

Increased FVIII and physiological coagulation inhibitors deficiency that we found increase the risk of venous thrombosis in patients with sickle cell disease. It is known today that high level of FVIII multiplied by six the risk of thrombosis [6] although this risk is multiplied up to 10 times in case of deficiency in coagulation inhibitors protein C, protein S and antithrombin [7, 8].

Especially since we found a combination of at least 2 of haemostatic abnormalities in 63 % of patients increasing the risk of thrombosis knowing that the risk of thrombosis in case of multiple risk factors is more important than the sum of risks of this factors taken alone [9].

Increased levels of protein C and antithrombin in patients with sickle cell trait may be due to chance and we found no studies in the literature confirming this observation.

Many investigators have shown biomarker evidence for activation of coagulation. Thrombin generation is increased in SCD evidenced by increased prothrombin fragments 1.2, thrombin antithrombin complexes, plasma fibrinogen products and D dimer. Decreases in physiological anticoagulants levels would promotes this hypercoagulable state and have been reported in SCD at steady state [10].Physiological anticoagulants tend to decrease to even lower levels during crises episodes. In 2004 Solovey et al. [11] observed in a SCD mouse model that 3 h of exposure to a hypoxic environment resulted in an increase in pulmonary veins tissue factor expression, suggesting that hypoxia in VOC of SCD may play a role in activation of procoagulant protein and consumption of physiological anticoagulants.

Other studies have found abnormalities of hemostasis explaining hypercoagulability in sickle cell disease and among these we find the exposure of membrane phospholipids pro coagulants including phosphatidylserine on the surface of sickle cell [4, 12] but also increase of microparticles expressing tissue factor in sickle cell disease [13].

Hydroxyurea is used in sickle cell patients, adults and children, to reduce the frequency of painful crises. Assigned only at the beginning of its use in patients with sickle cell disease to reactivation of fetal hemoglobin synthesis, this beneficial effect was also found associated with many other processes, such as reducing excessive adhesion of erythrocytes endothelium but also a restoration of a normal distribution of membrane phosphatidylserine [14] and a decrease in markers of hypercoagulability [15].

Koc et al. [16] found that the introduction of a hydroxyurea treatment decreased in average 54 % of the FVIII level compared to its one before treatment.

This could explain the results of the normal blood count and thrombophilia parameters of the patient under hydroxyurea in connection with the disappearance of vaso-occlusive crises. This observation about a single patient should be confirmed on a larger cohort.

Conclusion

Deficits at variable rates in physiological coagulation inhibitors and the increased activity of factor VIII are abnormalities of hemostasis that reflect the existence of a pro thrombotic state during SCD.

These abnormalities suggest a dose effect in the number of S alleles, and they are more frequent in homozygotes than in/S β thalassemia patients and heterozygotes AS.

This pro thrombotic state can so explain the increase of thromboembolic events in the SCD.

Factor VIII clotting consistently high in SCD may well be a prime therapeutic target in the treatment of thrombotic manifestations of this disease.

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