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RESISTANCE TO ACTIVATED PROTEIN C AS A RISK FACTOR OF STROKE IN A THALASSEMIC PATIENT

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ABSTRACT

It is well known that thalassemic patients exhibit an increased frequency of thrombotic events. Most individuals with resistance to activated protein C (APCR) are the result of a point mutation replacing Arg 506 with Gln in the factor V aminoacidic sequence (factor V Leiden). Recently APCR has been shown to account for up to 50% of cases of thrombophilia. In this report, we describe a 10 year old thalassemic intermedia patient heterozygous for Factor V R506Q who developed a stroke following transfusion. Coagulation laboratory values were all within the normal range and there was no evidence of a lupus anticoagulant. Computerized brain tomography showed an ischemic area in the left temporo-parietal region. At follow-up, plasma from the patient demonstrated APCR and molecular diagnostic testing revealed heterozygosity for factor V R506Q. We suggest that the heterozygosity for factor V Leiden could increase the thrombotic risk in thalassemia intermedia. We believe it may be beneficial to screen all intermedia thalassemic patients for APCR especially before starting a transfusional regimen.

Key words: thalassemia, FV Leiden, APCR, thrombophilia

It is well known that thalassemic patients exhibit an increased frequency of thrombotic events including transient ischemic attacks, strokes, peripheral arterial venous thrombosis, and an increase in pulmonary emboli found at autopsy.[1] The cause of the hypercoagulable state seen in thalassemic patients has been attributed to a wide variety of hemostatic alterations including platelet hyperaggregability, absence of a spleen due to therapeutic splenectomy[2,3] and reduced levels of physiologic anticoagulants such as antithrombin III (ATIII), protein C (PC) and protein S (PS).[4,5] Hypercoagulability is more evident in untransfused thalassemia intermedia patients than in thalassemia major patients. At least two potential causes have been suggested: a greater exposure of phosphatidylserine in the outer leaflet of red blood cells[6] and a lower level of heparin cofactor II (HCII).[7]

Recently, resistance to activated protein C (APCR), has been shown to account for up to 50% of cases of thrombophilia.[8] It was shown that this APCR is associated with a mutation in the factor V (FV Leiden), in which Arg 506 in one of the APC cleavage sites has been replaced by Gln.[8,9] The prevalence of APCR in asymptomatic individuals is 2%-5%, and in Italy the mutation has been found in approximately 2% of normal population and 23% of patients with deep vein thrombosis.[10]

In this report, we describe a 10-year-old thalassemic intermedia patient heterozygous for Factor V R506Q who developed a stroke following transfusion.

Case Report

A ten-year-old girl was admitted to our pediatric department with right hemiparesis of sudden onset. The patient is the fourth child in a family of 5 children: two affected by β-thalassemia (the subject and her monozygotic twin sister), two males with thalassemic trait and one homozygous normal male. Analysis of β-globin gene molecular defects gave the results reported in Table 1. There was no positive family history for venous and arterial thrombosis with the exception of the twin sister who developed a cerebral microangiopathy 25 days after bone marrow transplantation (BMT) as treatment of her thalassemia, one year prior to this report. Forty-one days after BMT, the sister died due to respiratory failure by *Cytomegalovirus pneumonitis*.

Four days prior to admission, the patient received 250 mL of packed red blood cells over a period of 4-5 hours. The hematocrit prior to the transfusion was 22% and the platelet count 350×10^9/L. This was her third transfusion of a recently initiated monthly transfusion regimen. The patient had not had a splenectomy. Several hours after her transfu-

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sion she complained of headache and fatigue and suddenly developed right hemiparesis 24 hours later.

Physical examination revealed drowsiness and slurred speech. There were no signs of meningeal irritation. A central right facial nerve palsy was noted. Deep tendon reflexes were asymmetrically exaggerated with a right positive Babinski sign.

Laboratory values included a hematocrit of 36%, a platelet count 411×10^9/L, a normal PT, aPTT, and TT, a fibrinogen of 280 mg/dl, and D-dimers were not elevated. AT III, PC, PS and HCII were all within the normal range and there was no evidence of a lupus anticoagulant (LA) (Table 1). Cholesterol and triglyceride values were not increased. Computerized brain tomography showed an ischemic area in the left temporo-parietal district related to an arterial thrombosis. Treatment with dexamethasone and glycerol was initiated and three days later there was a symptomatic improvement and complete remission of symptoms after 30 days.

At follow-up, plasma from both the child and the mother demonstrated APCR, and DNA analysis revealed both the mother and child to be heterozygous for the factor V R506Q mutation (Table 1). Of the other four members of the family, only one brother was heterozygous for factor V R506Q. It is assumed that the monozygotic twin sister was also positive for factor V R506Q.

Discussion

An extensive search for the underlying mechanisms of thrombosis in thalassemic patients had revealed multiple alterations in the clotting system, with little capacity to predict individual thrombotic risk. A higher thrombotic risk has been described in patients with thalassemia intermedia especially in untransfused cases.[11] This has been attributed to abnormal exposure of phoshatidylserine on the red blood cells which lead to the formation of thrombin through the prothrombinase complex. So, this procoagulant surface of red blood cells produces chronic *in vivo* platelet activation, as evidenced by shortened platelet survival and enhanced excretion of thromboxane A2 metabolites. Moreover untransfused thalassemia patients have lower levels of HCII related to increased red cell turnover which can be normalized once this turnover has been suppressed by 2-3 months of hypertransfusion.[12]

Recently, it has been confirmed that APCR is the most prevalent cause of venous thrombosis yet identified.[8] Although some preliminary reports suggest APCR is also associated with arterial thrombosis, more detailed studies are required to solve this question.

In our case, because of the exclusion of more prevalent causes of acquired and congenital thrombotic risk factors, we tested for the presence of APCR. APCR is often asymptomatic, thus a negative family history for thrombosis does not exclude its presence. Although its causative role in the development of idiopathic thrombosis is generally accepted, thrombosis often develops when additional risk factors (e.g. surgery, trauma, obesity) occur.

This seems to apply to the family reported here. The mother had no additional risk factors and had no history of thrombosis. We believe that the blood transfusion leading to a pronounced (> 50%) increase in hematocrit may be proposed as a possible trigger for the development of cerebral thrombosis in addition to the fact that the patient had an underlying disease, i.e. thalassemia intermedia.

A large increase in hematocrit was proposed previously as a possible mechanism for the development of stroke in several thalassemic patients. Therefore in our thalassemia intermedia patient, APCR represents a genetic predisposition for thrombosis in addition to the other risk factors of an acute rise in hemoglobin. The fact that the twin sister of our patient also developed a cerebral microangiopathy, probably triggered by cyclosporin and other risk factors due to BMT, demonstrates the importance of testing APCR in chronic clinical conditions associated with an increased thrombotic risk.

Subjects	*Type of thalassemia*	*Factor V Arg^{506}/Gln*	*APCR*	*AT III (%)*	*PC (%)*	*LA*
Patient, age 10 yrs	β° IVS 1-1/β⁺IVS 1-110	+/–	0.61	75	70	negative
Father, age 44 yrs	β° IVS 1-1	–/–	1.04	89	95	ND
Mother, age 42 yrs	β+ IVS 1-110	+/–	0.45	94	73	ND
Twin sister, dead	β° IVS 1-1/β⁺IVS 1-110	+/–	ND	ND	ND	ND
Brother, age 14 yrs	β° IVS 1-1	+/–	0.65	ND	ND	ND
Brother, age 12 yrs	β° IVS 1-1	–/–	1.10	ND	ND	ND
Brother, age 7 yrs	β° IVS 1-1	–/–	1.15	ND	ND	ND

APCR: Resistance to activated protein C; LA: Lupus Anticoagulant; ND: not determined

Table 1. Type of thalassemia and investigations of thrombophilia in the patient and her family.

In conclusion, considering the high prevalence of thalassemia intermedia in the mediterranean population and the elevated risk of thrombosis in thalassemia intermedia, we believe it may be beneficial to screen all intermedia thalassemic patients for APCR especially before starting a transfusional regimen.

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