Suspicion of Coexistence of Hemophilia A and Type 2N von Willebrand Disease

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Citation

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Abstract

Introduction: Hemophilia A and von Willebrand disease are the most common hereditary hemostasis disorders and very few cases of coexistence have been reported. Composite heterozygosity combining a mutation of the factor VIII (FVIII) hemophilia A type gene and a mutation of the Type 2N von Willebrand factor (vWF) gene exists and can make the therapeutic management of the patients more complex, leading us to undertake targeted screening.

Methods: This study involved 48 patients monitored in the clinical hematology department of Abidjan for bleeding disorders due to FVIII deficiency. Patient data were collected from June 2018 to April 2019. We analyzed vWF via 3 biological approaches: Willebrand antigen (vWF: Ag), Willebrand activity (vWF: Ac) and vWF capacity for binding to FVIII (vWF: FVIIIB). FVIII (vWF: FVIIIB) was determined by the ELISA method using the ASSERACHROM® vWF kit: FVIIIB, Stago, Asnières, France.

Results: The average age of the patients was 14 years old. The hemorrhagic signs observed were: 71% associations [hematomas + hemarthrosis + cutaneous hemorrhages], 19% associations [hematomas + hemarthrosis] compared with 6% of isolated cutaneous-mucosal hemorrhages. Analysis of vWF binding to factor VIII allowed us to observe a decrease in binding capacity in 7 patients (15%) below 65%. Antigenic and functional assays for vWF were normal; vWF:FVIIIB/vWF:Ag ratios had decreased.

Conclusion: This study revealed a binding deficiency of less than 65% in 7 patients with vWF:FVIIIB, suggesting the existence of Type 2N heterozygous von Willebrand disease. The suspicion of composite genetic impairment with the addition of a Type 2N vWF gene mutation in our hemophiliac A patients must be definitively argued, beyond simply analyzing the FVIII gene, by looking into the vWF gene.

INTRODUCTION

Hereditary bleeding disorders affect more than 7.5 million people worldwide. Von Willebrand disease (VWD) is the most common disease but hemophilia is the most commonly diagnosed severe bleeding disorder [1,2,3]. The global prevalence of VWD in different populations is estimated as being 0.01 to 1% [4], while the incidence of hemophilia A is estimated as 1 in 5,000 births [5,6,7].

VWD is defined by a quantitative and / or qualitative defect in von Willebrand factor (vWF) due to a mutation in the gene encoding for vWF. VWF is a glycoprotein involved in primary hemostasis, wherein it mainly promotes platelet adhesion at the site of vascular injury. It also plays a role in coagulation by transporting the anti-hemophilic factor A also known as factor VIII (FVIII) [8]. VWF protects FVIII from proteolysis by activated protein C in particular [9], thus stabilizing its bloodstream half-life [10,11]. There are three types of VWD: Types 1 and 3 are quantitative defects and Type 2 is a qualitative defect [12]. Of the various Types 2 described, Type 2N (Normandy 2) VWD is characterized by a markedly decreased binding of vWF to FVIII. This leads to a deficiency in plasma FVIII, a phenotype that mimics hemophilia A, but with an autosomal transmission mode instead [11,13]. The FVIII gene is normal, whereas the vWF gene is mutated. Type 2N VWD was first described nearly

30 years ago in patients with normal bleeding time, low plasma FVIII levels, and near normal to normal vWF levels [14,15]. In vitro assessment shows that the binding capacity of FVIII to vWF (vWF: FVIIIB) is reduced to less than 80% [16].

Patients with classical Type 2N VWD are either homozygous for the typical 2N mutation or heterozygous composite combining different forms of 2N mutations. These patients have a binding capacity of less than 20% [17]. Besides the classical forms, there are other composite heterozygote forms with more complex phenotypes. These forms combine the 2N mutations with another mutation. The second mutation can be a Type 1 VWD, or a mutation of the gene encoding for FVIII (F8), or a Type 3 VWD [18]. These patients have a moderately low vWF:FVIII (30-65%) [16].

Composite heterozygosity associating Type 2N mutations with FVIII mutations has been reported in large scale screening programs but rarely identified on the basis of clinical suspicion [19,20]. Although hemophilia A and VWD are the most common hereditary hemostasis disorders, very few cases of coexistence have been reported [21]. Lindsay et al. reported the coexistence of both Type 2N VWD and hemophilia A mutations [22]. The mixed phenotype complicates care for these patients, which has led us to undertake targeted screening for them.

PATIENTS AND METHODS

Patients

Our study recruited 48 outpatients being monitored at the clinical hematology department of Yopougon University Hospital in Abidjan, Côte d'Ivoire for bleeding disorders. These patients had an elongated activated partial thromboplastin time (APTT) leading to the diagnosis of low FVIII levels. Rerunning the FVIII procoagulant activity assay and the antigenic and functional assays for vWF in the hematology laboratory at Nîmes University Hospital in France allowed us to diagnose hemophilia A in these patients with normal vWF levels. All patients gave their informed consent to participate in this study. For the children, consent was obtained from the accompanying family member.

Methods

This is a descriptive cross-sectional study. Data were prospectively collected from June 2018 to April 2019. Blood samples were taken at Yopougon University Hospital in Abidjan, Côte d'Ivoire. After venipuncture as atraumatic as possible, the blood was taken on citrate anticoagulant at a rate of nine parts of whole peripheral blood to one part of anticoagulant. Citrated, platelet-depleted plasmas were made by double-centrifugation at 2,500 g for 10 minutes. They were then aliquoted and frozen to -80 ° C until further processing. Some aliquots were sent on dry ice to the hematology laboratory at Nîmes University Hospital, where vWF antigen (vWF: Ag), vWF activity (vWF: Ac) and vWF: FVIIIB were assessed.

The procoagulant activity of factor VIII (FVIII: C) was measured with a one-step chronometric method using an Option 4 plus semi-automated coagulometer (Biomerieux). The vWF: Ac and vWF: Ag were measured turbidimetrically on the Stago STA-R Max analyser. The binding capacity of the patient's vWF to purified FVIII was performed with an ELISA method using the ASSERACHROM vWF kit (Stago, Asnières, France). A plastic support coated with F(ab')2 fragments of rabbit anti-human vWF antibodies, captured the vWF contained in the plasma to be tested. Recombinant FVIII (FVIIIr) added to the medium binds to the solid phase immobilized vWF. Bound FVIIIr is detected with a human anti-FVIII monoclonal antibody conjugated to peroxidase. The quantity of bound peroxidase is measured using a substrate for peroxidase called 3,3', 5,5'-tetramethylbenzidine (or TMB). The Optical Density (OD), after stopping the reaction with a strong acid, is a function of the initial concentration of vWF capable of binding to FVIII. A vWF binding capacity to factor VIII of less than or equal to 20% indicates Type 2N von Willebrand disease. A moderately low rate suggests the existence of 2N heterozygosity.

RESULTS

The study population consisted of two thirds children and one third adults. The average age was 14 years old. The hemorrhagic signs observed were generally multiple with 71% hematomas + hemarthrosis + mucocutaneous hemorrhages, 19% hematoma + hemarthrosis, and 6% isolated mucocutaneous hemorrhages (Table 1).

Analysis of vWF binding to factor VIII allowed us to observe normal binding, defined as greater than 80%, in 30 patients (63%). For patients with reduced vWF binding to factor VIII, 23% had an activity in the 65% - 80% range and 15% an activity in the 0% - 65% range (Table 2). The clinico-biological characteristics of the 7 patients with FVIII binding below 65% are shown in Table 3. The von

Willebrand antigenic (vWF: Ag) and functional (vWF: Ac) assays are normal. The vWF: FVIIIB / vWF Ag ratios are reduced (norms> 0.74 according to Casonato et al. [11]).

Table 1

Hemorrhagic signs observed

Hemorrhagic signs	Frequency (n)	Percentages
Hematomas	2	4.17%
Hematomas + hemarthrosis	9	18.75%
Mucocutaneous hemorrhages	3	6.25%
Hematomas + hemarthrosis + mucocutaneous hemorrhages	34	70.83%
Total	48	100%

Table 2

Patient distribution according to vWF:FVIIIB results

vWF:FVIIIB (%)	Frequency (n)	Percentages
[0-65]	7	14.6%
]65-80]	11	22.9%
]80-150]	30	62.5%
Total	48	100%

Table 3

Clinical and biological data of patients with vWF: FVIIIB < 65%

Patients *n=7*	P-25	P-3	P-31	P-33	P-41	P-52	P-53
Age (Year)	3	14	12	1	9	34	28
Hemorrhagic signs	Hematoma	Hematoma	hemarthrosis Gum bleeding	Skin bleeding following dermatosis	Hematoma hemarthrosis	Gum bleeding	Gum bleeding
ABO blood group	O positive	O positive	B positive	B positive	A positive	A positive	A positive
FVIII:C (%)	< 1	1.3	< 1	< 1	< 1	1.4	2.4
vWF:Ag (%)	89	138	158	148	278	171	173
vWF:Ac (%)	66	95	160	126	175	96	128
Ratio vWF:Ac / vWF:Ag	0,74	0,68	1,01	0,85	0,63	0,56	0,74
vWF:FVIIIB (%)	48	61	55	41	38	45	62
Ratio vWF:FVIIIB/ vWF:Ag	0.54	0.44	0.34	0.27	0.13	0.26	0.36

DISCUSSION

Hemophilia A is a hereditary coagulation disorder transmitted in an X-linked recessive mode. According to the residual functional activity of FVIII (FVIII: C), there is severe HA (FVIII: C < 1%), moderate HA (FVIII: C = 1 -5%) and minor HA (FVIII: C = 5 -40%). Factor VIII circulates linked to von Willebrand factor in the blood, which protects it from proteolytic degradation by activated protein C [23,24]. VWD is characterized by mutations in the vWF gene, transmitted in autosomal mode, which alter the concentration and/or function of vWF [25]. Type 2N VWD is defined by a binding defect of vWF to FVIII. It is induced by a vWF mutation altering the binding interface of vWF (domains D' and D3) to FVIII. This binding defect can coexist with hemophilia A, thus combining a vWF gene mutation with an FVIII gene mutation in the same patient, even though these two pathologies are rare diseases. It should be noted that males have a single FVIII gene on the X chromosome and two vWF genes on the autosomal chromosome 12 (12p13.31).

The first part of our study did not show any quantitative (vWF: Ag) or qualitative (vWF:Ac) defects in vWF. Consequently, the participants in this study did not have classical VWD. We therefore explored vWF binding to FVIII in the 48 patients.

The age of our study population ranged from 1 to 65 years old (average age 14 years old). This average was similar to that of the Ivorian population which is still very young according to the results from the general census of Population and Housing conducted in 2014 by the National Institute of Statistics in Côte d'Ivoire [26]. Our data is similar to Hasina data in Madagascar with an average age of 12 in 2014 [27]. However, previous studies conducted in Côte d'Ivoire by Koffi in 1999 [28] and Kadja in 2001 [29] reported average ages of 8.5 years and 9.3 years old, respectively. This difference might be explained by an increase in the life expectancy of hemophiliacs which today is very close to that of the general population [30], due to improvements in their care.

Hematomas and hemarthrosis are the most commonly observed clinical signs related to the factor VIII deficiency in this study.

Evaluation of the binding capacity of vWF to FVIII revealed a binding defect in some patients, seven of whom had a binding capacity of less than 65%. This suggested the existence of heterozygous 2N VWD [16]. The vWF:FVIIIB / vWF: Ag ratio, which ought to be greater than 0.74 [11], was decreased in these patients. The decrease is in favor of Type 2N VWD heterozygosity [11,22]. In general, heterozygous

2N VWD patients with a moderately low vWF: FVIIIB activity, no vWF: Ag and vWF: Ac deficiency, have an FVIII level that remains normal or slightly decreased [16,31,32]. These patients are asymptomatic [22]. In this study, patients with a binding capacity of less than 65% and a vWF: FVIIIB / vWF: Ag ratio of less than 0.74 had normal von Willebrand factor for antigen (vWF: Ag) and activity (vWF: Ac), but low levels of FVIII ranging from 1% to 2.4%. This made them potential carriers of heterozygous Type 2N VWD + hemophilia A (Table 3).

This mixed presentation of HA and Type 2N VWD had previously been reported by Lindsay et al. in 2014 [22] and Casonato et al. in 2001 [33]. The 2N heterozygosity of VWD may be one of the reasons for the manifestation of variable hemorrhagic phenotypes in the same patient. It may transform hemophilia A induced by a mutation of the FVIII that causes moderate deficiency in FVIII, into hemophilia A with a more severe biological and clinical phenotype. We observed both hematomas or hemarthrosis and hemorrhages of the skin and mucocutaneous membranes (gum bleeding) in all seven suspected patients.

CONCLUSION

Although carriers of Type 2N VWD rarely appear to develop bleeding symptoms, proper screening would nevertheless seem interesting in patients with other diseases characterized by an FVIII defect such as HA. Proper screening would allow further exploration to help our understanding of the finally expressed phenotype. In these cases, exploration of the FVIII gene, in order to identify the responsible mutation and especially its ability to induce a more or less severe FVIII deficiency, would allow us to assess the aggravating impact of VWD 2N on the phenotype. In our hemophiliac A patients, suspicion of composite genetic impairment in addition to a 2N mutation of the vWF gene must be thoroughly explored, beyond simply analyzing the FVIII gene, by looking into the vWF gene.

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