Variant von Willebrand's Disease and Pregnancy

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The clinical course and coagulation profile of a pregnant patient with variant von Willebrand's disease were followed from the second trimester through puerperium. The clinical course was characterized by a normal delivery and absence of abnormal bleeding or need for replacement therapy. The coagulation profile demonstrated an increase in factor VIII procoagulant activity, factor-VIII-related antigen, and platelet aggregation activity in response to ristocetin prior to delivery. Postpartum, these factors decreased to prepregnancy values with distinctly different patterns. Factor VIII procoagulant activity continued to rise for 5 days after delivery and then decreased with a half-life of approximately 6 days. Factor-VIII-related antigen began to decrease just prior to delivery, displaying a half-life of approximately 6 days. Ristocetin cofactor activity, however, dropped immediately postpartum and displayed a half-life of approximately 6 hr. The ristocetin cofactor activity was associated with factor-VIII-related antigen, which displayed a significantly smaller molecular weight than does normal factor-VIII-related antigen. Larger aggregates of factor-VIII-related antigen did not appear during the pregnancy, and ristocetin cofactor activity could not be demonstrated in fragments of less than 0.8 x 10^6.

VON WILLEBRAND'S DISEASE (vWd) is a heterogenous group of bleeding disorders inherited as an autosomal dominant trait. It is associated with variable tendencies for abnormal and prolonged bleeding.1-3 The two predominant types of vWd, classic (type I) and variant (type II),1-3 are associated with prolonged bleeding time (BT) and decreased platelet adhesiveness (PA), factor VIII procoagulant activity (FVIII:C), factor-VIII-related antigen (FVIII:RAg), and ristocetin cofactor activity (RCF). Variant vWd is distinguished from classic vWd primarily by a qualitative abnormality in FVIII:RAg, which leads to a deficiency of large molecular weight (mol wt) factor VIII complexes in plasma and platelets (type IIA). A subtype of variant vWd (type IIB) displays an enhanced RCF along with intermediate mol wt FVIII:RAg complexes in plasma and large complexes in platelets.4-5

Current models depict factor VIII (FVIII) as an aggregated series of homologous oligomers, including FVIII:C and FVIII:RAg, with RCF as an integral function of larger aggregates of FVIII:RAg2,6 and separable only after proteolytic digestion.7-9 An alternative model depicts RCF as a separate molecule that aggregates with the FVIII macromolecule complex.10,11 Still another alternative suggests FVIII:C, FVIII:RAg, and RCF are only minimally aggregated in vivo.12-14 RCF is an important determinant in the diagnosis of vWd.1,2 How RCF is involved in the correction of PA and BT is still not clear;15 however, the suggestion has been made that an as yet undescribed characteristic of FVIII is responsible.16

Few cases of vWd in pregnancy have been thoroughly documented.17,18 Previous studies of vWd in pregnancy have generally focused on FVIII:C, BT, and the tendency for postpartum hemorrhage.17 Some studies17,19,20 report levels of FVIII:RAg, but only two reports documenting RCF in vWd were found.21,22 These studies indicate that coagulation factors, including FVIII:C and FVIII:RAg, gradually increase during gestation with peaks occurring at or near delivery. BT and PA are not consistently corrected, even though plasma FVIII-associated properties are at or above mean levels for the normal nonpregnant population.

We report a complete study of one case of type IIA vWd in pregnancy. The clinical course is documented along with a coagulation profile from the second trimester through puerperium. BT, PA, RCF, FVIII:RAg, FVIII:C, partial thromboplastin time (PTT), and prothrombin time (PT), as well as estradiol levels, were measured throughout the course. Two normal volunteers were similarly studied at the same time.

MATERIALS AND METHODS

Patient

The patient is a white 22-yr-old female with type IIA vWd.4,4 A family history of the disease was well documented through four generations.19 Prior to pregnancy, coagulation data showed FVIII:C (15% of normal) and FVIII:RAg (47%), prolonged BT (>15 min, normal <7 min), RCF undetectable, and slightly reduced PA (50%-70%, normal 88.1% ± 7.8%). Cross-immunoelectrophoresis indicated a more anodically migrating FVIII:RAg than is usual for normal plasma. Primary bleeding problems included epistaxis and bleeding after dental extraction. Physical examination was unremarkable. Hemoglobinopathy and telangiectasia were absent.

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Supported in part by the National Hemophilia Foundation; the Physicians Medical Education and Research Foundation, Knoxville; and NIH Grant FR 5541.

Submitted February 9, 1981; accepted June 23, 1981.

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©0006-4971/81/5805-0003$01.00/0
The patient, first known to be pregnant at the beginning of the second trimester, was followed regularly at the East Tennessee Hemophilia Clinic. The course of pregnancy was normal. Delivery was spontaneous and uncomplicated and proceeded without the need for replacement therapy; blood loss was estimated to be 300 ml or less. After labor, the patient received eight doses of Methergin (methyl-ergonovine maleate), 0.2 mg every 6 hr. No excess bleeding occurred postpartum and the patient was discharged on the seventh postpartum day. No bleeding occurred until the 17th postpartum day, when abnormal vaginal bleeding required replacement therapy. Ten units of cryoprecipitate were given and oral contraceptives were started.

Two normal pregnant volunteers were concomitantly followed. They were white, 35 and 30 yr of age, and had no history of bleeding disorders. The patient and volunteers gave their informed consent before participating in the study.

**Citrated Plasma**

Venous blood was drawn by the two-syringe technique from antecubital veins and added to one-ninth volume of 0.109 M sodium citrate. Polypropylene syringes and tubes were used throughout. Samples were immediately centrifuged at 1500 g for 20 min at 4°C. Plasma was decanted and platelets removed by recentrifuging at 12,000 g for 20 min prior to aliquoting, snap-freezing in acetone-dry ice, and stored at −60°C.

**Pooled Normal Plasma**

Pooled normal plasma, the standard for each assay described, was prepared using equal volumes of fresh plasma from six female and six male donors. All donors were known to be healthy individuals who neither smoked nor took birth control pills, and had taken no other medication within 10 days of donation.

**FVIII:C**

FVIII:C was determined using the modified PTT (one-stage) method. A minimum of four replicate determinations were performed for each sample. Briefly, 10 μl of antiserum, described below, were placed in 15 ml of melted 1% agarose (I.B.F., 4°C on 8 x 10 cm glass plates). Anti-FVIII-RAg serum was purchased from Cal Biochem-Behring (San Diego, Calif.). The plates were washed, dried, and stained as described above.

**SDS Agarose Gel Electrophoresis**

The multimeric composition of FVIII:RAg was determined on three samples pre- and postpartum from the vWD patient by Drs. Ruggeri and Zimmerman (Dept. of Molecular Immunology, Research Institute of Scripps Clinic, La Jolla, Calif.) The methods they have described were used.

**Microtiter Assay for RCF**

RCF in plasma was assayed by the method of Ramsey and Evatt. Fifty microliters of plasma were placed in the first well of a U-well microtitration plate (Cooke Engineering Co.) and 25 μl of PBS in all other wells. Serial dilutions were made up to 1:256. Formalin-fixed platelets, prepared by the method of Bowman et al., were diluted in PBS to a concentration of 4 x 10^9/ml and 25 μl added to each well. Twenty-five microliters of ristocetin (7.5 mg/ml) were added to each well. Controls consisted of plasma from a known vWD patient, pooled normal plasma, and nonaggregating reagents: PBS, platelets, and ristocetin. After the plates had been mixed for 10 min, platelet aggregates were allowed to settle for 20 min, and the plates were read immediately. Results were taken from the last well to show aggregation and were expressed as percent of normal control.

**Screening Coagulation Tests**

PTT, PT, and TT were performed using the methods and reagents described by Sibley.

**Bleeding Time (BT)**

BT was performed by the method of Ivy (normal 4–7 min).

**Platelet Adhesiveness (PA)**

PA was measured by the method of Salzman. Venipuncture was performed with a 19-gauge butterfly infusion set (Abbott Laboratories, North Chicago, Ill.). A control sample of blood was collected in a Vacutainer tube (Beckton Dickinson, East Rutherford, N.J.) containing 0.06 ml of EDTA solution, 4.5 mg/ml. A glass bead column prepared locally was then attached to the butterfly tubing and a second Vacutainer tube containing EDTA was connected to the columns, drawing the blood through the column into the tube. Platelet counts were made by phase contrast microscopy on both samples. Platelets adhering to glass beads were reported as the percent of control platelet count (normal is 88% ± 7.8%).

**Gel Filtration on Sepharose 6-B**

Plasma samples, taken within 24 hr of delivery from the patient with vWD and from a normal pregnant and a normal nonpregnant volunteer, were chromatographed on Sepharose 6-B (Pharmacia, Upsala, Sweden) in 0.05 M Tris, 0.15 M saline, with 0.01 M trisodium citrate, pH 7.2. The column (0.9 x 60 cm) was loaded with 0.5 ml citrated plasma and developed with an ascending flow of 2 ml/hr at 4°C. Absorbance at 280 μm was monitored on a UVicord-S monitor with 0.5 cm cuvette (LKB, Upsala, Sweden). Fractions of 0.53 ml were collected, and RCF was immediately determined as described above with the exception that arithmetic, instead of log, dilutions were used. The column was calibrated during each run by using Laurell immunoelectrophoresis to determine the elution profile of the following human plasma proteins: IgM (mol wt 970,000), Fg (mol wt 340,000), IgA (mol wt 180,000),
and AT III (mol wt 59,000). FVIII:RAg was similarly determined, and the mol wt estimated using a standard curve of \( K_a \) versus log mol wt of the standard proteins. The exclusion limit for Sepharose 6-B is approximately \( 4 \times 10^6 \). Antiserum was either prepared as described below or purchased from Cal-Biochem/Behring, La Jolla, Calif.

**Antiserum**

Antiserum monospecific for FVIII:RAg was prepared by injecting FVIII:anti-FVIII immunoprecipitins that had been generated in agarose and reconstituted with parallel troughs. Troughs were cut 1 cm apart and alternately filled with 100 μl of Hemofil (Hyland Laboratories, Costa Mesa, Calif.) (10 mg/ml) or with antiserum prepared against cryoprecipitate and rendered monospecific by absorption with plasma from an individual with severe vWD. Precipitin lines were excised and cleared of soluble proteins by soaking for 5 days in 0.05 M Tris buffered in 0.5 M saline. Buffers were changed twice each day. The gel was macerated and mixed with an equal volume of Freund's adjuvant and homogenized prior to injection. A total of 0.4 mg protein per injection was employed with two injections made 10 days apart. The antiserum thus produced was monospecific for FVIII:RAg, inhibited RCF, and did not affect FVIII:C activity.

**Estradiol Analysis**

The concentration of estradiol 17β (E2) was measured by double antibody-polyethylene glycol, competitive binding, radioimmunoassay (Radioassay Systems Laboratories, Carson, Calif.). Estrogens were extracted from 0.05 ml of citrated plasma with 5.0 ml of ethyl acetate: hexane (3.2/1 by vol). Then 3.5 ml of extract was removed, dried, and reconstituted with 0.1 ml 0.7 PBS for radioimmunoassay of estradiol 17β. A standard curve was plotted on 3-cycle semilog paper. Unknown patient levels were determined by direct interpolation.

**RESULTS**

Changes in FVIII-related properties, PA, BT, and estradiol levels in the vWD patient and the two normal pregnant women are illustrated in Fig. 1 A and B, respectively. Gradual increases in FVIII:C and FVIII:RAg were found during gestation in the vWD patient and in both normal volunteers. FVIII:C and FVIII:RAg maintained roughly at a 1:1 ratio until the last month of pregnancy, when FVIII:RAg increased above FVIII:C levels in the vWD and one normal subject. Although FVIII:C and FVIII:RAg in the vWD patient reached levels that are normal for a nonpregnant person, these factors peaked at only half the levels for the normal pregnant subjects studied. Normal levels for RCF were reached in vWD only in the last month of gestation and peaked just prior to delivery. As with FVIII:C and FVIII:RAg, RCF in vWD reached levels that are normal for a nonpregnant person but did not attain normal pregnancy levels. Estradiol levels were within normal limits during gestation in vWD.

After delivery, FVIII:RAg steadily decreased in the vWD and one normal patient and remained constant in the other normal patient; ratios of FVIII:C and FVIII:RAg returned to approximately 1:1 by the end of the puerperium. In the patient with vWd, however, RCF dropped precipitously; the half-life was approximately 6 hr or less compared to a half-life of 6 days or more for the FVIII:RAg during the same period. Estradiol dropped to one-tenth of normal nonpregnant levels within the first 24 hr postpartum.

For the vWD patient, a secondary rise in FVIII:RAg and RCF corresponded with peak FVIII:C levels at 6–7 days postpartum. BT remained consistently prolonged (normal is \(<7\) min) throughout the vWD pregnancy, but was shortened in 8 min just prior to delivery.

In the vWD patient, platelet adhesion remained just below normal levels (normal is \(88% \pm 7.8\%\)) throughout most of the pregnancy and increased to within normal limits during the last month of gestation.

The global assays, PTT, PT, and TT, as well as hemoglobin and hematocrit, remained normal throughout gestation and postpartum in all three pregnancies.

The multimeric composition of vWD FVIII:RAg was studied via SDS agarose gel electrophoresis at 1 day before delivery and at 1 and 3 days after delivery. Only small aggregates of FVIII:RAg were found. In each sample, the mol wt ranged up to approximately \(5 \times 10^6\) (Fig. 2) with a predominance of 0.8 and \(3.4 \times 10^6\) mol wt aggregates.

Gel filtration on Sepharose 6-B showed that RCF was associated strictly with FVIII:RAg in the vWD pregnant patient (Fig. 3). RCF from a normal pregnant individual or pooled normal plasma (nonpregnant) eluted at the column void volume (\(V_0 \approx 4 \times 10^6\) mol wt). RCF from vWD pregnant individual's sample, however, eluted at 1.14 \(V_0\) corresponding to a mol wt of roughly \(3 \times 10^6\). RCF could not be demonstrated in eluates corresponding to a mol wt of less than \(0.8 \times 10^6\) for either vWD or normal plasma.

**DISCUSSION**

Understanding of the molecular pathology that underlies vWD is still scanty. The study of vWD in pregnancy may provide a useful model for correlating molecular changes with clinical signs, since FVIII:C, FVIII:RAg, RCF, and other coagulation factors do increase during pregnancy. These factors also increase in response to hormonal therapy and stress in both normal and vWD individuals. Some results of the present study agree with previous findings, particularly the gradual increases noted during gestation for various FVIII-related properties. The continued rise in FVIII:C postpartum...
contrasting with the decline in FVIII:RAg has been noted for both normal and vWD pregnancies. The late rise in RCF is in agreement with the single previously reported case, but the sharp drop postpartum is more clearly documented in the present case.

For this case and for most of the pregnant vWD patients previously reported, increases in FVIII-related properties and no abnormal bleeding were noted during pregnancy; however, some did not demonstrate such a favorable response. According-
ly, pregnant vWd patients should be monitored very closely during pregnancy, labor, and in the postpartum period since, if FVIII-related properties do not increase, they may bleed abnormally. Variant vWd patients may be at an even higher risk since, as shown in this case, the function and multimeric composition of FVIII:RAg may not become normal. Adequate replacement therapy may prevent this hazardous state of affairs during these times.

The discrepancy between acquisition and loss of FVIII and RCF highlights the lack of understanding of the molecular basis underlying vWd. The concept that RCF exists as a molecular entity independent of FVIII:RAg is not supported by gel filtration data, since RCF elutes strictly in association with FVIII:RAg regardless of source (Fig. 3).

Lack of appropriate posttranslational modification has been suggested as the underlying biochemical defect that is responsible for the lack of RCF and the small multimeric composition of FVIII:RAg. Changes in the multimeric composition of FVIII:RAg are probably not responsible for the acquisition or loss of RCF. The predelivery sample, with 100% RCF, displayed the same small multimeric composition of FVIII:RAg as the postpartum samples with less than 10% RCF (Fig. 2). Neither can the modifications necessary to elicit RCF be held to account for production of a normal, large multimeric composition of FVIII:RAg.

The steep drop in RCF postpartum also suggests an active degradation of this activity, thus indicating that even the posttranslational modification imparting RCF might be incomplete. As an example, RCF might be acquired through a modification by which a carbohydrate moiety, including galactose but lacking a terminal sialic acid, is inserted. The incorporation of the galactose would impart RCF, but the lack of sialic acid would make the molecule susceptible to degradation.
Additionally, PA was only slightly decreased in our patient before pregnancy. Whether this is indicative of a further property of FVIII:RAg, distinct from those necessary for normal RCF and BT, or simply reflects the inherent variability of this test remains uncertain.

Taken together, the above data suggest that FVIII:RAg from this patient does not correct RCF or BT, even at normal levels. Thus, a qualitative change must be inferred to occur in the FVIII:RAg molecule to account for the correction of these properties near term. The qualitative change does not produce completely normal FVIII:RAg, since correction of these functions was not accompanied by correction of the multimeric composition of FVIII:RAg. Our data support the concept that at least two defects are responsible for the molecular pathology characteristic of variant vWd; one defect leads to loss of RCF, the other to the small multimeric composition of FVIII:RAg.

ACKNOWLEDGMENT

The authors would like to thank Dr. Zaveria M. Ruggeri and Dr. Theodore S. Zimmerman, Department of Molecular Immunology, Research Institute at Scripps Clinic, La Jolla, Calif., for performing SDS agarose gel electrophoresis on the patient’s samples (Fig. 2). The authors would also like to acknowledge the help and advice of Marian A. Miles, B.S.N., of the East Tennessee Hemophilia Clinic, and Rose Clift, Teresa Williams, Pam Monteen, Bo Riewerts, and Dianne Trent of the Department of Medical Biology. Thanks are also offered to MEDIC, Knoxville, for providing recently outdated platelets, and to the American National Red Cross for supplying the cryoprecipitate and antithrombin III.

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