von Willebrand Disease : A Clinico-haematological Spectrum

Lt Col DK Mishra*, Dr A Chaturvedi †, Lt Col A Sharma ‡, Air Cmde H Subramanya**,
Lt Col Harsh Kumar††, Lt Col R Varadarajulu, vsm##, Col KP Anand***

Abstract

Background : Bleeding disorders are commonly seen in clinical practice. von Willebrand Disease (vWD), is the commonest and yet a profoundly under diagnosed cause, having a wide spectrum of clinical presentation. Of its three types, type 1 vWD (70% of the total vWD cases) has the mildest and a highly variable clinical and laboratory presentation.

Methods : A series of ten cases of vWD were comprehensively evaluated using recommended diagnostic parameters and therapeutic interventions.

Results : All major types of vWD were represented. A female preponderance, with primary presentation in the form of mucocutaneous bleeds was observed. A positive history of consanguineous parental marriage and family history of bleeding disorder was elicited in two and three patients respectively. Nine patients were found to be anemic and thrombocytopenia was present in only one. Bleeding time by modified template (SIMPLATE) method, along with activated partial thromboplastin time (APTT) was increased in all ten cases and of these, nine had low factor VIII: C levels. Ristocetin induced platelet aggregation studies were abnormal in all the five cases it was performed. vWF:RCO activity determined in one individual was shown to be low. vWF:Ag assay was done in four cases revealing a near complete absence of von Willebrand factor antigen in one and mildly decreased levels in the other three. vWF multimer assay was advised in three cases. DDAVP, plasma derived vWF, blood products and local antifibrinolytics were used as primary modalities of treatment

Conclusion : Thus, strong clinical suspicion, thorough clinical evaluation and judicious use of investigations including repeated investigations at different times are needed for making a diagnosis of vWD.

MJAFI 2004; 60 : 337-341

Key Words : Bleeding disorders; von Willebrand Disease

Introduction

The need to confirm or exclude a bleeding disorder is common in clinical practice. It involves making a clinical assessment and an initial set of haemostatic screening tests and occasionally more specific tests to arrive at definitive diagnosis. vWD is the most common bleeding disorder [1], with an estimated prevalence of 1% [2]. Notwithstanding the growing recognition of this disease there is still marked underdiagnosis of this entity[3,4], as it has a wide spectrum of clinical presentation and large variability in laboratory values [5,6]. Most (70%) cases are type I (partial quantitative defects) with a mild clinical presentation and haematologic basis, with laboratory values fluctuating over time and occasionally falling within the normal range[5]. We present here a series of 10 cases of vWD highlighting the spectrum of clinical manifestations and hematologic parameters.

Material and Methods

Ten cases of vWD underwent a comprehensive clinico-hematologic evaluation. The clinical details principally from the medical history, focused on confirmation of a bleeding disorder, the individual having inherited the disorder, and the pathology primarily involving the platelet dependent phase of hemostasis. The presence of disorders or situations, which could cause or exacerbate any bleeding tendency viz. liver dysfunction, uremia, pregnancy, drug intake or immediate history of viral infection, were ruled out.

Hematologic laboratory assessment; apart from a complete blood count, liver and renal function tests included the coagulation profile of the individuals. The initial or screening tests[7] carried out were platelet count, peripheral blood smear examination for platelet morphology and aggregation (on the Wright Geimsa stained finger prick smears) bleeding time (BT modified template/SIMPLATE method), clotting time (CT by Lee and White method), prothrombin time (PT), activated partial thromboplastin time (APTT), thrombin time (TT) and plasma fibrinogen levels. Further investigations [7] included

*Classified Specialist (Pathology and Haematology), †Classified Specialist (Medicine and Haematology), Army Hospital (R and R), Delhi Cantt, †Post Graduate Resident, Department of Pathology, ††Associate Professor, Department of Blood Transfusion, Armed Forces Medical College, Pune 411 040, ***Senior Advisor (Medicine), Command Hospital (Eastern Command), Calcutta, **PMO, HQ Eastern Air Command, C/o 99 APO, ##Classified Specialist (Medicine and Neurology), Command Hospital (Air Force), Bangalore.

mixing studies of APTT and PT with control plasma, aged serum and adsorbed plasma with 2 hour incubation at 37°C, factor VIII: C and factor IX assays, platelet aggregation studies with ADP, collagen, epinephrine, arachidonic acid and ristocetin (RIPA)-using light transmission aggregometry. Where indicated following tests [7] were carried out or advised; von Willebrand factor antigen levels (vWF:Ag) using enzyme linked fluorescent assay (ELFA), ristocetin cofactor assay (RiCoF Assay), vWF multimer assay (1.3% SDS PAGE electrophoresis) was also advised where indicated.

Results

Ten cases of vWD were studied. There was a female preponderance with M:F ratio of 3:7. The mean age at presentation was 16.1 years (range 7 to 35). The clinical presentation was primarily with muco-cutaneous bleeds. Six of ten cases had a positive history of epistaxis and three of ten had significant gum bleeds. The bleeds had been spontaneous, and generally controlled with prolonged application of pressure. Two (Patient No 2 and 9) of the five

females who had attained menarche gave a history of menorrhagia. Positive history of easy bruisability on mild trauma or at play was present in five of ten cases. Two (Patient No 3 and 4) out of 10 patients had a positive history of significant umbilical stump bleed at delivery. These two patients had also required FFP and blood transfusion at some point of time for management of bleeds. Another patient (Patient No 2) had been given FFP as a prophylaxis prior to ENT surgery. A negative history of recent viral infection, drug intake or liver and renal disease was elicited in all cases.

Two (Patient No 4 and 9) out of nine cases were born out of consanguineous marriage, but in both individuals the parents had not shown any features suggestive of a bleeding diathesis. This history could not be elicited from one individual. Two (Patient No 1 and 2) of the patients were married and had an uneventful obstetric history. A positive family history of bleeding diathesis could be elicited in 3 cases (Patient No 1, 4 and 9) with the first two having 1st degree siblings affected by similar complaints. The third gave

Table 1
Clinical profile of patients

Patient No.	Sex	Age (yrs)	Presenting complaint	History of transfusion	Parental marriage	F/H bleeding diathesis
1.	F	35	Epa	Nil	NC	First order
2.	F	37	Ebb/Ep/Mc	Prophylactic	NC	Nil
3.	F	7	Gbd/Ep/Ubc	Therapeutic	NC	Nil
4.	F	13	Gb/Ep/Ub	Therapeutic	C	First order
5.	F	12	Eb	Nil	NC	Nil
6.	F	14	Eb	Nil	NC	Nil
7.	M	8	Eb/Ep	Nil	NC	Nil
8.	M	10	Eb/Ep	Nil	NC	Nil
9.	F	18	Ep/M	Nil	C	Second order
10.	M	12	Gb	Nil	NC	Nil

1. a:Epistaxis, b:Easy bruisability, c:Menorrhagia, d:Gum bleed, e:Umbilical bleed, NC:Non-consanguineous, C:Consanguineous

2. On examination case 3 also revealed features of growth retardation

Table 2
Laboratory investigation profile of patients

Patient No.	Hb (g%)	Plt/c (x10 ³ /mm ³)	BT (min)	APTT (control in sec/test in sec)	Mixing study ^a	Factor VIIIc assay (%)	RIPA ^b (%)	vWF:Ag (%)
1.	12.0	250	>15	29/39	Corrected	39	-	-
2.	11.2	185	>15	29/38	Corrected	45	At 1.5 mg/ml present 0.5 mg/ml absent	44
3.	8.4	90	>15	29/38	Corrected	28	-	40
4.	6.7	185	>15	29/75	Partially corrected	1.3	At 1.5 mg/ml absent 0.5 mg/ml absent	3.3
5.	11.4	300	15	29/48	Corrected	6	Flat ristocetin	-
6.	11.0	194	14	33/40	Corrected	40	-	-
7.	11.4	210	15	30/38	Corrected	60	Ristocetin defective	38
8.	10.0	220	11	40/48	Corrected	42	-	-
9.	11.8	184	>15	40/56	Corrected	12	Ristocetin absent	-
10.	11.0	240	>15	35/54	Corrected	70	-	-

1. a:Mixing study-50:50 normal pooled plasma with patient plasma after 2 hours incubation at 37°C, b:RIPA-Ristocetin induced platelet aggregation

a history of a second-degree relative affected by similar complaints.

On examination, all cases were found to be haemodynamically stable with vital parameters within normal limits. One patient (Patient No 3), a seven year girl was found to have retarded growth with a weight of 15 kg (expected mean weight 20.5 kg) and a height of 108 cm (expected-118.2 cm), which were both 2 SD below the normal for her age [8]. She had suffered from recurrent episodes of significant bleeds, requiring multiple transfusions of whole blood and FFP.

Investigations revealed anemia in 9 cases (Hb<12g%) with 8 having mild anemia (8-12g%) and one individual having moderate anemia with a Hb value of 6.7 g%. Red blood corpuscle (RBC) indices in these cases revealed a microcytic hypochromic picture and the peripheral blood smear (PBS) confirmed the same. Thrombocytopenia (platelet count: 90,000/ μ l) was present in only one (Patient No 3) case with the PBS showing a mild increase in platelet size (MPV). Other hematological investigations and hepatic and renal function tests were within normal limits. The initial coagulation screen and related specific tests carried out were then analysed. Bleeding time was raised beyond 15 minutes in eight of the ten cases, with the other two (Patient No 6 and 8) cases showing increased bleeding time of 14 and 11 minutes respectively. CT, TT and PT were normal in all 10 cases. APTT was increased in nine of ten, with a modal increase of 9 seconds (range of 7-46 seconds from the control). Except one case (Patient No 4), another repeatedly transfused individual, born of a consanguineous marriage, showed partial correction, the other eight showed full correction on mixing studies with normal pooled plasma. F VIII: C assay was carried out in all ten cases of which nine cases had FVIII: C levels below 50% (range 1.3% - 45%). Of these one (Patient No 5) individual had a moderate deficiency of 6% and another (Patient No 4) had a severe deficiency of 1.3%.

Platelet aggregation studies (RIPA) were abnormal in all 5 cases in which they were carried out. In one (Patient No 4), presence of aggregation was absent at both concentrations of 1.5mg/ml and 0.5mg/ml of ristocetin, confirming type 3 vWD, vWF:RCO activity level determined in the same case, was found to be 54%. vWF: Ag assay carried out in 4 cases revealed a nearly absent level of 3.3% by ELFA (enzyme linked fluorescent assay) in the individual (Patient No 4) with absent RIPA, whereas the other three (Patient No 2,3 and 7) patients had levels of 44%, 40% and 38% respectively (>50% arbitrarily taken as normal reference value for the population). vWF multimer analysis was advised in three (Patient No 2,3 and 6) of our patients to subtype the disorder. Tests for clot stability, clot retraction tests, fibrinogen level assay, factor IX assay and urea stability tests carried out to rule other coagulopathies were negative in all cases. Of the three (Patient No 2,3 and 4) individuals in which blood grouping was done, two were O+ and one had A+ blood group.

Discussion

The von Willebrand factor is a heterogeneous multimeric plasma glycoprotein, discrete domains of

which facilitate platelet adhesion to vascular sub-endothelium [5]. It also serves as a plasma carrier for F VIII [5]. A modest reduction in plasma vWF concentration or selective loss in the high molecular weight multimer decreases platelet adhesion and causes clinical bleeding [5]. Erik von Willebrand, a Finnish internist investigating a family on Aland Islands in 1928 [5], first described this disease. vWD is a diagnostic challenge. Due to heterogeneity of clinical presentation and underlying pathologic basis, at times molecular investigations for detection of mutations to correctly subtype and manage the disease are also required [4].

Seventy percent of vWD patients have a type 1 or the mild form of the disorder, especially difficult to diagnose as most cases present only after surgery or trauma. Variations are also related to ABO blood group type, systemic inflammation and pregnancy [5,9]. Misdiagnosis of type 2N as mild hemophilia A and type 2B as autoimmune thrombocytopenia is also well known [5]. Laboratory findings are variable. The most common diagnostic pattern is a combination of prolonged BT, normal platelet count, PT, with increased APTT, decreased plasma vWF concentration, a parallel reduction in its biologic activity (ristocetin cofactor assay), and reduced FVIII activity [1,6].

Our study revealed similar clinical and laboratory variations in the presentation of vWD. Two third of our patients were females [10], conforming to published literature. The presenting complaints in vWD, mainly mild types 1 and 2; are most commonly epistaxis, skin bruises, haematomas, prolonged bleed from trivial wounds, gum bleeds, excessive menstrual bleeds and rarely gastrointestinal bleeds which are generally of a serious nature. Type 3 vWD is serious in nature and can present with cephalhaematomas in newborns, haemarthroses, haematuria and intramuscular, intracerebral and retroperitoneal hemorrhages [1,5,11]. A significant percentage of patients with mild vWD may have a negative bleeding history [11]. Similarly in our set of cases, mucocutaneous bleeds were the predominant form of presentation in all ten individuals. Prolonged and excessive umbilical bleed at birth suggestive of an inherited and severe bleeding disorder was present in two patients (Patient No 3 and 4). A positive family history of a bleeding diathesis was present in three patients (Patient No 1,4 and 9). One of the patients (Patient No 3) was small for age, with history of transfusion from the age of two years. Early presentation of vWD with severe anemia can lead to growth retardation.

Optimal testing strategies in vWD have not yet been validated [5]. A complete blood count with platelet count along with initial screening tests of coagulation which

include BT, PT and APTT are necessary to identify other causes of bleeding as well as indicate the severity of anemia [5,11]. These investigations revealed iron deficiency anemia to be present in nine cases, which reflects the recurrent and prolonged bleeding episodes in a setting of insufficient dietary iron intake and increased menstrual blood loss in our set-up. Platelet counts are within the normal range in vWD [5], except in type 2B vWD where mild variable thrombocytopenia may be seen and may be the only indication of vWD. In this setting, misdiagnosis of type 2B as autoimmune thrombocytopenia is well known [5]. Role of platelet counts in subtyping the disorder increases in case of an absent RIPA test, which has a characteristic response in type 2B vWD. In our study type 2B vWD was seen in one (Patient No 3) patient. PBS confirmed the findings of iron deficiency anemia as well as excluded Bernard-Soulier syndrome (giant platelets) [12,13] in all ten cases.

Normal TLC, DLC, reticulocyte count along with liver and renal function tests ruled out systemic inflammatory conditions and other causes of altered vWF levels.

BT performed in a standardized manner [12,13] is often prolonged in vWD, but there are exceptions particularly in type 1 disease. In our study we found all ten cases of vWD to have raised BT. In three patients (Patient No 2,3 and 4) initial BT done by finger prick method was within normal limits but with SIMPLATE was markedly prolonged, underscoring the importance of carrying out BT test in a standardized manner. CT, PT and TT are normal in vWD [5] as was also seen in all our cases. APTT values due to a corresponding deficiency of FVIII: C levels may be deranged in vWD, but not infrequently in type 1 and 2 vWD, will have normal initial APTT since FVIII levels may be sufficiently high (>30 U/L) to show a normal APTT response. In our study, APTT was increased in all ten cases but in one individual (Patient No 4), it was partial. FVIII: C levels are usually low but may be normal or near normal [1] (N is 50-150%). Type 2N vWD is due to abnormal FVIII binding sites and its differential diagnosis is haemophilia A, which is distinguishable by the finding of normal vWF: Ag levels and limitation of the disorder to males in inherited haemophilia A. The other subtypes of vWD have a proportionate decrease in vWF and FVIII: C, whereas there is a markedly low FVIII: C affinity in type 2N vWD.

Platelet aggregation studies (using ADP, epinephrine, arachidonic acid and collagen) excluded functional platelet disorders. RIPA using ristocetin concentrations of 1.5mg/ml and 0.5 mg/ml shows a low to absent response in all vWD types except type 2B vWD where a brisk platelet aggregation is seen even at low concentrations. In our study, all five individuals (Patient

No 2,4,5,7 and 9) evaluated with ristocetin showed a flattened aggregation response curve with none of these patients showing a type 2B pattern.

vWF: Ag is the next test generally carried out. Normal vWF: Ag levels do not exclude vWD. vWF levels are just below normal in type 1, normal in type 2A and 2M, usually decreased in 2B and 2N and nearly to completely absent in type 3 vWD. In this study, vWF: Ag levels were tested in four patients (Patient No 2,3,4,7). One individual (Patient No 4) had nearly absent levels (3.3%), which was suggestive of type 3 vWD, whereas other three had mildly decreased (44%, 40% and 38%) levels against a normal of 50-200%. In latter case, a 32-year female (Serial No 2) in whom a strong clinical suspicion of vWD was present, repeated F VIII and VWF: Ag levels estimations were done. The third evaluation showed abnormal levels of these parameters, highlighting the fact that these parameters may be normal intermittently, requiring repeated testing to establish a diagnosis of vWD.

vWF : RCo (RiCoF) activity specifically useful in subtyping type 2 vWD, is a screen for vWF concentration and function, but is difficult to standardize and is not very precise. In type 2A it is disproportionately low with respect to vWF: Ag levels. Type 2B (as also type 2M and type 1) has a proportional decrease. vWF : RCo activity is essentially absent in type 3 vWD. This test was performed in one of our patients (Patient No 4) and revealed a low normal value of 54% (normal > 50% activity). Low values for vWF : RCo and antigen are hard to evaluate because of the wide range of antigen levels and various confounding variables like ABO type where levels are 25% less for O blood group [5].

Multimer analysis, helpful in classifying vWD at the time of original diagnosis was advised in three patients (Patient No 2,3 and 6) to determine the exact subtype of vWD because of its therapeutic implications [3,5].

The aim of therapy in vWD is correction of the bleeding time and coagulation abnormalities [14]. Desmopressin / DDAVP, available in intranasal and intravenous forms, is the treatment of choice in type 1 vWD, but is not recommended in type 2B where it is known to aggravate thrombocytopenia [15]. The intravenous preparations can be administered subcutaneously. The intranasal form produces results equivalent to i/v DDAVP [17]. Though DDAVP is ineffective in the treatment of type 3 vWD, it may enhance the response to cryoprecipitate, thereby being of use when full correction of bleeding time is required [18]. Plasma derived vWF, factor VIII concentrates (Humate-P or Alphanate) and cryoprecipitate, are the treatment of choice in most patients with types 2 and 3 vWD. ϵ -aminocaproic acid and tranexamic acid are used

for local antifibrinolysis in mucosal bleeds. The role of platelet transfusion is not yet defined. Oral progestational agents may be useful in women, especially when menstrual bleeding constitutes the major clinical symptom [15].

In our study, apart from prescribing haematinics to the patients with anemia, DDAVP was prescribed in three cases (Patient No 2,3 and 4) with a resultant decrease in bleeding symptomatology. Intranasal DDAVP was administered, in the dosage of 150 µg (75µg/nostril) for optimal results. This DDAVP preparation was also advised for home management of minor bleeds. The patient having type 3 vWD (Patient No 4) also required blood transfusions for correction of her anemic state. In all cases local antifibrinolytic agents for managing mucosal bleeds, along-with cryoprecipitate and fresh frozen plasma were advised in settings of an emergency or planned surgical procedure. Precautionary avoidance of contact sports was also impressed upon.

The prognosis in mild forms of vWD is excellent; wherein patients can expect a normal life span, but the prognosis in severe, type 3 vWD is more guarded [14]. Thus, the cornerstone of diagnosis of vWD rests on a strong clinical suspicion, comprehensive clinical evaluation, with a judicious use of screening and specific haematologic work up which could include repeated laboratory investigations at times.

References

1. Rodgers GM, Greenberg CS. Inherited coagulation disorders. In: Lee RG, Foerester J, Lukens J, Paraskevas F, Greer JP, Rodgers GM, editors. Wintrobe's-Clinical Haematology. 10th ed. Williams and Wilkins, 1999:1692-1700.
2. Rodeghiero F, Castaman G, Dini E. Epidemiological investigation of the prevalence of von Willebrand disease. Blood 1987;69:454-9.
3. Philips MD, Santhouse A. von Willebrand disease: Recent advances in Pathophysiology and treatment. Am J Med Sci 1998;316(2):77-86.
4. Schneppenheim R, Thomas KB, Krey S et al. Identification of a candidate missense mutation in a family with von Willebrand Disease Type II c. Hum genetics 1995;95:681-6.
5. Sadler JE, Blinder M. von Willebrand Disease: Diagnosis, classification and treatment. In: Colman RW, Hirsh J, Marder VJ, Clowes AW, George JN, editors. Haemostasis and Thrombosis - Basic principles and clinical practice. 4th ed, Lippincott, William and Wilkins, 2001:825-37.
6. Italian working group : Spectrum of von Willebrand's disease : A study of 100 cases. Br J Haematol, 1977;35:101-3.
7. Laffan MA, Manning RA. Investigation of haemostasis. In: Lewis SM, Bain BJ, Bates I, editors. Dacie and Lewis-Practical haematology. 9th ed, Churchill Livingstone, 2001;16:339-90.
8. Ghai OP. Growth and development. In: Ghai OP, editor, Essential Paediatrics. 4th ed, 1997;1:6-7.
9. Gill JC, Endres-Brooks, Bauer PJ, Marks WJ, Montgomery RR. The effect of ABO blood group on the diagnosis of von Willebrand factor. Blood, 1987;69:1691-5.
10. Miller CH, Graham JB, Goldin LR, Melston RC. Genetics of classic von Willebrand Disease. Phenotypic variation within families. Blood 1979;54:117-36.
11. Rick M. Diagnosis and management of von Willebrand syndrome. Med Clin North Am 1994;78:609-23.
12. Rodgers RPL, Levin JA. Critical reappraisal of the bleeding time. Semin Thromb Haemost 1990;16:1-20.
13. Lind SE. The bleeding time does not predict surgical bleeding. Blood 1991;77:2547-52.
14. White CG, Montgomery R. Clinical aspects of and therapy for von Willebrand Disease. In: Hoffman R, Benz EJ, Shatill SJ, Furie B, Cohen HJ et al, editors. Haematology :Basic Principles and practice. 3rd ed. Churchill Livingstone, 2000;114:1947-58.
15. Hambelton J, George J. Hemostasis and Thrombosis. In: George JN, Williams ME, editors. American Society of Hematology: Self-Assessment Program. 1st ed. Blackwill Publishing, 2003;13:266-9.
16. Kohler M, Hellstern P, Miyashita C et al. Comparative study of intranasal, subcutaneous and intravenous administration of desamino-D-arginine vasopressin (DDAVP). Thromb Hemost 1986;55:108.
17. Lethagen S, Harris AS, Sjorin E, Nilsson IM. Intranasal and intravenous administration of desmopressin: Effect of Factor VIII/vWF, pharmacokinetics and reproducibility. Thromb Haemost 1987;58:1033.
18. Catteno M, Moia M, Delle-valle P et al. DDAVP shortens prolonged bleeding times of patients of severe von Willebrand disease treated with cryoprecipitate: evidence for a mechanism of action independent of released von Willebrand factor. Blood 1989;74:1972.