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Clinicopathological Profile of the von Willebrand Disease in a Tertiary Care Centre in Varanasi

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Abstract

Objective The von Willebrand disease (vWD) is one of the most common inherited bleeding disorders in India; however, the diagnostic tests and its interpretation require specialized laboratory and personnel which are not readily available in the eastern part of North India. The purpose of this study is to estimate the relative prevalence of vWD and study the clinical and laboratory features including advanced diagnostic tests.

Methods All patients referred to the pathology department for evaluation of bleeding were evaluated for vWD during a period of 4 years. Clinical and laboratory features were analyzed and reported.

Results A total of 1,126 cases of bleeding manifestations were evaluated, and 237 cases of inherited bleeding disorders were diagnosed; vWD was diagnosed in 38 (16%) of these 237 cases. Advanced diagnostic tests were done in all of these cases.

Conclusion The vWD is among the most common inherited bleeding disorders in the country, second only to hemophilia A. Type-1 vWD was the most frequent with 25 cases (65.7%), followed by type-2N with 7 cases (18.4%).

Keywords
- von Willebrand disease
- subtypes
- RIPA

Introduction

The von Willebrand disease (vWD) was first described by Dr. Eric von Willebrand in 1926 in a 13-year-old female with severe menorrhagia and called it hereditary pseudohemophilia.[1] The gene for this factor (*vWF*) is located on the short arm of chromosome 12. It is synthesized by endothelial cells and megakaryocytes and has multiple posttranslational modifications.[1] Many studies have described vWD as the most common inherited bleeding disorder, affecting approximately 0.1 to 1% of the total population, whereas symptomatic vWD affects only 0.01% of the population.[1] vWD is among the most common inherited bleeding disorders in India. It is classified in multiple subtypes and needs complex laboratory tests for accurate diagnosis. The aim of this study is to estimate the prevalence of vWD in patients presenting with bleeding in a tertiary care center in Northeast India, as this region is historically underdeveloped and this center is one of very few centers providing tertiary care. The objectives include laboratory evaluation of all patients presenting with bleeding to estimate the prevalence of vWD subtypes and describe the clinicopathological features among subtypes. This study is among the first few studies from this region with an in-depth evaluation of bleeding disorders and the results are compared with other studies also.

The International Society of Thrombosis and Hemostasis (ISTH) divides vWD into three types. The clinical, genetic, and laboratory profiles vary according to subtypes of vWD.[2]

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Type 1: partial quantitative deficiency.
Type 2: qualitative defects in vWF, subtypes include the following:

- 2A: selective decrease in large functional vWF multimers and decreased platelet adhesion.
- 2B: increase in platelet (gp1b)-vWF binding, causing depletion of large vWF multimers.
- 2M: decrease in vWF-dependent platelet adhesion, no reduction of large vWF multimers.
- 2N: impaired binding of vWF to factor VIII (FVIII), lowering FVIII levels.

Type 3: complete quantitative deficiency of vWF and decreased FVIII.

Clinical features include bleeding from mucous membranes, skin, and menorrhagia. Muscle and joint bleeding occurs in severe disease mimicking the features of hemophilia. Bleeding is mostly mild to moderate in severity.[3] No single test is sufficient for the diagnosis of vWD. Laboratory evaluation of vWD is done by compiling results of several tests. Initial evaluation must include complete blood count, platelet counts, prothrombin time (PT), and activated partial thromboplastin time (APTT). If these tests are normal or show isolated prolongation of APTT, vWD-specific tests are done. These include vWF antigen levels, Ristocetin cofactor activity assay (vWF:RCo), vWF:RCo/vWF:Ag ratio, FVIII coagulant assay (FVIII:C), vWF multimer analysis, low-dose ristocetin-induced platelet aggregation (LD RIPA), vWF-FVIII binding assay (vWF/FVIII:B), vWF-collagen binding assay (vWF:CB), vWF propeptide ratio (vWFpp)/vWF:Ag, the DDAVP challenge testing (DDAVP responsiveness), and lastly genotyping can be done for diagnosis.[1]

Materials and Methods

The study is a prospective study conducted from time period of January 2014 to December 2018. Inclusion criteria include all patients referred to pathology department with bleeding manifestations. Detailed clinical history was taken including age of onset, nature, amount and frequency of bleeding, family history, medication history, menstrual history in females, and blood loss during dental/surgical procedures.

Sodium citrate of 3.2% was used as anticoagulant for coagulation and platelet studies, in 1:9 ratios and processed within 4 hours. Complete blood count with platelet count was done.

Ethylenediamine tetraacetic acid (EDTA) samples were tested for a complete hemogram with platelet count. Platelet studies were done on platelet-rich plasma and coagulation studies were done on platelet poor plasma. Laboratory analysis for vWD comprised of tests like PT, APTT, FVIII:C, and these were performed manually. Bleeding time (BT) was done by modified Ivy's method. vWF:Ag was performed by enzyme-linked immunosorbent assay (ELISA; (Diagnostica Stago, France). RIPA by platelet aggregometry with ristocetin concentration of 1.5 mg/mL. LD RIPA at a concentration of 0.5 mg/mL (RIPA-LD). vWF CB (vWF:CB) was done by ELISA (Diagnostica Stago).

Table 1 Distribution of inherited bleeding disorders

Diagnosis	No.	Percentage
Hemophilia A	151	63.7
Hemophilia B	31	13.1
von Willebrand disease	38	16.0
Platelet disorders	10	4.2
Factor XIII	5	2.1
Factor X	1	0.4
Factor VII	1	0.4

Results

A total of 1,126 cases of bleeding manifestations were evaluated, and 237 cases were diagnosed as inherited bleeding disorders in a time period of 4 years. Hemophilia A was the most common inherited bleeding disorder, diagnosed in 151 patients (63.7%), Hemophilia B in 31 patients (13%). vWD was diagnosed in 38 (16%) of these 237 cases. The distribution of these 237 cases is tabulated in ►**Table 1**.

vWD patients' age ranged from 8 months to 55 years, although more prevalent in younger age group (mean age: 15 years). Females predominate over males and male-to-female ratio is 1:5 (6 males and 32 females diagnosed as vWD). Family history was not consistently positive in vWD patients, possibly due to social stigma. History of blood transfusions was present in six patients. Out of the 38 diagnosed cases of vWD, 37 presented with spontaneous bleeding and one presented with excessive posttraumatic bleeding.

Type-1 vWD was the most frequent with 25 cases (65.7%) followed by type 2N with 7 cases (18.4%). Only four cases were found to be of type 3 (10.5%). Type 2B was not seen. The distribution of the subtypes of vWD is shown in ►**Table 2**.

Distribution of clinical manifestations among subtypes was evaluated. Mucosal bleedings, such as epistaxis, menorrhagia, and gum bleeding, were frequently present and hemarthrosis was exceedingly rare, only presenting in one each of type 3 and type 2N, both classically associated with reduced FVIII. Type-3 vWD showed severe disease with clinical features of bleeding like epistaxis and menorrhagia in females in almost all patients.

Laboratory evaluation of vWD was done, screening tests showed normal PT in all patients and prolonged APTT in type

Table 2 von Willebrand (vWD) subtypes

Subtype	No. of cases	Percentage
vWD 1	25	65.7
vWD 3	4	10.5
vWD 2A	1	2.6
vWD 2M	1	2.6
vWD 2N	7	18.4

Table 3 Laboratory features of vWD: screening tests

vWD	PT (mean in second)	APTT (mean in second)	BT (second)
Control	12.5–13.5	26–30	120–300
1	14.2	30.9	402
3	14.4	62.9	915
2A	14.8	26	525
2M	13.8	33.3	690
2N	13.8	55.5	902

Abbreviations: APTT, activated partial thromboplastin time; BT, bleeding time; PT, prothrombin time; vWD, von Willebrand.

3 (mean: 62.9 seconds) and type 2N (mean: 55.5 seconds). The mean values are shown in ►**Table 3**. Platelet count was adequate in all these cases, thus excluding any platelet-related deficiency. Although type 2B and pseudo-vWD show mild thrombocytopenia, we did not identify any of these two cases in our study. The diagnostic or confirmatory tests were done as tabulated in ►**Table 4** and **5**.

FVIII:C-level evaluation was done and it was reduced in severe disease such as type 3. When normal, it also helps in excluding the diagnosis of hemophilia A which has overlapping presentation. Since multimer study was not performed for subtyping, FVIII assay helped in diagnosing type 2N which showed reduced FVIII and reduced FVIII binding.

FVIII binding (VIII B) is evaluated to diagnose type 2N which shows disproportionately reduced FVIII binding (less than or equal to 20%; Diagnostica Stago). Present study shows FVIII B value of 16% in type 2N. Also, it is extremely reduced in type-3 vWD.

vWF:Ag level refers to quantitative estimation of vWF:Ag and were extremely low in type-3 vWD. However, some type-1 and type-2 subtypes showed normal or near-normal values. To avoid missing these cases vWF:Ag levels should not be the sole determinant of vWD, and few functional assays were also included in the evaluation.

The vWF:CB is a more vWD-specific assay and gives estimate about the vWF function. The most functional multimers are high molecular weight multimers (HMWM) which

Table 4 Laboratory features of vWD used for diagnosis of subtypes

vWD	FVIII	vWF:Ag	vWF/ F VIIIB	vWF:CB	RIPA	LD RIPA	CB/Ag	Multimer analysis
1	Low/normal	Low	Normal	Low	Normal/low	Low	Normal	All low
3	Very low	Not measurable	Not used	Very low	Absent	Absent	Not used	Absent
2A	Normal/mild low	Mildly low	Normal	Very low	Normal/low	Absent	Low	Absence of large and intermediate
2B	Normal/mild low	Normal/mild low	Normal	Low	Increased	Increased	Low	Absence of large
2M	Normal/mild low	Low	Normal	Low	Normal/low	Absent	Low/ normal	Normal
2N	Low	Normal	Low	Normal	Normal	Absent	Normal	Normal

Abbreviations: Ag, antigen; CB, collagen binding; FVIII, factor VIII; LD, low dose; RIPA, ristocetin-induced platelet aggregation; vWD, von Willebrand.

Table 5 Laboratory features of vWD (diagnostic tests): results

vWD	FVIII:C (mean in %)	vWF:Ag (mean in %)	vWF/FVIIIB (mean in %)	vWF:CB (mean in %)	RIPA (mean)	LD RIPA (mean)	CB/AG (mean in %)
Normal range	50–150	70–150	60–160	64–160	11.5–17 seconds	28–34 seconds	0.8–1.2
1	84–163 (111)	50–70 (61.2)	87–149 (116)	56–63 (60)	12.2–28.5 (15.2)	90–120 (117.6)	Normal
3	Undetectable	3–6 (5.0)	5–8 (6.5)	1–3 (2.2)	> 2 minutes	> 2 minutes	Not detectable
2A	60	48	106	30	26.2	> 2 minutes	Reduced (0.6)
2M	76	35	108	51	29.3	> 2 minutes	Normal
2N	3–25 (8.3)	9–36 (17.2)	14–18 (16)	70–89 (80)	13.4–17 (14.6)	> 2 minutes	Normal

Abbreviations: Ag, antigen; C, coagulant; CB, collagen binding; FVIII, factor VIII; LD, low dose; RIPA, ristocetin-induced platelet aggregation; vWD, von Willebrand.
Note: All values are mean values, reference ranges are the laboratory reference ranges.

are absent in subtypes 2A and 2B, thus resulting in extremely low vWF:CB in these subtypes. In the present study, values of vWF:CB were the lowest in type 3 and, among type 2, the lowest in 2A, slightly low in 2M, and normal in 2N.

vWF:CB/AG gives an estimate about qualitative defect versus quantitative defect as CB is a functional assay and is reduced in absence of HMWM (subtypes 2A, 2B), whereas vWF:Ag is a quantitative assay. If all the results of quantity and function are uniformly low, then the probability of quantitative deficiency like type 1 or 3 is considered. If the results are not uniformly low and functional assays, like CB, are reduced disproportionately to Ag levels, the ratio of CB/AG is reduced. As in the present study, it is reduced in type 2A allowing differentiation from quantitative defects like types 1 and 3. Also type 2A (loss of HMWM, so reduced CB/AG ratio) can be differentiated from 2M (normal ratio) by this ratio in absence of multimer analysis.

Ristocetin-induced platelet aggregation: this test depends on function and number of vWF and normally aggregation will occur at ristocetin concentration of 1 mg/mL and above. Quantitative and qualitative defects will show reduced aggregation at 1 mg/mL, whereas type 2B shows increased sensitivity to LD ristocetin.

Discussion

vWD is one of the most common inherited bleeding disorder, although its incidence among patients referred at our center was second only to hemophilia A. In the present study, 16% of the referred patients were found to have vWD. In another Indian study by Ghosh et al, 81 patients out of 761 evaluated were found to have vWD (10.6%).[4] A previous study at our center of previous 4 years' identified vWD in 40 (17%) out of 230 patients of inherited bleeding disorders.[5] The prevalence of vWD in our region of eastern Uttar Pradesh, India, and adjoining states seems to be consistent as seen by the present study and previous study at the same center but of different periods and patients. ►**Table 6** compares the studies on vWD, and the percentage of vWD among inherited bleeding disorder ranges from 8.6 to 28.6% in these studies. The ratio of vWD to hemophilia A ranges from 0.1 to 0.23 in published Indian studies.[4,6] The present study showed ratio of vWD to hemophilia A of 0.25 which is consistent with other published data.

Type-1 vWD was the most frequent with 25 cases (65.7%) followed by type 2N with 7 cases (18.4%). Only four cases were found to be of type 3 (10.5%). This contrasts with other studies from Indian literature, whereas the western literature also states type-1 as the most common subtype of vWD. Indian studies have possibly reported lower prevalence of type-1 vWD due to lower health awareness and ignorance of mild disease. However, study done by Gupta et al describes type 2 to be most common and describes possible misclassification of type 2 as type 1 in the absence of functional assays and multimer analysis.[7,8]

Majority of patients in the present study were females and the most common symptom was menorrhagia, similar to few other studies which stresses the importance of testing for inherited bleeding disorders in patients of menorrhagia.[4,5] Platelet-type bleeding from mucous membranes was seen predominantly while only one case of hemarthrosis was seen in a type-3 vWD patient due to concurrent low FVIII levels in type 3.

Laboratory evaluation was performed including screening tests like PT, APTT, and BT and advanced diagnostic tests, such as vWF:Ag assay, FVIII:C assay, functional assays like RIPA, LDRIPA, vWF:CB, and vWF/FVIII binding assay, were performed for diagnosis and subtyping. Levels were compared with the diagnostic criteria and categories defined. However multimer analysis and genetic studies were not performed. Another recent advance in the continuously evolving laboratory techniques is the vWF:GPIbM (recombinant/mutant glycoprotein) assay. This test eliminates need of ristocetin by introducing gain-of-function mutations in GPIbα receptor, so that it can spontaneously bind to vWF without ristocetin and has been reported to be more accurate; however, the availability of this test is very limited and not yet performed in India.[9]

Another recent study has proposed cutoff value less than 30 IU/dL for diagnosis of vWD, as it is associated with bleeding and mutations.[9] But levels greater than 30 labeled as low vWF, presenting with abnormal bleeding that should be investigated for vWD type 1. Type-2 vWD can present with near-normal Ag levels, so functional assays will be needed for diagnosis. Also, genotyping has an important

Table 6 Distribution of von Willebrand (vWD) and its subtypes in published studies

Study (year)	No. of patients	No. of vWD patients (%)	Type 1 (%)	Type 2A (%)	Type 2B (%)	Type 2M (%)	Type 2N (%)	Type 3 (%)
Gupta et al (2005)[7]	224	64 (28.6)	14 (21.9)	24 (37.5)	–	4 (6)	–	21 (32.8)
Trasi et al (2005)[11]	796	58 (7.3)	18%	9.5%	4.7%	1.2%	3.6%	59.5%
Gupta et al (2007)[8]	872	94 (16.8)	20 (21.3)	38 (40.4)	–	04 (4.3)	–	32 (34.04)
Ahmad et al (2008)[6]	1,576	136 (8.6)	29 (21.3)	–	–	–	–	33 (24.3)
Kumar et al (2010)[5]	230	40 (17.34)	17 (42.5)	10 (25)	–	–	1 (2.5)	12 (30.0)
Srivastava and Rodeghiero (2005)[12]	200×10^6	211 (0.000001)	–	–	–			95 (51.9)
Present study	237	38 (16)	25 (65.7)	1 (2.6)	–	1 (2.6)	7 (18.4)	4 (10.5)

role in diagnosis of type 2N.[2] Another important point is to make sure that repeat testing is always done for confirming the results.[10–12]

Conclusion

vWD is among the most common inherited bleeding disorders in the country second only to hemophilia A but due to the complex phenotype of disease and necessity of advanced tests and trained personnel, the diagnosis is sometimes difficult. This is especially true for India where majority of hematology laboratories do not have these facilities. The current study is one of the very few Indian studies that estimate the prevalence of vWD and describes the clinical and laboratory features including the advanced diagnostic tests. Treatment of vWD includes desmopressin, cryoprecipitate, plasma derived or recombinant vWF concentrates, and antifibrinolytics.

Ethics

This study was approved by institutional ethics committee.

Conflict of Interest

None declared.

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