Prevalence & spectrum of von Willebrand disease from western India

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Received June 15, 2004

Background & objectives: von Willebrand disease (VWD) is one of the most common inherited bleeding disorders in the west. Limited studies from India showed a prevalence of approximately 10 per cent of VWD among the cases with hereditary bleeding disorders. VWD remains an underdiagnosed entity in India. The prevalence of different subtypes of VWD is also not known which is essential for a proper management of these cases. The present study was thus undertaken to know the prevalence of VWD and its various subtypes in the western part of our country.

Methods: A total of 796 consecutive patients presented with various bleeding manifestations were analysed. The initial screening and confirmation tests for the diagnosis of VWD included bleeding time (BT), screening coagulation tests i.e., prothrombin time (PT), activated partial thromboplastin time (APTT), thrombin time (TT), factor VIII: C assay, ristocetin-induced platelet aggregation (RIPA) and VWF antigen (VWF:Ag) estimations. VWF multimer analysis, ristocetin cofactor activity (RCOF), VWF collagen binding assay (VWF: CBA), factor VIII : VWF binding assay were also done to classify and subtype these cases.

Results: The patients were subtyped as per the International Society of Thrombosis and Haemostasis (ISTH) criteria. Of the 796 patients screened, 58 were diagnosed as VWD. Of the 15 families with a positive family history of bleeding, 26 additional cases were diagnosed as VWD. Majority of the patients were type 3 (59.5%) with severe clinical manifestations, about 18 per cent of type 1 VWD patients were detected in this group while the prevalence of the qualitative variants of VWD i.e., type 2 VWD was found to be 19 per cent and the prevalence of various subtypes were type 2A (9.52%), type 2B (4.76%), type 2M (1.2%), type 2N (3.6%).

Interpretation & conclusion: The high prevalence of type 3 and a low prevalence of type 1 VWD which is in contrast to the western reports, suggests the low awareness of the disease as also the underdiagnosis of the mild cases in our country.

Key words India - prevalence - subtypes of VWD - von Willebrand disease

von Willebrand disease (VWD) is one of the most common inherited bleeding disorders, caused by a qualitative or quantitative deficiency of the von Willebrand factor (VWF). The various forms of VWD can be differentiated by different patterns of genetic transmission and abnormalities of VWF in plasma and platelets. It has been classified into three different types: type 1 being partial deficiency of VWF, type 2 being a qualitative defect in the VWF, and type 3 being a total loss of the VWF. This bewildering phenotypic heterogeneity of VWD has made it difficult to identify and diagnose the right type of variant or subtype and hence the actual incidence of VWD in the population is difficult to know.
Several groups have reported a frequency of 0.7 to 1.6 per cent of VWD in the population in western countries\(^1\)\(^4\). Although not many studies are available on the prevalence of VWD in India, some of the tertiary centres or hospitals have reported a prevalence of approximately 10 per cent of VWD among the cases with hereditary bleeding disorder cases\(^5\)\(^9\). VWD therefore remains an underdiagnosed entity in India. Further, the prevalence of different subtypes of VWD is also not known. The present study was carried out to know the prevalence of VWD and its various subtypes in western India.

### Material & Methods

The study was conducted in Institute of Immunohaematology, Mumbai which is a tertiary care centre. Patients from the Haematology Department, King Edward Memorial (KEM) Hospital, Mumbai and referred cases from other hospitals in and around states of Maharashtra, Gujarat and Madhya Pradesh with bleeding manifestations like prolonged bleeding from injuries and following tooth extraction, epistaxis, menorrhagia, gum bleeding, ecchymoses, haemarthrosis, etc., were investigated for VWD. A total of 796 consecutive patients referred between January 2000 and September 2003 were investigated. The mean age of these patients was 24.2\(\pm\) 11.4 (mean\(\pm\)SD). Of the 796 patients, 594 were males and 202 females. A detailed proforma containing the nature of bleeding, family history and mode of inheritance was filled along with detailed physical findings. The study was approved by the Institutional Ethics Committee. The consent from the patients as well as family members for participation in the study obtained prior to collection of blood samples.

Blood (9 ml) was collected in 3.13 per cent trisodium citrate\(2\text{H}_2\text{O}\) (9:1 proportion of blood: anticoagulant) and 1 ml in EDTA bulbs. Platelet rich plasma (PRP) was prepared by spinning the citrated blood sample at 150-200g for 10 min and platelet poor plasma (PPP) at 2000g for 10 min. The leucocytes were preserved at -70\(^\circ\)C for DNA analysis. The complete blood count (CBC) was performed in the EDTA sample using an automated cell counter (Sysmex K 1000, Japan). Bleeding time (BT) was calculated using modified Ivy\(^10\) methods. Screening coagulation tests i.e. prothrombin time (PT), activated partial i.e. thromboplastin time (APTT), thrombin time (TT) and factor VIII : C one stage assays\(^10\) were performed in a semiautomated coagulometer (ST Art, Diagnostica Stago, France). Ristocetin-induced platelet aggregation (RIPA) was performed by aggregometry (Chrono-log Corporation, Havertown PA, USA). VWF: antigen levels were estimated by ELISA\(^11\) using commercial kits (Diagnostica Stago, France) and VWF: ristocetin cofactor activity (RCOF) by using fixed platelets\(^10\). VWF: collagen binding assay (VWF:CBA) was performed by slight modification of the technique described earlier\(^12\). Briefly, 100 \(\mu\)l (10 \(\mu\)g/well) of pepsin digested type III collagen from human placenta (Sigma, USA) was added to 96-well microtitre plates (Maxisorp, NUNC, Denmark) and incubated overnight at 4\(^\circ\)C. After blocking with 3 per cent gelatin (Sigma, USA) plasma dilutions (1:80 and 1:160) from patients as well as standards were added and incubated at 37\(^\circ\)C for 2 h. After washing with phosphate buffered saline (PBS, \(pH\) 7.2), anti-human VWF-horse radish peroxidase (HRP) conjugate (DAKO, Denmark) was added and incubated for 1 h at 37\(^\circ\)C, followed by addition of the substrate i.e., 1,2 -o- phenylenediamine hydrochloride (OPD) and incubated for 30 min at 37\(^\circ\)C. Finally, the plates were read at 492 nm in an ELISA reader (Titretek, Germany). The readings were plotted in log linear graph paper and compared with the standard curve. VWF: FVIII binding assay\(^13\) and VWF multimer analysis\(^14\) were performed as described earlier. The subtyping and the classification of the VWD cases was done according to International Society of Thrombosis and Haemostasis (ISTH) criteria\(^15\).
Family studies were carried out in patients with a positive family history of bleeding.

**Results**

In the study group, there were 38 females and 46 males. The age at which the patients reported varied from 5 days to 77 yr. Of the 796 patients investigated for bleeding disorders, 58 patients (7.29%) were diagnosed with VWD. Seventeen of the 58 patients (29.31%) gave a positive family history of bleeding tendencies. Upon investigating these 15 families, 26 additional cases of VWD could be diagnosed. Type 3 VWD was the predominant subtype (59.5%), followed by type 2 (19%) of which type 2 A was more frequent (9.52%) as compared to other type 2 subtypes (Table I).

A variety of bleeding manifestations were observed in these patients (Table II). Mucocutaneous bleeds were common in these patients. Mild to moderate bleeding was seen in types 1 and 2 VWD patients while severe bleeding and haemarthrosis was more common among type 3 VWD patients. Table III shows the various laboratory parameters studied in the different groups.

**Discussion**

A wide spectrum of VWD subtypes was obtained in the present study. A maximum of type 3 VWD patients (60%) were detected in this series. This suggests that these patients with severe bleeding tendencies need constant medical attention, which prompted them to seek medical advice. Type 1 VWD was seen in 18 per cent which is patients in contrast to the 60-70 per cent prevalence of type 1 VWD reported in studies from other countries\(^{16-22}\). A large variation was seen in the number of type 1 and type 3 VWD patients. The type 1 VWD have infrequent and mild bleeding episodes and hence consider their bleeding tendency as normal, unless they come across a major haemostatic challenge like tooth extraction or surgery for which they get themselves investigated. These milder forms of VWD are often missed out on diagnosis or may not be detected at all as screening coagulation tests may be in the normal range. A large number of patients could also be asymptomatic or might have not come across a haemostatic challenge; however, they could bleed profusely if they face a major haemostatic challenge. Lack of diagnostic facilities may be another factor for a low detection rate of the mild cases.

Two cases with acquired VWD were diagnosed. One of the patients had seborrhic dermatitis and vitiligo while the other was a case of hypothyroidism. One patient could not be classified because of the unusual multimer pattern. Hence the type of VWD and the actual defect causing the same may be known by determining the causative mutation. Other rare variants of VWD like platelet type VWD were not detected in this study.

Mucocutaneous bleeding was more predominant with ecchymoses, epistaxis and gum bleeding being common among these patients. Bleeding symptoms were found to be of mild to moderate nature in type 1 and type 2 VWD patients while severe life-threatening
Table III. Laboratory parameters in different groups

<table>
<thead>
<tr>
<th>Patients (no.)</th>
<th>Platelets (X 10^3 µl)</th>
<th>BT (min)</th>
<th>PT (sec)</th>
<th>APTT (sec)</th>
<th>TT (sec)</th>
<th>F VIII:C (%)</th>
<th>RIPA (%)</th>
<th>VWF:AG (%)</th>
<th>VWF:RCOF (%)</th>
<th>VWF:CBA (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>VWD type 1 (15)</td>
<td>320.16±70.12</td>
<td>6.24±2.34</td>
<td>14.03±1.89</td>
<td>46.94±12.24</td>
<td>16.26±2.34</td>
<td>20.5±18.34</td>
<td>65±205</td>
<td>28.26±19.76</td>
<td>67.54±34.56</td>
<td>76.53±7.87</td>
</tr>
<tr>
<td>Type 2 (16)</td>
<td>290.34±35.18</td>
<td>6.84±3.24</td>
<td>13.09±1.54</td>
<td>51.45±11.20</td>
<td>17.24±1.84</td>
<td>18.38±5.67</td>
<td>42±14.20</td>
<td>58.54±17.34</td>
<td>16.38±8.76</td>
<td>24.67±9.65</td>
</tr>
<tr>
<td>Type 3 (50)</td>
<td>296.12±90.18</td>
<td>11.45±4.36</td>
<td>14.34±1.34</td>
<td>98±13.08</td>
<td>15.84±1.94</td>
<td>2.5±1.1</td>
<td>5.86±4.84</td>
<td>8.94±3.84</td>
<td>50.84±3.47</td>
<td>49.89±5.87</td>
</tr>
<tr>
<td>Acquired/unclassified (3)</td>
<td>310.18±84.23</td>
<td>8.45±3.25</td>
<td>14.34±1.25</td>
<td>96±2.31</td>
<td>16.2±2.04</td>
<td>12.45±10.12</td>
<td>30.86±6.24</td>
<td>18.45±8.34</td>
<td>26.34±8.97</td>
<td>54.78±5.78</td>
</tr>
<tr>
<td>Others (712)*</td>
<td>3674±96.4</td>
<td>4.56±2.34</td>
<td>16.24±2.23</td>
<td>56±24.06</td>
<td>16.24±2.56</td>
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</table>

* Specific tests are not performed in all the other samples except when required
bleeding occurred in some patients with type 3 VWD. Since FVIII is usually slightly reduced in type 1 VWD, manifestations of a severe coagulation defect like haemarthrosis and muscle haematomas were rare in type 1 VWD patients, whereas in type 3 VWD patients the severity of bleeding resembled that of patients with haemophilia. It was interesting to note that the symptoms varied among affected members of the same family and also over time in a single individual. Menorrhagia was found in 14/29 (48.28%) of women in the reproductive age group and could be a major pointer for VWD in females. Though post-partum bleeding is rare in patients with VWD because of the normalization of FVIII/VWF levels at the end of pregnancy, two patients with post-partum bleeding were found in the present study; one with type 1 VWD and other with type 2A VWD, both required replacement therapy for management. Bleeding after dental extraction was the most frequent post-operative bleeding manifestation.

The inheritance pattern of VWD in these 84 patients revealed that there were more patients with recessive inheritance (79%). Another important aspect noted in this study was the presence of consanguinity among the parents (35%). Consanguineous marriages are very common in certain states in India. It was found that 47.7 per cent of patients with recessive inheritance of VWD showed consanguinity. This shows that parents of these VWD patients were heterozygous for the affected VWF gene, which resulted in a homozygous disease condition in the offspring.

Most epidemiological studies conducted in Caucasian population have shown VWD as the commonest coagulation disorder accounting for up to 1 per cent of the population. Our data from a tertiary care centre in western India probably has picked up most of the severely affected patients, i.e., with type 3 VWD, leading to substantial underdiagnosis of milder type 1 VWD who never reached the hospitals. An epidemiological study in the general population needs to be done to assess the magnitude of this disease in our country.

References


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