Prevalence and Clinico-Hematologic Scenario of Von Willebrand Disease in a Population of Filipinos with Bleeding Tendency

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Von Willebrand Disease (VWD) is the most common inherited bleeding disorder. It is defined as a deficiency of von Willebrand Factor (VWF) function causing impaired hemostasis. VWF functions as a carrier protein for coagulation factor VIII (FVIII) and as adhesive protein for vessel wall-platelet interaction. Studies in a predominantly pediatric population reveal that the prevalence of VWD is 0.8 - 1.3%. There are 3 main types of VWD based on the quantitative or qualitative defects in VWF.

Ninety-nine patients with bleeding manifestations were referred to the National Hemophilia Center from all over the Philippines. Patients who fulfilled the inclusion and exclusion criteria with at least 2 symptoms consistent with VWD underwent initial screening tests: a complete blood count with actual platelet, blood typing, bleeding time, prothrombin time and activated partial thromboplastin time. Laboratory tests to diagnose VWD included FVIII, VWF antigen (VWF:Ag) and ristocetin cofactor (VWF:RCo). Thirty-four patients (34.34%) with bleeding manifestations had VWD. Patients with or without VWD were comparable as to age, sex distribution, family history of bleeding, blood type and bleeding manifestations.

Among the patients with VWD, 11 (32.35%) had Type 1 VWD and 23 (67.65%) had Type 2 VWD. No Type 3 VWD was observed. The mean FVIII, VWF:Ag and VWF:RCo were decreased in Type 1 VWD. In Type 2 VWD, the mean VWF: Ag was normal while the mean FVIII and VWF: RCo were decreased.

The study suggests that among patients with bleeding tendency in the Philippines, there is a high proportion of patients with VWD.

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INTRODUCTION

Von Willebrand Disease (VWD) is the most common inherited bleeding ailment. Following an autosomal dominant mode of inheritance, it is the most common bleeding disorder with an estimated prevalence of 1 - 3% in the general population while the prevalence is 0.8 to 1.3% in the pediatric population.[1-4)] Individuals with VWD have defects in, or reduced levels of von Willebrand factor (VWF), an adhesive plasma protein crucial for effective primary hemostasis.[5)] The protein circulates in the plasma complexed to Factor VIII (FVIII) and is required for the normal adhesion of platelets to the sub-endothelium and for in vitro ristocetin-induced platelet aggregation. The clinical manifestations of the disease range from severe cases requiring replacement therapy to stop or prevent hemorrhages to mildly affected patients. Diagnosis of VWD is based on a panel of laboratory tests, in the background of hemorrhagic manifestations, that measure the amount and function of von Willebrand factor (VWF). These tests include Factor VIII (FVIII), von Willebrand factor antigen (VWF:Ag), Ristocetin cofactor (VWF:RCo), collagen binding (VWF:CB) and factor VIII binding (VWF:FVIIIB).

Numerous studies have shown that there is ethnic variation in the VWF level.[6,7)] The study found that there was a significant difference in VWF:Ag levels between Indians and Africans, and between Caucasians and Africans, whereas no significant difference between Indians and Caucasians was noted. VWF:Ag levels between blood groups have likewise shown significant variations.[8)] VWF:Ag levels are higher in persons with blood groups A and B compared to blood group O. This is because persons with blood group O are associated with a relatively low level of protein glycosylation thus VWF is less glycosylated. These sources of variation will influence the diagnosis and prevalence of VWD in different populations. The prevalence of the disease is important in a country in order to guide clinicians in the diagnosis of the disease and appropriate treatment may

be instituted.
The objective of this study is to determine the prevalence of VWD in a population of Filipinos with bleeding manifestations. Also, the study will determine the clinico-hematologic profile of Filipino patients diagnosed to have VWD.

PATIENTS AND METHODS

Study Population

The study subjects were referred to the National Hemophilia Center by participating hematologists from all over the Philippines. The patients were examined by a board certified hematologist (Principal Investigator). Patients who fulfilled the inclusion criteria and had at least 2 symptoms consistent with VWD were included and underwent the initial screening tests. Inclusion criteria were: Age between 1 and 35 years old of either sex, no hepatosplenomegaly, no documented hematologic disorder, no intake of aspirin or coumadin; nor received heparin in the past month. Symptoms of VWD were: epistaxis after excluding specific causes, hemorrhage after tooth extraction, hemorrhage after surgery, prolonged bleeding after superficial cuts, post-partum hemorrhage after ruling out specific causes, menorrhagia when specific causes have been ruled out and easy bruising.
The following laboratory tests were done on included subjects: complete blood count with actual platelet count, blood typing, bleeding time (Ivy Template Method), prothrombin time, activated partial thromboplastin time, factor VIII activity, Von Willebrand Factor antigen. The diagnosis of VWD was based on the criteria of the International Society of Thrombosis and Hemostasis.[9)]

The Ristocetin Cofactor Test

The Ristocetin Cofactor tests were performed using the Dade Behring Kit. The manual agglutination method was performed, by preparing doubling dilution of patient's plasma in normal saline solution to give 1:20 through to 1:160, which corresponded to a titre of 20 through to 160.
Onto each field of the glass plate, 50µl of plasma dilution was pipetted along with 50µl of von Willebrand ricof latex reagent. This was mixed well and agitated for 1 minute and then left to stand for another minute.
The titre of the specimen is evaluated by the dilution in which there is a distinct agglutination when compared to the blank mixture, which is composed of normal saline solution only. The ristocetin cofactor content is expressed in percentage by multiplying the titre of the specimen with the limit of detection indicated in the vial of the reagent. In our study the value of the von Willebrand ricof content of the reagent was 0.7%.

Factor VIII Assay

Calibration or preparation of a standard curve for this assay for subjects and control was performed using the automated coagulation analyzer (CA-550, Sysmex, Japan) with Standard Human Plasma (SHP) according to the parameters entered in the instrument. A series of dilution for this calibration were chosen in the default dilution already incorporated in the instrument. A normal and an abnormal control were analyzed with every batch tests. A clot-based one stage FVIII assay was used in this study.

Von Willebrand Antigen

Calibration of this test was performed using the same instrument used for FVIII determination. Three calibration settings were available for this particular parameter, the Medium, Low and High level of VWF:Ag. In this study, only two clinically related settings, the Medium and Low level of VWF:Ag, were used for calibration.

Medium Settings:	Screening for VWD (bleeding risk) Range limit of the instrument is 10-200%
Low Settings:	Screening for severe bleeding risk and VWD Type 3 (<2%) Range limit of the instrument is 2-20%
High Settings:	Sample suspected or known to have a high VWF: Ag associated with the risk for thrombosis (Post surgery, liver disease, after DVT). Range limit of the instrument:100-600%

Establishment of Reference Ranges

Reference ranges were established for the following laboratory exams: bleeding time, prothrombin time, activated partial thromboplastin time (aPTT), FVIII, VWF:Ag and VWF:RCo. The reference ranges were based on plasma from 46 subjects aged between 1 to 35 years old, with no concomitant disease, no family history of bleeding, no intake of aspirin or coumadin, have not received heparin in the past month, no intake of contraceptive pills for female subjects and the hemorrhagic symptoms. Ten (10) males and 15 females with blood type O and 10 males and 11 females with blood type other than O were included. Plasma from these subjects was tested using SE-9500, Sysmex, Japan (CBC and platelet) and CA-550 (for coagulation assays). The geometric mean was determined for the blood exams and the 5th percentile was accepted as the cut-off.

Statistical Analysis

The chi-square test or Fisher's exact test was used for qualitative variables while the unpaired t-test was used for quantitative variables. All statistical tests used to analyze the data were two-tailed at alpha = 0.05 level of significance. The data was encoded (based on a coding manual) in Excel and analyzed using Stata version 8.2 (Stata Corp LP, USA).

RESULTS

One hundred two (102) patients with bleeding manifestations were referred to the National Hemophilia Center (NHC) by participating Pediatric and Adult Hematologists. All patients were seen and examined by the supervising investigator to determine inclusion into

Table 1 *Comparison of clinical characteristics of patients with von Willibrand's Disease and without disease*

Variable	With VWD N - 34 (%)	No VWD N - 65 (%)	P value
Age (years) - mean±SD	11.44±8.25	11.06±7.84	0.82
Sex			
Male	13 (35.24)	31 (47.69)	0.40
Female	21 (61.76)	34 (52.31)	
Family history of bleeding			
Positive	13 (38.24)	26 (40.00)	0.86
Negative	21 (61.76)	39 (60.00)	
Blood type			
O	24 (70.59)	28 (43.08)	0.03
A	4 (11.76)	18 (27.69)	
B/AB	6 (17.65)	19 (29.23)	
Bleeding manifestations			
Epistaxis	22 (64.71)	39 (60.00)	0.65
Hemorrhage	7 (20.59)	18 (27.69)	0.44
Prolonged bleeding	6 (17.65)	18 (27.69)	0.27
Menorrhagia	5 (14.71)	12 (18.46)	0.64
Easy bruising	24 (70.59)	57 (87.69)	0.04

the study. Three patients were excluded from the study because of presence of other hematologic disorders. A total of 99 patients fulfilled the inclusion and exclusion criteria.

There were 34 patients with VWD and 65 patients without the disease. The prevalence of von Willebrand disease in the population of patients who presented with bleeding manifestation is 34.34%. ***Table 1*** shows the clinical characteristics of participating patients. The mean age was similar in the subjects with VWD (11.44 ±8.25) and those who did not have the disease (11.06 ±7.84), and it was not significantly different (p=0.82). Likewise, the distribution of sex (p=0.40) and family history of bleeding (p=0.86) were not significantly different between the two groups. In both groups, Blood type O (VWD - 70.59%, no VWD - 43.08%) was more common compared to the other blood types. However, the distribution of blood type in the two study populations was significantly different. Blood type A (27.69%), B and AB (29.23%) were significantly (p=0.03) more among those without VWD compared to those with VWD (Blood type A -11.76%, Blood type A and AB - 17.65%). Easy bruising was the most common bleeding manifestation in both groups. Patients without VWD had statistically more (p=0.04) easy bruising compared with those patients with VWD.

Among the patients with VWD, 11 patients (32.35%) were classified as type 1 VWD. The FVIII, VWF: Ag and VWF: RCo of these patients were decreased and levels were similar. There were 23 patients (67.65%) who were classified as type 2 VWD. These patients had mildly low to low-normal FVIII and VWF: Ag whiles the VWF: RCo was definitely low. This pattern suggests either type 2A, 2B or 2M VWD. Among those patients who were classified as type 2, FVIII was definitely low and the VWF:Ag and VWF:RCo were mildly low to normal. This pattern suggests type 2N. However, multimer analysis of patients who were classified as type 2 must be done in order to definitely determine the subtype and to confirm the diagnosis of VWF type 2. No patients were classified as type 3 VWD.

Table 2 shows the comparison of blood parameters of the patients with VWD. Hemoglobin, WBC, platelet, bleeding time, prothrombin time and aPTT were normal in the two groups.

The mean FVIII, VWF:Ag and VWF:RCo were all decreased in patients with type 1 VWD. In patients with type 2 VWD, the mean FVIII and VWF:RCo were decreased but the mean VWF:Ag was normal.

Table 2 *Comparison of blood parameters in patients with von Willibrand Disease*

Variable	Type 1 VWD N - 11 Mean (±SD)	Type 2 VWD N - 23 Mean (±SD)
Hemoglobin (g/dL)	12.30 (0.71)	11.97 (0.79)
WBC (x 10^9/L)	5.89 (2.81)	5.73 (3.14)
Platelet (x 10^9/L)	199.09 (110.08)	191.65 (97.46)
Bleeding time (sec.)	6.91 (2.93)	6.89 (3.50)
Prothrombin time (sec.)	12.94 (1.21)	13.11 (1.46)
aPTT (sec.)	41.40 (8.14)	40.13 (8.39)
FVIII (%)	36.60 (20.00)	45.98 (20.80)
VWF:Ag %	30.41 (9.60)	63.03 (21.74)
VWF:RCo %	25.45 (15.10)	37.74 (15.47)

DISCUSSION

From the results of the study, the prevalence of VWD among Filipino patients with bleeding manifestations was 34.34%. The results are in contrast to the study in India, where the prevalence of VWD was 7.29% among subjects with bleeding disorders.[12] Community based studies in children with bleeding manifestations revealed lower prevalence of 0.8 to 1.0%.[13] However, this study utilized only VWF:RCo in addition to personal and family history of bleeding.

Only two subtypes of VWD were identified in the study, type 2 being more common than type 1. The study of Trasi S, et al, showed that half of the subjects are type 3 followed by type 2. On the other hand, type 1 VWD is the most common (75%) subtype in the west.[14]

Since the time the disease was described by Dr. Erik von Willebrand in 1926, it remains to be a clinical diagnostic problem to many. Despite the growing recognition of this disease there is still marked under-diagnosis. Often people with the disease may only have easy bruising or some may experience unnecessary hysterectomy because of unexplained heavy bleeding.[8] The present study showed that bleeding manifestations were not significantly different between patients with VWD and those without VWD. In fact, easy bruising was statistically higher in those without VWD. The result tells us that bleeding manifestations is not a reliable basis for making a diagnosis of VWD.

Our study showed female predominance for the disease. The study of Mishra et al showed a M:F ratio of 3:7.[10] Fifty (50%) of patients with the disease in this study had a positive family history of bleeding. If an autosomal dominant mode of inheritance is suggested, all patients should have a family history of bleeding. Research concerning the inheritance in VWD is still underway to clarify a great deal of information that is not yet understood about the disease. Another way of getting VWD is through molecular defects, which include point mutations and deletions. There may be no history of the disease because the patient got the abnormal gene through a new mutation. Chromosome 12 changes at conception or soon after.

Thrombocytopenia is not common in VWD but has been reported in Type 2B VWD. Our study showed that 8 subjects with thrombocytopenia had normal template bleeding time. Patients with type 2B VWD may have thrombocytopenia presumably due to the binding of VWF and formation of small platelet aggregates, which are cleared from circulation. Patients with platelet-type of VWD may also have thrombocytopenia.[11]

Typical VWD has prolonged bleeding time. However, our study showed that majority of the patients, who has the disease had normal bleeding time. Many studies have shown that the tests of plasma von Willebrand factor activity do not correlate well with the bleeding time.[11]

aPTT is usually performed as part of the initial evaluation for VWD. There were 4 patients in the study who has VWD with normal aPTT. Not infrequently in type 1 and 2 VWD, will have initial normal aPTT since FVIII levels may be sufficiently high to show a normal aPTT response.[10] Though this test is sensitive to decreased levels of FVIII, it frequently is normal in patients with mild VWD. The evaluation of FVIII levels also may be within the normal range in some patients with variants of type 1 VWD.[10]

Accurate diagnosis of VWD is very important so that appropriate therapy may be instituted. Most of these patients who present in the clinics of general pediatricians or hematologist may be mislabeled as vascular purpura or mild hemophilia. When these mislabeled patients go into bleeding during surgery or when post-partum bleeding occurs, they will be managed with frequent blood transfusion. As a result, patients may have iron overload from frequent transfusions. Bleeding in VWD may be managed with DDAVP. This will reduce the risk of transfusion related events.

CONCLUSION

Thirty-four subjects (34.34%) out of 99 Filipinos with bleeding tendencies were documented to have VWD. Type 2 VWD is the most common subtype of VWD. Bleeding manifestations are not adequate to make a diagnosis of VWD.

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