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Original Research Article

To Determine the Incidence of Various Subtypes of VWD with Specified VWF:RCof/ VWF:Ag Ratio's In Pediatric Patients

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Abstract

Background: VWD is the most common inherited bleeding disorder characterized by defects in the concentration, structure, or function VWF. There are three main types of VWD that differ according to the degree of disease severity and inheritance pattern (Type 1, Type 2A, 2B, 2M, 2N and Type 3). ***Methodology***: This is 05 years study (Feb 2016–Jan 2021) conducted in hematology section. In present study, amongst total inherited bleeding disorders, Incidence of VWD is 27%, Hemophilia (50%), inherited platelet disorders (14%) and remainder are undiagnosed. Amongst VWD, Type 1 VWD is the most common subtype studied, comprising 66% of total cases. Type 2 & 3 VWD comprised 23% and 10% of total cases showing good correlation with the literature and other studies. ***Discussion***: The European cross-sectional study yielded a population-based estimate of 0.05 per 100,000 for type 3 VWD. Estimates by VWD type from Europe and Western Pacific reported higher prevalence estimates for type 1 disease than type 2 or type 3: 2.7–7.2 per 100,000 for type 1 VWD, 0.8–2.5 per 100,000 for type 2, and 0.1–0.3 per 100,000 for type 3. ***Conclusion***: VWD usually presents with mild bleeding symptoms (except in type 3 VWD) the diagnosis is often delayed. Prompt diagnosisand management can help to avoid potentially life-threatening bleeding events and unnecessary exposure to blood products.

Keywords: Von willibrand disease, Hemophilia, Inherited bleeding disorders.

INTRODUCTION

Von Willebrand disease (VWD) is an inherited bleeding disorder characterized by defects in the concentration, structure, or function of von Willebrand factor (VWF), a glycoprotein that acts as a carrier protein for factor VIII (FVIII) and facilitates platelet adhesion at vascular injury sites [1, 2].

VWD is caused by either decreased quantity or abnormal function of a large multimeric protein, VWF. The protein ranges in size from 450 kDa to over 10,000 kDa and is located at chromosome 12p13.2. VWF is made in the endothelium and by megakaryocytes. This protein has two roles: the binding of platelets to exposed collagen at sites of vascular injury, and the binding and stabilization of factor VIII [3].

There are three main types of VWD that differ according to the degree of disease severity and inheritance pattern. Types 1 and 3 are characterized by quantitative defects in the VWD protein: type 1 VWD is characterized by partial quantitative VWF deficiency, resulting in a mild to moderate bleeding phenotype, whereas type 3, the most severe form of VWD, results from near-complete absence of VWF [1].

Type 2 VWD represents a group of disease phenotypes resulting from qualitative defects in VWF, affecting formation of multimers (types 2A and 2B), platelet adhesion (type 2M), or FVIII binding (type 2N).2,4 Bleeding complications in patients with VWD vary depending on the level of residual VWF activity, disease type, age, and sex [3, 4].

Patients most often experience mucocutaneous bleeding, including epistaxis, easy bruising, and heavy menstrual bleeding as well as bleeding after surgery/trauma [4].

OBJECTIVES

1. To determine incidence of various subtypes of VWD
2. To sub classify VWD into various categories

Limitations

1. Genetic testing were not available
2. Family History of bleeding was difficult to obtain in some cases

MATERIALS AND METHOD

This is 05 years study (Feb 2016 – Jan 2021) conducted in hematology section, MMCH, KSA. Institutional review committee provided ethical approval and permission. The local policy was followed for obtaining consent

Inclusion Criteria

1. As per the Hospital policy, children upto 14 years were included
2. VWD is diagnosed based on VWF:Ag, VWF:Rcof and FVIII levels
3. Rosners Index is used to interpret Mixing studies

Exclusion Criteria

1. No Age wise categorisation
2. Hemophilia were excluded

Statistical Analysis of Data

Mean +/- SD was used to express all data. Utilizing the unpaired studentst test, statistical analysis was conducted. Statistically significant data has a p value less than 0.05.

Primary Diagnostic Tests

VWF levels rise with anxiety, needle phobia, strenuous exercise and pregnancy Tests should be performed at least twice on separate occasions before making diagnosis

APTT: Normal or prolonged

FVIII: C

- APTT-based 1-Stage Assay
- FVIII half-life regulated by VWF and so level may be low in all types
- May be normal

Plasma VWF Antigen Level

- ELISA - Rabbit anti-VWF binds to VWF in patient plasma and produces colour reaction
- Or Latex Agglutination – Latex particles with Anti-VWF bind to VWF in patient plasma and cause agglutination.
- False high results with Rheumatoid Factor

VWF Ristocetin Cofactor (Rcof) Activity

- Ristocetin binds to the VWF A1 domain causing a conformational change in VWF which aids VWF binding to platelet GP1b receptors, resulting in platelet crosslinking.
- The test reflects VWF-Gp1b binding by assessing ristocetin cofactor activity.
- It measures the agglutination of platelet in a solution containing an excess of ristocetin along with dilutions of patient plasma. The activity level is determined by comparison to a reference plasma.
- Result is also dependent on presence of HMW multimers and intact Gp1b binding sites.
- Non-physiological – some patients will test low in the absence of a bleeding phenotype.

VWF Collagen Binding (CB) Assay

- Collagen binds to the VWF A3 domain
- Successful binding is dependent on the presence of intact HMW mutimers
- Tested by chemiluminescent immunoassay
- Uses particles coated with collagen peptides.

Secondary Classification Tests

Ristocetin-Induced Platelet Agglutination (RIPA)

- In normal individuals, low concentrations of ristocetin are insufficient to initiate VWF-dependent platelet agglutination
- If agglutination does occur at low concentrations of ristocetin (0.5mg/ml) it suggests the pathological enhancement of VWF-Gp1b interactions seen in Type 2B VWD or Platelet-Type VWD.

Genetic Analysis

- Increasingly appropriate and should be considered as an aid in many circumstances

RESULTS

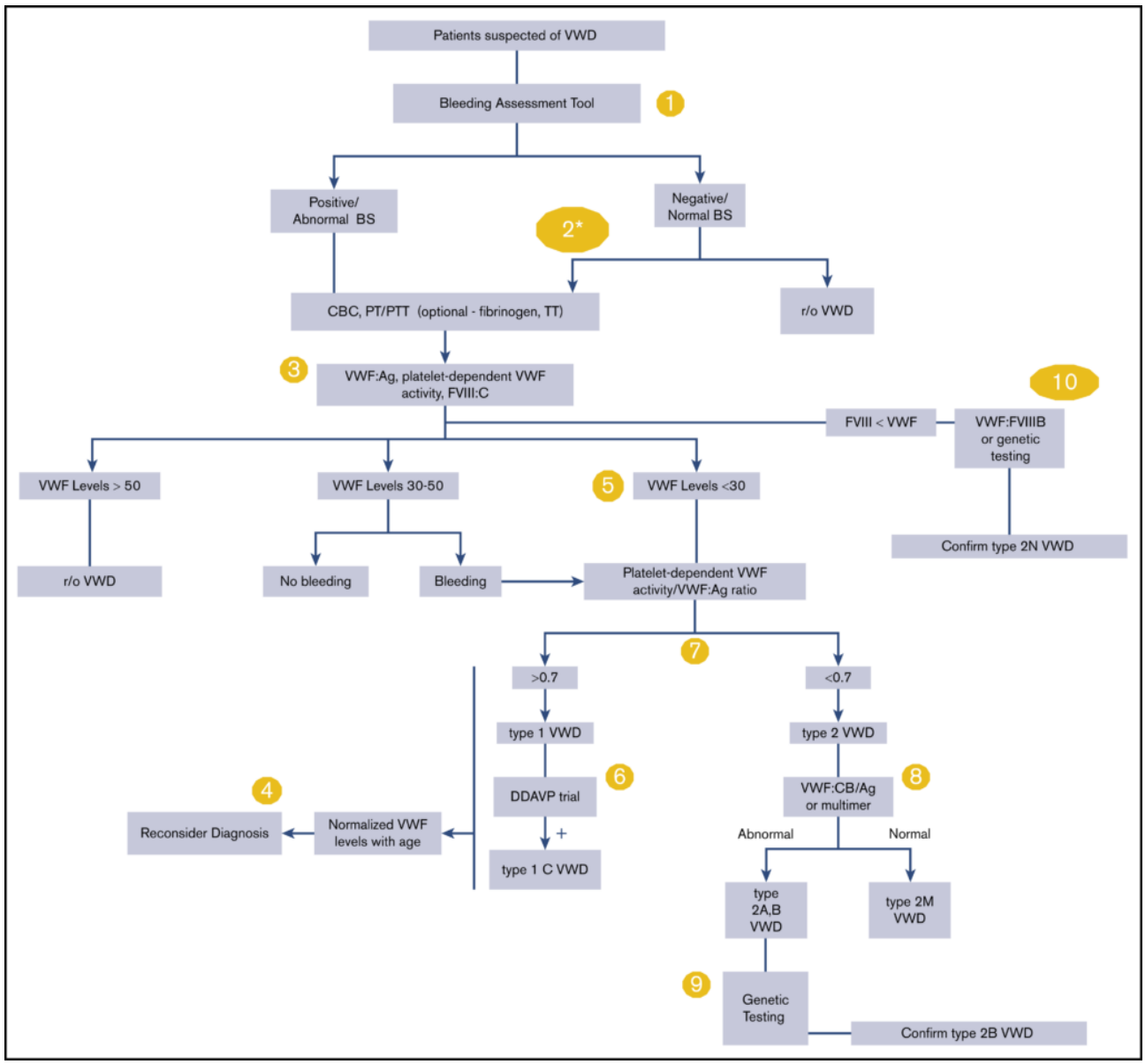
Table: Incidence of various types of VWD

APTT	Mixing test Rosners Index		VWD Type 1	VWD Type 2 (VWF:RCoF/VWF:Ag <0.7) 07 cases (23%)				VWD Type 3
	< 10	>15		Multimers		+ RIPA (Low dose) Low PLT	Isolated Low FVIII VWF:Ag Normal	
				-	+			
				2A	2M			
30	29 (96%)	1 (04%)	20 (66%)	02 (07%)	01 (04%)	03 (10%)	01(04%)	03 (10%)

In present study, Out of 30 cases of VWD, 20 cases shows correction on mixing studies (ie Rosners Index < 10) indicating factor deficiency and one case showed no correction on mixing studies indicating nonspecific inhibitor.

07 cases (23%) were classified as type 2VWD as the VWF:RCof/ VWF:Ag <0.7. of which 2 cases were Type 2A VWD (Absent multimers on Gel electrophoresis), 1 case of Type 2M VWD (normal multimers), 03 cases showed thrombocytopenia and showed Aggregation on Low dose Ristocetin (RIPA) on Light transmission Aggregometry (LTA). 20 cases of Type 1 VWD were noted comprising 66% of total cases correlating well with literature. 3 cases were of Type 3 VWD with factor level less than 01%.

ISTH Based Classification of VWD is Easy to follow and Helps in Interpretation of Borderline Results



ISTH classification of VWD

DISCUSSION

VWD is the most common inherited bleeding disorder seen in children and it affects approximately 1% of the population. It was first described in Aland Islands by Erik von Willebrand [1, 2].

Two paediatric studies, one involving the screening of 1218 healthy Italian children and another involving 600 American children, found 10 (0.82%) Italian and eight (1.3%) American children with VWD [5].

Laboratory results according to type of von Willebrand's disease

Type	Defect	Laboratory abnormality VWF:Ag	VWF:Rco	Multimers	RIPA	RIPA-LD	Factor VIII
1	Quantitative	↓	↓	↓*	↓ or normal	Absent	↓ or normal
2A	Qualitative	↓ or normal	↓↓	↓ HMW	↓↓	Absent	↓ or normal
2B	Qualitative	↓ or normal	↓	↓ HMW	normal	↑	↓ or normal
	↑ affinity for platelets						
2M	Qualitative	↓ or normal	↓↓	normal	↓	Absent	↓ or normal
2N	Qualitative	↓ or normal	↓ or normal	normal	↓ or normal	Absent	↓↓
	↓ affinity for factor VIII						
3	Quantitative	↓↓↓	↓↓↓	↓↓↓	↓↓↓	Absent	↓↓↓

**Uniform decrease in multimer pattern. ↑ Increased; ↓ Decreased. HMW High molecular weight multimers; LD Low dose; RIPA Ristocetin-induced platelet aggregation; VWF:Ag von Willebrand factor antigen; VWF:CBA von Willebrand factor collagen binding assay; VWF:Rco von Willebrand factor ristocetin cofactor. Adapted with*

The European cross-sectional study yielded a population-based estimate of 0.05 per 100,000 for type 3 VWD [5].

Country-specific prevalence estimates for VWD were reported from 17 sources: five from Europe, three from Americas, and nine from other regions. Referral-based prevalence estimates for overall VWD ranged from 4.4 to 16.5 per 100,000 in Europe, 12–16 from 0.1 to 8.8 per 100,000 in Western Pacific, and from 1.8 to 2.0 per 100,000 in Eastern Mediterranean [6, 7].

Cross sectional Study from KSA using ad hoc data collection based on questionnaire and laboratory values showed 1500 cases with suspected bleeding disorder in students aged 17-22 yrs. Case definition– Decreased VWF:Ag/Activity and FVIII [7].

Estimates by VWD type from Europe and Western Pacific reported higher prevalence estimates for type 1 disease than type 2 or type 3: 2.7–7.2 per 100,000 for type 1 VWD, 0.8–2.5 per 100,000 for type 2, and 0.1–0.3 per 100,000 for type 3. Higher prevalence estimates were also reported for adults than for children in the National Register of Congenital Coagulopathies in Italy [8, 9].

Population-based estimates, which were mostly conducted in pediatric populations, were higher than referral-based estimates, varying between 800 and 1000 per 100,000 in Europe, 1500 per 100,000 in Eastern Mediterranean, 108.9 and 1300 per 100,000 in the Americas and 2200 per 100,000 in Africa [10].

In present study, amongst total inherited bleeding disorders, Incidence of VWD is 27%, Hemophilia (50%), inherited platelet disorders (14%) and remainder are undiagnosed.

The differences between our study and other studies is due to the fact that, our hospital is tertiary care Hemophilia and thalassemia centre, so all the cases from primary health centres are referred here. Amongst VWD, Type 1 VWD is the most common subtype studied, comprising 66% of total Cases. Type 2 & 3 VWD comprised 23% and 10% of total cases showing good correlation with the literature and other studies.

In our study, mixing study was done for Raised APTT cases and interpretation was done based on Rosners index

$$\text{Rosners Index} = \frac{\text{1:1 mix APTT – Control APTT X 100}}{\text{Patient APTT}}$$

Cut offs <10 is correction ie factor deficiency, >15 is inhibitor

CONCLUSION

VWD usually presents with mild bleeding symptoms (except in type 3 VWD) the diagnosis is often delayed. Prompt diagnosis and management can help to avoid potentially life-threatening bleeding events and unnecessary exposure to blood products.

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