The Role of Collagen Binding Assay in Classification of von Willebrand Disease

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Abstract

Background	von Willebrand disease (VWD) is the most common, genetically, hereditary and clinically heterogeneous bleeding disorder caused by qualitative or quantitative defects in von Willebrand factor (VWF) that leads to imperfect VWF interaction between platelets and injured blood vessel wall which deteriorate primary hemostasis.
Objective	To assess the role of collagen binding assay in the classification of VMD.
Methods	A cross sectional study was conducted on 52 suspected known patients with VWD with no consideration to their age or gender who attended the National Center of Hematology. They were submitted to von Willebrand factor antigen (VWF:Ag), factor VIII (FVIII), ristocetin co factor assay (VWF:RCo), ristocetin induced platelet aggregation (RIPA), and collagen binding assay (VWF:CB) at the same center.
Results	The patients mean age was 14.42 ±1.64 years, median 10 years, and range of 1-40 years. More than half of cases below 10 years with M:F ratio 1:1.26. Two out of 9 cases of subtype I was diagnosed as subtype II, 4 out of 7 cases of subtype II sub classified as subtype2M and the rest 3 cases sub classified as 2A, 11 out of 36 of subtype III diagnosed as 8 cases for subtype I, and 3 cases for subtype II.
Conclusion	VWF:CB assay is an important and effective supplementary test, in addition to three test panel FVIII, VWF:Ag, VWF:RCo, and multimere analysis, for sub classification of VWD. VWF:CB assay has a role in reclassified VWD patients with highly variable clinical presentation and laboratory values. Type III VWD is most frequent diagnosed type among symptomatic patients due to high consanguinity rate which detected earlier with severe bleeding tendency.
Keywords	Von Willebrand disease, collagen binding assay, VWF:RCo.
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List of abbreviation: FVIII = Factor VIII, RIPA = Ristocetin induced platelet aggregation, VWD = von Willebrand disease, VWF = von Willebrand factor, VWF:Ag = VWF antigen, VWF:CB = VWF collagen binding assay, VWF:RCo = VWF ristocetin co factor assay

Introduction

Von Willebrand disease (VWD) is the most common, genetically and clinically heterogeneous bleeding disorder caused by qualitative or quantitative defects in VWF that leads to imperfect von Willebrand factor (VWF) interaction between platelets and injured blood vessel wall which deteriorate primary hemostasis [1,2].

VWF is produced in vascular endothelium and megakaryocytes. It acts as an adhesive protein,

which binds to several ligands that are essential for the hemostatic process. VWF stimulate platelet adhesion to the sub endothelium to make platelet aggregation support and bind to factor VIII (FVIII) to avoid its premature degradation [3,4].

Incidence of VWD in all developing countries recognized subtype III VWD as the most common subtype then subtype I and II. About 60-70% cases of subtype III VWD are associated with consanguineous marriages of parents [5].

There are three main subtypes of VWD; subtype I and III exist with quantitative VWF deficiency while subtype II presents with qualitative VWF deficiency that subdivide into four variants 2A, 2B, 2M, and 2N. The determination of the exact subtype is important in treatment and prognosis [6].

The diagnosis of VWD depend on the history of mucocutaneous bleeding, a family history of bleeding, and a laboratory evaluation, which include VWF antigen (VWF:Ag), FVIII, ristocetin co factor assay (VWF:RCo), = VWF collagen binding assay (VWF:CB) and ristocetin induced platelet aggregation (RIPA) [7].

This study obejectives was to assess the role of collagen binding assay in the classification of VMD.

Methods
Patients
A cross sectional study was conducted on 52 patients who attended the National Center of Hematology collected over a period of 10 months from December 2015 till September 2016.

The VWF:Ag, FVIII, VWF:RCo, and RIPA results were done at the National Center of Hematology for 28 newly diagnosed cases, while 24 cases obtained from patients files with no consideration to their age and gender.

The FVIII, VWF:RCo, and RIPA results were obtained from the patient files at the National Center of Hematology that diagnosed VWD by depending on VWF:RCo/Ag ratio. When VWF:RCo/Ag ratio ≥ 0.6 diagnosed as subtype I and <0.6 diagnosed as subtype II, while

subtype III exhibited markedly reduced FVIII and VWF:Ag, and absent VWF:RCo8. The VWF:CB assay and VWF Ag were done at the National Center of Hematology.

Materials
VWF:Ag and VWF:CB were assessed by Asserachrom kit (Diagnostica Stago/ France) via sandwich ELISA maneuver by Reader device, 3.5 ml of venous blood was collected from VWD patients in plastic tube containing 9:1 ratio of blood to 3.2% trisodium citrate anticoagulant. Pediatric volume of 2.5 ml in appropriate ratio provided that the blood to anticoagulant then put this sample in the centrifuge to separate the plasma and subject for the test [8].

Statistical analysis
The statistical analysis of this prospective study performed with the statistical package for social sciences (SPSS) 21.0 and Microsoft Excel 2013. Numerical data were described as mean and standard error. Analysis of variance (ANOVA) used for comparison among three groups. While, categorical data described as count and percentage, Chi-square test used to estimate the association between variables. The lower level of accepted statistical significant difference is below to 0.05.

Results
The study was performed on 52 patients with mean age of 14.42±1.64 years, median 10 years, and range of 1-40 years. More than half of cases below 10 years with M:F ratio 1:1.26. About 70% of cases were of type III VWD.

Forty five patients presented with 2nd degree consanguineous marriage of their parents. By adapting the results of FVIII, VWF:RCo, and RIPA obtained from the patients files at the National Center of Hematology, 9 cases were subtype I, 7 cases were subtype II, and 36 cases were subtype III (Table 1). After applying collagen binding assay and VWF:Ag results to those parameters, the classification of the patients as shown in (Table 2).

Table 1. VWF Ag, FVIII, VWF:RCo and PTT in VWD types at presentation

Classification at presentation	N	VWF:Ag (IU/dl)	FVIII (IU/dl)	VWF:RCo (IU/dl)	VWF:RCo/Ag (IU/dl)
Subtype I	9	26.40±4.87	51.43±11.48	25.21±3.89	2.20±1.29
Subtype II	7	21.89±4.50	44.33±7.32	6.47±0.97	0.32±0.05
Subtype III	36	6.75±1.26	16.93±5.08	0.00±0.00	0.00±0.00
P value		<0.001**	0.010*	<0.001**	<0.001**

*Statistically significant, ** High statistically significant (P<0.001)

Table 2 VWF Ag, FVIII, VWF:RCo and VWF:CB in VWD types in reviewed data

Reviewed data	N	VWF:Ag (IU/dl)	FVIII (IU/dl)	VWF:RCo (IU/dl)	VWF:RCo /Ag	VWF:CB (IU/dl)	VWF:Ag (IU/dl)	VWF:CB/ Ag
Subtype I	15	20.04 ±3.06	46.97 ±8.77	15.42 ±4.99	1.20 ±0.65	20.11 ±3.12	17.66 ±2.67	1.38 ±0.15
Subtype II	12	22.42 ±2.77	47.75 ±6.55	7.12 ±2.04	0.30 ±0.07	11.98 ±1.64	21.44 ±2.69	0.61 ±0.096
Subtype III	25	3.39 ±0.40	7.40 ±4.95	0.00 ±0.00	0.00 ±0.00	3.18 ±0.12	3.3 ±0.32	1.29 ±0.22
P value		<0.001**	<0.001**	<0.001**	0.055[NS]	<0.001**	<0.001**	0.050 [NS]

NS: Not statistically significant (p>0.05), ** High statistically significant (P<0.001)

Some cases of VWD had change in their classification by using VWF:CB assay as well as to VWF:Ag, FVIII, and VWF:RCo. Two out of 9 cases of subtype I was diagnosed as subtype II, 4 out of 7 cases of subtype II sub classified as subtype2M and the rest 3 cases subclassified as 2A, 11 out of 36 of subtype III diagnosed as 8 cases for subtype I, and 3 cases for subtype II (Table 3 and 4).

Discussion

In the current study, the patient age ranged between 1-40 years and they were grouped according to 10 years interval, more than half of cases (55.77%) were ≤ 10 years of age, and the least percentage of cases were in the third decade of life. This due to that subtype III, which characterizes by the severe bleeding tendency due to markedly reduced of VWF and FVIII levels; (<10% for both) [9,10], which was the commonest subtype.

That result was similar to a study done by Sanders et al. [11], stating that subtype III VWD will be presented early in life.

Table 3. VWF:Ag, FVIII, PTT, VWF:RCo and VWF:CB, of VWD patients who had re-classification

Changed data	N	VWF:Ag7 (IU/dl)	FVIII (IU/dl)	VWF:RCo (IU/dl)	VWF:R Co/Ag	VWF:CB (IU/dl)	VWF:Ag (IU/dl)	VWF:CB /Ag	PTT (Sec)
Subtype I	2	29.45 ±5.65	42.50 ±17.5	20.35 ±2.65	0.68 ±0.03	12.46 ±4.74	29.45 ±5.65	0.41 ±0.09	33 ±6
Subtype III	11	15.86 ±2.39	38.59 ±9.70	0.00 ±0	0.00 ±0	12.02 ±1.16	13.64 ±1.71	1.06 ±0.17	36.55 ±1.15
P value		0.388 NS	0.119 NS	0.844 NS	0.424 NS	0.438 NS	0.439 NS	0.399 NS	0.061 NS

NS: Not statistically significant (p>0.05)

Table 4 VWF:Ag, FVIII, PTT, VWF:RCo and VWF:CB, of subtype II VWD patients who had changed their sub classification

Subtype II	N	VWF:Ag (IU/dl)	FVIII (IU/dl)	VWF:RCo (IU/dl)	VWF:RCo/Ag	VWF:CB (IU/dl)	VWF:Ag (IU/dl)	VWF:CB/Ag
2M	4	20.60 ±1.98	53.0 ±7.68	6.30 ±1.04	0.31 ±0.06	18.15 ±1.64	19.53 ±2.63	0.97 ±0.15
2A	3	22.98 ±9.60	38.7 ±11.70	6.53 ±1.58	0.34 ±0.07	7.10 ±1.19	23.10 ±9.51	0.40 ±0.12
2A or 2B	5	23.54 ±4.42	49.0 ±13.70	20.35 ±2.65	0.68 ±0.3	9.96 ±1.89	21.98 ±3.91	0.44 ±0.06
P value		0.003*	0.008*	0.232 NS	0.018*	0.138 NS	0.006*	0.029*

NS: Not statistically significant (p>0.05), * statistically significant

In agreement with the present study, an Iranian [12], Turkish [13], and Indian studies [14] found that subtype III was the commonest subtype whereas a Canadian [10] and European [9,11,15] studies found that subtype III was the latest frequent subtype. This controversy result due to high rate of consanguinity in Middle East and India compared to Western countries; which has an impact mainly on subtype III, since it is inherited as autosomal recessive trait [11,14] as well as subtype III VWD has severe symptoms and required medical consultation while subtype I is subclinical case.

After collagen binding assay and VWF:Ag were applied, there was change in the classification of VWD subtypes. Two out of 9 cases of subtype I was diagnosed as subtype II, 4 out of 7 cases of subtype II sub classified as subtype2M and the rest 3 cases sub classified as 2A, 11 out of 36 of subtype III diagnosed as 8 cases for subtype I, and 3 cases for subtype II.

These results were parallel to an American [16] and Australian [17] studies, which stated that a high background diagnostic error rate was identified in laboratories that performed the standard three test panel FVIII, VWF:Ag, and VWF:RCo; and after the application of collagen binding test, there was nearly 20% error rate for type I VWD misidentified as type II VWD, nearly 30% for type II VWD misidentified as type I VWD, and an error rate of 90% in type III misidentified as type I and type II VWD [17].

During current research, it was observed that most of changed cases presented with unexpected findings regarding age of presentation, history of bleeding tendency, and FVIII at presentation.

For example, young female presented with mild bleeding tendency at 12 years old, the result of her investigation as follow; VWF:Ag was 29, FVIII was 65, and VWF:RCo was absent that diagnosed as subtype III VWD. When applied collagen binding assay the diagnosis is sub classified to type I.

Second case, female patient presented with mild bleeding tendency at 9 years old, found that VWF:Ag was 31, FVIII was 10, and VWF:RCo was absent also diagnosed as subtype III. When applied collagen binding assay the diagnosis is sub classified to type II.

Third example, one year male infant presented with severe bleeding tendency, the VWF:Ag was 35, FVIII was 45, and VWF:RCo was 23 diagnosed as subtype I. When applied collagen binding assay the diagnosis is sub classified as type II.

There are multiple studies worldwide on VWD that considered VWF:CB assay as an effective test, in addition to the three test panel FVIII, VWF:Ag, and VWF:RCo, in classification of VWD [16,18,19].

Although VWF:RCo test display poor assay reproducibility, poor low VWF level sensitivity, high relative intraassay and interassay variability, and time consuming; however, it is

widely applied for the diagnosis of VWD and sub classification particularly in the VWF dysfunction of type 2 VWD such as types 2A , 2B, and 2M [20].

As conclusions; the collagen binding assay cannot replace the ristocetin co factor assay per se but collagen binding assay should be added to the test panel to reduce the classification errors, if the results of VWF:RCo values were highly variable among different center, collagen binding should replace the VWF:RCo in order to classify VWD.

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Author contributions:

Dr. Jabbar collected the samples, organized the data, performed ELISA test and analyzed the results. Dr. Al-Mudallal helped in study design and supervising the work. Dr. Shabeeb helped in ELISA technique working. Dr. Al-Obaidy helped in the samples collection and performed VWF:RCo and RIPA tests. Dr. Al-Mamoori helped in progress of this study and manuscript organization and editing.

Conflict of interest

The authors have no conflicts of interest.

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References

1. Ng C, Motto DG, Di Paola J. Diagnostic approach to von Willebrand disease. Blood J. 2015; 125(13): 2029-38. doi: 10.1182/blood-2014-08-528398.
2. De Jong A, and Eikenboom J. Developments in the diagnostic procedures for von Willebrand disease. J Thromb Haemost. 2016; 14(3): 449-60. doi: 10.1111/jth.13243.
3. De Meyer SF, Deckmyn H, Vanhoorelbeke K. von Willebrand factor to the rescue. Blood. 2009; 113(21): 5049-57. doi: 10.1182/blood-2008-10-165621.
4. Sadler JE, Mannucci PM, Mazurier C, et al. Update on the pathophysiology and classification of von Willebrand disease: a report of the Subcommittee on von Willebrand Factor. J Thromb Haemost. 2006; 4(10): 2103-14. doi: 10.1111/j.1538-7836.2006.02146.x.
5. Ghosh K, Shetty S. Epidemiology, diagnosis, and management of von Willebrand disease in India. Semin Thromb Hemost. 2011; 37(5): 595-601. doi: 10.1055/s-0031-1281048.
6. National Heart, Lung and Blood Institute. The diagnosis, evaluation, and management of von Willebrand disease. NIC Publications, 2007. https://www.nhlbi.nih.gov/files/docs/guidelines/vwd.pdf. Accessed at 2016-7-21.
7. Lillicrap D, James P. von Willebrand disease : an introduction for the primary care physician. World Fed Hemoph. 2009; 47: 1-7.
8. Cleveland Clinic Laboratories. Collagen binding activity assay for von Willebrand disease. http://clevelandcliniclabs.com/wp-content/assets/pdfs/technical-briefs/collagen-87899.pdf. Accessed at 2016-5-3
9. Abshire TC, Federici AB, Alvárez MT, et al. Prophylaxis in severe forms of von Willebrand's disease: results from the von Willebrand Disease Prophylaxis Network (VWD PN). Haemophilia. 2013; 19(1): 76-81. doi: 10.1111/j.1365-2516.2012.02916.x.
10. James PD, Lillicrap D. von Willebrand disease: clinical and laboratory lessons learned from the large von Willebrand disease studies. Am J Hematol. 2012; 87(Suppl 1): S4-11. doi: 10.1002/ajh.23142.
11. Sanders YV, Fijnvandraat K, Boender J, et al. Bleeding spectrum in children with moderate or severe von Willebrand disease: Relevance of pediatric-specific bleeding. Am J Hematol. 2015; 90(12): 1142-8. doi: 10.1002/ajh.24195.
12. Lak M, Peyvandi F, Mannucci PM. Clinical manifestations and complications of childbirth and replacement therapy in 385 Iranian patients with type 3 von Willebrand disease. Br J Haematol. 2008; 111(4): 1236-9. doi: 10.1111/j.1365-2141.2000.02507.x.
13. Hampshire DJ, Abuzenadah AM, Cartwright A, et al. Identification and characterisation of mutations associated with von Willebrand disease in a Turkish patient cohort. Thromb Haemost. 2013; 110(2): 264-74. doi: 10.1160/TH13-02-0135.
14. Trasi S, Shetty S, Ghosh K, et al. Prevalence and spectrum of von Willebrand disease from western India. Indian J Med Res. 2005; 121(5): 653-8.
15. Sanders Y, Giezenaar M a, Laros-van Gorkom B, et al. von Willebrand disease and aging: an evolving phenotype. J Thromb Haemost. 2014; 12(7): 1066-75. doi: 10.1111/jth.12586.
16. Adcock D, Bethel M, Valcour A. Diagnosing von Willebrand Disease: A Large Reference Laboratory's Perspective. Semin Thromb Hemost. 2006; 32(5): 472-9. doi: 10.1055/s-2006-947860.
17. Favaloro E, Bonar R, Kershaw G, et al. Reducing errors in identification of von Willebrand disease: the experience of the royal college of pathologists of Australasia quality assurance program. Semin

Thromb Hemost. 2006; 32(5): 505-13. doi: 10.1055/s-2006-947865.

18. Favaloro E. Toward a new paradigm for the identification and functional characterization of von Willebrand disease. Semin Thromb Hemost. 2009; 35(1): 60-75. doi: 10.1055/s-0029-1214149.

19. Federici AB, Canciani MT, Forza I, et al. Ristocetin cofactor and collagen binding activities normalized to antigen levels for a rapid diagnosis of type 2 von Willebrand disease-single center comparison of four different assays. Thromb Haemost. 2000; 84(6): 1127-8.

20. Favaloro E. An update on the von Willebrand factor collagen binding assay: 21 Years of age and beyond adolescence but not yet a mature adult. Semin Thromb Hemost. 2007; 33(8): 727-44. doi: 10.1055/s-2007-1000364.