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Collagen binding assay in the diagnosis of von Willebrand Disease

Ammad H. Fida[1], Huma Amin[2], Shahla[3], Zafar T.[4], Asif Naveed[5], Shahida Mohsin[1]*

[1]Department of Hematology, University of Health Sciences, Lahore.
[2]Yusra Medical College, Rawalpindi.
[3]Hemophilia Center, 31 Aziz Avenue, Jail Road, Lahore.
[4]Hemophilia Center, Rawalpindi.
[5]King Edward Medical University.

Correspondence Address: *Dr. Shahida Mohsin, Department of Hematology, University of Health Sciences, Lahore.

Abstract

The accurate diagnosis and classification of von Willebrand disease (vWD) is of utmost important because it is not only determines the hemorrhagic risk, but also help in subsequent clinical management. Present laboratory test panels face limitation in diagnoses and sub classification of vWD. The aim of our study was to assess the utility of collagen binding assay for typing and subtyping of vWD along with a combination of other tests. This was a descriptive study carried out in University of Health Sciences Lahore. Sixty four already diagnosed cases of vWD, vWF: RCo were included in this study. vWF: Ag and vWF: CBA were performed by ELISA while FVIII was determined by CA-50.All patients included in this study were found to have very low levels of vWF: Ag, FVIII and CBA.vWF:Ag level was found to be <5% of normal and CBA/Ag ratio was >1 which strongly suggested the presence of Type 3 vWD in all the patients. Linear regression scatter plot between vWF: Ag and vWF: CBA showing concordant correlation. We concluded from the results of our study that all of the patients had Type 3 vWD. vWF:CBA can be useful addition in package of tests used for typing and subtyping of vWD. Large sample size studies are required to establish this as fact.

Keywords: Collagen, von Willebrand disease (vWD)

Introduction

Von Willebrand factor (vWF) is a plasma protein responsible for platelet adhesion and aggregation. It acts as a bridge between platelet receptors (GpIb, GpIIb-IIIa) and collagen at the sites of endothelial vascular injury (Ruggeri et al., 2003).vWF therefore readily participates in promotion of blood coagulation and thrombus formation. vWF has also another function of stabilization and binding of FVIII in circulation. This is done by protecting FVIII from proteolytic degradation by activated protein C (Sadler et al., 2006; Favaloro., 2006; Mazurier., 1992). Von Willebrand disease (VWD) is the most common inherited bleeding disorder and is characterized by low levels and/or abnormal function of von Willebrand factor (VWF) (Favaloro. 2009). International Society on Thrombosis and Hemostasis divided vWD in

six types i.e. Type 1, 2A, 2B, 2M, 2N and type 3 vWD (Sadler. 2006).Type 1 and type 3 represent quantitative deficiencies in vWF. (Sadleret al., 2006; Favaloro. 2006). Type 2 vWD is further subdivided into 4 subtypes (2A, 2B, 2M and 2N) Type 2A vWD defines a specific deficiency of HMW (high molecular weight) vWF. Type 2 B defines individuals with hyperadhesive vWF thus favoring thrombocytopenia. In Type 2M vWD there is defect in binding of vWF to platelet GPIb where as in Type 2N vWD there is dysfunctional binding of vWF to FVIII, so these individuals will present with relatively low level of plasma FVIII (Othman et al. 2008).

The correct determination of both concentration and function of von Willebrand Factor (VWF) is important for an accurate diagnosis and classification of von Willebrand disease (vWD). A variety of laboratory assays may be required but unfortunately no single combination of tests is comprehensive enough to detect all of the variants of von Willebrand Disease (VWD).Laboratory investigations usually includeinitial plasma testing of factor VIII coagulant(FVIII:C), VWF protein antigen (VWF:Ag), and ristocetin co-factor (VWF:RCo) assay(Favaloro. 2009).vWF: Ag tests the amount of vWF present in the plasma,ristocetin cofactor activity tests the capability of vWF to bind to platelets(Pareti et al. 1987). The VWF: RCo classically is a time-consuming assay, based onthe measurement of platelet agglutination in an aggregometer. Besides, ithas the disadvantage ofan unacceptable poor reproducibility(Caron et al. 2006).

Collagen-binding assay (VWF: CBA) is a newer test for detecting the function of VWF protein (Favaloro. 2009). This functional assay detects the property of vWF adhesion to collagen required for the discrimination between vWD type 1 and 2 (Brown et al. 1986). The ability of vWF: CBA to discriminate between functional and dysfunctional vWF is considerably better than of vWF: RCo because of less inter-assay and inter-laboratory variability (Michiels et al. 2006)It is asimple, reproducible and non-time consuming test as compared with vWF: RCo. CBA is considered superior when compared in combination of vWF: Ag and vWF: RCo in detection of HMW multimers of vWF which are absent in Type 2A and 2B. CBA and vWF: Ag (in combination) has capability to detect all forms of vWD with the exception of Type 2N and 2M (Favaloro. 2009).

In this study we have madean effort to develop a test combination (FVIII, vWF: Ag and vWF: CBA) for not only diagnosis of vWD but also for typing and subtyping of vWD.

Materials and methods

This was a descriptive study, a total of 64 already diagnosed cases of vWD were included without age and sex discrimination. Patients were diagnosed on the basis of BT, PT, APTT, FVIII levels and vWF: RCo. Detailed history was taken and diagnosis of vWD was rechecked in the Department of Hematology, before including each patient in this study.

Samples were obtained by aseptic technique. Venous blood was transferred to the B.D blue topped (3.2% sodium citrate) vacutainers. Platelet poor plasma was separated by centrifugation at room temperature for 15 min at 2500g. Plasma samples were aliquoted separately containing 0.5ml plasma in each Eppendorf tube. Plasma for FVIII, vWF: Ag and vWF: CBA were stored at-80 C and were used for assay in batches. FVIII was measured by using the CA-50 quantitatively by one stage assay. vWF: Ag, a specific test to detect the level of vWF in the plasma, was performed by using the standard ELISA (Enzyme Linked Immunosorbent Assay) technique. While vWF: CBA was measured by using

sandwich Enzyme Linked Immunosorbent Assay (ELISA).

Blood groups were also determined by using the standard test tube method by both forward and reverse grouping technique. This informationwas used to establish the relationship between different blood groups and level of vWF protein.

All the data was entered and analyzed by using SPSS 19. Mean ± standard deviation was given for quantitative variables like FVIII, vWF: Ag and vWF: CBA.

Results

Demographic data of patient included in the study is outlined in Table-1.

Mean levels of Factor VIII, vWF: Ag and vWF: CBA in all sixty four patients are summarized in Table-2. The level of FVIII was found to be very low as compared to the reference range (50-150%). vWF: Ag and vWF: CBA measured by ELISA technique showed very low levels in both male and female patients (Table-2). All patients in our study group belonged to the type 3 vWD. Type 3 vWD is represented by the complete absence or presence of vWF in negligible amount.

Very low to absent values of vWF antigen in all the patients were found in relation to very low level of FVIII. A linear regression scatter plot between FVIII and vWF: Ag showed that level of FVIII decreases indirect relation with vWF: Ag. The correlation between FVIII and vWF: Ag as found to be significant at P= 0.01 (Fig. 1).

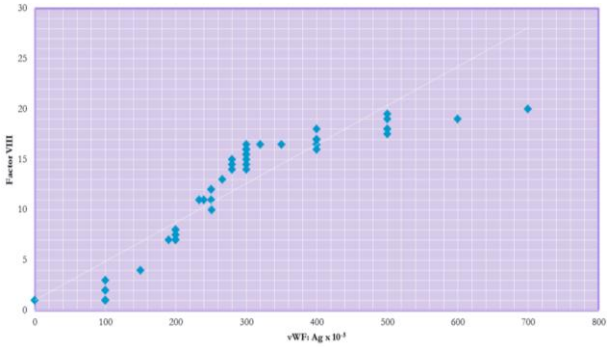

Figure 1: Linear regression scatter plot of FVIII levels and vWF: Ag concentration in 64 vWD patients after x 10^5 transformation.

Table 1: demographic data of patients.

Variable	
Total number of patients (n)	64
Number of male patients	32
Number of female patients	32
Male:female ratio	1:1
Age range of patients (years)	1-58
Mean age (years)	14.46

Table 2: Mean levels of factor VIII, VWF: AG and VWF: CBA.

Study variables	Sex of patient	No. of patients	Mean
Factor VIII (%)	Male	32	3.8 + 2.64
	Female	32	4.29 + 2.81
vWF: Ag (U/ml)	Male	32	0.028 + 0.011
	Female	32	0.03 + 0.014
vWF:CBA(U/ml)	Male	32	14.91 ± 1.63
	Female	32	15.16 ± 1.819

Table 3: relation of vWF Ag, functions and their ratios with different blood groups.

Blood group	% of Total	vWF : CBA	vWF : Ag	vWFCBA : vWFAg
A	23.43	15.36±1.48	0.0036±0.0014	>1
B	15.62	15.96±1.14	0.0040±0.00014	>1
O	54.68	14.86±2.24	0.0025±0.0011	>1
AB	6.25	14.95±1.51	0.0031±0.009	>1

Similarly linear regression scatter plot was also obtained between vWF: Ag and vWF: CBA to determine the correlation between these two variables (Figure 2).

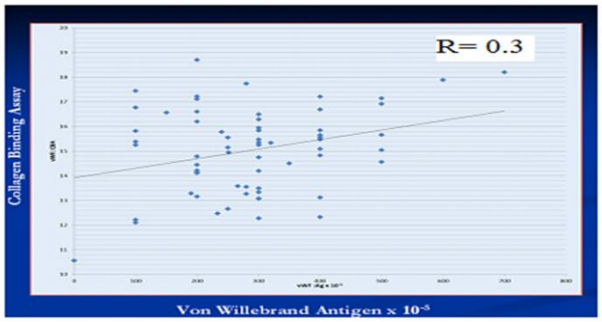

Figure 2: Linear regression scatter plot between vWF: Ag and vWF: CBA showing concordant correlation.

Relation of different blood groups with vWF is given in Table-3. Blood group O was found to be the most common group in our study patients followed by blood group A and blood group B. Patients with blood group O had lowest levels of VWF Ag as compared to other blood groups. The ratio between vWF: CBA/ vWF: Ag was found to be far more greater than 1 (Table-3).

Discussion

Differential diagnosis of vWD is crucial because treatment varies with subtypes. To avoid misdiagnosis in identifying the most frequent quantitative and qualitative VWD variants, a combination of standard and vWF specific coagulation assays is usually required including FVIII: C, vWF: Ag, vWF: RCo, RIPA, vWF: CBA and vWF multimeric assays (Favaloro et al. 2006). vWF:CBA appeared not only to be as sensitive as VWF:RCo, but also more capable of identifying the decrease/absence in large and intermediate VWF multimers displayed by type 2A and 2B vWD (Casonato et al. 2001).

In this study we aimed to investigate whether VWF: CBA improve the diagnosis of VWD.A panel of tests, including vWF: Ag, Factor VIII and vWF: CBA and vWD was used for diagnosis. Besides, an attempt was also made to subtype the disease.

In our study results mean level of vWF: Ag in male and female patients was found to be very low (0.028U/ml and 0.03U/ml) respectively. The lowest level of vWF: Ag which was detected in this study was 0.00001U/ml i.e. up to five decimal figures. Such low levels of vWF: Ag was considered to be undetectable. Previously it was documented that virtual absence of vWF in plasma favors Type 3 vWD, however in practice to diagnose Type 3 vWD measured levels of plasma vWF should be less than 5% of normal (Favaloro. 2009).

Low level of FVIII in accordance with low vWF:Ag level has also been found in another study (Favaloro. 2008). The levels of FVIII were found to be directly proportional with vWF levels .i.e. very low and a significant correlation was found. Mean levels of FVIII in male and female patients were calculated as 3.8 ± 2.64 and 4.29 ±2.81 respectively. Various studies have showed that <5% of normal level of FVIII was present in most severe form of vWD i.e. Type 3 vWD (Goodman. 2001).

This finding of high prevalence of type 3 vWD in our study group is also supported by previous local studies. Shahbaz et al. conducted a study on population of Northern Pakistan and found Type 3vWD to be most

frequently occurring type of vWD amongst the population selected by them (Shahbaz et al., 2008).The diagnosis of higher number of patients of Type 3 vWD could be due to the fact that these patients were most severely affected and symptomatic while on the other hand patients with Type 1 vWD had milder symptoms so as a result lesser number of patients seek medical advice and remain undiagnosed throughout their lives. The high incidence of Type 3 vWD may also be due to the high consanguinity rate in Pakistani population. Consanguinity and family marriages among the parents were considered to be the important aspect of the disease (Mohsin et al., 2012). Shahbaz et al. in Iran also reported that most of the patients were off springs of consanguineous marriages. A study conducted in northern Pakistan showed consanguinity in 40.6% of cases (Shahbaz et al., 2008).

To detect the capability of vWF to bind to collagen, CBA was performed. This is a sensitive and relatively newer test. Our results showed that the level of vWF: CBA has also been markedly reduced. Mean level of CBA in male and female patients was calculated as 14.91± 1.63U/ml and 15.16± 1.81 U/ml respectively in our study with reference range of CBA in general population of 50-194 U/dl.

Normally there is close correlation between plasma concentration of vWF: Ag and the protein's functional ability as assessed by its collagen binding activity (vWF: CBA) (Favaloro. 2006). A ratio of 1.00 indicates perfect correlation between quantity and functional activity and an abnormal high discrepancy in this ratio suggests a dysfunctional vWF profile (Saddler et al., 2006). Ratio between vWF: CBA and vWF: Ag was calculated and was found to be far greater than 1 showing that there is very minimal amount of vWF:Ag present that too had very limited collagen binding activity.

Low to undetectable level of vWF: Ag, FVIII and vWF: CBA in our results favor diagnosis of type 3 vWD in all the patients in the present study. This puts a limitation to observe the utility of vWF:CBA in improving the diagnosis of vWD at the phenotypic level and subtyping vWD in this study. Therefore the suggestion of its inclusion in the group of first-choice tests together with vWF:Ag, with or without vWF:RCo could not be established.

We conclude that all of the patients included in our study had Type 3 vWD. There was not a single patient with Type 1 or 2 vWD. This limits the usage of ratio between FVIII, vWF: Ag and vWF: CBA in our study for sub typing of Type 2 vWD. Furthermore, more studies with large sample size are required.

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