A PILOT STUDY TO DETECT VON WILLEBRAND DISEASE IN WOMEN WITH MENORRHAGIA

By

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LIST OF ABBREVIATIONS

ADP	Adenosine diphosphate
APTT	Activated partial thromboplastin time
AvWS	Acquired von Willebrand Syndrome
BT	Bleeding time
CT	Closure Time
DDAVP	Desmopressin
DUB	Dysfunctional uterine bleeding
EDI	Electro-Immunodiffusion
EDTA	Ethylenediamine Tetra-Acetic Acid
ELISA	Ezyme Link Immunosorbant Assay
FBC	Full Blood Count
FVIII:C	Factor VIII
Hb	Haemoglobin
HES	Hydroxyethyl starch
HMWK	High molecular weight kalleikrein
HRP	Horseradish peroxidase
HRT	Hormone Replacement Therapy
HUSM	Hospital Universiti Sains Malaysia
INR	International Ratio
IQR	Inter Quarter Range
KCCT	Koalin Cephalin Clotting Time

LIA	Latex Immunoassay
LRP	Low density lipoprotein receptor related protein
MBL	Menstrual blood loss
MGUS	Monoclonal gammopathy of undertermined significance
PBAC	Pictorial Blood Assessment Chart
Plt	Platelet
PPP	Platelet Poor Plasma
PRP	Platelet Rich Plasma
PT	Prothrombin Time
PTTK	Thromboplastin Time Using Koalin
RIPA	Ristocetin Induce Platelet Aggregation
SBT	Skin Bleeding Time
SD	Standard Deviation
TXA_2	Tromboxane A_2
vWD	von Willebrand's disease
vWF	von Willebrand factor
vWF: Ac	von Willebrand Factor Activity
vWF: Ag	von Willebrand Factor Antigen
vWF:CBA	Collagen Binding Assay
vWF-CP	vWF- cleaving protease
vWF: RCof	vWF Ristocetin Cofactor Assay

ABSTRAK

Penyakit Von Willebrand's adalah penyakit keturunan yang melibatkan masalah dengan pembekuan darah. Penyakit ini didapati sebanyak 1% di dalam populasi keseluruhan. Penyakit von Willebrand ini adalah disebabkan kekurangan atau keabnormalan fungsi von Willebrand faktor yang akan menyebabkan proses pembekuan darah terganggu.
Haid berpanjangan dan berlebihan(menoragia) bermaksud pendarahan melebihi 80ml dan melebihi 7 hari dan ia merupakan masalah utama perempuan di dalam perigkat umur reproduktif. Ia merupakan tanda atau gejala bagi wanita yang mempunyai masalah dengan pembekuan darah. Maka, kekerapan penyakit ini serta jenis-jenis vWD ini dikaji di kalangan wanita ini.

Tiga puluh orang wanita yang datang ke klinik sakit puan, HUSM dengan masalah pendarahan tanpa keabnormalan pada sistem reproduktif di pilih. Sejarah berkaitan dengan masalah pendarahan ditanya dengan terperinci. Ujian Full blood count, prothrombin time (PT), activated partial prothrombin time (APTT), ABO blood grouping, Faktor VIII (FVIII:C), von Wilebrand faktor antigen (vWF: Ag), von Willebrand faktor aktiviti (vWF: Ac) dan collagen binding assay (vWF: CBA) dijalankan. Kemudian ujian ristocetin induced platelet aggregation (RIPA) dijalankan kepada mereka yang mempunyai keputusan ujian von Willebrand yang abnormal.

Daripada 30 orang pesakit, 97% adalah Melayu dengan median umur 42 tahun. Empat (13.3%) daripada pesakit mempunyai keputusan ujian von Willebrand yang abnormal. Dua daripadanya di sahkan sebagai vWD Type 1, seorang sebagai kekurangan von

Willebrand berkaitan dengan kumpulan darah O dan pesakit yang terahkir disahkan menghidap vWD Type 2 samada Type 2A atau 2M. Kajian ini mendapati tiada perkaitan di antara umur, permulaan pendarahan, tempoh pendarahan, sejarah transfusi darah atau tanda pendarahan yang lain dengan penyakit von Willebrand.

Hasil daripada keputusan ujian von Willebrand pada 4 pesakit, functional assay von Willebrand (vWF: Ac dan vWF: CBA) mempunyai nilai signifikan yang rendah berbanding dengan vWF: Ag dan FVIII:C. Kesan rawatan dengan hormon dan kumpulan darah ke atas ujian von Willebrand tidak menunjukkan kesan yang signifkan.

Kesimpulannya, ini adalah kajian yang pertama mengenai penyakit von Willebrand di kalangan wanita Malaysia yang mempunyai masalah pendarahan tanpa keabnormalan pada sistem reproductive organ. Insiden penyakit von Wllebrand adalah tinggi. Memandangkan ujian sarigan tidak dapat membantu , kami ingin mencadangkan bahawa ujian von Willebrand hanya dijalankan kepada pesakit sebelum sebarang prosedur yang invasive dilakukan.

ABSTRACT

Von Willebrand disease (vWD) is the most common inherited bleeding disorders, found in approximately 1% of the general population, without ethnic differences. vWD results from a qualitative or quantitative defect in von Willebrand factor (vWF) resulting in impaired primary homeostasis. Menorrhagia is defined objectively as > 80ml menstrual blood loss per cycle or menses lasting longer than 7 days and is a common gynecologic problem in women of reproductive age. However, in 50% of cases, no pathology is detectable. Menorrhagia is valuable predictors of bleeding disorder in women. The frequency of vWD in women with menorrhagia ranges from 5-50% in different studies. The aim of this study is to know the prevalence of vWD in women with menorrhagia of unknown cause and identify the subtypes of the disease.

Thirty women who came to Obstetric and Gynecology Clinic, HUSM with menorrhagia without uterine pathology was selected for this study. A detailed history related to menorrhagia was acquired from them. Full blood count (FBC), prothrombin time (PT), activated partial prothrombin time (APTT), ABO blood grouping, factor VIII activity (FVIII: C), von Willebrand factor antigen (vWF: Ag) and von Willebrand factor activity (vWF: Ac) and collagen binding assays (vWF: CBA) were measured in all patients. Subsequently ristocetin induced platelet aggregation (RIPA) was performed for those who had abnormal von Willebrand studies.

Out of 30, 97% were Malay with median age of 42 years old. Four (13.3%) patients have abnormal parameters of von Willebrand studies. Two were diagnosed as 'possible' vWD

Type 1, one patient as von Willebrand deficiency related to blood group O and the other one as 'possible' vWD Type 2 either subtype 2A or 2M. There is no association between age, onset of menorrhagia, duration of menstruation, history of blood transfusion or other bleeding tendencies with the development of the disease.

Based on von Willebrand studies, von Willebrand functional assays (vWF: Ac and vWF: CBA) was significantly lower than vWF: Ag and FVIII: C in this 4 patients. Effect of hormone replacement therapy and blood group were analyzed and both did not show statistically significant in contributing to von Willebrand profiles.

In conclusion, to date this is the first reported cases of vWD among Malaysian women with menorrhagia without uterine pathology. vWD is highly prevalence and though coagulation screening test is not helpful, we would like to suggest that a von Willebrand studies is directed to patient at least before planning for an invasive procedure.

CHAPTER 1

GENERAL INTRODUCTION

1.0 GENERAL INTRODUCTION

Von Willebrand's disease (vWD) is now recognized to be the most common inherited bleeding disorder, found in approximately 1% of general population (Werner *et al.*, 1993). Individuals with vWD have defects in, or reduced levels of von Willebrand factor (vWF), an adhesive plasma protein essential for primary homeostasis. It is generally an autosomal dominant and heterogenous disorder with variable clinical expression as a result patients are typed according to pathophysiology. vWD is very heterogenous, and may be defined by a reduction or absence of high molecular weight (HMW) vWF, by a reduction or absence of all forms of vWF, or by the loss of a specific vWF function (Sadler *et al.*, 2000, 1994; Laffan *et al.*, 2004; Favaloro *et al.*, 2001, 1999). There are three major types: Type 1 is due to a partial quantitative deficiency of a normal vWF and accounts for 70-80% of all vWD; Type 2 (20% of vWD) includes several qualitative defects in vWF and patient with Type 3 vWD (5-10%) is due to complete deficiency of vWF and a secondary severe deficiency of FVIII. This condition is associated with mucocutaneous bleeding and they may present with echymoses, epistaxis, menorrhagia or bleeding following trauma or at the time of surgery.

The clinical aspects of vWD in females can be pronounced because of the additional challenges to the haemostatic system from monthly menstruation and from childbirth. Menorrhagia is defined objectively as > 80ml menstrual blood loss (MBL) (Rees *et al.*, 1987). Although menorrhagia is a common gynecological symptom, a specific cause is identified in less than 50% of affected women. However another 50% of cases, no

pathology is detectable and such women are diagnosed as having dysfunctional uterine bleeding (DUB) (Rees *et al.*, 1987).

Recently, there has been growing recognition that vWD is not uncommon in women with menorrhagia, with prevalence estimates of 5-20% (Jody *et al.*, 2005), compared with less than 1% in women without menorrhagia (Kouides *et al.*, 2002). Despite the high prevalence of VWD in the general population, women who are referred for investigation of menorrhagia are not routinely screened for this coagulation disorder.

This study was carried out to assess the frequency of vWD among women presenting with menorrhagia in gynecology clinic at Hospital University Sains Malaysia (HUSM). We also assessed whether information from the patient's history was a predictive of this disorder. Standard panels of coagulation tests to identify and subtype vWD were used.

CHAPTER 2

LITERATURE REVIEW

2.0 LITERATURE REVIEW

2.1 INTRODUCTION ON VON WILLEBRAND DISEASE

2.1.1 Definition

vWD is a disease of bleeding disorder due to deficiency of vWF functional activity. The abnormality may be quantitative and/or qualitative.

2.1.2 Background

vWD is first described by Dr. Erik von Willebrand in 1962 after studying a large family Foglo on the island of Aaland in the Gulf of Bothnia, the coast of Sweden. vWD is now recognized to be the most common inherited bleeding disorder, manifesting as an increased tendency for spontaneous or trauma related bleeding in 1-3% of the general population (Murray *et al.*, 1996).

2.1.3 Biosynthesis and structure of vWF

vWF is a multifunctional plasma protein that plays a prominent role in the events that lead to the normal arrest of bleeding. vWF is produced by endothelial cells and either stored in intracellular organelles known as Weibel-Palade bodies or secreted constitutively by megakaryocytes and stored in platelet alpha-granules (Ruggeri *et al.*, 2001). The gene coding for vWF is 178 000 bases long, is located on chromosome 12 and contains 52 exons. vWF is originated from the vWF precursor (pro-vWF) and synthesized as a large precursor protein (360 kDa, 2813 amino acids), which consists of a 22-amino acid signal peptide, a pro-polypeptide (100 kDa, 741 amino acids) also known as vWF antigen II, and a mature subunit (270 kDa, 2050 amino acids). The largest proportion of the vWF species is propeptide vWF and in immature form, is secreted

through the constitutive pathway. The remainder fully processed and functional vWF is stored in the Weibel-Palade bodies and secreted via the regulated pathway. The two polypeptides have distinct biological functions. About 1% of plasma vWF contains the pro-peptide, possibly due to incomplete processing. Mature vWF both mediates the adhesion of blood platelets at sites of vascular injury and functions as a stabilizing carrier protein of factor VIII, an essential co-factor in the coagulation system. In addition, vWF may help to anchor endothelial cells to the extracellular matrix. The pro-peptide of vWF is required for the proper post-translational multimerization and subsequent storage of vWF. Dimers are formed in the endoplasmatic reticulum by covalent dimerization of the subunits at their C-termini. Multimers are formed in the Golgi apparatus and the secretory vesicles by covalent multimerization of these dimers at the N-terminus (Figure 2.1).

The pro-peptide of vWF is required for normal multimer formation and is cleaved off by furin, a dibasic paired amino acid-cleaving enzyme. vWF is released from endothelial cells as ultra large multimers and circulates in the plasma as a series of multimers of very high molecular weight (500–20 000 kDa). Proteolysis by the specific metalloproteinase, ADAMST13 is involved in the break down into the smaller multimers, and shear stress enhances the susceptibility to proteolytic cleavage. Recently, it was shown that human plasma contains a vWF-degrading enzyme, vWF-cleaving protease (vWF-CP) and that the cleavage site of this enzyme is located between amino acid residues 842Thr and 843Met in the A2 domain of the vWF subunit. vWF-CP cleaves ultra large multimers, normally stored in Weibel–Palade bodies of vascular endothelium, from which they are secreted luminally into plasma and abluminally into the subendothelium. Each vWF subunit shows a characteristic pattern of homologous A, B, C and D domains, which are

independent building blocks in many other proteins. The pro-peptide contains a D1 and D2 domain. The mature subunit consists of D'–D3–A1–A2–A3–D4–B1–B2–B3–C1–C2 domains, and a C-terminal part of 151 amino acids that has no internal homology (Figure 2.1). The average plasma concentration is 10 μg/mL; the average concentration in platelets is 280 ng/10^9 platelets (Rohdeghiero *et al.,* 1992). The normal range of plasma vWF, however is broad and strongly influenced by the blood group with 25% lower level in blood group O and higher level in persons with the AA,BB, or AB loci (Sarode *et al.,* 2000). The deficiency of vWF associated with blood Type O is not caused by a mutation in the vWF gene per se, instead, clearance of vWF protein may be enhanced in people with this specific blood type. The mechanism by which this clearance occurs in humans is not yet characterized fully (Julie *et al.,* 2001). Platelet vWF is independent of the ABO blood type.

The levels of vWF are variable which are affected by a variety of genetic, physiologic and pharmacologic factors including age, blood group and hormonal status. vWF and FVIII levels are increased by age, strenuous exercise, inflammation(vWF is an acute phase reactant), hyperthyroidism and high levels of estrogen during pregnancy or use of oral contraceptive. Enhance levels of vWF are associated with a risk for thrombotic complications (Philipp *et al.,* 2003).

The variation in vWF levels during the normal menstrual cycle is not well defined, with conflicting results in both longitudinal and cross sectional study (Miller *et al.,* 2002). In studies showing a cyclic variation, the lowest vWF and FVIII levels occurred at variable

times during the first half of the cycle, with the lowest vWF antigens and activity levels during the early follicular phase (day 9 and 10)(Kadir *et al.*, 1999).

2.1.4 Functions of vWF

After its release from the endothelium and platelets, vWF serves as two essential biological functions: mediates the adhesion and aggregation of platelets at sites of vascular injury and it modulates the survival and function of factor VIII. In addition, proper haemodynamic conditions (a high shear rate) and highly polymerized vWF molecules are required to achieve efficient platelet adhesion and aggregation.

2.1.4.1 Factor VIII binding

vWF circulates in plasma with factor VIII as a non-covalent complex. These interactions extend the survival of factor VIII in the circulation. Any change in plasma vWF level is coupled with a concordant change in the plasma concentration of factor VIII. Low vWF levels, as in vWD, are also associated with low plasma factor VIII levels. Similarly, qualitative vWF defects, as in vWD Type 2N, may abolish proper factor VIII-vWF interaction, as a consequence decreasing the plasma factor VIII level. However the plasma vWF level is not influenced by the factor VIII level.

The N terminal D' domain of vWF comprises the primary Factor VIII binding site (Lenting *et al.*, 1998). Only in its dimeric form in this Cys-rich domain able to bind factor VIII, indicating a complex mode of protein-protein interaction. vWF not only binds to factor VIII and thereby controls its biological survival, but also protects factor VIII from premature proteolytic cleavages and inactivation. Each vWF monomer is able to bind one

factor VIII molecule. In vivo, however, not all vWF monomers are bound to factor VIII. Relatively small proportions (1-2%) of the available vWF monomers are occupied by the factor VIII (Vlot *et al.*, 1998).These complexes are relatively stable. Upon triggering of the coagulation system, one of the vWF-binding sites on the factor VIII molecule is cleaved off, notably by thrombin, resulting in the dissociation of the complex and the subsequent conversion of factor VIII into its active configuration (factor VIIIa) (Saenko *et al.*, 1997).

The vWF modulates the function of factor VIII at various levels. It inhibits the interaction between factor VIII and different proteases of the coagulation system, including factor IX, Factor X, and protein C, as well as negatively charged phospholipids membranes, thereby preventing the premature activation of the coagulation system. These interactions are likely to play a significant role in controlling the haemostatic response to vascular injury.

Recent reports have shown that vWF might also control the biological half-life of factor VIII by controlling its interaction with low-density lipoprotein receptor-related protein (LRP)(Schwarz *et al.*, 2000). LRP is a multifunctional scavenger receptor involved in the clearance of a spectrum of ligands, including proteases, protease-inhibitor complexes, lipases and apolipoproteins from the circulation. LRP also binds factor VIII and thereby mediates its cellular internalization and degradation. These processes are inhibited by vWF. vWF may also control factor VIII clearance by a mechanism independent of the premature proteolytic breakdown of factor VIII.

2.1.4. 2 Platelet thrombus formation

At intermediate and higher rates of shear, vWF undergoes a conformational change from a closed structure to an extended form that can reach several microns in length and which has multiple platelet and collagen binding sites. In this extended form, vWF is able to bind platelets through the GPIb-IX-V complex and thereby support thrombus formation, even at very low rates of shear when immobilized on the surface. This extended form of vWF can also be induced using the antibiotic ristocetin or snake venom toxin botrocetin (Hoffbrand *et al.*, 2006).

vWF's role in this process is dependent on the fast on rate of association to the GPIbα subunit in the GPIb-IX-V complex. The stable recruitment of platelets into the thrombus requires interaction with other platelet agonists or very high rates of shear to activate platelet integrins. GPIb-IX-V binds to a number of other ligands. GPIb-IX-V binds to the integrin $\alpha_M\beta_2$ on the surface of leucocytes and this interaction along with binding of platelet P-selectin to PSGL-1, is implicated in the attachment and transmigration of leucocytes through a mural thrombus. GPIb-IX-V also binds to P-selectin and this has been shown to support rolling of platelets with activated endothelium in low pressure vessels.

Collagen activates platelet via GPVI leading to an increase in affinity of the integrins αIIbβ3 and α2β1 for vWF/fibrinogen and collagen respectively. These mediate stable adhesion and potentiate activation through further activation of GPVI and also release of ADP and TxA2. vWF and fibrinogen, in combination with ADP, TxA2 and thrombin,

mediate thrombus formation (aggregation) and stabilization (clot retraction) (Hoffbrand *et al.*, 2006).

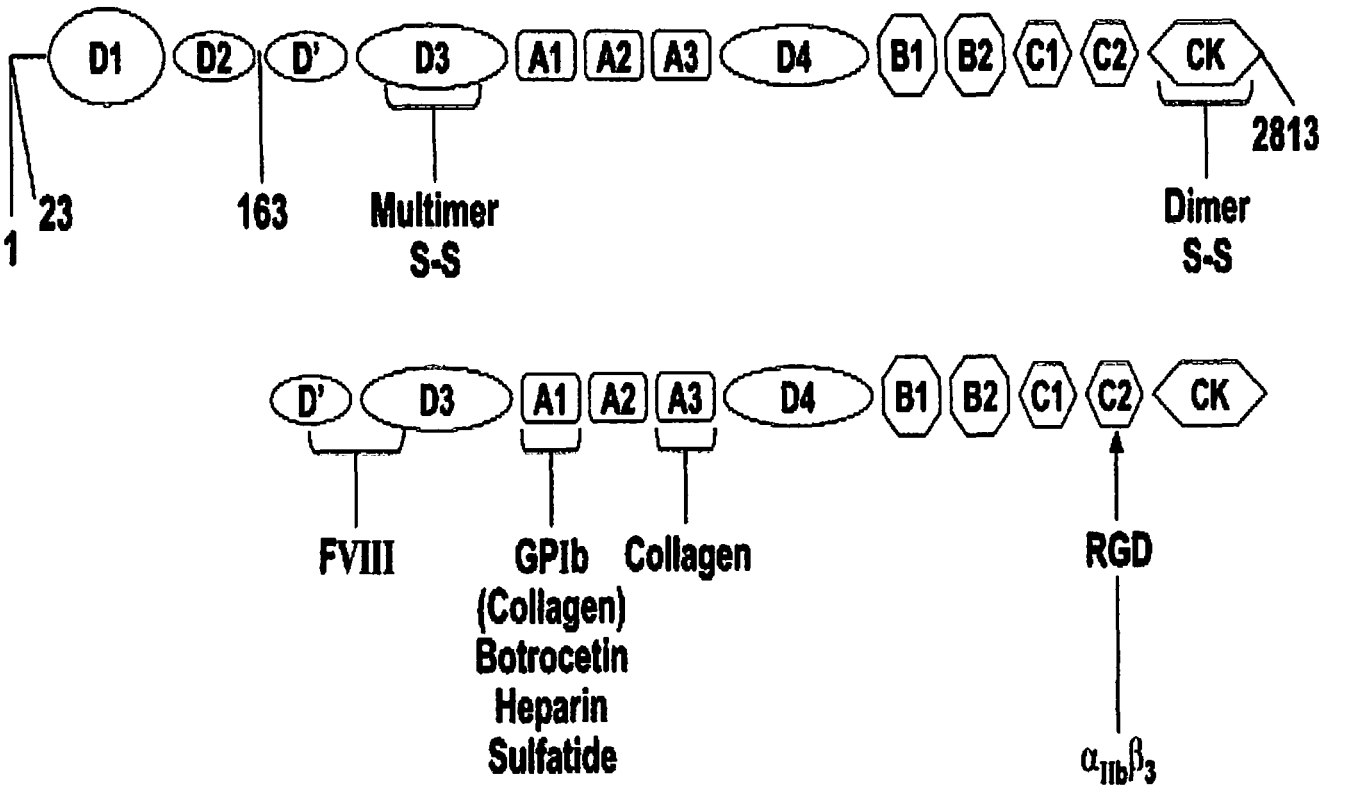

Figure 2.1 Domain structure and processing of vWF. The domain structure (A-D) of prepro vWF and mature are shown (Adapted from Vlot *et al.*, 1998).

2.1.5 Prevalence

vWD is the most frequent inherited bleeding disorder. Nilsson *et al* (1984), reported a frequency of approximately 125 cases per million in Sweden, twice as frequent as for haemophilia. Rodeghiero *et a,l (*1987), during a large Italian epidemiological study in children, found the prevalence to be 0.82%. More recent studies in different populations among American adults confirm a prevalence of the disease of approximately 1-2% (Budde *et al.*, 2001). Among the different vWD types, Type 1 is the most frequent (60–80%); all Type 2 vWD variants are 15–30%, while Type 3 is diagnosed in 5–10% of vWD patients (Awidi *et al.*, 1992).

2.1.5.1 Pathophysiology and classification

Inherited vWD has been subdivided into three types, which reflect its pathophysiology. Type 1 and 3 vWD reflect, respectively, the partial or virtually complete deficiency of vWF while Type 2 vWD reflects a qualitative deficiency of vWF. The revised classification introduced in 1994 by Sadler et al, recommends that Type 2 vWD is subdivided into four subtypes (2A, 2B, 2M, 2N) according to specific details of the phenotypic features (Table 2.1).

2.1.6.1 Type 1

Type 1 is the most frequent form of vWD, inherited as an autosomal dominant trait in most cases; recessive transmission of Type 1 vWD defects has also been shown (Eikemboom *et al.*, 1998). Patients with Type 1 vWD are characterized by mild to

moderate bleeding symptoms, normal or variably prolonged BT and low levels of vWF:Ag, vWF:RCo and FVIII and normal multimeric structure.

Definite diagnosis requires documentation of all three factors: inheritance, a history of bleeding, and low levels of normal vWF. Limitations of these criteria are the broad normal range for vWF levels and the variation of these levels over time (Blomback *et al.*, 1992).

Patients with Type 1 vWD are very heterogeneous. Type 1 vWD may be classified in three subtypes: Type 1 'platelet normal', with a normal content of functionally normal vWF; Type 1 'platelet low', with low concentration of functionally normal vWF; and Type 1 'platelet discordant', with normal concentrations of dysfunctional vWF (Mannucci *et al.*, 1985). Patients with low levels of platelet vWF have more prolonged BT and usually more severe symptoms than those with normal platelet vWF, because they have also low levels of vWF stored in endothelial cells (Fedrrici *et al.*, 1993).

The diagnosis of Type 1 vWD may also be complicated by several factors. ABO blood groups modify vWF levels in plasma (Gill *et al.*, 1987). A dominant Type 1 vWD has been identified with mutated cysteine residues on the D3 domain of vWF (Eikemboom *et al.*, 1996).

Table 2.1 Classification of vWD

Type 1 vWD refers to partial quantitative deficiency of vWF
Type 3 vWD refers to virtually complete deficiency of vWF
Type 2 vWD refers to qualitative deficiency of vWF
Type 2A refers to qualitative variants with decreased platelet-dependent function that is associated with the absence of high-molecular-weight multimers (HMW)
Type 2B vWD refers to qualitative variants with increased affinity for platelet GPIb leading to loss of HMW vWF multimers in plasma.
Type 2M vWD refers to qualitative variants with decreased platelet-dependent function not caused by the absence of HMW vWF multimers
Type 2N vWD refers to qualitative variants with markedly decreased affinity for factor VIII
From an updated version by Sadler et al, 1994

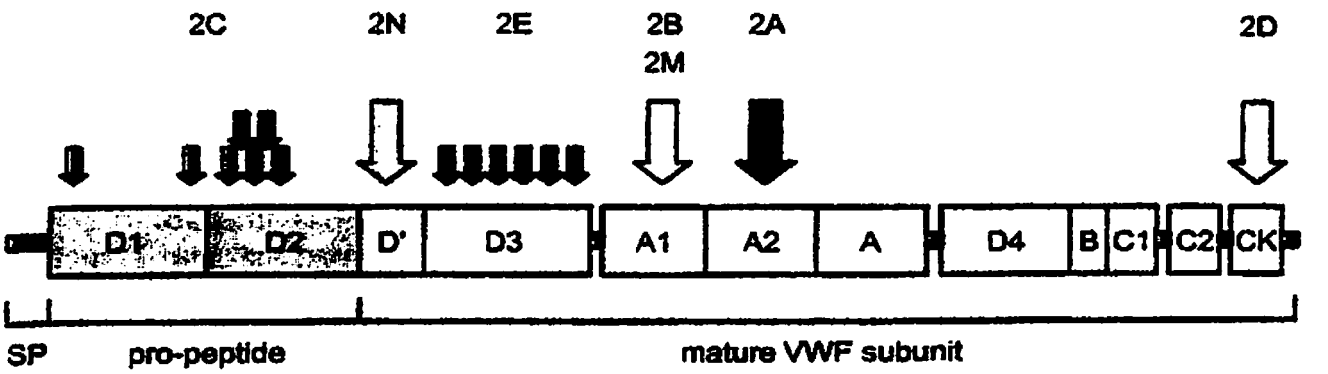

Figure 2.2 Clustered distribution of vWF mutations causing specific subtypes of vWD Type 2.

2.1.6.2.1 Type 2

In type 2 vWD, there is qualitative abnormality of vWF (Sadler *et al.*, 1994). Patients may be having normal levels of vWF protein, but the protein is dysfunctional. These variant accounts for 15-30% of cases There are four major subtypes with distribution estimated as 30% Type 2A, 28% Type 2B, 34% Type 2N, and 8% Type 2M (Sadler *et al.*, 2000).

Type 2A is the most frequent subtype among Type 2 vWD (Ruggeri *et al.*, 1980). It is inherited mainly with an autosomal dominant pattern but a recessive pattern is also described (Askura *et al.*, 1997). Patients with Type 2A vWD are identified by normal to slightly reduced vWF:Ag levels and markedly low vWF: RCo, with an abnormal multimeric pattern characterized by loss of the high molecular weight multimers and increase of the intensity of low molecular weight multimers. Type 2A vWD are due to specific mutations located within the A2 domain of vWF subunit, and data obtained by expression studies show that two mechanisms are responsible for this defect (Meyer *et al.*, 1997)(Figure 2.2). One class of mutations, referred to as group 1, causes defective intracellular transport of vWF and impairs the assembly, storage and secretion of large vWF multimers in both plasma and platelets. Group 2 mutations do not interfere with vWF assembly or secretion, but render the multimers more sensitive to proteolysis in plasma (Lyons *et al.*, 1992).

Another cause of high molecular weight deficiency in Type 2A vWD is a defective post-translational processing that includes defects of dimerization at the vWF C-terminus in the subtype previously described as type IID (Enayat *et al.*, 2001), and defects of further

polymerization of vWF dimers to multimers in their N-termini. The latter multimeriztion defects can result either from mutations in the D1 and D2 domains of the vWF propeptide, which is necessary to catalyse intermolecular disulphide binding at the D3 domain of mature vWF in the subtype previously indicated as IIC, or from mutations in the D3 domain itself in the subtypes indicated as IIE and IIF as well as in Type IIC Miami (Schneippenheim *et al.*, 2001).

Type 2B can be identified because of heightened response to ristocetin and absence of large multimers from plasma (Ruggeri *et al.*, 1980). The multimeric structure of platelet vWF and of vWF produced by cultured endothelial cells is normal (De Groot *et al.*, 1989). The inheritance pattern is mainly autosomal dominant, but cases with apparently recessive pattern have also been described (Federici *et al.*, 1989).

A large degree of phenotypic heterogeneity has been identified since the original description. Typical features for 2B vWD are mild thrombocytopenia with increased mean platelet volume, prolonged BT, low to normal FVIII, low to normal vWF:Ag, low vWF: RCo and heightened RIPA. Thrombocytopenia can be more pronounced during pregnancy (Pareti *et al.*, 1990). In some families, spontaneous platelet aggregation occurs (Federici *et al.*, 1997). More than 20 different missense mutations and one small insertion have been identified in Type 2B, all located within the A1 domain of the vWF subunit, and the abnormal binding to platelet glycoprotein has been confirmed by expression studies in the majority of these mutants (Meyer *et al.*, 1997)(Figure 2.2).

Type 2M ('M' for multimers) includes variants in which binding to platelets is impaired but the vWF multimeric distribution is normal. This phenotype may be produced by mutations that inactivate specific binding sites for ligand on platelets or collagens. Laboratory results generally are similar to those in Type 2A, but there are high molecular weight forms. The Type 2M mutations that have been characterized are located within domain A1 of the vWF subunit and show reduced binding to platelet GpIb in studies of expressed mutants (Hillery *et al.*, 1998)(Figure 2.2). Among this subgroup of vWD variants, patients with type 2M 'Vicenza' have low levels of vWF antigen but have larger than normal multimers (supranormal) in plasma, similar to those observed in plasma after desmopressin infusion and in endothelial cells and platelets (Manucci *et al.*, 1988). Candidate missense mutation in Type 2M 'Vicenza' have been identified in domain D3 (Schneppenheim *et al.*, 2000).

Type 2N vWD ('N' for Normandy) is characterized by normal levels of vWF: Ag and vWF:Rco, and normal multimeric structure, but low plasma FVIII levels. It therefore resembles haemophilia A, but its inheritance pattern is not X-linked but autosomal recessive. Low FVIII levels, at variance with haemophilia, are due to decreased plasma half-life of FVIII, which cannot bind to vWF as a consequence of an intrinsic abnormality of vWF (Nishino *et al.*, 1989). Type 2N can be caused by several missense mutations, all localized to the D' and D3 domains of vWF subunit (Figure 2.2). Co-inheritance of a Type 2N mutatiom with a Type 1 vWD allele may contribute to the variable expressivity of Type 1 vWD (Mazurier *et al.*, 1981).

2.1.6.3 Type 3

Type 3 (severe) vWD is caused by impaired biosynthesis of vWF and is characterized by undetectable levels of vWF in plasma and platelets. As vWF is also the carrier of FVIII, plasma levels of FVIII are very low (1–5%). As a consequence, patients with Type 3 vWD have a severe bleeding tendency, characterized not only by mucocutaneous haemorrhages but also by haemarthroses and haematomas as observed in severe haemophilia. The inheritance pattern of Type 3 vWD is autosomal recessive and its prevalence is 1–5 per million populations (Sadler *et al.*, 2000).

Alloantibodies against vWF may arise in 5–8% of patients treated with FVIII–vWF concentrates (Mannuci *et al.*, 1995); gene deletions predispose to the formation of alloantibodies (Shelton *et al.*, 1987). The presence of antibodies has been demonstrated *in vitro* by the capacity of patient plasma to inhibit RIPA of normal PRP in a time-dependent manner (Mannuci *et al.*, 1981). *In vivo*, the antibodies are responsible for a poor clinical response to replacement therapy. In some patients with high antibody titers, replacement therapy not only is ineffective but also may trigger life-threatening anaphylactic reactions (Mannuchi *et al.*, 1995). The most common mutations in Type 3 vWD are total or partial deletions, nonsense, splicing and frame shift mutations, found throughout the 52 exons of the vWF gene (Zhang *et al.*, 1995).

2.1.7 Clinical manifestations

Patient with vWD may have variable clinical expression. Affected individual may experience mucocutaneous such as epistaxis, easy bruising and menorrhagia. Additional symptoms include haemorrhage after tooth extraction, haemorrhage after surgery, prolonged bleeding after superficial cuts and postpartum haemorrhage. The bleeding tendency however is highly variable and depends on the type and severity of the disease. In many patients with Type 1 or Type 2 vWD, the bleeding tendency may be mild or absent. In contrast with patient Type 3 vWD have a moderately severe haemorrhagic tendency; mucosal bleeding is very frequent and may be life-threatening. In addition, due to the severe FVIII defect, haematoma and haemarthrosis can occur.

2.2 MENORRHAGIA

Menorrhagia is defined as menstrual blood loss of 80ml or more per period. It is seen in 5% of women of reproductive age and 12% of gynecological referrals are because of this. Menorrhagia is one of the most common gynecological complaints in contemporary gynecology (Warner *et al.*, 2001). Current gynecological surveys report that 30% of all premenopausal women perceive menses to be excessive.

Menorrhagia has been attributed to a number of different general or local causes but in more than 50% of cases no organic pathology is found. Increased menstrual blood loss has been reported in women with inherited bleeding disorders, especially vWD and carriers of hemophilia. Other less common inherited bleeding disorders, including deficiencies of prothrombin, fibrinogen, FV, FVII, FX and FXI may also be associated with menorrhagia. The frequency of vWD in women with menorrhagia ranges from 5% to 20% in different studies (Andra *et al.*, 2004), where as menorrhagia was reported by 60-95% of women with vWD (Kirtava *et al.*, 2003). Despite this, a recent survey of obstetrician-gynecologists found that only 6% believed vWD or other bleeding disorders were a frequent cause of menorrhagia (Dilley *et al.*, 2002).

VWD-associated menorrhagia may be most severe during the first few years after menarche (Silwer *et al.*, 1973). In one study of women with objectively confirmed menorrhagia, 65% of women with vWD reported an onset at menarche compared to only 9% of those without a bleeding disorder (Kadir *et al.*, 1991). The frequency of vWD in

adolescents with menorrhagia is not well defined, because most studies evaluating prevalence did not include adolescent women (Woo *et al.*, 2002). Several small retrospective reviews identified vWD in 2.8-5% of adolescent women requiring urgent evaluation or hospitalization for menorrhagia (Bevan *et al.*, 2001).

The prevalence of menorrhagia in different types of vWD is not well defined. Several studies include only one woman with Type 1 disease, 79-93% of whom reported a history of excessive menstrual bleeding (Kouides *et al.*, 2000). Severe menorrhagia which required blood-product replacement occurred in 86% of women with Type 2 and 3 vWD unresponsive to DDAVP (Foster *et al.*, 1995). Other less common inherited bleeding disorders, including deficiencies of prothrombin, fibrinogen, FV, FVII, FX, and FIX may also be associated with menorrhagia.

Menstrual blood loss can be assessed by using a special scoring system, a Pictorial Blood Assessment Chart (PBAC) (Higham *et al.*, 1990) (APPENDIX 2.) which show a reasonable accuracy for assessment menstrual blood loss and diagnosis of menorrhagia compared to alkaline haematin method. An accurate prevalence value of menorrhagia in vWD appears to be 74% based on a pictorial chart score of over 100 (Kadir *et al.*, 1999).

2.3 LABORATORY ASSESSMENT FOR APPROPRIATE DIAGNOSIS AND SUBCLASSIFICATION OF VWD

vWD is heterogenous disorder and patient are type according to pathophysiology. The correct diagnosis and sub-classification of patient's vWD is crucial because the presenting biological activity of vWF determined the hemorrhagic risk, and since subsequent clinical management will differ accordingly.

After obtaining a suggestive history and family history, a panel of test must be done to diagnose vWD. Many preanalytical variables can cause substantial problems for the identification of vWD. The blood is normally collected into a sodium citrate anticoagulant tube. Poor collection technique or difficult collection may lead to partial sample clotting and loss of high molecular weight of vWF because of entrapment or platelet adhesion. The sample transportation and the storage of the samples must be done at ambient temperature for a short period (< 4 hours) as it will not generally affect the diagnosis of vWD. However in contrast, low temperature (refrigerated) transport and /or storage will give rise to loss of high molecular weight vWF and misidentification of Type 1 or Type 2 vWD may occur in normal/non vWD individuals and Type 2 may be misdiagnosed in Type 1 vWD individuals. Blood collection in EDTA is more resistant to such artifacts, can be used as an alternative specimen for some tests (vWF: Ag, vWF: CBA) but not suitable for FVIII:C. Plasma vWF levels also fluctuate in individuals according to individual variation (different results are possible on sequential-day samples), diurnal variation (levels appear higher later in the day), hormonal influenced (e.g, fluctuations within menstrual cycles, and higher levels in pregnancy. ABO blood

group (O-group vWF levels generally lower than non-O blood group levels), age (levels increased with age) and stress.

In order to diagnose vWD, it is important to detect within the blood of the prospective individual, a reduction in level of either the vWF or associated function. Studies requiring testing on only one occasion may underestimate the prevalence of vWD due to normal fluctuation in vWF levels (Philip *et al.,* 2003). Repeated testing is often necessary to confirm or exclude the diagnosis. Because of vWD heterogeneity, different combinations of abnormalities can be determined by the laboratory test panel, and it is the specific combination of test results that will help to define the defect.

Individual laboratory tests differ substantially in their relative power to diagnose vWD or to identify its various forms. The laboratory assays used in the diagnosis of vWD can be divided by:

2.3.1 Laboratory screening assays

2.3.2 Laboratory assay used to confirm and/or classify vWD

If all the results are normal, and if the initial clinical suspicion was low to begin with (presenting history was not striking), no further investigations may be warranted. Alternatively, if the history is striking or if results of preliminary testing were abnormal, further investigation is warranted and should be specifically tailored to the individual being tested. Repeat testing is usually indicated to both exclude a potential testing and

collection artifact, and also to confirm findings because of assay variability (Favaloro *et al.*, 1999).

2.3.1 Laboratory screening assays

Laboratory screening assays is a 'clotting screen', a basic test of coagulation and often performed to investigate a case of hypo-coagulopathy, platelet-function related disorders and vWD or may detect an unsuspected hazard which increases the risk of postoperative bleeding.

2.3.1.1 Routine coagulation tests

These typically comprise the prothrombin time (PT) and activated partial thromboplastin time (APTT). These are standard 'global' clot–based that are usually performed by using automated coagulation analyzer with results reported as a clotting times in seconds.

2.3.1.1.1 Prothrombin time (PT)

The test measures the clotting time of plasma in the presence of an optimal concentration of tissue extract (thromboplastin) and indicates the overall efficiency of the extrinsic clotting system. The test is now known to depend also on reactions with factors V,VII and X and on the fibrinogen concentration of the plasma (Quick *et al.*, 1973).This test is particularly relevant when patient are being monitored on oral anticoagulant (eg:warfarin) therapy, it is not relevant for individuals being assessed for vWD.