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Determination of von willebrand factor antigen in patients with undiagnosed menorrhagia

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Abstract
This prospective study is about screening of von willbrand antigen in patients with undiagnosed menorrhagia, because the underlying cause may be haematological disorder in 50% of cases. To estimate the incidence of von willbrand disease in patients with undiagnosed menorrhagia. During the period of 2 months from February to april 2005, total of 50 patients were included in this study that were referred to Al-yarmok teaching hospital. The age range of these patients was 18-45 year. Full history and physical examination done, ultrasound examination to exclude local causes of menorrhagia, thyroid function done. 30 healthy volunteer age matched were included as control group. For each patient and control group the following tests were done: Bleeding time, Prothrombin time, Activated partial thromboplastin time, Factor 8 level, Von willbrand factor antigen level.

Results

1. There was no obvious or statistically significant difference in mean bleeding time and activated partial thromboplastine time observed between healthy controls and patients with menorrhagia.
2. The mean Von Willebrand factor antigen (%) was significantly lower in patients with menorrhagia compared with healthy controls.
3. The mean Von Willebrand factor antigen was slightly lower at the older age group compared to younger age group.
4. The proportion of patients with prolonged activated partial thromboplastine time was obviously higher among those with abnormally low Von Willebrand factor antigen compared to those with acceptable level.
5. The relative frequency of cases with abnormally low factor VIII activity was higher among subjects with other bleeding manifestations compared to those with no manifestations.
6. The relative frequency of cases with abnormally low factor VIII activity was higher among subjects with positive family history of bleeding tendency.
7. The relative frequency of cases with abnormally low Von Willebrand factor was obviously higher among subjects with other bleeding manifestations compared to those with no bleeding manifestations, also it was higher among cases with positive family history of bleeding tendency and those with past history of severe bleeding episodes.

Conclusions: The prevalence of Von Willebrand disease is increased in women with menorrhagia. So it is reasonable to believe that Von Willebrand disease is the underlying cause for meborrhagia in a small but significant proportion of women. This study revealed that 6% of women with menorrhagia had Von Willebrand disease.

Keywords: Menorrhagia, von willebrand, disease

Introduction

Von Willebrand Factor: von Willebrand factor (vWF) is a multimeric glycoprotein which is encoded by a gene on chromosome 12, which was cloned simultaneously by several groups. [1, 2]. The primary gene product is an extremely long protein monomer comprising 2813 amino acids. This is predominantly produced in vascular endothelial cells and also megakaryocytes [1, 2, 5]. The multimers are either secreted constitutively into the plasma or stored in platelet a-granules and Weibel-Palade bodies in the endothelial cells and released upon demands [2, 7, 8]. The primary product of the vWF gene is a 2813 amino acid protein made of a signal peptide of 22 amino acids (also called a pre-peptide), a large pro-peptide of 741 amino acids and a mature vWF molecule containing 2050 amino acids [5]. The pro-peptide, previously known as von Willebrand antigen II is detectable in plasma and is essential for dimer formation, dimers are then assembled into tetramers, which then further

polymerize to form series of multimers with molecular weight ranging from 1 x 106 to 20 x 106 Dalton [1, 2]. Different protein regions, corresponding to four types of repeated domains (D_1, D, D3, A1, A2, A3, D4, B, C_1, and C_2) of cDNA are responsible for the different binding functions of molecule [5]. The A_1 domain contains a protease-sensitive domain that may play a regulatory role in vWF function. A3 domain contains an important collagen-binding domain, C_1 domain has an RGD square capable of interacting with platelet glycoprotein II b/ III a, and the D and D3 domains contain a factor VIII binding sequence [4]. Purified vWF visualized by electron microscopy appears either as filamentous structure with a diameter of 2 - 3 nm and length of up to 1300 nm, close to the diameter of platelets, or asloosely coiled molecule with a diameter of 2 - 300 nm. The building block of VWF multimers is a dimer made up of two single-chain-pro vWF molecules joined through disulphide bonds within their C-terminal region [5]. Glycosylation begins in the endoplasmic reticulum with 12 potential N-linked glycosylation sites present on the mature subunit and 3 on the peptide. Extensive additional post-translational modification of v WF occurs in the Golgi apparatus, including the addition of multiple O-linked carbohydrate structures multimerization through the formation of disulfide bonds at the N-terminal of adjacent dimers [3]. vWF is secreted from the cell along a constitutive and a regulated pathway. The regulated secretion of vWF from its storage site in the Weibel-Palade body is triggered by a number of secretagogues, including thrombin, fibrin, histamine and C5b 9 complement complex while vasopressin analog desmopressin causes marked release of vWF *in vivo* [3, 5]. The latter is used for rapid stimuli-induced release (e.g. by desmopressin through its binding on the vasopressin V_2 receptor of the endothelial cells) from specialized storage organelles of the endothelial cells known as Weibel-Palade bodies [5]. The majority of circulating vWF is present in plasma, with approximately 15 % of circulating vWF present intracellularly within platelets. Ultra-large multimers released into the plasma are further degraded to smaller multimers, presumably through the activity of vWF clearing protease [4].

Functions of vWF: VWF has two main functions in haemostasis:

1. As a carrier of factor VIII: vWF protect factor VIII from proteolytic degradation, prolonging its half-life in circulation and efficiently localizing it at the site of vascular injury [1, 5, 7, 9]. Each vWF monomer has one binding domain located in the first 272 amino acids of the mature subunit (D domain) which can bind on factor VIII molecule [5, 9]. Although factor VIII and vWF are entirely distinct entities with separated functions, they circulate together as a complex in which vWF protects factor VIII from degradation, so that a deficiency in vWF or a reduction in its ability to bind factor VIII may also results in a low plasma level of factor VIII [2, 4]. Indeed plasma level of factor VIII is usually proportional to levels of VIIIc or VIII antigen in normal individuals, but in cases of quantitative vWF deficiency, the ratio of VIIIc levels may be moderately increased, thus factor VIIIc levels may be normal or near normal in Type I vWD. This finding may be also observed after transfusion therapy in vWD.
2. The second function of vWF is its role in platelet attachment to subendothelial surface, platelet spreading and platelet-platelet interactions, i.e. aggregation of platelets at the site of injury [3, 5]. The main steps in platelets function are; adhesion, activation with shape changes and aggregation. When the vessel wall is damaged, the subendothelial structures including basement membrane, collagen and microfibrils are exposed and the surface bounded vWF binds to Gp Ib on the circulating platelets resulting in an initial monolayer of adhering platelet, binding via Gp Ib initiating the activation of the platelet via a G-protein mechanism.

Factors Affecting the Level of vWF: There are numbers of physiological and pathological situations can alter the levels of vWF when testing, and these factors are:

1. **Menstrual Cycle:** Estrogen may raise the level of vWF and causes fluctuation during the menstrual cycle [6]. So menstrual cycle should be considered during testing vWF, because under-diagnosis of vWD could result if raised estrogen levels, which occur during the early follicular phase (before day 7 of the cycle), give a falsely high reading of vWF, thus causing a false negative test for vWD. However recent data indicates that the lowest vWF level are on day 1 to 4 of the cycle, i.e. during menses, suggesting that this is the best time to test; in another word the lowest level is in the early follicular phase.
2. **Pregnancy:** vWF usually raised in pregnancy, factor VIII levels are often higher in pregnancy [4, 6].
3. **Blood Group:** Individuals with blood group A, B, or AB can have higher levels of vWF than those with blood group O [6]. Genetic component accounts for about 30%-60% of the variance in plasma vWF, and ABO blood type is one major genetic determination of vWF level. The average vWF level for person with blood type O is 25-35 allow that in person with blood group type A, B, or AB, and ABO type accounts for approximately 30% of their genetic variance of vWF [2, 11, 12]. The deficiency of vWF associated with blood type O is not caused by mutation in the vWF gene per se, but the clearance of the vWF protein may be enhanced in people with this specific blood group [4, 8]. Human plasma vWF has ABO blood group antigens on the asparagines-linked sugar chains, it is quite unique because only limited human plasma glycoprotein (vWF, FVIII, and a part of a2-macroglobulin) covalently link these antigens. The concentration of vWF has been known to be influenced by these blood groups and is significantly lowered in person with blood group O [13].
4. **Stress:** Physical and mental stress can raise the levels of vWF in the circulation [6].
5. **Race:** Many European studies, the diagnosis of vWD was examined separately for Caucasian and African-American women. vWD was found in 15.9 % of white women with menorrhagia and only 1.4% of African-American women with menorrhagia [10]. So for an unknown reason the prevalence of vWD was lower among black women and this indicates the importance of considering inherited bleeding disorder menorrhagia [14]. as a cause of
6. **Acute Infection:** Plasma vWF Ag concentration in patients with acute infections of bacterial, viral or parasitic origin are significantly elevated with a mean greater than 3 folds above normal. In individual patients the elevation of vWF correlated strongly with the elevation of serum C-reactive protein (CRP). Thus vWF

is an acute phase reactant in human [4, 15].

7. **Hypothyroidism:** A low thyroid hormone level appears to decrease the vWF level, which may result in acquired vWD, interestingly there had been reports of females presenting with menorrhagia who were ultimately discovered to have both hypothyroidism and a concurrently low vWF level. Both the menorrhagia and the low vWF level subsequently being corrected with thyroid hormone replacement [11, 16, 17].
8. **Age:** Levels of vWF increase with age, rising approximately for each decade increase in age. The absence of a family history is often due to the family's lack of knowledge, and negative family history does not role out a hereditary bleeding disorder [3, 31]. Thrombocytopenia is a common feature of Type II B vWD, and is not seen in any other forms of vWD, and most patients only experience thrombocytopenia at times of increased vWF production or secretion such as during physical effort, pregnancy, newborn infants, postoperatively or if an infection develops. The platelets count rarely drops sufficiently to contribute to clinical bleeding [3].

Diagnosis of von Willebrand Disease: After obtaining a suggestive personal history and a family history. The preliminary tests required are full blood count and a coagulation screen. The history may reveal the following; Increased or easy bruising., Recurrent epistaxis., Menorrhagia, Postoperative bleeding. Family history of a bleeding diathesis., Bleeding from wounds., Gingival bleeding., Post-partum bleeding [2, 4, 18].

Whom to screen?

1. Adolescents presenting with severe menorrhagia should be screened for vWD, as many as one third of adolescents presenting with menorrhagia since menarche have been found to have Vwd [51, 52, 53, 54].
2. Screening is necessary among adult women with significant menorrhagia with-out another cause because it is not unusual to encounter adult women with menorrhagia who have a mild form of vWD [52, 55].
3. Hysterectomy for excessive menstrual bleeding should not be performed without the consideration of bleeding disorder women with vWD have in the past undoubtedly been given the diagnosis of dys-functional uterine bleeding and have had hysterectomy for therapy with resultant risks from bleeding at the time of surgery
4. The diagnosis of vWD must be suspected in any pregnant patient with an abnormal bleeding tendency particularly with recurrent postpartum bleeding .The course of pregnancy in the majority of patients with vWD is benign and the most frequent complication is bleeding during labor or postpartum bleeding. Also there is a high proportion of postpartum hemorrhage in type I patients especially after the first 24 hours. Post delivery, this may occur despite a normalization of factor VIII level in the third trimester.

Patients and Methods: During the period of two months from February to April 2005, total of 50 patients (non pregnant women) were included in this study that were referred to Al-Yarmouk teaching hospital in Baghdad. The age range was 18-45 years.

The following data were documented for each patient: Patient name, age, occupation, address, past medical history, past surgical history, drug history, gynecological and obstetrical history which include (history of infection, IUCD, pain, parity), history of menorrhagia (>80 ml blood loss) and its duration, any other bleeding manifestation, and any family history of bleeding disease. Complete physical examination was done for each patient, also gynecological examination done in department of obstetric and gynecology at Al-Yarmouk teaching hospital. Ultrasonic examination also was done to exclude local cause of menorrhagia. Thyroid function tests T3, T4 were done to exclude hypothyroidism that may cause menorrhagia.

Control Group: A total of 30 healthy volunteers age matched were included in this present study as control group. Pooled plasma from at least 4-6 healthy individuals were prepared and divided into aliquots each contains 1ml stored at -40 °C to be used simultaneously with patient plasma for a period not exceeding 10 days [13]. A Blood sampling and processing: A 5.6 ml of venous blood was collected using clean aseptic venipuncture technique from each patient and control which was then processed as follows: A 3.6ml of venous blood added into a clean disposable capped plastic tube containing 0.4mi of 3.2% of trisodium citrate dihydrate in a ratio of 1 volume citrate to 9 volumes of blood, er gentle repeated inversion of the blood containing tube several times to ensure good mixing manually. The specimen was immediately centrifuged at 4000 rpm (2000g) for 15 minutes to prepare PPP, the obtained plasma was aspirated refully using automatic pipettes then disposed into two Esposable capped plastic tube (1ml of plasma for each). One to be stored at -20 for further assessment when required and the her sample was used to perform PT, PTT, F VIII assay, VWF say. All these tests were done as early as possible on the eshly prepared plasma with a minimum delay of less than one B Two-ml of venous blood added in a tube containing EDTA ir platelet count. Bleeding time was done for those patient immediately by y's method. DPreparation of normal pooled plasma: Normal pooled plasma was prepared by pooling plasma from 20 mal healthy volunteers. Platelet poor plasma from each volunteer was prepared in similar way as mentioned above. The platelet poor plasma were mixed and divided into aliquot each containing 0.6ml of plasma and stored at -40 °C for a maximum 10 days and was used for e drawing of calibration curve for factor VIII.

Bleeding Time: Principle: A standard incision is made on the volar surface of the forearm and the time that incision bleed is measured. Cessation of bleeding indicates the formation of hemostatic plug which is in turn dependent on an adequate number of platelet and on the ability of the platelet to adhere to the subendothelium and to form aggregate. We used Ivy's method which is similar to the template method i.e. we start by placing a sphygmomanometer cuff around the patient arm above the elbow and inflate to 40 mmHg and keeping it at this pressure through-out the test and then cleaning the area with ethanol swab and allows to dry and then choosing an area on the Volar surface of the forearm which is devoid of visible superficial veins but in Ivy's method instead of using standardized incision of the template, we use two separate punctures 5-10 mm apart made with quick succession using a disposable lancet of 2.5mm cutting depth and 1mm width.

PLATELET COUNT: A 1 to 20 dilution of blood was made by adding 0.1 µl of blood to 1.9 ml of the diluent fluid (10

g/l) ammonium oxalate in a 75x12 mm glass tube. Mixing was done using mechanical mixer for 10-15 minutes. Using Pasteur pipette the improved Neubauer counting chamber was filled then the chamber was left untouched in a moist Petri dish for 20 minutes to give time for platelet to settle, then the preparation was examined under the light microscope. The number of platelet in one or more area of 1mm was counted. The normal control of platelet in this study was 210-300x10/L using the following equation for calculation: Platelet count per liter = N x 200 × 106 Prothrombin Time (PT).

Principle: 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 depends on reaction with factor V, VII, X, II and fibrinogen concentration of the plasma.

Reagents: Isimat (thromoplastin and calcium) (biomerieux Sa France) The reagent prepared by reconstituting thromboplastin vial with 4ml of sodium azide NaN3, 0.5g/l with gentle shaking.

Method: The test was done by incubating 0.1 ml of test plasma in a glass tube measuring 10x75mm for 2minutes at 37°C then 0.2ml of pre-incubated thromboplastin reagent added and a stop watch was started simultaneously to record the clotting time in seconds. Normal range as obtained from the control group was 12-14 seconds.

Activated Partial Thromplastin (APTT)
Principle: The test measures the clotting time of plasma after the activation of contact factor but without added tissue thromboplastin and so indicate the overall efficiency of the intrinsic clotting system. The test depend not only on contact factors and factor VIII and factor IX but also on the reaction with prothrombin and fibrinogen, it is also sensitive to the presence of circulating anticoagulant (inhibitors and heparin)

Reagents: Cephalite kit for APTT (Biomerieaux Sa France) The reagent was prepared by reconstituting a vial of cephalite with 5ml of distilled water - CaCL2 0.025mol/l.

Method: The test was done by incubating 0.1ml of test plasma with 0.1ml of cephalite in a water bath at 37 °C for 3 minutes then 0.1 ml of pre-warmed calcium chloride was added and a stop watch started simultaneously to record clotting time in seconds. The control group was 29-37 seconds.

Factor Viii Assay
Principle: It is based on PTT, this assay consist of the measurements of the clotting time, in the presence of cephalin and activator of a system in which all the factors are present, constant and in excess except factor VIII which is derived from the sample being test. [6:53 pm, 13/07/2021] Amir Masawod: METHOD: The test was done by following the instruction of the manufacture. The test included: The preparation of 3 dilutions of patient and control plasma in Michalis buffer as follows: 1:5 (0.2 ml of plasma +0.8ml buffer and it equal to 200% activity) 1:10 (0.5 ml of the first dilution +0.5 ml buffer and it is equal to 100% activity) • 1:20 (0.5 ml of the second dilution +0.5 ml buffer it equal to 50% activity) The test was done by putting a glass tube measuring 10x75 mm in a water bath at 37 °C containing 0.1 ml of STA _deficient VIII and 0.1 ml of each dilution, the content mixed and incubated for 3 minutes, 0.1 ml of cephalite in water bath at 37° C for 3 minutes then 0.1 ml of pre warmed calcium chloride was added and stop watch started simultaneo.

G vWF ASSAY
Principle: When a beam of monochromatic light is allowed to traverse a suspension of microlatex particle to which specific antibodies have been attached by covalent binding and if the wavelength of the light is much greater than the diameter of the latex particle, the light is only slightly absorbed. In the presence of antigen being tested for, the antibody-coated latex particle agglutinate to form aggregate of diameter greater than the wavelength of the light, more of the latter is absorbed. This increase of absorption is proportional to the antigen level present in the test sample.

Reagents: Using commercially available kit (Diagnostic stago) 92600 ASNIERES-SUR-SEINE (France) The kit is Bar-code box. This Bar code contains the following information: LOT number, kit code number, reagent code number, and expiration date.

Reagent 1: glycin buffer
Reagent 2: suspension of microlatex, particles with rabbit antihuman vWF antibodies, then stabilized with Bovine albumin. • Reagent 3: solution containing glycin for dilution of latex reagent (reagent 2) Reagent 1 is allowed to stand at room temperature before opening for 15 minutes, then swirling the vial gently. Reagent 2 should pour the entire content of vial of reagent 3 into vial of reagent 2 of the same kit. We should ensure that all drops of reagent 3 are transferred. Swirling the reagent 2 vial to mix well with out creating any bubbles. [6:53 pm, 13/07/2021] Amir Masawod.

Method
0.1ml of the tested plasma is mixed with 0.2 ml of reagent 1, and the mixture is left in curette of spectrophotometer at room temperature for 5 minutes with continuous shaking, after the end of the 5 minutes we add 0.3 ml of the mixture of reagent 2 and 3 and immediately put it in the spectrophotometer to read (TO) and start stop watch for another 5 minutes then read (T5). The result of T5-TO should be obtained. Use 5 tubes do the calibration curve. The first tube is non diluted calibrator, the second tube is 400 µl mixed with 200 µl of Michaelis buffer (2:3), the third tube is 200 µl calibrator mixed with 200µl of Michaelis buffer (1:2), the fourth tube is 100 µl calibrator mixed with 900 µl of Michaelis buffer (1:10), the fifth tube is Michaelis buffer only. Note the calibrator here is the pooled plasma. We proceed the test as mentioned above on all dilutions then we draw a graph on a log paper as follows: 100% equals to T5-TO of tube 1 70% equals to T5-TO of tube 2 0% equals to T5-TO of tube 5, 50% equals to T5-TO of tube 3, 30% equals to T5-TO of tube 4 We draw the calibration curve on linear graph paper (linear interpolation); the abscissa (x-axis) is the vWF antigen percentage, while the ordinate (y-axis) is the optical density for 5 minutes. The optical density of the tested plasma is drawn against 100% of the x axis and a horizontal line is drawn to cut the calibration curve at point from which a vertical line is drawn to the x axis to

determined the vWF antigen percentage. Normal range of vWF antigen is 70-120% Note: the optical density of the spectrophotometer was read at a wave length of 540 nm.

Results

1. The results were based on analysis of 50 female patients with age ranging from 18-45 years. The frequency distribution of cases with menorrhagia by age is shown in table (2). There were (20%) of patients aged less than 25 years and 34% of them were aged between 25-34 years, and 46% were above the age of 35 years.
2. Bleeding manifestations and positive family history: All of the patients 50 (100%) presented with menorrhagia, 7 of them (14%) have other bleeding manifestations and 1 patient (2%) has family history of bleeding or coagulation disorders .One patient (2%) has past history of severe bleeding episode as post-partum bleeding as shown in table 3.All patients in this study were examined by gynecologist and ultrasound to exclude any local causes of menorrhagia. There were no identifiable organic causes for menorrhagia.

Table 1: Frequency distribution of cases of menorrhagia by age

Age groups	N	%
<25	10	20
25-34	17	34
35+	23	46
total	50	100

Table 2: The relative frequency of selected bleeding manifestations and positive family history

Bleeding manifestations and history(n=50)	N	%
Other bleeding manifestations	7	14
Family history of bleeding or coagulation disorder	1	2
Past history of severe bleeding episode	1	2

Coagulation Screening Tests, Results Of Coagulation Screening Tests Of Control Group (TABLE 4): Bleeding time: the range of bleeding time was 2.1-7.5 minutes with a mean of 3.5 1.5 minutes. Prothrombin time: the range of prothrombin time was 12-14 secondswith a mean of 12.8 ± 0.5 seconds Partial thromboplastin time: the mean partial thromboplastin time was 35.4 1.4 seconds • Von willebrand factor antigen %: the range was 70-120 with a mean of 95.5 ± 12.2. Factor VIII activity %: the range was 85-150 with a mean of 122 15.5.

Results of Coagulation Screening Tests of Patients Group bleeding time: the range of bleeding time was 1.5-13 minutes with a mean of 3.6 ± 2.1 minutes.

Prothrombin time: the range of prothrombin time was 12-18 seconds with a mean of 13.6±1.4 seconds.

Partial thromboplastin time: the range of partial thromboplastin time was 23-47 seconds with a mean of 35.9 ± 4.8 seconds. • Von willebrand factor antigen %: the range was 22-120 with a mean of 76.9 ± 18.4.

Factor VIII activity %: the range was 25-173 with a mean of 106.2+39.9. There was no statically significant differences in mean bleeding time and PTT observed between healthy controls and cases with [7:09 pm, 13/07/2021] Amir Masawod: menorrhagia with p value of 0.92 and 0.59 respectively. The mean von Willebrand factor antigen (%) was significantly lower in cases with menorrhagia (76.9%) compared to healthy controls (95.5%), table 4 and figure 1. The histogram in figure 2 shows a significant shift to the left of the normal curve of Von Willbrand factor antigen in cases with menorrhagia compared to healthy controls with a p value of <0.001. The histogram shows shift to the left of the normal curve of factor VIII activity in cases with menorrhagia compared to healthy controls with a p value of 0.042.

The Differences in Mean of Von Willebrand Factor Antigen % And Factor Viii Activity In Relation To Age: The mean von Willebrand factor antigen was sligh. [7:09 pm, 13/07/2021] Amir Masawod: III. 5 The Differences In Mean Von Willebrand Factor Antigen And Factor Viii Activity Between Cases With Blood Group O And Cses With Other Blood Group:

Clotting Parameter: There was no important or statistically significant difference in relative frequency of prolonged bleeding time (>7.5 minutes) between cases with menorrhagia (10%) and healthy contr. Higher among cases (38%) compared to healthy controls (3.3%) The finding of abnormally low factor VIII activity at the reported cut-off value of <50% was slightly more frequent in cases (8%) compared to healthy controls (0%), but the difference was also small to be statistically significant. However at the control standardized cut-off value of <91%, the rate of abnormally low factor VIII activity was significantly higher among cases (36%) compared to healthy controls (3.3%), table 7 and figure 7. The ROC analysis was used to mark the study variables according to their ability to better discrimination between cases with menorrhagia and healthy controls. Von Willbrand factor antigen activity is the first followed by PT test.

The Difference in Mean of Selected Variables in Cases With Menorrhagia with Abnormally Low Von Willebrand Factor Antigen And Those With Acceptable Level: No important or statistically significant difference in mean bleeding time, PT, PTT, factor VIII activity %, blood platelets, serum T3, serum T4, serum total billirubin, SGOT, SGPT, SGPT and serum alkaline phosphatase between menorrhagia cases with low von Willebrand and those with acceptable range. Also no significant linear correlation observed between those variables and von Willebrand factor antigen activity. A noticeable exception was observed for PTT, which was slightly higher among cases with low Von willbrand factor antigen and it showed a moderately strong negative (inverse) and statistically significant linear correlation. (Table 9, figure 4) The mean serum T3 was also lower among cases with low von Willebrand and there is a moderately strong positive (direct) correlation. Factor VIII activity showed no important or statistically significant linear correlation with von Willebrand factor antigen activity.

The Association between Prolongd PTT and Abnormally Low Level of VWF Antigen and Factor Viii Activity with Menorrhagia: The proportion of cases with prolong PTT was obviously higher among those with abnormally low vWF antigen (42.1%) compared to those with acceptable level (25.8%). The association however failed to reach the level of statistically significant as shown in table 10. The relative frequency of cases with abnormally low factor VIII showed no important difference between those with low Vwf antigen % compared to those with acceptable vWF antigen %.

The Rate of Abnormally Low Vwf Antigen By Selected Independent Variables In Cases With Menorrhagia: The relative frequency of cases with abnormally low vWF was higher among subjects with other bleeding manifestations (71.4%) compared to those with no bleeding manifestation (32.6%), it was also higher among cases with positive family history of bleeding tendency and those with past history of severe bleeding episodes, but these association failed to reach the level of statistically significant possible because of small group size. [7:11 pm, 13/07/2021] Amir Masawod:

The Rate of Abnormally Low Level Factor Viii Activity By Selected Independent Variables In Cases With Menorrhagia: The relative frequency of cases with abnormally low factor VIII activity was highe among subjects with other bleeding manifestations (57.1%) compared to manifestations (32.6%). those with no It was also higher among cases with positive family history of bleeding tendency and those with past history of severe bleeding episodes.

Statistical Analysis: Data were translated into a computerized database structure and an expert statistical advice was sought for Statistical analysis was computer assisted using SPSS ver13 (statistical package for social sciences). Frequency distribution for selected variables was done first .P value less than the 0.05 level of significance was considered statistically significant. The statistical significance of difference in mean of a normally distributed quantitative variable between two groups was assessed by independent sample T-test, while between more than two groups the ANOVA test was used. The strength, direction and statistical significance of linear correlation between two quantitative variables was assessed by Pearson's correlation coefficient. The Chi-sequare test was used to assess the statistical significance of association between two categorical variables .The control standardized cut-off values for defining abnormally low level of factor VIII and von Willebrand factor was based on the statistical definition of the range of normal values based on a sample of healthy controls. Range of normal values based on a sample of healthy controls =sample mean +/-2SD.

Discussion

Von Willebrand disease (vWD) is the most frequent inherited bleeding disease. An estimated prevalence ranging from 4-10 per 100 000 inhabitant was reported. It was assumed that the number of people with symptomatic vWD requiring specific treatment would be at least 100 millions In this study it was revealed that (6%) of women with menorrhagia had vWD This difference in the prevalence regardless to the group size may be due to the unavailability of most of the investigations that can give more precise and accurate picture for vWf profile as vWF: RCOF, multimer analysis, Ristocetin induced platelets aggregation while in this study the diagnosis is based on (BT, PTT, VWF Ag, f VIII C%) In this study as all of the previous studies the patients population who are involved are women with menorrhagia as a presenting symptom and all of them had been excluded to have underlying local uterine abnormality by pelvic examination and pelvic ultrasound. There is an important point in the diagnosis of vWD and this point is the threshold that upon which we depend in defining the patients with less than normal vWF Ag. Indeed the threshold level for the diagnosis were different in the European and North America studies i.e. in the European studies they use a value below the lower limit of normal range as diagnostic and this level was 50 IU/L in contrast, most North America studies used two standard deviations below the control mean or the 2.5th. While in this study the diagnosis of menorrhagia is based on history of excessive menstrual blood loss for more than three consecutive menstrual periods. As perception of menorrhagia is influenced by cultural, social and demographic factors and suggestive diagnosis is often inaccurate and the need for objective measurement of menstrual blood loss is well documented recently there has been growing recognition that VWD is not uncommon in women with menorrhagia with prevalence estimates of 5-20%, compared with less than 1% in women without menorrhagia. The present study showed that the level of vWF is related to the blood group and the lowest level was in blood group O. and this finding agrees with that of others studies.

Conclusions

The prevalence of von Willebrand disease is increased in women with menorrhagia. So it is reasonable to believe that VWD is the underlying cause for menorrhagia in a but significant proportion of women. So testing of this bleeding disorder should be considered when investigating women with menorrhagia especially those with no obvious pelvic pathology (normal pelvic examination and pelvic ultrasound). Awareness of vWD as a cause for menorrhagia is important for the following reasons: Health implications like future surgery and child birth**,** Affective medical treatment of menorrhagia with desmopressin nasal spray, OCP, antifibrinolytic agents and avoidance of major surgical interventions screening and early diagnosis of vWD in relatives.

Recommendations: Based on the results obtained in this study we recommend that:

1. There should be a screening for vWD in women with menorrhagia which is important to prevent unnecessary surgical intervention
2. Screening tests of vWD should be available in centers as Ristocetin cofactor; Ristocetin induced platelet aggregation and multimer analysis.
3. Further study should include larger number of women with bleeding disorder to obtain more accurate data and to determine the prevalence of VWD and its distribution.
4. Screen a large number of healthy individuals to establish the normal range of vWF and F VIII activity in our population.
5. Further investigations (eg. F VII assay) are recommended in menorrhagia cases to define the causes of raised PT in these cases.

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