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Identification of Bleeding Disorders in Women with Menorrhagia in Gezira state, Sudan (2017-2018)

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Abstract

Menorrhagia (Heavy menstrual Bleeding) (HMB) is defined as bleeding in excess of 80 ml per menstrual cycle when measured objectively or bleeding more than 7.0 days or both. It is common among patients with bleeding disorders and can be a presenting symptom. The aim of this study was to identify bleeding disorders in women with menorrhagia in Gezira state teaching hospitals, Sudan. This is prospective study, conducted at the period from June 2017 to June 2018. Seventy-six women with menorrhagia and 15 normal women (used as control) were enrolled in this study. 2.5 ml of venous blood were collected in trisodium citrate container, 2.5 ml in EDTA container during menstruation and after 14 days of ending menstruation. Bleeding time, activated partial thromboplastin time, platelets count and platelets aggregation were performed. Luteinizing hormone (LH), Follicle-stimulating Hormone (FSH), progesterone, factor VIII and Von Willebrand factor antigen during and post menstruation Measured. The data was analyzed by using statistical analysis program SPSS. The mean women age was 25.0 (+/- 3.0) years, ranged from 14 – 45 years. About 5.3% of participants has family history of bleeding and 93.5% has no family history. The result showed that there was significance difference between the mean of hemoglobin (9.340 +/- 2.066) with (p = 0.019), platelets count mean (311.29 +/- 30.000) with (p = 0.027), bleeding time (4.89 +/- 1.856) minutes with (p =0.005), APTT(38.822 +/- 8.8926) seconds with (p =0.002), LH (P =0.075), FSH (P =0.000), progesterone (p =0.03) and VWF Ag (p =0.000) and no significance difference between Factor VIII (P =0.291) in patients and controls. According to the results of this study the possibly causative agents of menorrhagia were Von Willebrand factor antigen (5.3%), abnormal platelets aggregation (3.9%) (Platelets dysfunction), hormones abnormalities (1.6%), low platelets count (thrombocytopenia) (3%), 86.2% others causes (unknown).

Keywords: Heavy menstrual Bleeding, Von Willebrand factor, SFH, LH and platelets.

Introduction

Coagulation markers are greatly different in women due to different physiological conditions such as age, ethnicity, blood group and phases of the menstrual cycle (Kadir *et al*., 1999). Various studies report that hemostatic factors reach the lowest levels during menstrual and early follicular

phase, especially for Von Willebrand factor, antihemophilic factor A (Factor VIII) and platelet function tests, hemostatic parameters showed variation in different phases of menstrual cycle. Fibrinogen and fibrinogen degradation products were significantly increased in the luteal phase as compared with the follicular phase. Platelets functions were altered during the ovarian cycle under the influence of progesterone and estrogen on Von Willebrand factor (Verschueren., 2017). Hemostasis is achieved through a delicate equilibrium between the coagulation and the fibrinolytic cascades. Abnormalities of platelet function, such as Von Will ebrand disease, appear to be more prevalent in women with menorrhagia than in the general population (Bashawri and Ahmed, 2007). The prevalence of von Willebrand disease in women menorrhagia varies from 5 to 24 percent (Von Will ebrand, 2013). Menorrhagia is the most common gynecological disorders which involves abnormal, prolonged or heavy bleeding or both from uterus. Hemostatic disorders one of the ignored causes of menorrhagia. Presenting symptoms in women with bleeding disorders which is a variable presentation ranged from mild to severe typically with easy bruising or mucocutaneous bleeding, the women are apparently more symptomatic with menorrhagia. There are little data suggesting that a lower quality of life occurs commonly in women with menorrhagia and Von Willebrand disease than in those with menorrhagia alone (Lavin *et al*., 2018). To date there is very limited related published studies done in Gezira State in Sudan about this topic, so this study conducted to identify the bleeding disorders in women with menorrhagia in Gezira state.

Methodology

This was a prospective hospital-based study, conducted at Gezira state, Obstetrical and Gynecological Teaching Hospitals, during the period from June 2017 to June 2018. A total of seventy-six women with menorrhagia were included in this study and fifteen normal women used as control group. Ethical approval was taken from Ministry of Health Gezira State. Inform consent, patients' privacy and confidentiality were assured and patients are not going to pay for tests done for this study, all investigation results were freely and timely given back to the doctor and patients. All women with organic gynecological (masses, fibroid, endometrialpolyps, malignancy) and obstetrical causes (pregnancy) were excluded from this study.

Blood sample collection and VWF antigen ELISA assay

A total of 2.5 ml of blood sample were taken into EDTA anticoagulant tubes from each participant, centrifuged for 15.0 minutes at 1000 X g 2- 8 ℃, and plasma was taken into another tube for Von Will brand factor antigen screening using ELISA Kits (CUSABIO Company).

Test procedure

VWF antigen assay, is quantitative measurement that measure the VWF amount using enzyme linked immune sorbent assay (ELISA) Kits, were purchased from CUSABIO Company. This kits based in the biotin double antibodies technology to assay the human von Willebrand factor Ag (vWF Ag). The wells are pre-coated with vWF monoclonal antibody. Standard solution were prepared by doubling dilution of the original standard concentration by the standard dilution in small tubes with standard No .7 has 400 ng/ml, standard No .6 has 200 ng/ml, standard No.5 100ng/ml. standard No .4 has 50 ng/ml ,standard No 3 has 25 ng/ml, standard No2 has 12.5 ng/ml ,standard No .1 has 6.25 ng/ml concentration .

Assay procedure of VWF Ag

Firstly all reagents were prepared; working standard and samples as directed by manufacture.

Then the assay layout sheet was used to determine the number of wells to be used.

After that 0.100 ml of standard and sample were added per well and covered with the adhesive strip provided. Then incubated for 2 hours at 37˚C. A plate layout was provided to record standard and samples assayed.

Then 0.100 ml of Biotin-antibody (1x) (Warmed up to room temperature and mixed gently until solution appears uniform) was added to each well and covered with a new adhesive strip and incubated for one hour at 37˚C.

After that each well was aspirated and washed, then repeated two times for a total of three washes. Then washed by filling each well with washing buffer (0.200ml) using a squirt bottle, multi –channel pipette , manifold dispenser, or auto washer, and we let it stand for 2 minutes to complete removal of liquid at each step for good performance . After the last wash, we removed any remaining wash buffer by aspirating or decanting.

Then 0.100 ml of HRP- avidin (1x) was added to each well. And covered with a new adhesive strip and incubated for 1 hour at 37˚ C. The aspiration/ wash process was repeated for five times. Then 90 microlitre of TMB substrate was added to each well and incubated for 15-30 minutes at 37 ˚C. Lastly 0.050 ml of stop solution was added to each well and gently taped through mixing.

Then the optical density of each well was determined within 5 minutes, using amicroplate reader set to 450 nm.

Complete blood count

Sysmex XE-2100 (TOA Medical Electronics, Kobe, Japan) was used to measure Hematological parameters (WBCs and RBCs, Hb, platelets and RBCs indices).

Bleeding time Assay

After placing of sphygmomanometer cuff around the patient arm above the elbow, inflated to 40mm Hg, and kept pressed, an area around was cleaned with 70% ethanol and allowed to dry. By using a commercial template device two standard longitudinal incisions were made. With the edge of a filter paper, at 15 seconds intervals blood was blotted off from the cut. When bleeding has ceased, the edges of the incision was opposed carefully and an adhesive strip was applied to reduce the risk of keloid formation and an unsightly scar (Barbara *et al*., 2011)

Factor VIII assay

A total of 0.1ml of factor VIII deficient plasma was taken then 0.1 ml of APTT reagent was added, and shacked briefly and incubated for 3 min at 37℃, then 0.1ml of CaC_{l2} 0.25 mmo/L solution was carefully added. The point of coagulation was determined. APTT time against the plasma concentration was plotted in comparison with calibration curve to give concentration of factor VIII in percent.

Luteinizing hormone (LH) assay

A total of 0.050 ml of appropriate patients and control serum were assigned into wells. 0.100 ml of LH-Enzyme reagent (Fortress Diagnostics Limited, United Kingdom) were added to all wells. micro plate were swirled gently for 20-30 seconds, and incubated for 60 minutes at room temperature. the content of the micro plate were discarded by aspiration. 0.30 ml of washing buffer were added and incubated for 15 minutes, lastly, 0.050 ml of stop solution was added and gently mixed for 15 seconds, the absorbance in each well at 450 nm was read using reference wavelength of 620-630 nm.

Follicle-stimulating Hormone (FSH) assay

After bringing all reagents and serum to room temperature we pipette 0.05 ml of appropriate patients and controls serum into assigned wells, 0.1 ml of FSH-Enzyme reagent (Fortress Diagnostics Limited, United Kingdom) were added to all wells. After that we swirl the microplate gently for 20-30 seconds and incubated for 60 minutes at room temperature. After that 0.3 ml of wash buffer was added, lastly, 0.050 ml of stop solution was added and gently mixed for 15 seconds, then the absorbance was read at 450 nm, using reference wavelength of 620-630 nm.

Progesterone assay

After bringing all reagents and samples to room temperature the progesterone was assayed according to manufactures guidelines (Fortress Diagnostics Limited, United Kingdom).

Before proceeding with assay, all reagents, serum references and control brought to room temperature. Control and patient specimen were assayed in duplicate, 0.050 ml of the appropriate serum reference, control or specimen into the assigned wells. After that 0.050 ml of progesterone enzyme reagent was added to all wells. Then we swirled the micro plate gently for 10-20 seconds to mix. Then we added ml of progesterone Biotin reagent to wells. After that we swirled the micro plate gently for 10-20 seconds to mix, than covered and incubated for 60 minutes at room temperature. Then the contents of the micro plate were discarded by aspiration. After that 0.350 ml of wash buffer was added, repeated two additional times for a total of three washes. Then 0.100ml of working substrate solution was added to all wells. Then incubated at room temperature for 20 minutes, lastly 0.050 ml of stop solution were added to each well and gently mixed for 15-20 seconds. And the absorbance in each well was recorded at 450 nm using reference wavelength of 620-630nm

Results

Seventy six women with menorrhagia were enrolled in this study, the age groups of the study population were: (51%) aged from 14 – 30 years and (49%) aged from 31 – 45 years with mean 25.0 (+/- 3.0) years. About 6.5% of female have a family history of menorrhagia and 93.5% were found with no family history. About 5.3% of participants has family history of bleeding and 93.5% has no family history.

The result showed that there was significance difference between the mean of hemoglobin (9.340 +/- 2.066) with ($p = 0.019$), platelets count mean (311.29 +/- 30.000) with ($p = 0.027$), bleeding time (4.89 +/- 1.856) minutes with ($p =0.005$), APTT(38.822 +/- 8.8926) seconds with ($p =0.002$), LH ($P =0.075$), FSH ($P =0.000$), progesterone ($p =0.03$) and VWF Ag ($p =0.000$) and no significance difference between Factor VIII ($P =0.291$) in patients and controls (Table 1). The results of this study revealed that the main possible causative agents of menorrhagia were Von Willebrand factor antigen (5.3%), abnormal platelets aggregation (3.9%) (Platelets dysfunction), hormones abnormalities (1.6%), low platelets count (thrombocytopenia) (3%), 86.2% others causes (unknown) (Table 2).

Table 1: Difference between different parameters during and post menstruation in cases and controls

Parameter			N	Mean	Std. Deviation	P. value
Bleeding time	During	Cases	76	4.96	1.75	0.000
		Control	25	2.80	1.60	
	Post	Cases	76	4.10	1.48	0.000
		Control	25	1.90	.93	
Hb	During	Cases	76	9.35	2.19	0.005
		Control	25	11.31	1.26	
	Post	Cases	76	11.76	1.64	0.0713
		Control	25	11.89	1.24	
APTT	During	Cases	76	39.44	7.968	0.000
		Control	25	38.64	4.495	
	Post	Cases	76	35.07	5.594	0.000
		Control	25	37.14	4.649	
Platelets	During	Cases	76	274.59	105.54	0.073
		Control	25	300.52	61.00	
	Post	Cases	76	327.99	98.91	0.136
		Control	25	299.28	54.87	
Progesterone	During	Cases	76	.8400	0.30	.03424
		Control	15	.7733	0.33	
	Post	Cases	76	15.27	8.16	.93550
		Control	15	14.77	7.80	
FSH	During	Cases	76	8.07	5.55	0.000
		Control	15	6.93	2.03	
	Post	Cases	76	5.98	3.02	0.000
		Control	15	4.70	1.55	
VwF Ag	During	Cases	76	6.57	2.94	0.000
		Control	15	7.76	2.40	
	Post	Cases	76	7.58	3.20	0.000
		Control	15	8.83	2.44	
LH	During	Cases	76	8.05	7.96	0.075
		Control	15	5.42	2.08	
	Post	Cases	76	9.67	3.23	0.208
		Control	15	9.9673	3.45	
FVIII	During	Cases	76	56.53	15.06	0.291
		Control	15	63.68	18.22	
	Post	Cases	76	56.47	18.09	0.867
		Control	15	58.68	15.77	

Table 2: Number and percentage of the patients with menorrhagia and abnormal parameters

cause	Number of patients	percentage
Low VWF Ag	4	5.30%
Abnormal platelets aggregation	3	3.9%
Low Platelets count (Thrombocytopenia)	2	3.0%
Hormonal abnormalities (LH, FSH, VWF, Ag and progesterone)	1	1.6%
Others causes	66	86.2%
Total	76	100

Discussion

Menorrhagia is gynecological conditions which concern with excessive uterine bleeding occurring at regular intervals, or prolonged bleeding more than seven days, substantially decreases women's quality of life, social awareness, anemia, sexual problems, and time off work.

About 6.5% of female have a family history of menorrhagia and 93.5% were found with no family history, this result agree with study done in Egypt found that 90% of participants has no family history and 10% have family history (Sheriff *et al*., 2014).

According to results of this study the mean of bleeding time was (4.31 ± 1.59) minutes during menstruation and (4.829 ± 1.85) minutes post menstruation, the study also showed there was significant difference in bleeding time during and post menstruation cycle (P value = 0.005). The study showed there was significant difference in bleeding time between cases during menstruation and controls (P value = 0.00) and between post menstruation and control (P value = 0.00), this result agree with study done in Sudan found that significant difference in bleeding time (P value 0.000) (Ibrahim *et al*., 2018).

The mean of APTT in this study group was (38.8 ± 8 seconds), the result of APTT of cases also showed significant difference when compared with control subject (P value=0.029) this result agree with study done by (Ahmed, 2018)).

The study also showed the mean of hemoglobin concentration during menstruation was 9.3 g/dl and in post menstruation was 11.7 g/dl, when comparing the hemoglobin concentration between cases during menstruation and control subject the result showed significant differences (P value = 0.005) and there was no significant differences in cases post menstruation (P value = 0.713) (table 4.2), this result agree with result done by Dilley (Javaid *et al*., 2007).

In this study, about 3% of female during menstruation cycle showed low platelet count, this may be due to the changes of the level of hormone during menstrual cycles which affect the coagulation cascade by producing parallel change in prothrombtic tendency and fibrinolytic activity.

On the other hand, the study showed significant differences of the means of the FSH level in female during and post menstruation when compared with control subject (P value = (0.000) . Also in this study the percentage of FSH, LH and progesterone were: 3.2 %, 3.0 % and 1% during menstruation and the percentage decrease to 0.8% and 0% post menstruation, this may be due to that in the letual phase in the corpus luteum secretes progesterone for only 11 days which is causes inhibition of FSH and LH.

The study also showed the mean of Von Will brand antigens was (6.56 ng/ml) during menstruation and (7.57 ng/ml) post menstruation with significant differences when compared each group with control subject (P value= 0.000). The study also showed that about 6.8% of female during menstruation had low vWF Ag level and the percentage decreased to 5.3% in female post and during menstruation this result may be due to low level of hemostatic factors during menstrual cycle specially in early follicular phase which lead to acquired vWD and fibrinogen and fibrinogen degradation products increase in letual phase when compared with follicular phase.

Conclusion

This study concluded that the possible causative agents of menorrhagia in Gezira state were Von Willebrand factor antigen (5.3%), abnormal platelets aggregation

(3.9%) (Platelets dysfunction), hormones abnormalities (1.6%), low platelets count (thrombocytopenia) (3%), and the rest (86.2%) with other causative agents.

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