Measurement of Bleeding Time in Women with Menorrhagia, Wad Medani Maternity Teaching Hospital, Gezira state, Sudan (2017)

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B.S.c in Haematology, Sudan University of Science and Technology (2007)

A Dissertation

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Faculty of Medical Laboratories Science

May, 2018
Measurement of Bleeding Time women with Menorrhagia, Gezira state, Sudan (2018)

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Declaration

I authorized that my dissertation “Measurement of Bleeding Time in women with Menorrhagia, Wad Medani Maternity Teaching Hospital Gezira state, Sudan (2017)” submitted by me, under the supervision of Dr. Sanaa Elfatih Hussein and Dr. Albadawi Abd Elbagi Talha for the partial fulfillment for the award of Master degree in Medical Laboratory Sciences in Hematology & Immunohaematology. University of Gezira Faculty of Medical Laboratory Sciences Department of Haematology & Immunohaematology; Wad-Medani, Sudan and this is original and it was not submitted in part or in full, in any printed or electronic means, and is not being considered elsewhere for publication.

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Azza Abd elrahim Mohamed

Date: / / 2018
Dedication

TO....

my great mother
Acknowledgments

First of all, thank to Allah for giving me health and strength for completing this dissertation. Then, thanks to my supervisors Dr. Sanaa Elfatih Hussein and Dr. Albadawi Abd Elbagi Talha whom follow and support me in all stages of this dissertation. and gratitude to all who helped me in completing this dissertation and provided me with information and special thanks to Ustaz. Yousif Abd Alhameed, Mohamed Yousif for helping for completing this dissertation. Finally I would like to thanks everyone that helped me and not mentioned in this acknowledgment.
Measurement of Bleeding Time in Women with Menorrhagia in Wad Medani Maternity Teaching Hospital, Gezira state, Sudan (2017)

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Abstract

Menorrhagia is excessive uterine bleeding occurring at regular intervals or prolonged uterine bleeding lasting more than seven days. This is case control study was conducted in Gezira state aimed to measure the bleeding time in women with menorrhagia and compare to healthy normal females. A study questioner was designed to collect the information of twenty five women with menorrhagia were included in this study. Bleeding Time was performed using Bleeding Time technique during menstruation cycle and after fourteen days from the last menstruation cycle, the study results showed that a percentage of population study according to family history of bleeding was 76% no history, 24% have bleeding history, history of Epistaxis and Hematoma 8% yes 92% have no history. The fist onset of menorrhagia 76% in age between 14-25 year, 8% in age between 26 to 35 and 16% in age more than 36 years old their age mean was (27.96 ± 9.38) years and with range (14 – 46) years, 48% were married and 52% single ladies with menorrhagia. The bleeding time was (2.9 ± 1.5) min with range (1.3 – 6.3) min during menstruation in study group and (2.9 ± 1.5) min with range (1.1 – 6.2) min post menstruation in control group, the means of the bleeding time post menstruation cycle was (3.0 ± 1.5) min with range (1.5 – 6.7) min post menstruation sample in study group and (2.3 ± 1.0) min with range (1.0 – 4.2) min for post menstruation sample in control group. There were no significant differences between study population and control groups in during menstruation cycle sample (P= 0.690) and there were significant differences between pre and post menstruation sample in study population (P= 0.000). The study recommended that Bleeding Time should be used as routine test for menorrhagia patients to evaluate the bleeding tendency. Further study should be done to determine the underline cases of prolonged Bleeding Time before surgical intervention.
قياس وقت النزيف في النساء المصابات بغازارة الطمث، مستشفى ود مدني التعليمي للنساء والتوليد، ولاية الجزيرة 2018

عازه عبد الرحيم محمد

ملخص الدراسة

غازارة الطمث هو نزف طبيعي مفرط يحدث على فترات منتظمة أو نزف طبيعي مطول يستمر لأكثر من سبعة أيام. له تأثير سلبي على نوعية الحياة المادية والاجتماعية والعاطفية للممرأة. هذه هي دراسة الحالات التي أجريت في ولاية الجزيرة بهدف قياس وقت النزيف عند النساء اللواتي يعانين من غازارة الطمث ومقارنتها مع الإناث الأصحاء. أجريت هذه الدراسة في خمس وعشرين أهليًا لديها غازارة الطمث وسجلت المعلومات والضغوط باستخدام الاستبان. تم إجراء وقت النزف باستخدام تفكيك زمن النزيف أثناء الدورة الشهرية وبعد انتهاء الدورة. أظهرت نتائج الدراسة أن نسبة 76% لديهم تاريخ عائلي بالنزيف و24% ليس لديهم تاريخ مرضي. تاريخ الورم الذري والرعاش 8% نعم و92% ليس لديهم. بداية الغازارة الطمث كانت 76% في العمر بين 14 - 25 سنة 8% في العمر بين 26 و35 و16% في عمر أكبر من 36. كان متوسط عمر النساء (96 ± 38) سنة ونسبة (14 - 46) سنة. وقارة زمن النزيف (2.9 ± 1.5) دقيقة مع المدى (1.3 - 6.3) دقيقة خلال عينة الحبيض في مجموعة الحالات و (2.9 ± 1.5) دقيقة مع المدى (1.1 - 6.2) دقيقة لعينة ما بعد الطمث في مجموعة التحكم، كانت وثائقي وقت النزيف لدورة ما بعد الدورة الشهرية (3.0 ± 3.1) دقيقة مع المدى (1.5 - 10.1) دقيقة عينه ما بعد التعيين في مجموعة الحالات و (0.1 ± 7.5) دقيقة مع المدى (0.0 ± 10.1) دقيقة عينه ما بعد التشخيص في مجموعة المرضى. لم تكن هناك فروق ذات دلالة إحصائية بين مجموعة الحالات والمجموعة الضابطة خلال عينة الدورة الشهرية. (P = 0.690) وكذلك هناك فروق ذات دلالة إحصائية بين عينة ما قبل وبعد الحيض في مجموعة الحالات (P = 0.000). خصصت الدراسة إلى وجود زيادة زمن النزيف عند بعض النساء المصابات بغازارة الطمث. أوصت الدراسة بأجراء اختبارات معقولة مختصرة لمعرفة أسباب زيادة زمن النزيف. كما توصي بعمل اختيار زمن النزف عند هولاء المريضات خاصة عند التدخل الجراحي.
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<tr>
<td>AUB</td>
<td>Abnormal Uterine Bleeding</td>
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<td>FSH</td>
<td>follicle-stimulating hormone</td>
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<td>heavy menstrual bleeding</td>
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<td>GP</td>
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<td>molecular weight</td>
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<td>Nitric oxide</td>
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<td>GMP</td>
<td>guanosine monophosphate</td>
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<td>SPSS</td>
<td>Statistical Package for Social Science</td>
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<td>DNA</td>
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CHAPTER ONE
INTRODUCTION

1.1 Introduction:
Normal menstrual cycle is the presence of regular vaginal bleeding. This occurs as a result of
the shedding of the endometrial lining following failure of fertilization of the oocyte or
failure of implantation. The cycle depends on changes occurring after puberty within the
ovaries and fluctuation in ovarian hormone levels, which are themselves controlled by the
pituitary and hypothalamus within the hypothalmo–pituitary–ovarian axis (HPO).
In situations of disorders of sexual development or hormonal abnormalities, menstruation
may not begin.(Kenny and Bickerstaff, 2017)
Heavy menstrual bleeding, or menorrhagia, is defined as excessive menstrual blood loss that
occurs alone or in combination with other symptoms and has a negative impact on woman’s
physical, social, emotional, and material quality of life. Approximately 30% of women are
negatively affected by menorrhagia during their reproductive years (Qiu et al., 2014)
Abnormal uterine bleeding is a common problem in the adolescent population, where it
affects up to 37% of teenage girls(Amesse et al., 2013). Established risk factors for
menorrhagia include increased age, premenopausal leiomyomata, and endometrial polyps.
Parity, body mass index, and smoking are not risk factors. For some women, a cause of
menorrhagia is not identified.(Apgar et al., 2007)

Thrombocytes are derived from fragmentation of precursor megakaryocytes with average
lifespan of platelet is 5 to 9 days .They circulate in the blood of mammals and are involved
hemostasiss leading to formation of blood clots .if the number of platelet is too low or too
high it cause thrombocytopathy which could be either low number of platelets called
thrombocytopenia, a decrease in function called thrombasthenia . and increase in number of
platelets known thrombocytosis (O'connell et al., 2008).

Platelets are integral components of primary homeostasis. Upon vascular injury, Von
Willebrand factor (VWF) mediates platelet adherence to the exposed sub endothelial matrix.
Platelets are then activated and secrete the contents of their alpha (α) and dense (δ) granules
and other factors, i.e., thromboxane A2, platelet activating factor. The secreted factors in turn
interact with specific platelet receptors to mediate activation and recruitment of additional
platelets that bind to the adhered platelets, forming aggregates that lead to the formation of a
hemostatic plug. Inherited Platelet function disorders PFDs can be classified according to
their functional defects, although clear distinctions are not always evident. Defects can involve platelet secretion (e.g. α-, δ-granule storage pool deficiencies); signal transduction pathways (e.g. arachidonic acid, thromboxaneA2); signal transduction receptors (e.g., thromboxane A2, collagen); and platelet adhesion receptors (e.g., glycoprotein Ia/IIa). Of these, the dense (δ) granule storage pool deficiency (SPD) is the most common (Amesse et al., 2013)

Abnormalities of platelet function, such as von Willebrand’s disease, appear to be more prevalent in women with menorrhagia than in the general population. The prevalence of von Willebrand’s disease in women with menorrhagia varies from 5 to 24 percent. There are no data suggesting that a lower quality of life occurs more commonly in women with menorrhagia and von Willebrand’s disease than in those with menorrhagia alone. (Apgar et al., 2007)

Abnormal uterine bleeding is a common problem in the adolescent population, where it affects up to 37% of teenage girls. The bleeding etiology in this age group is often attributed to anovulatory menstrual cycles subsequent to immaturity of the hypothalamic-pituitary-ovarian axis. However, in nearly one half of all cases a definitive etiology remains elusive. Hemostatic disorders such as von Willebrand disease (VWD) and single coagulation factor deficiencies have been implicated as important etiologies for many years. Platelet function disorders (PFDs)—a heterogeneous group of inherited, qualitative platelet defects—have recently emerged as a frequent, equally important cause of abnormal uterine bleeding in adolescents. In this population subset, PFDs often manifest as heavy menstrual bleeding (HMB), formerly termed menorrhagia, defined as menstrual blood loss >80mL per menses. However, platelet dysfunction-associated HMB has not been methodically analyzed in adolescents, and there is limited published objective data on the disorder. For the last decade, a total of only 2 communications have examined in detail the clinical and laboratory features of platelet dysfunction-associated HMB in teenagers, and for both studies, data was obtained from a total of 10 adolescents combined. Indeed, most reports on HMB intermixed demographic data from adolescents with PFDs with either similarly affected adults or adolescents having disparate hematologic disorders, without exclusively evaluating adolescents with PFDs and HMB. Other reports and case series were predominately composed of patients diagnosed with syndromic platelet disorders, e.g., Bernard Soulier syndrome, Glanzmann’s thrombasthenia, or idiopathic thrombocytopenic purpura (Amesse et al., 2013)
1.2 Justification:
Menstrual problems are likely to be worse in women with bleeding disorders, menstrual bleedings were reported among 10%-70% of women with other bleeding disorders. Underlying bleeding disorders are generally missed and ignored in diagnostic criteria of menorrhagia to improve quality of life. This study was done to screen of bleeding disorders in young women with menorrhagia.

1.3 Objectives:

1.3.1 General Objectives:

To measure the Bleeding Time in women with menorrhagia in Gezira state.

1.3.2 Specific Objectives:

- To estimate the Bleeding Time during and post menstrual phase in women with heavy menstruation as case group.
- To compare Bleeding Time during menstrual phase between women with heavy menstruation and control group.
Chapter Two

2. Literature Review

2.1 Menstrual Cycle

The menstrual cycle consists of two phases of parallel events occurring at the ovaries and endometrium. Within the ovaries, these events are known as the follicular and luteal phases, while the concurrent endometrial events are known as the proliferative and secretory phases (Kenny and Bickerstaff, 2017)

2.1.1 The Follicular Phase

The follicular phase begins with the onset of menses and ends on the day of luteinizing hormone LH surge. Early in the follicular phase, the ovary secretes very little estrogen or progesterone. A rise in follicle-stimulating hormone FSH, however, stimulates estrogen production. The estrogen secreted by the developing follicle within the ovary stimulates uterine epithelial cells, blood vessel growth, and endometrial gland development to increase the thickness of the endometrium. The intense secretory capacity of the uterine glands provides a secretion that aids the implantation of the embryo (Bishop et al., 2010).

2.1.2 The Luteal Phase

Estrogen levels peak 1 day before ovulation, at which point a positive feedback system results in an LH surge. The start of the luteal phase is marked by the extrusion of the ovum approximately 36 hours after this LH surge with subsequent luteinization of the Graafian follicle to form the corpus luteum. The corpus luteum secretes progesterone to aid in the implantation of the embryo. In the absence of fertilization, a gradual decline in the production of progesterone and estrogen by the corpus luteum there is a loss of endometrial blood supply; this results in shedding of the endometrium approximately 14 days after ovulation occurred. The typical duration of menstrual bleeding is 3–5 days, with blood loss averaging 50 mL. Onset of menses marks the end of the luteal phase (Bishop et al., 2010).

2.2 Menstrual Cycle Abnormalities

The menstrual cycles ranges from 25 to 35 days, with an average 28-day duration. The average age of menopause in the United States is between 45 and 55 years of age with the median at age (Bishop et al., 2010).

Menorrhagia is defined as excessive cyclic uterine bleeding that occurs at regular intervals over several cycles, or prolonged bleeding that lasts for more than seven days (Apgar et al., 2007).
Menorrhagia is defined as a menstrual blood loss of more than 80 mL per menstrual cycle. A variety of organic endocrine, gynecologic, or other systemic causes may be responsible for menorrhagia. Menstrual problems are likely to be worse in women with bleeding disorders, as they are more likely to have heavy and painful menstrual periods and ovulation bleeding and pain. The excessive blood loss can cause anemia and tiredness., the most common inherited bleeding disorder, were reported to have heavy menstrual bleeding. Heavy menstrual bleedings were reported among 10%-70% of women with other bleeding disorders (Kılıç et al., 2013).

Underlying bleeding disorders were generally missed due to unavailability of detailed coagulation assays in routine laboratory procedures and lack of hematology consultation. The aim of this study was the screening of bleeding disorders in adolescents and young women presenting with menorrhagia. (Kenny and Bickerstaff, 2017)

2.3 The etiology of menorrhagia.

may be hormonal or structural, with common causes:

2.3.1 Fibroids: 30% of HMB is associated with fibroids.

2.3.2 Adenomyosis: 70% of women have Abnormal Uterine Bleeding AUB/HMB.

2.3.3 Endometrial polyps.

2.3.4 Coagulation disorders (e.g. von Willebrand disease).

2.3.5 Pelvic inflammatory disease (PID).

2.3.6 Thyroid disease.

2.3.7 Drug therapy (e.g. warfarin).

2.3.8 Intrauterine devices (IUDs).

2.3.9 Endometrial/cervical carcinoma. (Kenny and Bickerstaff, 2017)

2.4 Platelets:

Platelets are a nucleate blood cells that circulate in amounts of 150 to 400 × 10⁹/L, with mean counts slightly higher in women than in men and it trigger primary homeostasis on exposure to endothelial, sub endothelial, and plasma procoagulants in blood vessel injury. (Bain et al., 2011)

Platelets are produced in the bone marrow by fragmentation of the cytoplasm of megakaryocytes which is one of the largest cells in the body. The precursor of the megakaryocyte-the megakaryoblast-arises by a process of differentiation from the haemopoietic stem cell The megakaryocyte matures by endomitotic synchronous replication which means that they possess multiple chromosome copies within a single cell (i.e. DNA
replication in the absence of nuclear or cytoplasmic division) enlarging the cytoplasmic volume as the number of nuclear lobes increase in multiples of two. At a variable stage in development, most commonly at the eight nucleus stage, the cytoplasm becomes granular. Mature megakaryocytes are extremely large, with an eccentric placed single lobulated nucleus and a low nuclear to cytoplasmic ratio. Platelets form by fragmentation of megakaryocyte cytoplasm, approximately each megakaryocyte giving rise to 1000-5000 platelets. The time interval from differentiation of the human stem cell to the production of platelets averages approximately 10 days. Thrombopoietin is the major regulator of platelet production and is constitutively produced by the liver and kidneys. Thrombopoietin increases the number and rate of maturation of megakaryocytes via c-Mpl receptor. Platelet levels start to rise 6 days after the start of therapy and remain high for 7-10 days. Unfortunately, thrombopoietin is not available from the circulation. Therefore, levels are high in thrombocytopenia as a result of marrow aplasia and vice versa. The normal platelet count is approximately 250 x 10^9/L (range 150-400 x 10^9/L) and the normal platelet lifespan is 7-10 days. Up to one-third of the marrow output of platelets may be trapped at anyone time in the normal spleen but this rises to 90% in cases of massive splenomegaly for routine clinical practice. Platelets also have c-Mpl receptors for thrombopoietin and remove it from the circulation (Hoffbrand et al., 2006).

### 2.4.1 Platelet structure:

Platelets are extremely small and discoid, 3.0 x 0.5 m in diameter, with a mean volume 7-11 fL. The ultrastructure of platelets is represented in The glycoproteins of the surface coat are particularly important in the platelet reactions of adhesion and aggregation which are the initial events leading to platelet plug formation during haemostasis. Adhesion to collagen is facilitated by glycoprotein la (Grla). Glycoproteins Ib (defective in Bernard-Soulier syndrome) and IIb/IIIa (defective in thrombasthenia) are important in the attachment of platelets to von Willebrand factor (VWF) and hence to vascular subendothelium where metabolic interactions occur. The binding site for IIb/IIIa is also the receptor for fibrinogen which is important in platelet-platelet aggregation. The plasma membrane invaginates into the platelet interior to form an open membrane (canalicular) system which provides a large reactive surface to which the plasma coagulation proteins may be selectively absorbed. The membrane phospholipids (previously known as platelet factor 3) are of particular importance in the conversion of coagulation factor X to Xa and prothrombin (factor II) to thrombin (factor IIa) (Hoffbrand et al., 2006).
The platelet contains three types of storage granules: dense, α and lysosomes. The more frequent specific α granules contain a heparin antagonist, platelet-derived growth factor (PDGF), thromboglobulin, fibrinogen, VWF and other clotting factors. Dense granules are less common and contain adenosine diphosphate (ADP), adenosine triphosphate (ATP), 5-hydroxytryptamine (5-HT) and calcium. Lysosomes contain hydrolytic enzymes and peroxisomes contain catalase. During the release reaction described below, the contents of the granules are discharged into the open canalicular system.

Platelets are also rich in signaling and cytoskeletal proteins which support the rapid switch from quiescent to activation that follows vessel damage (Hoffbrand et al., 2006).

### 2.4.2 Platelet antigens:

Several platelet surface proteins have been found to be important antigens in platelet-specific autoimmunity and they have been termed human platelet antigens (HPA). In most cases, two different alleles exist, termed a or b alleles (e.g. HPA-la). Platelets also express ABO and human leukocyte antigen (HLA) class I but not class II antigens (Hoffbrand et al., 2006).

### 2.4.3 Platelet function:

The main function of platelets is the formation of mechanical plugs during the normal haemostatic response to vascular injury. In the absence of platelets, spontaneous leakage of blood through small vessels may occur. The immobilization of platelets at the sites of vascular injury requires specific platelet-vessel wall (adhesion) and platelet-platelet (aggregation) interactions. The blood flow conditions determine the specific receptor ligand interactions (Hoffbrand et al., 2006).

### 2.4.4 Platelet adhesion and activation:

Following blood vessel injury, platelets adhere to exposed sub endothelial matrix proteins via specific adhesive glycoprotein's (GP). Under condition high shear, e.g. arterioles, the exposed sub endothelial matrix is initially coated with VWF multitime. The platelets than make contact with VWF via GPIb-XI-V complex on platelets. This initiates platelet rolling in the direction of blood flow over the Exposed VWF with activation of GPIIb/IIIa receptor. Firm adhesion is established by the slower be stronger interaction of other glycoproteins including activated GPIIb/IIIa with VWF and GPVI integrin α1 / P2 with collagen and other component of the sub endothelial matrix. Under static or low shear conditions, platelets adhere predominant to collagen of the sub endothelium. Collagen bi initially to GPⅠa/Ⅱa, cross-links many of these integrin molecules, and in this way activates platelets.
This ligand receptor binding results in a complete cascade of signals which result in platelet activation. The events that follow are shape change and spreading, activation of GPIIb/IIla and granulesenetic Platelets become more spherical and extrude long pseudopodia which enhance platelet vessel wall and platelet-platelet interaction. The end result of spreading is a flattened spread out platelet with granules and organelles in the centre, resulting in a characteristic fried egg appearance. These changes are brought about by the action cytoskeleton. The granules are secreted from the centre of the cell(Hoffbrand et al., 2006).

2.4.5 Von Willebrand factor:

VWF is involved in platelet adhesion to the vessel wall and to other platelets (aggregation). It also carries factor VIII and used to be referred to as factor VIII related antigen (VIII-Rag). It is a large cysteine-rich glycoprotein, with multimers made up on average of 2-50 subunits, with a molecular weight (MW) of 0.8-20 x 106. VWF is encoded by a gene on chromosome 12 and is synthesized both in endothelial cells and megakaryocytes, and stored in Weibel-Palade bodies and platelet a granules respectively. Plasma VWF is almost entirely derived from endothelial cells, with two distinct pathways of secretion. The majority is continuously secreted and a minority is stored in Weibel-Palade bodies. The stored VWF can rise the plasma levels and it can be released under the influence of several secretagogues, like stress, exercise, adrenaline and infusion of desmopressin (1-deamieno-8-D-arginine vasopressin, DDAVP). The VWF released from Weibel-Palade bodies is in the form of large and ultra large multimers ,the most adhesive and reactive form of VWF. They are in turn cleaved in plasma to monomeric VWF and smaller multimers by the specific plasma metalloprotease, ADAMTS-13(Hoffbrand et al., 2006).

2.4.6 Platelet aggregation:

It is characterized by cross-linking of platelets through active GPIIb /IIIa receptors with fibrinogen bridges. A resting platelet has about 50-80 000 GPIIb/IIla receptors, which do not bind fibrinogen. VWF or other ligands. Stimulation of a platelet leads to an increase in GPIIb/IIla molecules, due to binding of a-granule membrane (rich in receptors) with the plasma membrane, activation of surface-exposed GPIIb /IIla, enabling platelet cross-linking with fibrinogen bridges. Binding brings about molecular conformational changes resulting in a firm connection and further activation of the platelet. Platelet release reaction and amplification. Primary activation by various agonists induces intracellular signaling, leading to the release of alfa and dense granules. a-Granule contents play an important role in platelet aggregate formation and stabilization and, in addition, the ADP released from dense
granules plays a major positive feedback role in promoting platelet activation. TXA2 is the second of the two major platelet positive feedback loops important in secondary amplification of platelet activation to firm a stable platelet aggregate. It is formed de novo upon activation of cytosolic phospholipase A2 (PLA2) which is the rate limiting step. This liberates arachidonic acid from the membrane phospholipids, and is metabolized by cycloxygenase to TXA2. It is a labile substance and lowers platelet cyclic adenosine monophosphate (cAMP) levels and initiates the release reaction. Thromboxane A2 not only potentiates platelet aggregation, but also has powerful vasoconstrictive activity. The release reaction is inhibited by substances that increase the level of platelet cAMP. One such substance is the prostaglandin prostacyclin (PGI2) which is synthesized by vascular endothelial cells. It is a potent inhibitor of platelet aggregation and prevents their deposition on normal vascular endothelium. (Hoffbrand et al., 2006)

2.4.7 Clot formation and retraction:
The highly localized enhancement of ongoing platelet activation by ADP and TXA2 results in a platelet plug large enough to plug the area of endothelial injury. In this platelet plug the platelets are completely degranulated and adherent to each other. This is followed by clot retraction which is mediated by GPIIb/IIIa receptors which link the cytoplasmic action filaments to the surface bound fibrin polymers. (Hoffbrand et al., 2006)

2.4.8 Platelet procoagulant activity:
After platelet aggregation and release, the exposed membrane phospholipid (platelet factor 3) is available for two reactions in the coagulation cascade. Both phospholipid-mediated reactions are calcium ion dependent. The first (tenase) involves factors IXa, VIIIa and X in the formation of factor Xa. The second (prothrombinase) results in the formation of thrombin from the interaction of factors Xa, Va and prothrombin (II). The phospholipid surface forms an ideal template for the crucial concentration and orientation of these proteins. (Hoffbrand et al., 2006)

2.4.9 Growth factor:
Platelet Derived Growth Factor, PDGF found in the specific granules of platelets stimulates vascular smooth muscle cells to multiply and this may hasten vascular healing following injury. (Hoffbrand et al., 2006)

2.4.10 Natural inhibitors of platelet function:
Nitric oxide (NO) is constitutively released from endothelial cells and also from macrophages and platelets. It has a short half-life of 3-5s. It inhibits platelet activation and promotes
vasodilatation. Prostacyclin synthesized by endothelial cells also inhibits platelet function and causes vasodilatation by raising cyclic guanosine monophosphate (GMP) levels. The transmembrane protein PECAM-1 is expressed also on endothelial cells. It is its own ligand and inhibits platelet activation by collagen. (Hoffbrand et al., 2006)

2.5 Previous studies

A study done by Philipp et al. 2003 in USA he found Seventy-four women (52 white, 16 black, six other) were studied. Bleeding time was prolonged in 23 women (31.5%). Maximal percent platelet aggregation was decreased with one or more agonists in 35 (47.3%) women (Philipp et al., 2003).

A study done by Oral et al., (2002) in Turkey he found the mean age of the patients was 13.9±1.6 (SD) years. A hematological abnormality that caused bleeding diathesis and acute menorrhagia was diagnosed in 7 of the 25 patients (28%). There were four cases of immune thrombocytopenic purpura, two cases of Van Willebrand disease and one case of acute promyelocytic leukemia. All seven patients with a coagulation disorder required blood transfusions and the mean hemoglobin level at presentation was 6.2 g/dl (Oral et al., 2002).

The study done by Rashmi et al., (2017) in from Northern India. he found Age of patients ranged from 13 years to 46 years. Eighteen patients had menorrhagia since menarche. Seven patients had family history of abnormal bleeding. Twenty three patients were found to have systemic haemostatic disorder (10 patients of Von Willebrand Disease (vWD), seven of Glanzmann-Thrombasthenia, one of Bernard- Soulier syndrome and five of immune thrombocytopenic purpura) (Kushwaha et al., 2017).
Chapter Three

3. Materials and Methods

3.1 Materials:

3.1.1 Study design:

This was a case control laboratory-based study.

3.1.2 Study area:

This study was conducted in Gezira State, Sudan, from September 2017 to March 2018. The Gezira state lies between latitudes (13-32 and 15-30) North and longitudes (22-32 and 20-34) East. It is bordered by Khartoum State to the North, Sinnar State to the South, Gadarif State to the East and White Nile State to the West. It has an area of 27,549 km². Total population is 2,796,330 in the census performed in 2008. Gezira state was inhabited by a mixture of races and tribes from inside and outside Sudan. The name comes from the Arabic word for Island. Wad Madani is the capital of the state. The Gezira is a well-populated area suitable for agriculture. The region has benefited from the Gezira Scheme, a program to foster cotton farming begun in 1925. At that time the Sinnar Dam and numerous irrigation canals were built. The Gezira became the Sudan's major agricultural region with more than 2.5 million acres (10,000 km²) under cultivation (Sudan.gov.sd, 2012).

3.1.3 Study period:

The study was done during the period between December 2017 – March 2018.

3.1.4 Study population and sample size:

Twenty-five Sudanese women with menorrhagia were included in this study.

3.1.5 Inclusion criteria:

- Sudanese women with age puberty age in Gezira state.
- Women diagnosed as menorrhagia.
3.1.6 Exclusion Criteria:

- Smoker’s women.
- Women with amenorrhea (lack of menstruation in the 3 months prior to the study)
- Women who use hormonal therapy.
- known cases of bleeding disorders.
- gynecological malignancy or fibroid.
- use of intra uterine device.
- liver disease and renal disease
- Use of anticoagulants within the last 2 months.
- use of non steroidal anti inflammatory agents and anti platelets drugs within 14 days of participation.

3.1.7 Data Collection:

Data were collected by using questionnaire.

3.1.8 Ethical approval:

Ethical approval was obtained from Faculty research committee.

3.1.9 Ethical permeation:

Ethical permeation was taken from Ministry of Health Authorities, Gezira state.

3.1.10 Ethical consents:

Written consent was taken from each participant.

3.2 Method of Bleeding Time:

3.2.1 principle:

A standard incision is made on the volar surface of the forearm and the time the incision bleeds is measured. Cessation of bleeding is dependent on an adequate number of platelets, the ability of the platelets to adhere to the sub endothelium directly and via adhesion molecules such Practical as VWF and fibrinogen. However the test has poor sensitivity for disorders such as VWD(Bain et al., 2011).

The bleeding time is a useful test for abnormal platelet function including the diagnosis of VWF deficiency. It will be prolonged in thrombocytopenia but is normal in vascular causes of abnormal bleeding. The test involves the application of pressure to the upper arm with a
blood pressure cuff, after which small incisions are made in the flexor surface forearm skin. Bleeding stops normally in 3.8 min. (Dacie and Lewis., 2009)

3.2.2 procedure:
Place a sphygmomanometer cuff around the patient's arm above the elbow, inflate to 40 mm Hg, and keep it at this pressure throughout the test. Clean the area with 70% ethanol and allow to dry. Choose an area of skin on the volar surface of the forearm that is devoid of visible superficial veins. Use a commercial template device to make one or two standard longitudinal incisions. If not available then press a sterile metal template with a linear slit 7–8 mm long firmly against the skin aligned along the long axis of the arm and use a scalpel blade with a guard so arranged that the tip of the blade protrudes 1 mm through the template slit. In this way make an incision 6 mm long and 1 mm deep. Modifications of the template and blade making two simultaneous cuts with a spring mechanism are commercially available. With the edge of a filter paper, at 15 sec intervals blot off the blood exuding from the cut. Avoid contact with the wound during this procedure because this may disturb the formation of the platelet plug. When bleeding has ceased, carefully oppose the edges of the incision and apply an adhesive strip to reduce the risk of keloid formation and an unsightly scar. (S. Lewis .,2006)

3.2.3 Normal Range :
Normal range is 2-7 min. An upper limit of 4 min has been reported in one study on men and women who had not used aspirin or other relevant drugs in the ten days before the test. Ideally, every laboratory should determine its own normal range and if possible ensure that the test is performed by the same operator. (S. Lewis .,2006)

3.3 Statistical analysis:
The data analyzed by using SPSS computer program version 16.
CHAPTER FOUR

Result and Discussion

4.1 Results:

A case control study was conducted during the period of December 2017 to March 2018. 25 women with menorrhagia and 25 healthy control subjects were conducted in the study,

Figure (4.1): Distribution of study population according to marital status:

From 25 study group 12 (48%) was a married and 13 (52%) were single
Figure (4.2): Distribution of study population according to history of Epistaxis:

Epistaxis and hematoma in this study found 2 (8%) yes of Epistaxis and hematoma and 23 (92%) have no bleeding disorder.
Figure (4.3): Distribution of study population according to family history of menorrhagia:

No family history 6 (24%) while 19 (76%) were found to have family history.
Figure (4.4): Distribution of study population according to Hematoma:

hematoma and Epistaxis in this study found 2 (8%) yes of Epistaxis and hematoma and 23 (92 %) have no bleeding disorder.
Age groups according onset of menorrhagia: 19 (76%) of women had menorrhagia at age 14 – 25 years, 2 (8%) in age 26-35 years, 4 (16%) more than 35 years.

Figure (4.5): Distribution study population according to first onset of menorrhagia/year:

Age groups according onset of menorrhagia: 19 (76%) of women had menorrhagia at age 14 – 25 years, 2 (8%) in age 26-35 years, 4 (16%) more than 35 years.
Figure (4.6):: Distribution of study population according to menstrual intervals (days):

The duration of menorrhagia/day between 7-10 days about 23 (92 %) while the others between 11 – 14 days about 1 (4 %) or more than 15 days about 1 (4 %).

Table 4-1: Means of bleeding time in the study population and control group during the menstruation cycle:

<table>
<thead>
<tr>
<th>Bleeding Time D</th>
<th>N</th>
<th>Mean/second</th>
<th>Std. Deviation</th>
<th>P. Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Study subject</td>
<td>25</td>
<td>174</td>
<td>92.999</td>
<td>0.690</td>
</tr>
<tr>
<td>Control</td>
<td>25</td>
<td>178</td>
<td>87.921</td>
<td></td>
</tr>
</tbody>
</table>

Table 4-2: Means of bleeding time in the study population during and post menstruation cycle:

<table>
<thead>
<tr>
<th>Bleeding Time</th>
<th>N</th>
<th>Mean/second</th>
<th>Std. Deviation</th>
<th>P. Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bleeding Time D</td>
<td>25</td>
<td>174</td>
<td>92.999</td>
<td>0.000</td>
</tr>
<tr>
<td>Bleeding Time P</td>
<td>25</td>
<td>177</td>
<td>89.334</td>
<td></td>
</tr>
</tbody>
</table>
4.2 Discussion:

Menorrhagia is gynecological conditions which concern with excessive uterine bleeding occurring at regular or irregular intervals, or prolonged bleeding more than seven days, substantially decreases women’s quality of life, social awareness, anemia, sexual problems, and time off work. This study was conducted to evaluate and compare of the bleeding time between the women with menorrhagia and normal healthy control.

The most cases were found to have no family history 6 (24%) while 19 (76%) were found to have family history. This agree with study done in Sudan (Mohamedahmed et al., 2015) he found 37 (74%) have no family history while 13 (26%) were found to have family history and quite lower than study done in Egypt (Sherif et al., 2014) whose found 82% of the participant hasn't family history.

In this study showed 2 (8%) has history of Epistaxis and hematoma and 23 (92%) have no bleeding disorder, this agree with study done in India (Kushwaha et al., 2017) Out of 104 patients, 32 patients has history of other bleeding tendencies associated with menorrhagia.

This study found onset of menorrhagia/year 14 – 25 frequency 19 (76%), 26 – 35 frequency 2 (8%) and more than 36 frequency 4 (16%).

Most cases were found to have duration of menorrhagia/day between 7-10 days about 23 (92%) while the others between 11 – 14 days about 1 (4%) or more than 15 days about 1 (4%). This is similar to study done in Sudan (Mohamedahmed et al., 2015) who reported the most cases were found duration of menorrhagia/day between 7-10 days about 43 (86%) while the others between 11 – 14 days about 3 (6%) or more than 15 days about 4 (8%).

Reading of the bleeding time was (2.9 ±1.5 ) min with range (1.3 – 6.3) min for during menstruation sample in cases group and (2.9 ±1.5 ) min with range (1.1 – 6.2) min for post menstruation sample in control group, the means of the bleeding time for post menstruation cycle was (3.0 ±1.5 ) min with range (1.5 – 6.7) min post menstruation sample in study group and (2.3 ±1.0 ) min with range (1.0 – 4.2) min post menstruation sample in control group.

The study result showed significant difference between the bleeding time during and post menstruation cycle sample (P = 0.000). This agreement with study done in Sudan (Abdalla, 2009) she found significant difference in bleeding time in woman (p value 0.000)
5.1 Conclusion:

Bleeding Time were prolonged in women during menstruation.

There were significant difference in Bleeding Time between study group and control.

5.2 Recommendation:

Bleeding Time should be used as routine test for menorrhagia patients to evaluate the bleeding tendency.

Further study should be done to determine the underline cases of prolonged Bleeding Time.
References:


Qiu, J., J. Cheng, Q. Wang and J. Hua (2014). Levonorgestrel-releasing intrauterine system versus medical therapy for menorrhagia: a systematic review and meta-

Appendix (1)

Questionnaire

Name ...........................................................................................................

NO…………………… Date ……/ ….. / ……………Time …………………

Age …………………. age group : 1. 14 – 30 ( ) 2. 31 – 46( )

Phone number ............................................................................................

Marital status single ( ) married ( )

Family history of bleeding yes ( ) No ( )

Patient bleeding of history yes ( ) No ( )

Epistaxis yes ( ) No ( )

Hematoma yes ( ) No ( )

Age of bleeding / years 14 – 25( ) 26 – 35 ( ) more than 36 ( )

Age of puberty 10 – 14 ( ) 15 – 25 ( )

Length of menstruation/ days 15 – 20 ( ) 20 – 25 ( )

25 – 28 ( ) 28 - 35 ( ) more than 36 ( )

Duration of menstruation / days 7 – 10 ( ) 11 – 15 ( ) more than 15 ( )

Results : ........................................................................................................

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