Assessment of plasma D.dimer Level in Women with Menorrhagia, Wad Medani Maternity Teaching Hospital, Gezira State, Sudan (2017)

Marwa Mohamed Hamed Ahmed

BSc. Collage of Medical Laboratory Sciences, University of Omdurman Islamic (2008)

A Dissertation

Submitted to the University of Gezira in Partial Fulfillment of Requirement for the Award of Master Degree of Sciences in Heamatology and Immunoheamatology

Faculty of Medical Laboratory Sciences

May, 2018
Assessment of plasma D.dimer Level in Women with Menorrhagia, Wad Medani Maternity Teaching Hospital, Gezira State, Sudan (2017)

Marwa Mohamed Hamed Ahmed

Supervision Committee:

<table>
<thead>
<tr>
<th>Name</th>
<th>Position</th>
<th>Signature</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dr. Sanaa Elfatih Hussein</td>
<td>Main supervisor</td>
<td>............</td>
</tr>
<tr>
<td>Dr. Al badawiAbdelbagi Talha</td>
<td>Co-supervisor</td>
<td>............</td>
</tr>
</tbody>
</table>

Date: 13/05/2018
Assessment of plasma D.dimer Level in Women with Menorrhagia, Wad Medani Maternity Teaching Hospital, Gezira State, Sudan (2017)

Marwa Mohamed Hamed Ahmed

Examination Committee:

<table>
<thead>
<tr>
<th>Name</th>
<th>Position</th>
<th>Signature</th>
</tr>
</thead>
</table>
| Dr. Sanaa ELFatih Hussein   | Chair- person     | ...........
| Dr. Abdel Rahman Ahmed Mhamed | External examiner | ............
| Dr. Abbas Abdalla Ibrahim   | Internal examiner | ............

Date: / / 2018
**Declaration**

I am Marwa Mohamed Hamed Ahmed. I hereby declare that my research Assessment of plasma D.dimer Level in Women with Menorrhagia in Gezira State is submitted by me. Moreover, I confirm this research been solely the result of my own work, and not been published before submission. I work in my research with full credibility and sincerity.

Name: Marwa Mohamed Hamed Ahmed

Date: May 2018
Dedication

To my family

To my wonderful supervisor;

Dr. sanaaElfatih Hussein who was with me when need.

To my special friends and colleagues who were integral parts of support group.

I dedicate this work
Acknowledgment

The greatest thank to Allah . I would like to express my deep graduate and thanks to every one who help me throughout this work at any step of it. Firstly, Most grateful to my supervisor Dr. Sanaa Elfatih Hussein for this expertise support and guidance. I would like to thanks my co-supervisor Dr. Albadawi Abdelbagi Talha, who guided me through all the work. Finally, thanks extend to all people whom the blood sample has been collected from. To all them very thanks with regards.
Assessment of plasma D.dimer Level in Women with Menorrhagia, Wad Medani Maternity Teaching Hospital, Gezira State, Sudan (2017)

Marwa Mohamed Hamed Ahmed

Abstract

Menorrhagia is a total blood loss exceeding 80 mL per cycle lasting longer than 7 days. It remains a public health challenge among women in the reproductive age group which interferes with a woman's physical, emotional, and material quality of life. It has many causes. It is common among patients with bleeding disorders and can be a presenting symptom. This case control study was conducted in Gezira state during the period from December 2017 to March 2018 to assess plasma D.dimer level in women with menorrhagia. Twenty-five women with menorrhagia and twenty-five healthy control were included in these studies and questionnaires were designed to collect the data.

The samples collected during and post-menstrual cycle, 5 ml of venous blood was obtained in tri sodium citrate container then centrifuge to get platelet poor plasma (PPP). D.dimer was assessed by cobas t 411, and the data were analyzed using statistical package social science (SPSS), computer program version 15 and express as means. The results showed that the mean±SD of the D.dimer in the cases was (242.6±182) versus (215.9±181; p-value=0.270) to control group, and D-dimer in case group during the cycle was (mean 242.6±182) versus (mean 240±129) post the cycle. There is a insignificant difference between the means of D-dimer in case group (women with menorrhagia) compared to control group (p-value=0.270). Also showed a significant difference between the means of D-dimer in test group during the cycle (p-value=0.000) post the cycle. From the result of this study, it is concluded that the level of D-dimer was not affected in women with menorrhagia. Further studies should be done with larger sample size and special test should be done to determine the underlying causes of menorrhagia.
تقييم مستوى دي دايمر في حالات جزيرة الدم لدى النساء المصابات بغزارة الطمث

بمستشفي ومدني التعليمي للنساء والتوليد، ولاية الجزيرة، السودان (2017)

مرورة محمد حمد أحمد

ملخص الدراسة
غزارة الطمث هي خسارة أكثر من 80 مل من الدم في كل دورة شهرية لأكثر من 7 أيام وهي مشكلة شائعة الحدوث عند النساء في عمر الانجاب وتعتبر تحديا للصحة العامة حيث تتداخل مع نوعية حياة المرأة الجسدية والمادية ولديها العديد من الأسباب وهي أكثر شيوعا بين النساء اللاتي يعانين من اضطرابات التزيف ويمكن ان تكون عرض لأمراض أخرى.

هذة دراسة تحليلية حالة وحالة ضابطة في ولاية الجزيرة في الفترة من ديسمبر 2017 حتى مارس 2018 تهدف

هذه الدراسة قياس مستوى دي دايمر عند النساء المصابات بغزارة الطمث تتوسط اعمارهن من 17-39، خمس وعشرون عينة أخذت من أنساء مع خمس وعشرون عينة من نساء غير مصابات كمجموعة تحكم، تم جمع العينات أثناء الدورة الشهرية أخذت 5 ملياير من الدم الوريدي وتم وضعه في إبرة يحتوي على مانع تجلط ثلاثي سترات الصوديوم واستخلص المصل الدموي لقياس مستوى دي دايمر بواسطة جهاز كوباس ت 411، وتم تحليل البيانات بواسطة برنامج الحزمة الإحصائية لعلوم المجتمع أداء 15.

وكانت النتيجة كالآتي: المتوسط ± الإحراز المعياري عند مجموعة المصابات (242±182) مقارنة بمجموعة التحكم (215±181) وكان الاحتمال الإحصائي 0.270، و أيضا 242.215 ) أثناء الدورة الشهرية و (240±129) بعد الدورة الشهرية عند مجموعة النساء المصابات بغزارة الطمث وكان الاحتمال الإحصائي 0.000، أظهرت النتائج عدم وجود فروقات ذات دلالة إحصائية في مستوى دي دايمر عند النساء المصابات بغزارة الطمث والمحذومة المضابطة (القيمة المعطية أكبر من 0.05، بينما أظهرت فروقات ذات دلالة إحصائية في مستوى دي دايمر أثناء وبعد الدورة الشهرية لدى النساء المصابات بغزارة الطمث (القيمة المعطية أقل من 0.05). خلصت هذه الدراسة إلى ان مستوى دي دايمر (د) لا يتأثر عند النساء المصابات بغزارة الطمث. اوصت هذه الدراسة أجراء اجراءات علاجية وإجراء اختبارات تخصيصية للإ soát الكامنة وراء غزارة الطمث.
Table of Contents:

<table>
<thead>
<tr>
<th>NO</th>
<th>Contents</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Supervision Committee</td>
<td>i</td>
</tr>
<tr>
<td></td>
<td>Examining Committee</td>
<td>ii</td>
</tr>
<tr>
<td></td>
<td>Declaration</td>
<td>iii</td>
</tr>
<tr>
<td></td>
<td>Dedication</td>
<td>iv</td>
</tr>
<tr>
<td></td>
<td>Acknowledgement</td>
<td>v</td>
</tr>
<tr>
<td></td>
<td>Abstract(English)</td>
<td>vi</td>
</tr>
<tr>
<td></td>
<td>Abstract(Arabic)</td>
<td>vi</td>
</tr>
<tr>
<td></td>
<td>Contents</td>
<td>vii</td>
</tr>
<tr>
<td></td>
<td>List of Tables</td>
<td>viii</td>
</tr>
<tr>
<td></td>
<td>List of Figures</td>
<td>ix</td>
</tr>
<tr>
<td></td>
<td>List of Abbreviations</td>
<td>X</td>
</tr>
</tbody>
</table>

**Chapter One (Introduction)**

1.1 Introduction 1
1.2 Justification 3
1.3 Objectives 3

**Chapter Two (Literature review)**

2.1 Menorrhagia 4
2.1.1 Definition of Menorrhagia 4
2.1.2 Epidemiology of Menorrhagia 4
2.1.3 Pathophisiology of Menorrhagia 4
2.1.4 Etiology of Menorrhagia 5
2.1.4.1 Endocrine Causes 5
2.1.4.2 Structural 5
2.1.4.3 Pregnancy complication 5
2.1.4.4 Infectious 6
2.1.4.5 Hematologic 6
2.1.4.6 Physiologic 6
2.1.4.7 Latrogenic 6
2.1.4.8 Systemic diseases 6
2.1.5 Measurement of Menorrhagia 6
2.2 Normal Hemostasis 7
<table>
<thead>
<tr>
<th>Section</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.2.1</td>
</tr>
<tr>
<td>2.2.2</td>
</tr>
<tr>
<td>2.2.3</td>
</tr>
<tr>
<td>2.3</td>
</tr>
<tr>
<td>2.3.1</td>
</tr>
<tr>
<td>2.3.1.1</td>
</tr>
<tr>
<td>2.3.1.2</td>
</tr>
<tr>
<td>2.3.2</td>
</tr>
<tr>
<td>2.3.2.1</td>
</tr>
<tr>
<td>2.3.2.1.1</td>
</tr>
<tr>
<td>2.3.2.1.2</td>
</tr>
<tr>
<td>2.3.2.1.3</td>
</tr>
<tr>
<td>2.3.2.1.4</td>
</tr>
<tr>
<td>2.3.2.2</td>
</tr>
<tr>
<td>2.3.2.2.1</td>
</tr>
<tr>
<td>2.3.2.2.2</td>
</tr>
<tr>
<td>2.3.2.2.3</td>
</tr>
<tr>
<td>2.4</td>
</tr>
<tr>
<td>2.4.1</td>
</tr>
<tr>
<td>2.4.2</td>
</tr>
<tr>
<td>2.5</td>
</tr>
</tbody>
</table>

**Chapter Three (Material and Method)**

<table>
<thead>
<tr>
<th>Section</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.1</td>
</tr>
<tr>
<td>3.1.1</td>
</tr>
<tr>
<td>3.1.2</td>
</tr>
<tr>
<td>3.1.3</td>
</tr>
<tr>
<td>3.1.4</td>
</tr>
<tr>
<td>3.1.5</td>
</tr>
<tr>
<td>3.1.5.1</td>
</tr>
<tr>
<td>3.1.5.2</td>
</tr>
<tr>
<td>3.1.6</td>
</tr>
<tr>
<td>3.1.7</td>
</tr>
<tr>
<td>3.1.8</td>
</tr>
<tr>
<td>3.2</td>
</tr>
<tr>
<td>3.2.1</td>
</tr>
<tr>
<td>Section</td>
</tr>
<tr>
<td>---------</td>
</tr>
<tr>
<td>3.2.2</td>
</tr>
<tr>
<td>3.2.3</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>4.1</td>
</tr>
<tr>
<td>4.2</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>5.1</td>
</tr>
<tr>
<td>5.2</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
</tbody>
</table>
List of Tables :

<table>
<thead>
<tr>
<th>NO</th>
<th>Table Name</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.7</td>
<td>Distribution of study population according to menstrual cycle length (in days)</td>
<td>24</td>
</tr>
<tr>
<td>4.8</td>
<td>Comparison between the means of D-dimer in women with menorrhagia and control group during menstruation</td>
<td>24</td>
</tr>
<tr>
<td>4.9</td>
<td>Comparison between the means of D-dimer in women with menorrhagia during and post menstruation</td>
<td>24</td>
</tr>
</tbody>
</table>
### List of Figures:

<table>
<thead>
<tr>
<th>NO</th>
<th>Figure Name</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Figure 4.1</td>
<td>Distribution of study group according age</td>
<td>18</td>
</tr>
<tr>
<td>Figure 4.2</td>
<td>Distribution of study group according to marital status</td>
<td>19</td>
</tr>
<tr>
<td>Figure 4.3</td>
<td>Distribution of study group according to marital status</td>
<td>20</td>
</tr>
<tr>
<td>Figure 4.4</td>
<td>Family history of menorrhagia among study group</td>
<td>21</td>
</tr>
<tr>
<td>Figure 4.5</td>
<td>History of epistaxis among study population</td>
<td>22</td>
</tr>
<tr>
<td>Figure 4.6</td>
<td>Duration of bleeding according to days among study group</td>
<td>23</td>
</tr>
</tbody>
</table>
List of Abbreviations:

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Complete name</th>
</tr>
</thead>
<tbody>
<tr>
<td>APC</td>
<td>Activated Protein C</td>
</tr>
<tr>
<td>DIC</td>
<td>Disseminated Intravascular Coagulation</td>
</tr>
<tr>
<td>FDPs</td>
<td>Fibrin Degradation Products</td>
</tr>
<tr>
<td>PAI-1</td>
<td>Plasminogen Activator Inhibitor type-1</td>
</tr>
<tr>
<td>PAI-2</td>
<td>Plasminogen Activator Inhibitor type-2</td>
</tr>
<tr>
<td>PE</td>
<td>Pulmonary Embolism</td>
</tr>
<tr>
<td>PPP</td>
<td>Platelet poor plasma</td>
</tr>
<tr>
<td>SD</td>
<td>Standard Deviation</td>
</tr>
<tr>
<td>SPSS</td>
<td>Statistical of Package Social Science</td>
</tr>
<tr>
<td>TF</td>
<td>Tissue Factor</td>
</tr>
<tr>
<td>Tpa</td>
<td>tissue plasminogen activater</td>
</tr>
<tr>
<td>Upa</td>
<td>Urokinase plasminogen activater</td>
</tr>
<tr>
<td>vWF</td>
<td>von Willebrand Factor</td>
</tr>
</tbody>
</table>
Chapter One

1. Introduction

1. Introduction:
Menorrhagia is defined as excessive uterine bleeding occurring at regular intervals or prolonged uterine bleeding more than seven days. Clinically, menorrhagia is defined as total blood loss exceeding 80 mL per cycle or menses lasting longer than 7 days (Chen YJ et al., 2015). It is one of the most common gynecologic complaints in contemporary gynecology (Hallberg L and Nilsson L, 1996).

In 2007, the National Institute for Health and Clinical Excellence (NICE) defined menorrhagia as “excessive menstrual blood loss which interferes with the woman's physical, emotional, social and material quality of life, which can occur alone or in combination with other symptoms.” The group further said that “any interventions should aim to improve quality of life measures (NICE, 2007).

Regardless of the bleeding disorder diagnosis, females experience a monthly reminder of their tendency to bleed heavily at least 12 times each year. While the manifestations of bleeding in women are not unique to women with bleeding disorders, they may be much more severe. Menorrhagia may lead to increased pain during menstruation, chronic anemia, hospitalizations, the need for blood transfusions, limitations in daily activities, time lost from work and school, and reduced quality of life. Other types of bleeding that are specific to females include hemorrhage during pregnancy and childbirth. The risk of life-threatening hemorrhage and hysterectomy in young women with bleeding disorders appears to be much greater than that of the general population (James A et al., 2006)

Heavy menstrual bleeding is a common problem. At least 5–10% of women of reproductive age seek medical attention for heavy menstrual bleeding (Lethaby A et al., 2000). Heavy menstrual bleeding is known to be associated with gynecologic abnormalities such as uterine fibroids, endometrial polyps, and adenomyosis. Moreover, heavy menstrual bleeding can also be associated with a wide range of hemostatic disorders (Clark A et al., 2003). Von Willebrand disease has been
recognized as an important etiologic or contributory factor (James AH et al., 2009), but platelet dysfunction and low factor XI levels are also prevalent (Kadir et al., 1991). There is evidence that fibrinolysis in the endometrium plays an important role in menstruation. In women with heavy menstrual bleeding, increased fibrinolytic activity was observed in the menstrual fluid, which suggested that this might be a contributory factor in the etiology of heavy menstrual bleeding (Knol HM et al., 2013).

Heavy menstrual bleeding (HMB) is associated with an increase in local fibrinolysis (Bonnar J et al., 1980). Plasminogen activators are a group of enzymes that cause fibrinolysis. An increase in the levels of plasminogen activators has been found in the endometrium of women with heavy menstrual bleeding compared to those with normal menstrual loss (Gleeson NC et al., 1994). Plasminogen activator inhibitors (antifibrinolytic agents) have therefore been promoted as a treatment for heavy menstrual bleeding. (Gleeson NC et al., 1994).

Moreover, antifibrinolytic agents, such as tranexamic acid, are effective in reducing menstrual blood loss (Oehler MK et al., 2003). Antifibrilolytic agent provide rational and effective treatment, reducing the degree of menstrual loss by about 50%. (McGavigan JC and Cameron, 2000; Milson I et al., 1991). Most of the studies in literature are with tranexamic acid as the anti-fibrinolytic agent. The effect of tranexamic acid on lowering endometrial tPA activity and menstrual fluid fibrinolysis has been reported in women with heavy menstrual bleeding (Van Eijkgen MA et al., 1992).

D-dimers, the fibrinogen degradation products of cross-linked fibrin, have emerged as the most useful of the procoagulant activity and ongoing fibrinolysis markers. During thrombus formation, fibrinogen is converted to fibrin monomers that are extensively cross-linked into a polymer network. This cross-linking of fibrin takes place in the region of the polymer termed the “D-domain.” Adjacent D-domains are covalently linked and constitute a fibrin specific feature of a thrombus, not found in fibrinogen or non-cross-linked fibrin degradation products (Dempfle, 2000). One of the terminal products of fibrinolysis is the covalently linked D-Domain called the D-Dimer fibrin fragment. Monoclonal antibodies to D-Dimer have been developed that can differentiate fibrin specific clot from non-cross-linked fibrin as well as fibrinogen. As opposed to other markers that only detect products of acute coagulation, D-Dimer assays expand the diagnostic window (Reber, 2000).
The D.dimer test utilize a monoclonal antibody recognizes a cross-linked fibrin epitope. Generation of D.dimer requires action of both thrombin and plasmin and thus specific for clot formation followed by lysis. (Dacie JV and Lewis SM, 2001).

1.2 JUSTIFICATION:
Menorrhagia is a common gynaecological disorder which involves abnormal, prolonged or heavy bleeding from the uterus. Fibrinolysis in the endometrium plays a role in heavy menstrual bleeding. It is unknown whether increased systemic fibrinolysis might also increase the risk for heavy menstrual bleeding. Measurement of D.dimer is important for determining the activation of fibrinolysis.

A. Few studies were done world wide to highlight the abnormalities in D-dimer in menorrhagia, there is no similar study done in our country.

1.3 Objectives:
1.3.1 General objective:
To measure D-dimer in women with menorrhagia

1.3.2 Specific objectives:
- To measure D-dimer level in women with menorrhagia during and post menstrual phase
- To compare D.dimer level between women with menorrhagia and control group.
CHAPTER TWO

2. Literature Review

2.1 Menorrhagia:
2.1.1 Definition:
Clinically, menorrhagia is defined as total blood loss exceeding 80 mL per cycle or menses lasting longer than 7 days (Chen YJ et al., 2015).

2.1.2 Epidemiology:
In US, 20% to 25% of healthy premenopausal have abnormal uterine bleeding. Rate of menorrhagia in non-western countries are unknown. Abnormal uterine bleeding of about 25% of gynecologic surgeries (Carlson KJ et al., 1993).

2.1.3 Pathophysiology of Menorrhagia:
The length of the menstrual cycle is an average of 28 days and consists of three phases: the follicular phase (cycle day (cd) 1–13), ovulation (~cd 14) and the luteal phase (cd 15–28). Estradiol concentrations are lowest on cd 1–3 and highest on cd 13–15 (late follicular phase and ovulation), followed by a decrease during the luteal phase. Progesterone concentrations are lowest on cd 1–8 (follicular phase) and highest on cd 21–25 (luteal phase) (Blomback et al., 1997; Mihm et al., 2011).

During normal cyclic menstrual bleeding, estrogen and progesterone from the ovary induce the production of prostaglandins, cytokines, and matrix metalloproteinases (MMPs). These are directly responsible for the cyclic regeneration of the functional layer of the endometrium (Sivridis E et al., 2004).

Abnormal uterine bleeding represents a disruption in this orderly progression. Thinning of the vascular smooth muscle cell layer of the spiral arterioles, shifts in prostaglandin secretion toward vasodilatory prostaglandins, and disturbances in the endometrial coagulation mechanisms are often found in women with heavy menstrual bleeding (Munro MG, 1999).

Menorrhagia may occur in ovulatory cycles that are typically regular. Heavy bleeding associated with irregular cycles is more likely to represent anovulatory bleeding, caused by a host of distinct conditions. Menorrhagia may occur without any identifiable structural, hormonal, hematologic, or other systemic abnormality (Munro MG, 1999).
2.1.4 Etiology of menorrhagia;

The etiology of menorrhagia can be classified according to the mechanism behind the excessive endometrial bleeding:

2.1.4.1 Endocrine

Lack of ovulation causes excessive endometrial growth and hyperplasia, leading to excessive bleeding as a result of unopposed estrogen stimulation. The most common cause of anovulation in women of reproductive age is polycystic ovary syndrome (PCOS). Other causes of anovulation include hyperprolactinemia and thyroid dysfunction, particularly hypothyroidism (Sweet, MG, et al., 2012;) however, these do not always result in menorrhagia. Dysfunction of the hypothalamo-pituitary-ovarian axis is another important cause of anovulation that is associated with menorrhagia, and is usually encountered in women at either end of the reproductive period (i.e., adolescents and perimenopausal women). Dysfunctional corpus luteum can be associated with menorrhagia due to lack of adequate progesterone production (Speroff L and Fritz M, 2005).

2.1.4.2 Structural

Includes uterine fibroids (leiomyomas), endometrial polyps, endometriosis, and adenomyosis (i.e., endometrial glandular growth into myometrium). Other less frequent causes include uterine and cervical cancers (Collins J and Crosignani, 2007).

2.1.4.3 Pregnancy complication

Excessive bleeding can be associated with pregnancy including miscarriage, gestational trophoblastic disease (choriocarcinoma), and, less commonly, ectopic pregnancy (Collins J and Crosignani, 2007).

2.1.4.4 Infectious

Endometritis and salpingitis (pelvic inflammatory disease) are less frequent causes (Collins J and Crosignani, 2007).
2.1.4.5 Hematologic

Menorrhagia can be a manifestation of several systemic diseases, including coagulation disorders (Collins J and Crosignani, 2007).

2.1.4.6 Physiologic

In the absence of an identified underlying organic cause, the term "dysfunctional uterine bleeding" is usually applied. Some believe that local endometrial functional derangements, such as altered synthesis of uterine vasodilatory prostanoids, reduced endothelin expression, increased fibrinolysis, perturbed endometrial regeneration, and overproduction of nitrogen oxide, underlie the occurrence of menorrhagia (Collins J and Crosignani, 2007).

2.1.4.7 Iatrogenic

Iatrogenic causes of menorrhagia are not uncommon (e.g., intrauterine contraceptive devices, anticoagulant therapy, tamoxifen, and hormonal therapies with exogenous estrogens). Herbal supplements (e.g., ginseng, ginkgo, and soy) may cause menstrual irregularities by altering estrogen levels or coagulation parameters (Nutescu EA et al., 2006; Lien LL and Lien EJ, 1996).

2.1.4.8 Systemic disease

Menorrhagia can be a manifestation of several systemic diseases, including chronic liver and renal disease (Collins J and Crosignani, 2007).

2.1.5 Measurement of Menorrhagia:

The gold standard for measurement of menstrual fluid loss in a research setting is the alkaline hematin technique. While reasonably accurate, this method requires that all feminine hygiene products from a menstrual cycle be collected and provided to the laboratory for testing, making it impractical for routine clinical assessment of bleeding. Various pictorial charts have been developed to assist in screening for menorrhagia, with reported predictive value of 75-85%. The charts require that the patient record the number of days of menstrual flow, as well as the number of
feminine hygiene products and level of saturation of the products used each day (Kouides P, 1998).

2.2 Normal Hemostasis

Hemostasis is defined as “the termination of a bleed by mechanical or chemical means or by the complex coagulation [clotting] process of the body…” Composing of a coordinated sequence of events, hemostasis consists of vasoconstriction of the blood vessel, platelet adhesion and aggregation, and thrombin and fibrin synthesis (Anderson K et al., 1994).

The general sequence of events in hemostasis is briefly presented by describing the three main phases: vascular, platelet and coagulation phases.

2.2.1 Vascular phase: Immediately, when blood vessels are injured, vasoconstriction of the arteries and veins begins. Within the injured vessel wall, exposure of subendothelial tissues, collagen, and basement membrane contribute to prothrombotic activities. Clotting activities include platelet aggregation and adhesion via release of adenosine diphosphate (ADP) and von Willebrand factor (vWF). Additionally, the release of a tissue factor (formerly known as tissue thromboplastin) during this phase initiates coagulation via the extrinsic pathway. At this point, the initial layer of the platelet plug is established at the site of the injury (Bick R, 2002; Little JW et al., 2008; Corton RS et al., 1999).

2.2.2 Platelet phase: “Platelets are cellular fragments from the cytoplasm of megakaryocytes” that survive in the vascular system for 8–12 days. They are essential for the clotting process in the blood. Primary hemostatic functions of platelets include: maintaining the health of the inner lining of the vascular wall; formation of a platelet plug during vessel wall injury; and initiation of the coagulation phase, which leads to the stabilization of the platelet plug (Little JW et al., 2008).

During the platelet phase, platelets become sticky and adhere to one another and to the site of injury after contact with exposed collagen and subendothelial tissue component vWF glycoprotein Ib. Additionally, Adenosine Di-phosphate (ADP) is released by exposed subendothelial tissues that cause platelets to aggregate, change shape, release dense and a-granule contents and synthesize thromboxane A2 that can further act as a feedback activator potentiating platelet responses by binding to
thromboxane receptor (TP). A product of platelets, thromboxane, causes another surge of platelet aggregation. (Little JW et al., 2008)

In summary, platelets adhere to the damaged subepithelial surface, change shape, become sticky, and aggregate to form a hemostatic platelet plug at the injured blood vessel site. Under these normal conditions, adequate numbers and function of platelets are required, resulting in the primary cessation of the bleed by the hemostatic platelet plug formation. (Bick R, 2002; Little JW et al., 2008; Corton RS et al., 1999)

2.2.3 Coagulation phase: Virtually simultaneously with the vascular and platelet phases, the extrinsic, intrinsic and common pathways, containing 12 circulating plasma proteins, (also termed plasma coagulation factors) are initiated. These plasma proteins are produced in the liver. More specifically, of the 12 plasma proteins, factors II, VII, IX and X are Vitamin-K dependent for synthesis. (Bick R, 2002).

The coagulation factors (F) are activated in a cascade-like manner within their respective pathways. The “faster” extrinsic pathway is initiated by FVII when exposed to a tissue factor (or a membrane protein) within the injured vessel; and the intrinsic pathway is initiated when FXII contacts with injury-exposed subendothelial tissues. Subsequently, coagulation factors in the intrinsic pathway activate one another: FXII activates FXI; FXI activates IX; and FIX activates FVIII. (Bick R, 2002).

Both pathways merge and FX is activated, yielding the activation of the common pathway. Subsequently, prothrombin is converted to thrombin; thrombin acts as a catalyst for the conversion of fibrinogen to fibrinogen. Fibrinogen is the precursor to fibrin. (Bick R, 2002; Little JW, et al., 2008; Corton RS, et al. 1999)

Finally, anticlotting mechanisms (broadly termed fibrin degradation products) in the fibrinolytic pathway are activated to prevent the formation of more clots and to allow for the dissolution of the definitive clot. The expected outcome is accomplished: repair of the injured blood vessel wall results and bleeding ceases (Sonis ST, et al., 2003; Corton RS, et al. 1999)

2.3 The Fibrinolytic System
The fibrinolytic system is an enzymatic cascade system whose activation leads to formation of a trypsin-like serine protease, plasmin, which is capable of degrading
fibrin as well as fibrinogen, factor V and VIII. It is believed that the main function of fibrinolysis is defence against thrombotic occlusion of vessels, dissolution of thrombi once they are formed (thrombolysis) and resolution of clots and fibrinous exudates occurring in various parts of the body (M. Pandolfi and A. Al-Rushood, 1991).

2.3.1 Activators of fibrinolysis
Plasmin splits fibrin into soluble fragments (fibrin degradation products-FDP) of different size. Plasmin results from activation of the proenzyme plasminogen, a Beta 2-glycoprotein with molecular weight of about 90 kD which is synthetised in the liver. It tends to be adsorbed to fibrin, the site at which the bulk of plasminogen activation occurs (M. Pandolfi and A. Al-Rushood, 1991).

Circulating blood contains two types of plasminogen activators: tissue type activator (t-PA) and urinary activator or urokinase (u-PA). Both activators may be present in native single chain forms: weakly active sc t_PA with a molecular weight of 60 kD, and sc u-PA (pro urokinase) with a molecular weight of 54 kD which is devoided of enzymatic activity (M. Pandolfi and A. Al-Rushood, 1991).

An important functional difference between the two activators is that t-PA binds to fibrin which it needs to activate plasminogen while u-PA does not bind to fibrin and is capable of activating plasminogen in absence of fibrin. During activation of fibrinolysis both native forms are converted into the active two chain t-PA and u-PA (tc t-PA and tc u-PA). (M. Pandolfi and A. Al-Rushood, 1991).

2.3.1.1 Tissue type Activator t-PA:
T-PA is produced and stored in the endothelium of certain blood vessels where it can be demonstrated histochemically (M. Pandolfi and A. Al-Rushood 1991)

From the vascular endothelium t-PA is continuously released into the circulation such release being enhanced by a variety of stimuli notably venous stasis and physical exercise. The concentration of t-PA antigen in plasma is low, about 5 ug/L. It is believed that t-P A plays the central role in maintaining the patency of the vascular tree by dissolving obstructing fibrin deposits or promoting the lysis of occluding thrombi (M. Pandolfi and A. Al-Rushood, 1991).

2.3.1 Urokinase plasmenogen activator U-PA is produced mainly by the kidney and excreted with the urine. Its main role is probably that of maintaining the urinary excretion pathways free from obstructing fibrin. Plasma contains minute amounts of u-PA mainly sc u-PA (M. Pandolfi and A. Al-Rushood, 1991).
2.3.2 Fibrinolytic Inhibitors:
Since plasmin is capable of degrading proteins other than plasmin it is necessary to confine its action to its primary substrate i.e. fibrin. Furthermore the effectiveness of the fibrinolytic system needs constant regulation since a too pronounced activity leads to a haemorrhagic diathesis, while a depressed activity leads to thrombosis(Aoki N, 1989; Kruithof EKO, 1988).
While circulating in blood as free enzymes the activity of t-PA is weak. When fibrin is formed, plasminogen and t-PA are bound to fibrin. Fibrin bound t-PA increases 200 times its catalytic activity and rapidly converts into plasmin the fibrin bound plasminogen. The result is a local fibrinolysis. Plasmin also converts the inactive sc u-PA into active tc u-PA which takes part in the dissolution of fibrin. However, the role played by u-PA in fibrin dissolution inside the vascular tree is probably ancillary.
The fibrinolytic process is restrained at two different levels by two classes of inhibitory agents present in plasma: the inhibitors of plasminogen activators, and the inhibitors of plasmin(Sprenger ED, Kluft C, 1987).

2.3.2.1 Inhibitors of Plasminogen Activators
2.3.2.1.1 plasminogen activator inhibitor (PAI-1)
Is contained in the vascular endothelium and in the platelet alpha granules; it immediately binds t-PA and u-PA. In normal conditions it binds 95% of the circulating t-PA forming a complex with molecular weight of 110 kD( Booth NA, et al, 1987). Changes in PAI-1 plasma levels rather than changes in t-PA have been recently related to diurnal variations of the fibrinolytic activity(Grimaudo V et al, 1988).

2.3.2.1.2 Plasminogen activator inhibitor (PAI-2)
PAI-2 has probably a secondary importance being secreted by placenta and being present only in plasma of pregnant women. However, PAI-2 has recently been found in the plasma of some men and non-pregnant women(Lecander I and Astedt B, 1989).

2.3.2.1.3 Activated Protein C
In addition to its anticoagulant activity, activated protein C (APC) stimulates fibrinolysis both in vitro and in vivo by binding PAI-I thus acting as an 'inhibitor of inhibitor'(Sakata Y et al, 1985).

2.3.2.1.4 Protein S
2.3.2.2 Inhibitors of plasmin

2.3.2.2.1 Alpha 2-antiplasmin (alpha2-AP)

The primary inhibitor of plasmin, (alpha 2-anti-plasmin ((alpha2-AP) rapidly forms a complex with circulating plasmin. In case of massive formation of plasmin as during thrombolytic treatment with streptokinase or in disseminated intravascular coagulation an exhaustion of available alpha2-AP occurs(Saito H,1988).

2.3.2.2.2 Alpha2-macroglobulin

The excess of plasmin is then complexed by alpha2-macroglobulin which functions as a reserve ('second defence line') inhibitor of plasmin(M Pandolfi and A Al-Rushood, 1991).

2.3.2.2.3 Cl inhibitor

is known to activate components of the complement C1s and C1r; it also inhibits clotting factor XIIa, Xla and kallikrein, Furthermore it reacts with plasmin and with t-PA although at such low rates that its significance as inhibitor of fibrinolysis is uncertain(Wiman B ,1986).

The balance between the pro fibrinolytic and antifibrinolitic activity is regulated by the synthesis of these agents and by their release into circulation. Synthesis and release have been found to be mediated by a number of substances such as thrombin, histamin, epinephrine(van Hingberg VWM ,1988).

2.4 D,dimers:

D dimers are generated when cross-linked fibrin is degraded and so they are not generated if non-cross linked fibrin or fibrinogen is broken down - they are, therefore, fundamentally different from Fibrinogen Degradation Products (FDPs)(van der Graaf et al.,2000)

2.4.1 D.dimer formation

In the first step of D-dimer formation, thrombin cleavage exposes a previously cryptic polymerization site on fibrinogen that promotes the binding of either another fibrinogen or a monomeric fibrin molecule of Fibrin monomers then bind to one another in an overlapping manner to form 2 molecule thick protofibrils(Doolittle RF and Pandi L ,2007).

Plasma remains fluid until 25% to 30% of plasma fibrinogen is cleaved by thrombin allowing time for fibrin to polymerize while simultaneously promoting thrombin activation of plasma factor XIII(Greenberg CS et al.,1988). Thrombin remains
associated with fibrin,(Weitz JI et al.,1998) and as additional fibrin molecules polymerize, it activates plasma factor XIII bound to fibrinogen(Meh DA et al.,1996). The complex between soluble fibrin polymers, thrombin, and plasma factor XIII promotes the formation of factor XIIIa before a fibrin gel is detected(Greenberg CS et al.,1985).

In the second step of D-dimer formation, factor XIIIa covalently cross links fibrin monomers via intermolecular isopeptide bonds formed between lysine and glutamine residues within the soluble protofibrils and the insoluble fibrin gel(Shen L and Lorand L,1993). D-dimer antigen remains undetectable until it is released from crosslinked fibrin by the action of plasmin. In the final step of D-dimer formation, plasmin formed on the fibrin surface by plasminogen activation cleaves substrate fibrin at specific sites(Medved L and Nieuwenhuizen W,2003). Fibrin degradation products are produced in a wide variety of molecular weights, including the terminal degradation products of crosslinked fibrin containing D-dimer and fragment E complex. It is uncommon to detect circulating terminal fibrin degradation products (D-dimer–E complex) in human plasma, whereas soluble high-molecular-weight fragments that contain the “D-dimer antigen” are present in patients with DIC and other thrombotic disorders(Gaffney PJ,2001).

These fragments may be derived from soluble fibrin before it has been incorporated into a fibrin gel, or alternatively may be derived from high-molecular-weight complexes released from an insoluble clot(Marder VJ et al.,1999),(Comberg A et al.,1992).

2.4.2 Causes of elevated of D.dimer:
Causes of an Elevated D-dimer Level D-dimers are detectable in the circulation of healthy individuals, as small amounts of fibrinogen are converted to fibrin physiologically(Righini M et al.,2008).

D-dimer levels are increased in almost all cases of acute VTE. However, D-dimer levels can also be increased during any process that increases fibrin production or breakdown. Such conditions include surgery, pregnancy, inflammation and cancer(Raimondi P et al.,1993). As a result, increased D-dimer levels are non-specific
for acute VTE. D-dimer levels have been shown to increase with age (Hager K and Patt D., 1995; Peiper CF et al., 2000).

2.5 Previous study:

- (P. Szczepaniak et al., 2015) examined women diagnosed with heavy menstrual bleeding (HMB) and healthy women found that D.dimer are similar in both groups.
- Study from Iraq conducted by (Mona A, Kashmola, 2005) 53 women with menorrhagia during menstrual phase, D.dimer positive in 16 out 53 cases (30.2%).
- Furthermore, there was significant correlation between amount of menstrual blood loss and fibrinolytic activity in menstrual fluid both for women who bleed normally and for women with menorrhagia, (Sperff L and Fritz M, 2005).
- Systemic review by (Knol et al., 2012) in normal menstrual women show there is little evidence about D.dimer levels, and some of that is contradictory, Koh et al., 2005 found the lowest levels at follicular [cd10-14] whereas (Giardina et al., 2005) found the lowest D-dimer levels in luteal phase [cd28].
- (T Repine and M. Osswald, 2003) found slightly elevated in D.dimer in women with menorrhagia and deficiency of plasminogen activator inhibitor-1.
Chaptor Three

3.Material and Method

3.1 Methodology

3.1.1 Study design:
Case-control follow up study aimed to assessment of D.dimer level in women with menorrhagia and healthy women in Gezira State.

3.1.2 Study area and Duration:
This study was conducted in Gezira State, Sudan from December 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 population:
Females in the reproductive age diagnosed as menorrhagia.

3.1.4 Sample Size:
Twenty five women with menorrhagia were included in the study and twenty five as control.

3.1.5 Study Criteria:
3.1.5.1 Inclusion criteria:
Women in the reproductive age diagnosed as menorrhagia,

3.1.5.2 Exclusion criteria:
- Women with bleeding disorders.
- Use of intra uterine device.
- Liver disease and renal disease.
- Use of anticoagulants within the last 2 months.
- Use of nonsteroidal anti-inflammatory agents, e.g., aspirin and anti-platelets drugs within 14 days of participation.

3.1.6 Data Collection:

The data was collected by using a questionnaire. A questionnaire was designed to include all needed information.

3.1.7 Data Analysis:

The data was obtained from questionnaire and the result of laboratory analysis was analyzed using SPSS (statistical package for social sciences).

3.1.8 Ethical Approval:

Ethical approval of this study was obtained from the ministry of health in Gezira State. Informed consent was obtained from participants before collection of samples. The specimens and information were collected from the individuals under privacy and confidentiality and were not used for any purposes rather than this study.

3.2 Material The following materials were utilized in this study:

- Lab coat.
- Gloves.
- Cotton.
- 70% isopropanol alcohol pad.
- Tourniquet.
- Syringes (5 ml).
- Tri sodium citrate anticoagulant vacocontainer tubes.
- Centrifuge
- Automatic micropipettes.
- Tips.
- Eppendorf tubes.
- Cobas t 411 device

3.3 Method:

3.3 Sample collection and preparation: blood was collected by a clean venipuncture in plastic tubes containing 0.109 M sodium citrate in a ratio of 9 parts of blood and 1 part anticoagulant. The venous sample was allowed to enter the syringe without pressure. Platelets poor plasma (PPP) was obtained immediately after blood collection by centrifugation blood samples at 2000 g for 15 minutes.

3.3.2 Laboratory analysis

Laboratory analysis was done as soon as possible and within not more than four hours from the time of collection in faculty of medical laboratories sciences, hematology lab. Analysis was done by using Cobas t 411.

3.3.2.1 Principle of test

Test principle Particle-enhanced immunoturbidimetric assay. Latex particles of uniform size are coated with monoclonal antibodies (F(ab')2 fragments) to the D-Dimer epitope. The antigen/antibody complexes produced by the addition of samples containing D-Dimer lead to an increase in the turbidity of the test reactants. The change in absorbance with time is dependent on the concentration of D-Dimer epitopes in the sample. The precipitate is determined turbidimetrically.
Chapter Four

4. Results and Discussion

4.1 Result

Plasma D-dimer level was measured in 25 women with menorrhagia as test group and 25 healthy women were enrolled into this study as control group between the ages of 17 and 39 years, the sample collected during and post menstrual cycle during the period of December 2017 to March 2018 in Gazeira state – Sudan. Among females with menorrhagia, their distribution according to age groups, showed 16 (64%) were less than 30 years old (Figure 4.1), 13 (52%) of them were single and 12 (48%) were married (Figure 4.2). The most cases were found to have not family history of menorrhagia 19 (76%) while 6 (24%) were found to have family history (Table 4.4) and (Figure 4.4), two women (8%) out of 25 women with menorrhagia, complained of epistaxis (Figure 4.5). The most cases 23 (92%) were found to have duration of menorrhagia/day between 7-10 days while the others between 11 – 14 days about 1 (4%) or more than 15 days about 1 (4%) (Figure 4.6). Most cases 19 (76%) have menstrual cycle length between 14-25 days (Table 4.1). (Table 4.2) shows the means of D-dimer in test group (women with menorrhagia) and control group. (mean + SD): (mean 242.6±182., 215.9 ±18; p-value0.270) respectively. And (Table 4.3) shows the
means of D-dimer in test group during the cycle (mean 242.±182) and (mean240±129; 
 p-value0.000) post the cycle .
Figure 4.1 Distribution of study group according to age

Figure 4.2 Distribution of study group according to marital status:
Figure 4.3 Family history of menorrhagia among study group
Figure 4.4 History of epistaxis among study group
Figure 4.5 Duration of bleeding according to days among study group
Table 4.1 Distribution of study population according to menstrual cycle length (in days)

<table>
<thead>
<tr>
<th>Frequency</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>14-25</td>
<td>19</td>
</tr>
<tr>
<td>26-35</td>
<td>2</td>
</tr>
<tr>
<td>More than 36</td>
<td>4</td>
</tr>
<tr>
<td>Total</td>
<td>25</td>
</tr>
</tbody>
</table>

Table 4.2 comparison between the means of D-dimer in women with menorrhagia and control group during menstruation

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>Mean</th>
<th>Std. Deviation</th>
<th>P. Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>D-Dimer D</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cases</td>
<td>25</td>
<td>242.5912</td>
<td>182.4463</td>
<td>0.270</td>
</tr>
<tr>
<td>Control</td>
<td>25</td>
<td>215.9308</td>
<td>181.7317</td>
<td></td>
</tr>
</tbody>
</table>

D:During menstrual cycle

Table 4.3 comparison between the means of D-dimer in women with menorrhagia during and post menstruation

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>Mean</th>
<th>Std. Deviation</th>
<th>P. Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>D-Dimer D</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D-Dimer D</td>
<td>25</td>
<td>242.5912</td>
<td>182.4463</td>
<td>0.000</td>
</tr>
<tr>
<td>D-Dimer P</td>
<td>25</td>
<td>240.1844</td>
<td>129.6856</td>
<td></td>
</tr>
</tbody>
</table>

D:During menstrual cycle  P:post menstrual cycle
4.2 Discussion
Menorrhagia is a common gynaecological disorder which involves abnormal, prolonged or heavy bleeding from the uterus is a total blood loss exceeding 80 mL per cycle lasting longer than 7 days. Such periods often interfere with work and activities of daily living and, if left untreated, can lead to iron deficiency anemia. The condition is most common among women of reproductive age, especially those approaching menopause. Common etiologies include hormonal disorders, bleeding disorders, uterine polyps, and uterine fibroids. It is common among patients with bleeding disorders and can be a presenting symptom. The prevalence of menorrhagia was highest between the age of 26-34 years the result with study from India by (T Kotagastis, 2005) but (coulter, A et al., 2003) reported in their study the prevalence of heavy bleeding among age group of 30-40 years these differance may be due to large sample size their study. The most cases were found to have not family history 37 (74%) while 13 (26 %) were found to have family history in agreement with study done by (Khalid et al., 20015), most of cases were found have not family history (74%) approximately similar to study done in Egypt (Sherif N., et al. 2014)
76% of cycle within the range of 14-25 days as show in table 4.7
In this study there is insignificant difference in D.dimer between women with menorrhagia and control group. This might indicate that the systemic fibrinolysis is not relevant in heavy menstrual bleeding, only the local fibrinolysis in agreement with study done by (S Wiewel et al., 2017), and also agreement with study done by (Stephen C. L., et al., 2007) In contrast to Study by Mona (M Shamden, 2005) from Iraq menthioned that the fibrinolysis could a cause of menorrhagia using the plasma D.dimer and Bisht et al by using serum fibrin/fibrinogen degradation (FDPs) as guide may be due to large sample size. High plasma D.dimer was seen in patients with prolonged duration of cycle (more than 10 days). Positive correlation between duration of bleeding and D.dimer level was observed by Mona (M ,Shamden ,2005)
The results of this study also indicate that, generally speaking, the concentrations of D.dimer in the plasma of women with menorrhagia are higher during the menstrual cycle than in the post menstrual cycle as show in (table 4.9), matched with study done by (Giardina et al., 2004)
Chapter Five
5. CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion
In conclusion, we showed that the D.dimer level was not increased in women with menorrhagia.

5.2 Recommendation
- Further studies should be done with larger sample size.
- Special test should be done to determine the underling causes of menorrhagia.
References:


Collins, J; Crosignani PG. Endometrial bleeding. ESHRE Capri Workshop Group, Hum Reprod Update. 13:421-431


Greenberg, CS; Miraglia, CC; Rickles, FR; Shuman MA (1995). Cleavage of blood fibrin degeneration product concentrations (D-dimers) in the course of ageing. Gerontology. 41:159-165


James, A; Ragni, M; Picozzi, V (2006). Bleeding disorders in premenopausal women: (Another) public health crisis for hematology? ASH Special Educational Symposium, 475-84


Kadir, RA; Economides, DL; Sabin, CA; Owens D; Lee, CA (1998.) Frequency of inherited bleeding disorders in women with menorrhagia. Lancet 351: 485–9.


Little, JW; Falace, D; Miller, C; Rhodus, NL (2008). Dental management of the medically compromised Patient. 7th ed. St Louis, MO. Mosby Elsevier.


Sakata, Y; Curriden, S; Lawrence, D; Griffin, JH; Loskutoff, DJ (1985). Activated protein C stimulates the fibrinolytic activity of cultured endothelial cells and decreases antiactivator activity. Proc Natn Acad Sci USA,82: 1121-5.

Stephen, C and L, Koh,(2006). The effect of levonorgestrel- releasing intrauterine system use on menstrual blood loss and the systems in women with menorrhagia. blood 87:9-11


Appendix (1)

Questionnaire

University of Gezira
Faculty of medical laboratory sciences
Department of Hematology and Immunohematology

Name……………………………………………………………………………………………………

No.……………… Date……../……./……………..

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

Phone Number………………

Marital status single (     ) married (     )

Result……………………………………………………………………………………