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The role of Glycodelin-A , soluble Fms like tyrosine kinase-1 , and Placental growth factor as predictor markers of first trimester pregnancy loss

A Thesis

Submitted to the College of Medicine , Baghdad University in Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy in Clinical Biochemistry

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Dedication

To my family, all of them , especially my wife for their encouragement , supporting and helping through all period of my study.

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List of Abbreviations

Abbrev.	Meaning
ACOG	American college of obstetricians and gynecologists
ANOVA	Analysis of variance
APA	American pregnancy association
CDC	Center of disease control
CRL	Crown rump length
CVS	Chorionic villus sampling
D&C	Dilation and Curettage
EECs	Endomaterial Epethelial Cells
ELISA	Enzyme linked immunosorbent assay
GdA	Glycodelin-A
GDM	Gestational diabetes mellitus
GS	Gesational sac
HCG	Human chorionic gonadotropin
HSG	Hystero salpingo gram
HDPs	Hypertensive disorders of pregnancy
HR	Heart rate
HRP	Horseradish peroxidase
IL	Interlukin
LMP	Last menstrual period

NK	Natural killer cells
NPV	Negative predictor value
PE	Pulmonary embolism
PE	Preeclampsia
PlGF	Placental growth factor
PPV	Positive predictor value
ROC	Receiver operator curve
RSA	Recurrent spontaneous abortion
SD	Standard deviation
sFlt-1	Soluble fms-like tyrosine kinase – 1
SPSS	Statistical packages social sciences
Th	T-helper
TIMP3	Tissue inhibitors of metalloproteinase 3
TVUS	Transvaginal ultrasound
UTI	Urinary tract infection
VEGF	Vascular endothelial growth factor
YS	Yolk sac

Abstract

Background :

The first trimester is begining from week one through 12 and includes conception , a miscarriage is the loss of pregnancy during the first 20 weeks , many pregnancies among healthy women will end in a miscarriage , sometimes , this happens before a woman is even aware of the pregnancy , in most cases , miscarriage isn't preventable , and it can happen for a variety of medical reasons , many of which aren't within a person's control .

Objective :

To study the role of Glycodeline-A , sFlt-1 , and PlGF as predictor markers of first trimester pregnancy loss .

Setting :

The present study was conducted at Baghdad Teaching Hospital during the period from April 2019 to March 2020 , the subject were selected from the patients attending the out patients clinic in Gynaecology consultation unit and the laboratory tests were done in Medical City /Teaching Laboratories .

Subjects :

From more than 200 individual slelect 85 pregnant woman identified the first day of LMP who are confirmed positive pregnancy test in blood , the age of them 21 – 35 year and gestational time 8 – 12 week , after taken the specimen blood from them we encountered these women until the end of week 12 of pregnancy , 40 of them considered as patients who have pregnancy loss in this period and the other 45 individuals who completed this period without problems considered as healty control .

Methods :

Enzyme Linkade Immunosorbent Assay (ELISA) test was used for the determination of serum Beta HCG , Glycodeline-A , sFlt-1 , and PlGF levels, eight to ten milliliters (mls) of peripheral venous blood was aspirated from each patient and control subject , transferred into plain tube , allows clotting for 30 minutes , then the serum was separated and stored at – 20 C^{o} till the time of measurements of the studied biochemical parameters : Beta HCG , Glycodeline-A , sFlt-1 , and PlGF .

Results :

- The current results revealed that serum levels of Beta HCG , Glycodeline-A , and PlGF were significantly lower in patients compared with healthy control ($P \leq 0.05$) , while serum level of sFlt-1was significantly higher in patients compared with healthy control ($P \leq 0.05$) .
- The present study showed there is no significant correlation between :

- Age and Beta HCG , Glycodelin-A , sFlt-1 , PlGF .
- sFlt-1 and Glycodelin-A , PlGF , Beta HCG .

and there is significant correlation between :

- Gest.age with Beta HCG , PlGF , Glycodeline-A .
- Glycodeline-A with PlGF , and Beta HCG .
- PlGF with Beta HCG .

Conclusion :

- The serum Beta HCG ,Glycodeline-A,and PlGF level showed significantly lower in patients compared with healthy controls .
- The serum level of sFlt-1 showed significant elevation in patients compared with healthy control.

Chapter One

Introduction

Chapter One

Introduction

1. First Trimester Pregnancy loss :

1.1 Definition :

Pregnancy is the state of carrying a developing embryo or fetus within the female body,this condition can be indicated by positive results on urine test,and confirmed through a blood test,ultrasound,detection of fetal heartbeat,pregnancy lasts for about nine months,measured from the date of the woman's last menstrual period (LMP),it is conventionally divided into three trimesters,each roughly three months long (**William et al ,2018**) .

The most important tasks of basic fetal cell differentiation occur during the first trimester,so any harm done to the fetus during this period is most likely to result in miscarriage or serious disability **(Valinda Riggins et al,2018)** .

The first trimester is from week one through 12 and includes conception, which is when the sperm fertilizes the egg, the fertilized egg then travels down the fallopian tube and attaches to the inside of the uterus, where it begins to form the embryo and placenta, gestational age can be confusing, most people think of pregnancy as lasting 9 months, and it’s true that the pregnant for about 9 months,but because pregnancy is measured from the first day of the last menstrual period about 3-4 weeks before actually pregnant a full-term pregnancy usually totals about 40 weeks from LMP roughly 10 months (**Eunice et al,2015**) .

A woman's reproductive system includes the uterus (including the cervix),two ovaries, two fallopian tubes,and the vagina, the fallopian tubes are a pair of hollowed tubes that run from each side of the uterus to the ovaries, once a month, an egg is released by one of the ovaries and travels down the fallopian tube,if the egg is fertilized in the tube by the male's sperm,

pregnancy begins, once the egg and sperm join, they rapidly develop new cells, this bundle of cells, called the embryo, normally implants on the inner wall of the uterus, once implanted, the embryo continues to grow inside a sac of amniotic fluid,sometimes called the "bag of water ," after several weeks, the embryo is called a fetus (**Jens Christian et al,2016**) .

Adequate formation of uteroplacental and fetal placental blood flow is the determining factor of physiological pregnancy and fetal development, successful uterine placental vascular morphogenesis and embryonic morphogenesis of fetal blood system are the basis of these processes .

There are two stages of vascular morphogenesis :

- vasculogenesis : primary formation and development of blood vessels de novo from committed mesodermal cells .
- angiogenesis : formation of new blood vessels from existing vascular structures, which reflect the formation of vascular system of a fetus and placenta during pregnancy (**Raul Artal et al,2019**)

A miscarriage is the loss of pregnancy during the first 20 weeks, according to the American Pregnancy Association (APA),many pregnancies among healthy women will end in a miscarriage,s ometimes, this happens before a woman is even aware of the pregnancy, in most cases, miscarriage isn’t preventable, a loss of pregnancy after week 20 of pregnancy is called a stillbirth, many times the cause for this isn’t known,it typically happens during the first trimester, or first three months of the pregnancy, and it can happen for a variety of medical reasons, many of which aren’t within a person’s control (**Pacagnella RC et al,2014**) .

Miscarriage,is a failed intrauterine pregnancy that ends before 20 weeks from the last menstrual period also known as spontaneous abortion and pregnancy loss, and it is the natural death of an embryo or fetus before it is able to survive independently, the first trimester begins on the first day of

last period and lasts until the end of week 12 of pregnancy, miscarriage in this period is common (**Saccone G et al,2017**) .

Most clinically apparent miscarriages (two-thirds to three-quarters in various studies) occur during the first trimester, many fertilized eggs miscarry, often before the pregnancy is known, the embryo typically dies before the pregnancy is expelled, bleeding into the decidua basalis and tissue necrosis causes uterine contractions to expel the pregnancy, early miscarriages can be due to a developmental abnormality of the placenta or other embryonic tissues,in some instances an embryo does not form but other tissues do, this has been called a "blighted ovum" **(David S et al,2018**) .

1.2 Epidemiology :

Women often see the common acute conditions during pregnancy, these conditions may be caused by pregnancy as obstetric problems or worsened by pregnancy as obstetrically aggravated problems, or they may require special consideration during pregnancy because of maternal or fetal risks as nonobstetric problems, primary differential diagnosis for common conditions during pregnancy and recognize the important findings of obstetric and urgent nonobstetric problems necessary at these conditions, and must be evaluate and treat most nonobstetric problems (**Vojel JP et al,2017**) .

Many women experience danger signs during pregnancy, as complications that unpredictable, these danger signs include vaginal bleeding, severe headache,vision problems, high fever, swollen hands and or face, and reduced fetal movement, these danger signs usually indicate the presence of an obstetric complication that may arise during pregnanc, complications of pregnancy are health problems that occur during pregnancy, they can involve the mother's health, the baby's health, or both, some women have health problems that arise during pregnancy, and other women have health problems before they become pregnant that could lead to complications, it is very important for women to receive health care before and during pregnancy to decrease the risk of pregnancy complications (**Alok A et al,2019**) .

1.3 Pathophysiology :

The pathophysiology of a spontaneous miscarriage may be suggested by its timing, chromosomal defects are commonly seen in spontaneous miscarriages, especially those that occur during 4-8 weeks' gestation, genetic problems are common in early first-trimester loss but may be seen throughout gestation, trisomy chromosomes are the most common chromosomal anomaly, insufficient or excessive hormonal levels usually result in spontaneous miscarriage before 10 week's gestation, infectious, immunologic, and environmental factors are generally seen in first-trimester pregnancy los, Anatomic factors are usually associated with second-trimester loss,factor XIII deficiency and a complete or partial deficiency of fibrinogen are associated with recurrent spontaneous miscarriage (**Montagnana M et al,2017**) .

1.3.1 Before Pregnancy :

Most of pregnant women have some risk of problems, they may have problems because of a health condition she had before get pregnant, and could also develop a condition during pregnancy, other causes of problems in a previous pregnancy,drug use before pregnancy, or being over age 35,if she is receiving treatment for a health problem, health care provider might want to change the way of health problem management, in addition, be sure to discuss any problems she had in any previous pregnancy, if health problems are under control and get good prenatal care, likely to have a normal, healthy baby (**Clement EG et al,2019**) .

1.3.2 During Pregnancy :

Pregnancy symptoms and complications can range from mild and annoying discomforts to severe, life- threatening, illnesses, sometimes it can be difficult for a woman to determine which symptoms are normal and which are not, problems during pregnancy may include physical and mental conditions that affect the health of the mother or the baby, these problems can be caused by or can be made worse by being pregnant, many problems are mild and do not progress, however, when they do, they may harm the mother or her baby, there are ways to manage problems that come up during pregnancy (**Danielle Betz et al,2019**) .

The following are some common maternal health conditions or problems that happen during pregnancy :

1.3.2.1 Anemia :

Anemia is having lower than the normal number of healthy red blood cells, treating the underlying cause of the anemia will help restore the number of healthy red blood cells, women with pregnancy related anemia may feel tired and weak, this can be helped by taking iron and folic acid supplements,health care provider will check iron levels throughout pregnancy (**Luft FC et al, 2016**).

1.3.2.2 Urinary tract infections (UTI) :

A UTI is a bacterial infection in the urinary tract, female may have a UTI if she have :

- Pain or burning when she use the bathroom .
- Fever, tiredness,or shakiness .
- An urge to use the bathroom often .
- Pressure in her lower belly .
- Urine that smells bad or looks cloudy or reddish .
- Nausea or back pain (**Allen RE et al,2017**) .

1.3.2.3 Mental Health Conditions :

Some women have depression during pregnancy, symptoms of depression are :

- A low or sad mood .
- Loss of interest in fun activities .
- Changes in appetite, sleep, and energy .
- Problems thinking, concentrating, and making decisions .
- Feelings of worthlessness, shame, or guilt .
- Thoughts that life is not worth living .

When many of these symptoms occur together and last for more than a week or two at a time, this is probably depression, depression that persists during pregnancy can make it hard for a woman to care for herself and her unborn baby, having depression before pregnancy also is a risk factor for postpartum depression, getting treatment is important for both mother and baby, if female have a history of depression, it is important to discuss this with her health care provider early in pregnancy so that a plan for management can be made (**Cerdira AS et al,2018**) .

1.3.2.4 Hypertension (High Blood Pressure) :

Chronic poorly-controlled high blood pressure before and during pregnancy puts a pregnant woman and her baby at risk for problems,it is associated with an increased risk for maternal complications such as preeclampsia external,placental abruption (when the placenta separates from the wall of the uterus),and gestational diabetes, these women also face a higher risk for poor birth outcomes such as preterm delivery, having an infant small for her gestational age, and infant death, the most important thing to do is to discuss blood pressure problems with provider before female become pregnant so that appropriate treatment and control of her blood pressure occurs before pregnancy, getting treatment for high blood pressure is important before, during, and after pregnancy (**Donald Wothe et al,2019**) .

1.3.2.5 Diabetes During Pregnancy :

Learn about types of diabetes during pregnancy, the percentage of women affected, and what CDC : center of disease control is doing to address this important health topic, managing diabetes very important and it can be help women to have healthy pregnancies and healthy babies (**Vivian U et al,2017**).

1.3.2.6 Infections :

Can complicate pregnancy and may have serious consequences for a woman,her pregnancy outcomes, and her baby, screening and treatment for these infections,and vaccinations against viruses, such as hepatitis B and human papillomavirus, can prevent many bad outcomes, a variety of bacterial, viral,and parasitic infections may complicate a pregnancy, infections can be harmful to both the mother and the baby, so it's important to seek treatment right away, some examples include:

- a urinary tract infection .
- bacterial vaginosis .
- cytomegalovirus .
- group B Streptococcus .
- hepatitis B virus,which can spread to the baby during birth .
- influenza .
- toxoplasmosis, which is an infection caused by a parasite found in cat feces,soil, and raw meat .
- fungel infection .
- Zika virus (**Munro Ke et al,2019**) .

1.3.2.7 Hyperemesis gravidarum :

Many women have some nausea or vomiting, or morning sickness, particularly during the first trimeter of pregnancy, the cause of nausea and vomiting during pregnancy is believed to be rapidly rising blood levels of a hormone called HCG : human chorionic gonadotropin, which is released by the placenta,however, hyperemesis gravidarum occurs when there is severe,persistent nausea and vomiting during pregnancy more extreme than morning sickness, this can lead to weight loss and dehydration and may require intensive treatment .

Most pregnancies occur without complications, however, some women who are pregnant will have complications that can involve their health, their baby's health, or both, even with complications, early detection and prenatal care can reduce any further risk to the pregnant and her baby (**Gunner T et al,2018**) .

1.4 Etiology :

While there are some things that increase the risk of miscarriage, generally it isn't a result of something that the pregnant did or didn't do, if she is having difficulty maintaining pregnancy, the doctor may check for some known causes of miscarriage, during pregnancy, the body supplies hormones and nutrients to the developing fetus, this helps the fetus grow, most first trimester miscarriages happen because the fetus doesn't develop normally,there are different factors that can cause this (**Magowan et al,2016**).

1.4.1 Genetic or chromosome issues :

Chromosomes hold genes, in a developing fetus, one set of chromosomes is contributed by the mother and another by the father, examples of these chromosome abnormalities include :

- Intrauterine fetal demise : The embryo forms but stops developing before that see or feel symptoms of pregnancy loss .
- Blighted ovum : No embryo forms at all .
- Molar pregnancy : Both sets of chromosomes come from the father, no fetal development occurs .
- Partial molar pregnancy : The mother's chromosomes remain, but the father has also provided two sets of chromosomes, errors can also occur randomly when the cells of the embryo divide, or due to a damaged egg or sperm cell, problems with the placenta can also lead to a miscarriage (**Meriwether et al,2018**) .

1.4.2 Underlying conditions and lifestyle habits :

Various underlying health conditions and lifestyle habits may also interfere with the development of a fetus, exercise and sexual intercourse do not cause miscarriages, working won't affect the fetus either, unless the pregnant exposed to harmful chemicals or radiation, conditions that can interfere with fetus development include :

- poor diet,or malnutrition .
- drug and alcohol use .
- advanced maternal age .
- untreated thyroid disease .
- issues with hormones .
- uncontrolled diabetes .
- infections .
- Trauma .
- problems with the cervix .
- abnormally shaped uterus .
- severe high blood pressure .
- food poisoning .
- certain medications (**Guillebaud D et al,2017**) .

1.5 Pregnancy risk factors :

Other factors that may increase the risk for complications include :

- being pregnant at age 35 or older .
- being pregnant at a young age .
- having an eating disorder like anorexia .
- smoking cigarettes .
- using illegal drugs .
- drinking alcohol .
- having a history of pregnancy loss or preterm birth .
- carrying multiples, such as twins or triplets .
- miscarriage (**Oats R et al,2016**) .

1.6 Miscarriage signs :

The symptoms of a miscarriage vary, depending on the stage of pregnancy, in some cases, it happens so quickly that may not even know pregnant before miscarry, there are some of the symptoms of miscarriage :

- heavy spotting .
- vaginal bleeding .
- discharge of tissue or fluid from vagina .
- severe abdominal pain or cramping .
- mild to severe back pain .

It’s also possible to have these symptoms without experiencing a miscarriage, but the doctor will want to conduct tests to make sure that everything is fine (**Hacker et al,2018**) .

1.7 Diagnosis approach :

Health care provider will perform a pelvic exam, an ultrasound test and blood work to confirm a miscarriage, if the miscarriage is complete and the uterus is empty, then no further treatment is usually required, occasionally, the uterus is not completely emptied, so a dilation and curettage (D&C) procedure is performed, during this procedure, the cervix is dilated and any remaining fetal or placental tissue is gently removed from the uterus, as an alternative to a D&C, certain medications can be given to cause the body to expel the contents in the uterus, this option may be more ideal in someone who wants to avoid surgery and whose condition is otherwise stable, blood work to determine the amount of a pregnancy hormone (hCG) is checked to monitor the progress of the miscarriage (**Beischer et al,2015**) .

Blood tests, genetic tests, or medication may be necessary if a woman has more than two miscarriages in a row (called recurrent miscarriage),some diagnostic procedures used to evaluate the cause of repeated miscarriage include pelvic ultrasound, hysterosalpingogram (an X-ray of the uterus and fallopian tubes),and hysteroscopy (a test in which the doctor views the inside of the uterus with a thin, telescope like device inserted through the vagina and cervix **(Symonds M et al,2017) .**

1.8 Differential Diagnosis for miscarriage or period :

Many times, a miscarriage can happen before the woman even know that she is pregnant, additionally,as with the menstrual period, some of the symptoms of a miscarriage involve bleeding and cramping, when trying to distinguish between a period and a miscarriage, there are several factors to consider :

- Symptoms : Severe or worsening back or abdominal pain as well as passing fluids and large clots could indicate a miscarriage .
- Time : A miscarriage very early in pregnancy can be mistaken for a period,however this is less likely after eight weeks into a pregnancy .
- Duration of symptoms : The symptoms of a miscarriage typically get worse and last longer than a period (**Bain et al,2011**) .

1.9 Miscarriage types :

There are many different types of miscarriage, depending on symptoms and the stage of pregnancy, the doctor will diagnose the condition as one of the following (**Doubilet PM et al,2018**) :

- Complete miscarriage : All pregnancy tissues have been expelled from the body .
- Incomplete miscarriage : passed some tissue or placental material,but some still remains in the body .
- Missed miscarriage : The embryo dies without knowledge,and don't deliver it .
- Threatened miscarriage : Bleeding and cramps point to a possible upcoming miscarriage (**Choobun T et al,2018**) .
- Inevitable miscarriage : The presence of bleeding, cramping, and cervical dilation indicates that a miscarriage is inevitable .
- Septic miscarriage : An infection has occurred within the uterus .
- Chemical Pregnancy : Despite the name, a chemical pregnancy is not a false pregnancy or a false positive on a pregnancy test, in fact, it's a very early miscarriage, doctors believe chemical pregnancies are usually caused by chromosomal abnormalities, some women who have a chemical pregnancy never even knew they were pregnant, as the bleeding from the pregnancy loss often occurs around the same time as a woman's period, that being said, home pregnancy tests are so good now at detecting hCG

levels,that many women often find out that they're pregnant very early (**Petrous S et al,2015**) .

- Ectopic pregnancy : Ectopic pregnancies happen when a fertilized egg implants someplace other than in the uterus, such as in one of the fallopian tubes, sometimes risk factors exist, but other times the cause is unknown,symptoms of an ectopic pregnancy may include severe abdominal cramping and dizziness.
- First-Trimester Miscarriage : First-trimester miscarriage, sometimes called spontaneous abortion, is very common but also heartbreaking for most moms, it's normal to have a lot of questions about signs of miscarriage, diagnosis, miscarriage causes, treatment, and risk factors, be sure to talk to the doctor,so the questions are answered and worries are addressed (**Rausch M et al,2017**) .
- Blighted Ovum : A blighted ovum is a miscarriage in which the baby doesn't develop, but a gestational sac continues to grow, and you may continue to experience pregnancy symptoms, a blighted ovum can be a missed miscarriage treated with a dilation and curettage, also known as a D&C, or it may end naturally (**Colleselli V et al,2018**) .
- Molar Pregnancy : Molar pregnancy is a rare condition that causes pregnancy tissue to overgrow and the fetus doesn't develop normally, molar pregnancies never develop normally, the cause is a chromosomal abnormality that occurs at the time of fertilization, this type of pregnancy requires close follow up with obstetrician .
- Second Trimester Miscarriage : Late miscarriages, such as those in the second trimester, can happen for a number of reasons, some of these causes might be chromosomal abnormalities, cervical insufficiency, congenital birth defects, placental problems, or other factors (**Slava V et al,2018**) .

- Preterm Delivery From Cervical Insufficiency : Incompetent cervix, or cervical insufficiency, is a medical condition in which the cervix dilates too early in the pregnancy, resulting in pregnancy loss or premature birth, risk factors for cervical insufficiency include having had a dilation and curettage (D&C),genetic disorders, and cervical trauma .
- Stillbirth : Stillbirth is the death of a baby in the womb before birth, potential causes and contributing factors to stillbirth include infection, placenta problems, birth defects, pregnancy complications, high blood pressure in the mother, umbilical cord issues, and maternal medical complications .
- Termination of Desired Pregnancy for Medical Reasons : Selective abortion is a divisive issue and a delic ate matter for parents to consider when prenatal screening results in the diagnosis of a severe chromosomal condition with a poor medical prognosis (**Melville NA et al,2017**) .

1.11 First Trimester Miscarriage :

An estimated many of known pregnancies end in miscarriage, the loss of a pregnancy before the 20th week,the actual number is likely higher, because many miscarriages occur very early on, before a woman knows she is pregnant, and may simply seem to be a heavy period on or near schedule, most clinically recognized miscarriages occur between the seventh and 12th week after a woman's last menstrual period, the chances of miscarriage decrease significantly once a heartbeat has been detected on ultrasound or by Doppler stethoscope (**Brown T et al,2016**),the vast majority of miscarriages (also called spontaneous abortions) cannot be prevented, they are random events that are not likely to recur, up to 70 percent of first trimester miscarriages, and 20 percent of second-trimester miscarriages, are caused by chromosomal anomalies, other known causes include infection, abnormalities of the uterus or cervix, smoking, substance abuse, exposure to environmental or industrial toxins,diabetes, thyroid disease, and autoimmune disease, older women are more likely to miscarry than younger women, serious physical trauma can also cause a miscarriage, in rare cases, women miscarry after diagnostic tests, such as chorionic villus sampling (CVS) or amniocentesis, most of the time,a specific cause for miscarriage is not identified (**Schreiber CA et al,2018**) .

The first symptoms of miscarriage are usually spotting or bleeding, followed by cramps in lower back or abdomen, other signs include fluid or tissue passing from the vagina, about 1 in 4 women experience some vaginal bleeding or spotting during their first trimester, if the bleeding is light and lasts only one to two days, it isn't associated with a greater risk of miscarriage, however, heavy bleeding is associated with miscarriage (**El Hashim H et al,2017**) .

First-trimester miscarriage is most likely a random event, blood tests on the parents may identify or rule out hormonal, immunological, or

chromosomal abnormalities, examinations of the uterus by ultrasound, hysteroscopy, hysterosalpingography, and/or an endometrial biopsy may also provide important information (**Kolte AM et al,2019**) .

Early pregnancy period includes several time intervals when the most significant for angiogenesis events occur, determining further course and outcome of pregnancy, during gestation up to 6 weeks, the primary fetal circulatory system and placental bed with the development of villi are formed,and extensive vascularization of placental villous tree occurs,the 6–8th weeks of pregnancy are marked by the start of transition to the placental circulation, as well as by the most expressed invasion of extravillous trophoblast into maternal spiral arteries (first wave of trophoblast invasion),the period of 11–13 weeks is considered to be borderline and is characterized by the completion of embryogenesis, the starting period of fetal development,fading of the first wave of trophoblast invasion and further increase in the volume of uteroplacental blood flow .

During trophoblast invasion into endometrium, interactions with components of the extracellular matrix, which is mediated by cell adhesion molecules, occur (**Pereza N et al,2017)** .

1.11 Causes for increased rate of Miscarriage :

It might be that the rate of spontaneous abortion has increased in the recent years, Several factors could be involved :

- More pregnancies are obtained in a subfertile or infertile population after correction of various disorders, obviously these pregnancies are more fragile and are at high risk for early loss .
- All kinds of technologies for assisted reproduction have a recognized high rate of early placental loss (**Giakoumelous S et al,2016**) .
- If there is a clear trend towards delayed childbearing,older women : over 35, have an overall loss rate of 31%,while under 34 it is only 17% .

- In the recent years, environmental problems have become prominent and pollution has been added as a cause of abortion, however,accidental irradiatio is also associated with an increased abortion rate, similarly, the rate of pregnancy loss increases with the effect of drug abuse, exposure to various chemicals and toxins in laboratories or factories,or to volatile anesthetics in operating rooms (**Stevens SM et al,2016**) .
- Human reproduction is adversely affected by stress, we live in a society in which emotional disturbances are prominent and affect all facets of daily life like (education, job, health,etc.) .
- Serious illnesses are better treated, and modern treatments allow such patients to become pregnant with, however, a high risk of demise (**Skeith L et al, 2018**) .

1.12 Blood tests :

1.12.1 Beta human corionic gonadotropin :

Human chorionic gonadotropin (hCG) is a chemical created by trophoblast tissue, tissue typically found in early embryos and which will eventually be part of the placenta, measuring hCG levels can be helpful in identifying a normal pregnancy, pathologic pregnancy, and can also be useful following an aborted pregnancy, there is also a benefit in measuring hCG in a variety of cancers including choriocarcinoma and extra-uterine malignancies (**Chen H et al,2019**) .

Human chorionic gonadotropin is a hormone produced primarily by syncytiotrophoblastic cells of the placenta during pregnancy,the hormone stimulates the corpus luteum to produce progesterone to maintain the pregnancy, smaller amounts of hCG are also produced in the pituitary gland,the liver, and the colon, certain malignancies can also produce either hCG or hCG-related hormone, trophoblastic cancers (hydatidiform mole, choriocarcinoma, and germ cell tumors) are associated with high serum levels of hCG-related molecules (**Feng Y et al,2018**) .

The hormone itself is a glycoprotein composed of two subunits, the alpha and beta subunits, there are multiple forms found in the serum and urine during pregnancy including the intact hormone and each of the free subunits, hCG is primarily catabolized by the liver ,although about 20% is excreted in the urine, the beta subunit is degraded in the kidney to make a core fragment which is measured by urine hCG tests (**Bordewijk EM et al,2019**) .

Serum tests for hCG are immunometric assays, this means that they use two antibodies that bind to the hCG molecule, a fixed antibody and a radiolabeled antibody which adhere to different sites on the molecule, sandwiching and immobilize the molecule to make it detectable, assays involve washing away the excess serum components and measuring the amount of remaining labeled hCG to give a quantitative result,there are more

than 100 different assays commercially available which results in significant variability in reported values (**Fox C et al,2019**) .

Urine assays are similar, although many detect total hCG levels greater than 20 mIU/mL, many over the counter urine pregnancy tests do not detect hyperglycosylated hCG, which accounts for most of the hCG in early pregnancy, resulting in a wide range of sensitivities of these tests, serum testing is much more sensitive and specific than urine testing, urine testing, however, is more convenient, affordable, comfortable for patients, has a fast turnaround (5 to 10 minutes),and does not require a medical prescription (**Moussa HN et al,2019**) .

HCG is an important hormone in pregnancy, and its clinical utility is primarily centered around its detection in early pregnancy, along with serial measurement during pregnancy and pregnancy related complications, levels of hCG can vary widely between women with normal pregnancies, typically, serum and urine concentrations of hCG rise exponentially in the first trimester of pregnancy, doubling about every 24 hours during the first 8 weeks, the peak is usually around 10 weeks of gestation and then levels decrease until about the 16th week of gestation where they remain fairly constant until term (**Gaskins AJ et al,2018**) .

Extrauterine (ectopic) pregnancies usually have a rate of rise that is low without the typical doubling, however,given the large range of normal hCG levels and inconsistent rate of rise of this hormone, checking serum levels is typically paired with ultrasound evaluation to improve sensitivity and specificity, return of hCG to zero following delivery or termination of pregnancy ranges from 7 to 60 days, trending the fall of hCG levels can be important in termination of molar pregnancies and also following the

termination of normal or ectopic pregnancies to be assured that the therapy has been successful (**Kolte AM et al,2019**) .

Detection of hCG is also useful in the evaluation of trophoblastic disease, including complete and partial hydatidiform mole, postmolar tumor, gestational choriocarcinoma, testicular choriocarcinoma, and placental site trophoblastic disease,all of these entities produce hCG,varying levels of which are reported on commercial assays (**Brezina PR et al,2016**),a total hCG level of greater than 100,000 mIU/mL in early pregnancy,for example, is highly suggestive of a complete hydatidiform mole, although many normal pregnancies may reach this level at their peak around weeks 8 to 11 of gestation, precise hCG measurements are important to assess the tumor mass, the successful treatment of malignancy, and to test for recurrence or persistence of disease (**Michael Weber et al,2019**) .

HCG in the serum increases with age in nonpregnant women, a cut off of 14 mIU/mL has been suggested for use in interpreting results in women over the age of 55,in all nonpregnant patients, testicular cancer, ovarian cancer, bladder cancer,or other malignancy should be evaluated as a source of persistently positive hCG testing (**Ikuma S et al,2019**) .

1.12.2 Glycodelin-A :

Glycodelin is a glycoprotein that belongs to the lipocalin superfamily, depending on glycosylation, glycodelin appears in various isoforms, in the uterus, glycodelin-A is the major progesterone-regulated glycoprotein secreted into uterine luminal cavity by secretory decidualized endometrial glands, successful pregnancy depends largely on adequate placentation and maternal tolerance of the fetus, glycodelin-A is a glycoprotein abundant in the decidua during early pregnancy, it plays an important role in placental development and fetomaternal defens, and it interacts by its unique carbohydrate side chains with the cell surface of various cell types in the human fetomaternal interface, particularly the trophoblasts and the immune cells, and modulates their functions and differentiation to permit successful pregnancy (**Zhang M et al,2017**) .

Glycodelin-A (GdA) is a glycoprotein secreted from the endometrial glands and decidual glandular epithelium, given its abundance and ubiquitous distribution in the first trimester uterus, GdA may be involved in early placental development via its modulatory effect on immune and trophoblast cells (**Kutteh WH et al,2019**) .

Glycodelin-A (GdA) is an abundant glycoprotein in the first-trimester deciduas, it is involved in fetomaternal defense and early placental development through its regulatory activities in various immune cells, glycodelin-A is a glycoprotein consistent with its peak expression in the decidua between 6 and 12 weeks of gestation, abnormal levels of glycodelin-A in the endometrium, uterine flushings, and/or maternal serum correlate with unexplained infertility, early pregnancy loss, and recurrent miscarriage (**Hazard FK et al,2015**) .

Glycodelin A (GdA), also known as placental protein 14 (PP14), is the most abundant glycoprotein during implantation and early pregnancy, in addition, GdA has been proposed to be involved in maintaining a healthy pregnancy,as its low levels are associated with habitual abortion, recurrent miscarriage, and unexplained infertility (**Kling C et al,2016**) .

Glycodelin A levels increase rapidly after implantation, reaching a maximum at 8 to 10 weeks of gestation and subsequently declining in a pattern that mimics the changes in hCG, GdA inhibits activation and proliferation, and induces apoptosis of T cells, by selectively inducing Th1 cell death, and it may shift the Th1/Th2 ratio at the feto-maternal interface, this is also achieved indirectly through enhanced expression of Fas in the Th1 cells, thus making them vulnerable to cell death through Fas ligand expressed on trophoblast, endometrial, and activated T helper cells (**Pereza N et al,2017**) .

GdA promotes secretion of the Th2 cytokines IL-6 and IL-13 from NK cells,and induces immunological tolerance of dendritic cells and apoptosis of monocytes, specific glycosylation is a prerequisite for the biological activities of GdA, reduction in α2-6 sialylation of GdA,as in gestational diabetes, is associated with impairment of its T cell apoptosis-inducing activities, this review integrates recent studies on GdA and its role as a paracrine regulator in early pregnancy (**Shi X et al,2017**) .

Abnormal levels of glycodelin-A in the endometrium, uterine flushings,and/or maternal serum correlate with unexplained infertility, early pregnancy loss, and recurrent miscarriage, in normal pregnancies glycodelin reaches its highest levels in both maternal serum and amniotic fluid at 10th–16th gestational weeks,recent studies have demonstrated that glycodelin has the potential to regulate various processes, including immunosuppression, fertilization, implantation, and placentation, glycodelin participates in early

placental development through its modulatory effect on immune cells and cytotrophoblasts (**Meuleman T et al,2019**) .

Glycodelin was shown to be one of the proteins that is significantly decreased in endometriosis while most of the genes were up regulated during normal window of implantation, the biochemical properties and functions of glycodelin, which can be sorted as induction of cellular differentiation, restriction of malignant cell proliferation, decreasing expression of oncogenes, glycodelin is released from endometrial glands and decidual glandular tissue in response to progesterone ,human chorionic gonadotropin, and relaxin, it was demonstrated that glycodelin has suppressive effects on mixed leukocyte populations, has modulatory effects on trophoblastic cell line and also has a suppressive effect on natural killer cells, serum glycodelin levels were reported to reach its highest level at 6th–12th gestational weeks, decreased to a plateau at the end of second trimester and remained low during the third trimester in a normal pregnancy, which is an indicative of pleiotropic effect of glycodelin in early pregnancy (**Mekinian A et al, 2016**).

GdA regulates the development of the placenta, which is closely related to the processes of angiogenesis, the angiogenic effect of GdA is mediated by the enhancement of vascular endothelial growth factor (VEGF),which is involved in placental angiogenesis,since glycodelin has been found in the glandular structures of many tissues, including seminal vesicles, lobular and ductar epithelium of the mammary gland, eccrine sweat glands and parabronchial glands, it is suggested that it plays the role of a marker of differentiation and morphogenesis in glandular tissues, glycodelin-A (GdA) has been proposed to represent a potential biomarker of endometrial function,but little is known about its expression during the different phases of the menstrual cycle and under pathological conditions,in the light of its potential importance also in embryo implantation (**Schleussner E et al,2015**).

1.12.3 sFlt-1 :

Placental soluble fms-like tyrosine kinase-1 (sFlt-1),an antagonist of vascular endothelial growth factor, is considered an etiological factor of endothelial damage in pregnancy pathologies, an increase in the sFlt-1 level is associated with alterations of endothelial integrity (**Pasquier E et al,2018**) .

Appropriate development and growth of the fetus depend on adequate vascularization of both fetus and mother at the feto maternal unit, this vascularization involves uterine vasodilation and vessel remodeling upon trophoblast invasion, as well as vasculo and angiogenesis within the placenta, the consequences of abnormal vascular development have been associated with various pregnancy related pathologies (**Robertson SA et al,2019**) .

sFlt-1 also known as soluble VEGFR1 : vascular endothelial growth factor receptor, which binds both circulating VEGF and PlGF (placenta growth factor),soluble fms-like tyrosine kinase-1 (sFlt-1 or sVEGFR-1) is a tyrosine kinase protein with antiangiogenic properties, a non membrane associated splice variant of VEGF receptor 1 (Flt-1), sFlt-1 binds the angiogenic factors VEGF (vascular endothelial growth factor) and PlGF (placental growth factor),reducing blood vessel growth through reduction of free VEGF and PlGF concentrations, in humans, sFlt-1 is important in the regulation of blood vessel formation in diverse tissues, including the kidneys, cornea, and uterus, abnormally high levels of sFlt-1 have been implicated in the pathogenesis of preeclampsia (**Kemp MW et al,2018**) .

Production of antiangiogenic factors is an integral part of the normal angiogenesis, as a result of the molecular dialog during vascularization, production of inhibitors serves as a hamper for excessive trophoblast invasion, as well as an obstacle for the further development of the vascular bed and for vascularization of pathologically changed tissue sites, angiogenic

factors are specifically expressed in endothelium and in the placenta during pregnancy, these include receptors VEGFR1 (sFlt1), VEGFR2 (Flk1, KDR) and VEGFR3 (Flt4),soluble forms of these receptors are able to bind growth factors in circulation, slowing or blocking angiogenesis (**Christiansen OB et al,2019**) .

Humoral factors involved in vascular formation processes are more accessible for maternal circulation, and the change in mother's blood reflects changes in the fetal blood circulation and tissues, in this regard, a complex study of angiogenesis related factors and their ratios is crucial for understanding and predicting vascular morphogenesis disorders during pregnancy, soluble fms-like tyrosine kinase-1 (sFlt-1) seems to interfere with the events that inhibiting local angiogenesis and/or by impeding trophoblast invasion (**Egerup P et al,2017**) .

Increased production of antiangiogenic factor soluble fms-like tyrosine kinase receptor-1 (sFlt-1) by the placenta contributes to the obstetric pathophysiology, sFlt-1 is expressed at very high levels in the trophoblast, and its production is highly increased in hypoxic conditions, the role sFlt-1 in pregnancy failures is the focus of ongoing investigation, however, decreased sFlt-1 in the maternal circulation during the first trimester has recently been proposed as a potential marker for identifying risk of pregnancy loss (**Weber Schoendorfer C et al,2017**) .

Two-stage theory of the pathophysiology of systemic vascular dysfunction, predisposing immunological, genetic,and preexisting maternal risk factors may affect abnormal cytotrophoblast invasion of spiral arteries (abnormal placentation) (First stage),the reduced uteroplacental perfusion

induces placental release of antiangiogenic factors (soluble fms-like tyrosine kinase 1 (sFlt1)) into the maternal circulation, which antagonizes proangiogenic factors, leading to endothelial dysfunction and systemic vascular dysfunction (Second stage) (figure 1.1) **(Int. J. et al,2019) .**

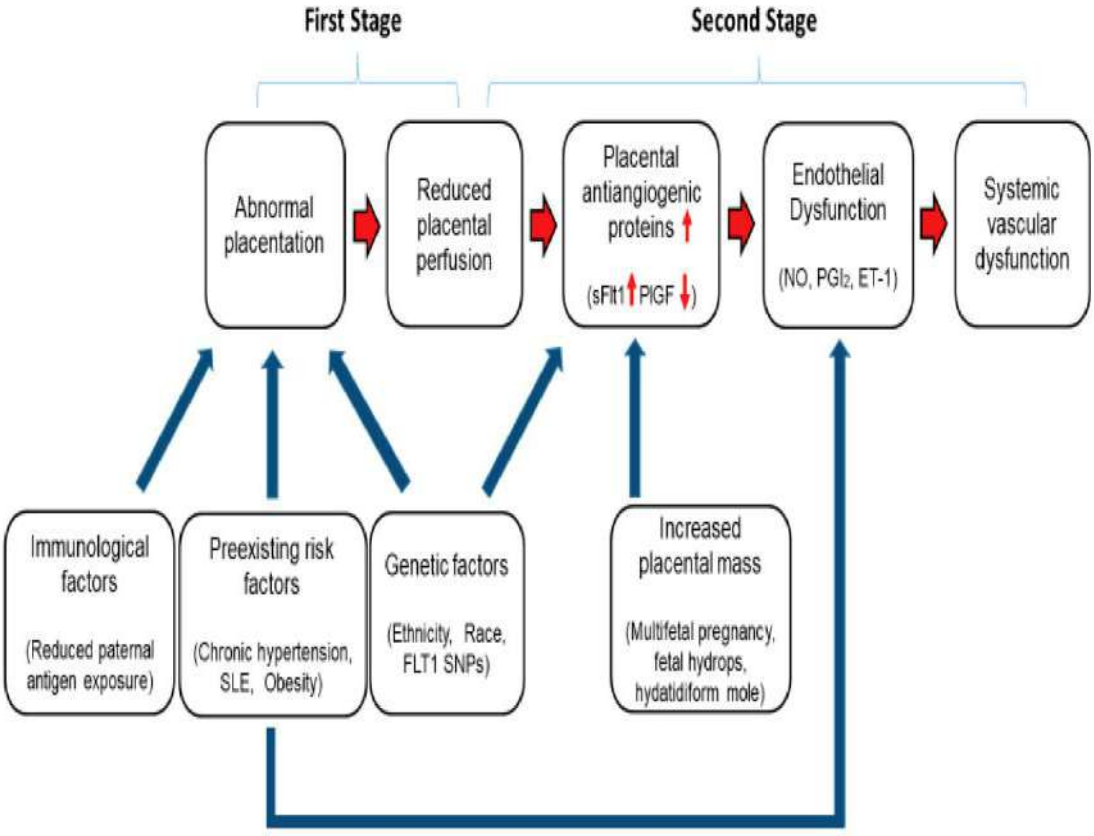

Figure 1.1 : Two-stage theory of the pathophysiology of systemic vascular dysfunction (Int. J. et al,2019) .

1.12.4 PlGF :

Placenta growth factor (PlGF) is a homodimeric growth factor that competes with VEGF for binding to VEGF R1/Flt-1, it therefore increases the availability of VEGF to bind to VEGF R2/KDR/Flk-1 and trigger angiogenesis,it can form heterodimers with some forms of VEGF and decrease the angiogenic effect of VEGF on VEGF R2, circulating PlGF levels increase during pregnancy, reaching a peak in mid-gestation, this increase is attenuated in preeclampsia,PlGF induces monocyte activation and migration as well as production of inflammatory cytokines and VEGF, these activities facilitate wound, bone fracture, and cardiac repair, but also contribute to inflammation in active sickle cell disease and atherosclerosis ,PlGF can also inhibit TIMP3 expression in the spleen, leading to immune triggering of hypertension (**Meng L et al,2018**) .

Vascular endothelial growth factor (VEGF),PlGF and sVEGF R1 are produced by trophoblasts, play key roles in regulating angiogenesis and are critical for successful placentation, both VEGF and PlGF promote angiogenesis by interacting with members of the VEGF receptor family found primarily on endothelial cells, these growth factors drive differentiation of,and are required to maintain, normal endothelial function, sVEGF R1, a soluble isoform of the transmembrane receptor for VEGF and PlGF, counteracts these angiogenic effects by binding circulating VEGF and PlGF and preventing activation of the membrane bound receptor, an increase in the production of sVEGF R1 may trigger the maternal endothelial dysfunction that results in the clinical findings of hypertension, proteinuria, and edema (**Agarwal A et al,2019**) .

PlGF, a member of the VEGF family, can promote placenta angiogenesis, increase vascular permeability, and enhance trophoblast cell activity, soluble fms-like tyrosine kinase-1 (sFlt-1) can decrease the serum concentration of PlGF, inhibit the biological function of PlGF, and impair the

permeability and integrity of vascular wall, leading to angiogenesis disorders, edema, urine protein, and hemoconcentration, increasing evidences showed that there were elevated serum concentration of sFlt-1 and declined serum concentration of PlGF in PE patients and that the degree of elevated or declined level was correlated with the severity of PE,because of this, sFlt-1 and PlGF were considered as the most promising serological indicators for PE diagnosis, Particularly, the sFlt-1/PlGF ratio could better reflect the antiangiogenic activity and could be used to predict the occurrence and prognosis of PE (**Coomarasamy A et al,2017**).

Predictive values varied largely across study countries, suggesting it may not necessarily be similar in different ethnic and geographical populations, PlGF is the most abundantly regulated angiogenic factor in uncomplicated first trimester deciduas, the discovering of the role of angiogenic-related factors (sFlt-1/PlGF) in the underlying pathophysiology of placental dysfunction, taking into account that angiogenesis related biomarkers are related to a particular placental insufficiency disease, these markers are important for early diagnosis and prognosis assessment (**Saccone G et al,2017**) .

1.14 Aim of the study :

To study the role of Glycodeline-A, sFlt-1, PlGF as predictor markers of first trimester pregnancy loss by :

- Investigate the serum levels of :

- Beta HCG
- Glycodelin-A
- sFlt-1
- PLGF

- Study the relationship between these markers in group of patients which have abortion at first trimester of pregnancy and group of healthy pregnancies control .

Chapter Two

Subjects Material and Methods

Chapter Two

Subjects, Materials and Methods

2.1 Patients :

The present study was conducted at Baghdad Teaching Hospital during the period from April 2019 to March 2020, it included more than 200 individual, we slelect from them 85 pregnant woman identified the first day of LMP who are confirmed positive pregnancy test in blood, matching age 21 – 35 year and gestational age 8 – 12 week, encountered them after taken blood sample during their attendance the Gynaecology consultation clinic at Baghdad Teaching hospital until the end of week 12 of pregnancy, 40 of them who have pregnancy loss at the this period are patients and the other 45 healty control who completed the same period without problems, a written consent was applied by each patient and an ethical approval had been approved by the Scientific Committee of the College of Medicine, University of Baghdad and the link with Ministry of Health and enviroment.

2.2 control :

Apparently healthy pregnancies whose their ages and gestational age were matched with patients group consisted of 45 individuals considered as control, all of them had received no treatment with no complain of other chronic or systemic disease, and all of them complete their period of pregnancy in this study without problems .

2.3 Exclusion criteria :

According to history which established by Consultant Gynaecologist or by available test results :

- Have autoimmune disease .
- Diabetic patients .
- Have genetic disease .
- Have thyroid problems .

2.4 Inclusion subject as patient :

Have pregnancy loss with unknown causes .

2.5 Study Protocols :

The study protocols include :

1. Determination of Beta HCG by enzyme-linked immunosorbent assay ELISA .
2. Determination of Glycodeline-A by enzyme-linked immunosorbent assay ELISA .
3. Determination of sFlt-1 by enzyme - linked immunosorbent assay ELISA .
4. Determination of PlGF by enzyme - linked immunosorbent assay ELISA .

2.6 Materials :

2.6.1 Instruments and equipments :

Table 2.1 : instruments and equipments

instruments or equipments	***Company***
Centrifuge	Hettich - Germany
Micropipeettes with disposable tips	GILSON-France
ELISA UNIT	Human - Germany
Refrigerator, Freezer	Japan
Water bath	Memmert - Germany
Eppendrofs and jeltube and planetube	Afma - Disposable
Disposable syringe 5 ml	

2.6.2 Kits :

Table 2.2 : Kits

Kit	*Company*
Beta HCG	Shanghia Yehua Biological Technology Co., Ltd., China .
Glycodeline-A	Shanghia Yehua Biological Technology Co., Ltd., China .
sFlt-1	Shanghia Yehua Biological Technology Co., Ltd., China .
PlGF	Shanghia Yehua Biological Technology Co., Ltd., China .

2.7 Methods :

2.7.1 Collection of blood sample:

Eight to ten milliliters (mls) of peripheral venous blood was aspirated from each patient and control subject, transferred into plain tube, allows clotting for 30 minutes, then the serum was separated by centrifugation at 2500 rpm for 10 minutes and stored at – 20 C^{o} till the time of measurements of the studied biochemical parameters : Beta HCG, Glycodeline-A, sFlt-1, PlGF By ELISA method .

2.7.2 Measurements :

2.7.2.1 Determination of Beta HCG ELISA method :

Beta HCG ELISA kit for quantitative determination of Beta HCG in human serum was supplied by Shanghia Yehua Biological Technology Co., Ltd., China .

2.7.2.1.1 Principle of the assay :

This kit is an Enzyme - Linked Immunosorbent Assay (ELISA), the plate has been pre-coated with Sheep B-HCG antibody, B-HCG present in the sample is added and binds to antibodies coated on the wells, and then biotinylated Sheep B-HCG Antibody is added and binds to B-HCG in the sample, then streptavidin-HRP is added and binds to the Biotinylated B-HCG antibody, after incubation unbound Streptavidin-HRP is washed away during a washing step, substrate solution is then added and color develops in proportion to the amount of Sheep B-HCG, the reaction is terminated by addition of acidic stop solution and absorbance is measured at 450 nm .

2.7.2.1.2 Normal range :

0.5 µIU/ml – 300 µIU/ml

2.7.2.1.3 Calculation of Result :

Construct a standard curve by plotting the average OD for each standard on the vertical (Y) axis against the concentration on the horizontal (X) axis and draw a best fit curve through the points on the graph, these calculations can be best performed with computer-based curve-fitting software and the best fit line can be determined by regression analysis .

2.7.2.1.4 Typical Data

This standard curve is only for demonstration purposes, a standard curve should be generated with each assay.

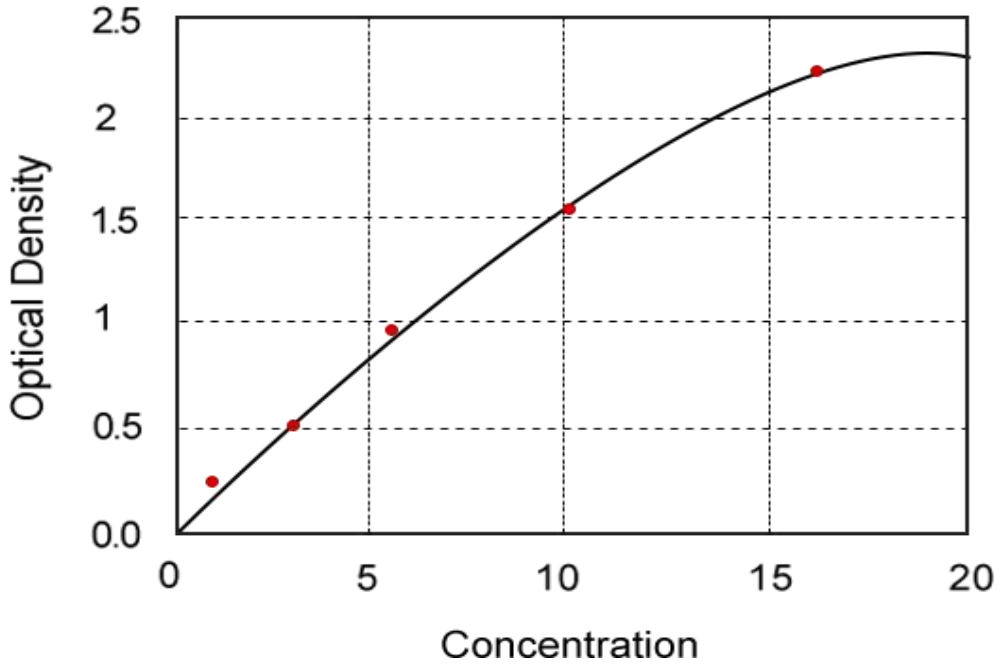

Figure 2.1 : standard curve chart for Beta HCG for reference only .

2.7.2.2 Determination of Serum Glycodelin-A ELISA method :

Glycodelin-A ELISA kit for quantitative determination of glycodeline-A in human serum was supplied by Shanghia Yehua Biological Technology Co., Ltd., China .

2.7.2.2.1 Principle of the assay :

This kit is an Enzyme-Linked Immunosorbent Assay (ELISA), the plate has been pre-coated with Bovine Glycodelin-A antibody, glycodelin-A present in the sample is added and binds to antibodies coated on the wells, and then biotinylated Bovine Glycodelin-A Antibody is added and binds to Glycodelin-A in the sample, then Streptavidin-HRP is added and binds to the Biotinylated Glycodelin-A antibody, after incubation unbound Streptavidin-HRP is washed away during a washing step, substrate solution is then added and color develops in proportion to the amount of Bovine Glycodelin-A, the reaction is terminated by addition of acidic stop solution and absorbance is measured at 450 nm .

2.7.2.2.2 Normal range :

1 ng/ml – 300 ng/ml

2.7.2.2.3 Calculation of Result :

Construct a standard curve by plotting the average OD for each standard on the vertical (Y) axis against the concentration on the horizontal (X) axis and draw a best fit curve through the points on the graph, these calculations can be best performed with computer-based curve-fitting software and the best fit line can be determined by regression analysis .

2.7.2.2.4 Typical Data :

This standard curve is only for demonstration purposes, a standard curve should be generated with each assay .

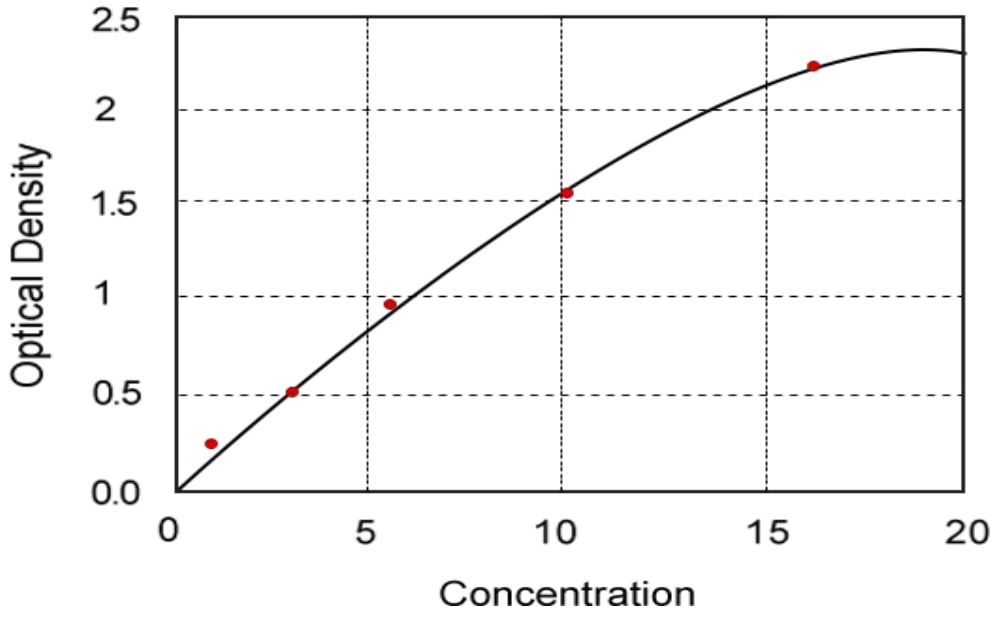

Figure 2.2 : standard curve chart for Glycodelin-A for reference only .

2.7.2.3 Determination of Serum sFlt-1 ELISA method :

sFlt–1 ELISA kit for quantitative determination of sFlt–1 in human serum was supplied by Shanghia Yehua Biological Technology Co., Ltd., China .

2.7.2.3.1 Principle of the assay :

This kit is an Enzyme-Linked Immunosorbent Assay (ELISA), the plate has been pre-coated with human SFLT-1 antibody, sFlt-1 present in the sample is added and binds to antibodies coated on the wells, and then biotinylated human sFlt-1 Antibody is added and binds to sFlt-1 in the sample, then Streptavidin-HRP is added and binds to the Biotinylated sFlt-1antibody, after incubation unbound Streptavidin-HRP is washed away during a washing step, substrate solution is then added and color develops in proportion to the amount of human sFlt-1, the reaction is terminated by addition of acidic stop solution and absorbance is measured at 450 nm .

2.7.2.3.2 Normal range :

0.5 ng/ml – 200 ng/ml

2.7.2.3.3 Calculation of Result :

Construct a standard curve by plotting the average OD for each standard on the vertical (Y) axis against the concentration on the horizontal (X) axis and draw a best fit curve through the points on the graph, these calculations can be best performed with computer-based curve-fitting software and the best fit line can be determined by regression analysis.

2.7.2.3.4 Typical Data :

This standard curve is only for demonstration purposes, a standard curve should be generated with each assay .

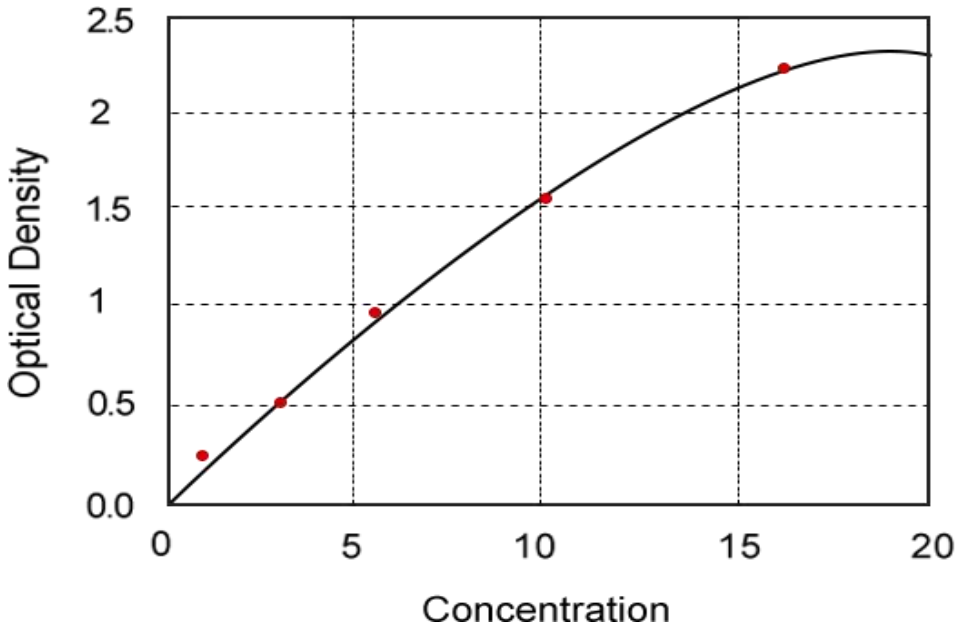

Figure 2.3 : standard curve chart for sFlt-1 for reference only .

2.7.2.4 Determination of Serum PlGF ELISA method :

PlGF ELISA kit for quantitative determination of PlGF in human serum was supplied by Shanghia Yehua Biological Technology Co., Ltd., China .

2.7.2.4.1 Principle of the assay :

This kit is an Enzyme-Linked Immunosorbent Assay (ELISA), the plate has been pre-coated with human PlGF antibody, PlGF present in the sample is added and binds to antibodies coated on the wells. And then biotinylated human PlGF Antibody is added and binds to PlGF in the sample, then streptavidin-HRP is added and binds to the Biotinylated PlGF antibody, after incubation unbound streptavidin-HRP is washed away during a washing step, substrate solution is then added and color develops in proportion to the amount of human PlGF, the reaction is terminated by addition of acidic stop solution and absorbance is measured at 450 nm .

2.7.2.4.2 Normal range :

8 ng/L – 1800 ng/L

2.7.2.4.3 Calculation of Result :

Construct a standard curve by plotting the average OD for each standard on the vertical (Y) axis against the concentration on the horizontal (X) axis and draw a best fit curve through the points on the graph, these calculations can be best performed with computer-based curve-fitting software and the best fit line can be determined by regression analysis .

2.7.2.4.4 Typical Data :

This standard curve is only for demonstration purposes, a standard curve should be generated with each assay.

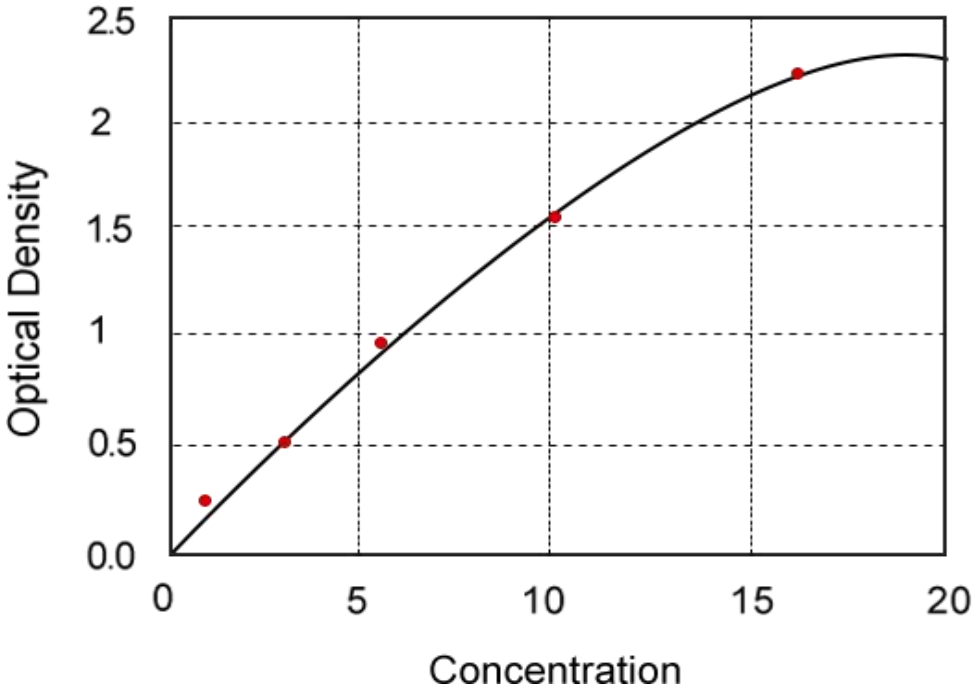

Figure 2.4 : standard curve chart for PlGF for reference only .

2.8 Statistical Analysis :

Data collected were coded and entered in the computer then analyzed using the available statistical software package of the SPSS-20 (Statistical packages for social sciences – version 20), data were presented as frequency, percentage, mean, standard deviation (SD), and range (minimum to maximum), to compare the significance of the difference in the mean values between patients and control student's t-test and / or ANOVA were applied, the comparison of p-value significance was determined as 0.05 as level of significance (sig .), the correlation between different parameters studied .

Pearson correlation was calculated for the correlation between two quantitative variables with its t-test for testing the significance of correlation, the correlation coefficient value (r) either positive (direct correlation) or negative (inverse correlation) with value :

- < 0.3 represent no correlation .
- 0.3 - < 0.5 represent weak correlation .
- 0.5 - < 0.7 represent moderate strength .
- > 0.7 represent strong correlation .

Receiver operator curve (ROC) used to see the validity of different parameters in separating cases from control, the true positive rate (Sensitivity) is plotted in function of the false positive rate (Specificity) for different cut-off points, each point on the ROC represents a Sensitivity/ Specificity pair corresponding to a particular decision threshold .

- Sensitivity : probability that a test result will be positive when the disease is present (true positive rate) .
- Specificity : probability that a test result will be negative when the disease is not present (true negative rate) .
- Positive Predictive Value (PPV) : probability that the disease is present when the test is positive .

- Negative Predictive Value (NPV) : probability that the disease is not present when the test is negative .
- Disease prevalence : whereas sensitivity and specificity and therefor the ROC and positive and negative likelihood ratio are independent of disease prevalence, positive and negative predictive values are highly dependent on disease prevalence or prior probability of disease, clinically, the disease prevalence is the same as probability .

When a test is used either for the purpose of screening or to exclude diagnostic possibility a cut – off value with a high sensitivity may be selected or when a test is used to confirm a disease a higher specificity may be required, P value considered when appropriate to be significant if less than or equal 0.05 . **(Wayne W Daniel, 2016) .**

Chapter Three

Results

Chapter Three

Results

3.1 Demographic features :

The results reppresented in this study were based on the analysis of results of 85 pregnant women divided into 40 woman have abortion at first trimester (8 – 12 weeks) compared them with 45 apparently healthy individuals considerds as controls under limited criteria , encountered these women until the end of week 12 of pregnancy .

3.1.1 Distribution of patients and Control according to age :

In the present study the age of pregnant women ranged between 21- 35 with mean ± S.D. of (27.125 ± 4.182) year , figure (3.1) showed that there was no statistical significant difference in the frequency of age groups between pregnant women have pregnancy loss and control group as healthy women Which is have mean ± S.D. of (27.244 ± 4.112) year (P ≥ 0.05) .

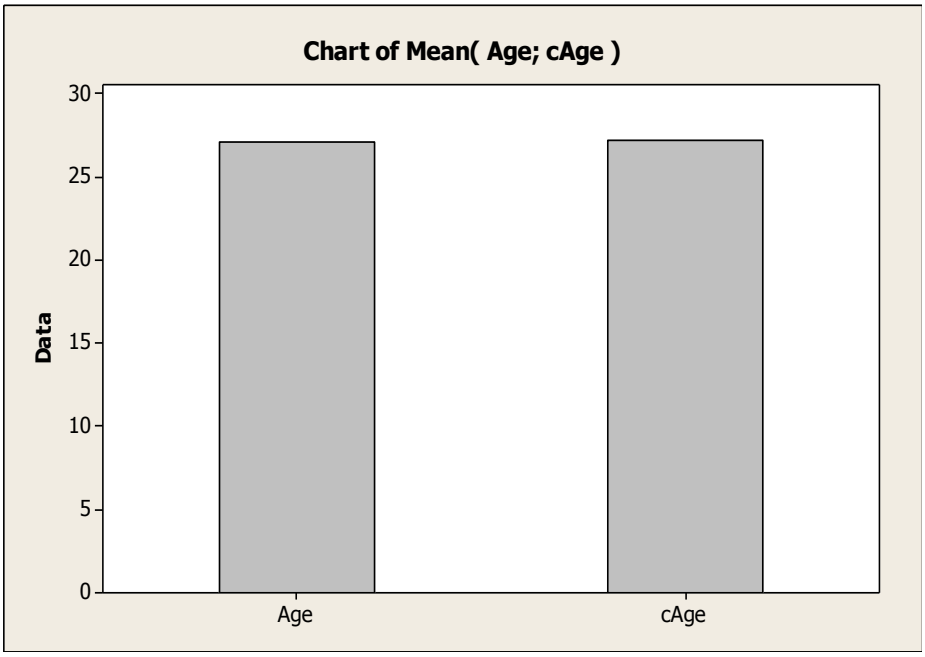

Figure 3.1:Distribution of patients and Control according to age .

3.1.2 Distribution of patients and Control according to gestational age:

Gestational age was found it's ranged between 8 -12 weeks with mean ± S.D. of (9.600 ± 1.296) week , figure (3.2) too showed that there was no statistical significant difference in the frequency of gestational age between pregnant women have pregnancy loss and control group as healthy women Which is have mean ± S.D. of (9.533 ± 1.099) week (P ≥ 0.05)

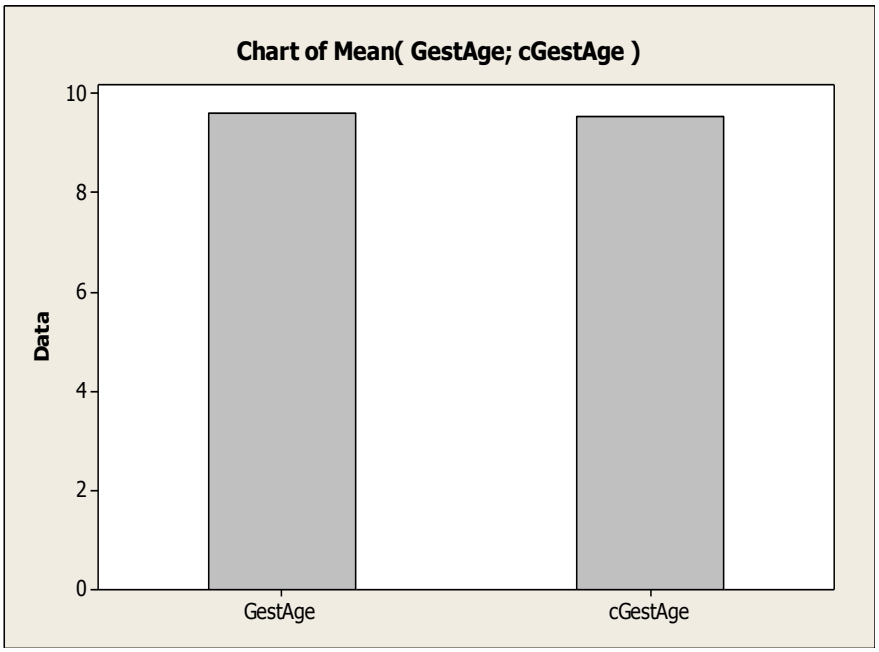

Figure 3.2:Distribution of patients and Control according to gestational age .

3.2 General description of lab features of patients which have abortion and healthy control group :

Table (3.1) shows the characteristics of lab. features of patients and control group , this table shows tbeta hCG mean ± SD in control group (2309.940 ±300.711) and in patients group (1024.774 ±4 5.939), glycodeline-A mean ± SD in control group (193.741 ± 20.056) and in patients group (70.944 ± 5.687) , sFlt-1 mean ± SD in control group (22.269 ± 1.916) and in patients group (118.215 ± 7.642) , PlGF mean ± SD in control group (340.294 ± 38.701) and in patients group (141.435 ± 28.410) .

3.3 Laboratory Findings and Comparision between patients which have abortin and healty control by their parameters :

The laboratory results of beta hCG showed that there is a higher value of beta hCG in Control group than patients which is have abortion with highly statistical significant difference ($P \leq 0.05$) and for the Glycodeline - A showed that there is a higher value of Glycodeline-A in Control group than patients which is have abortion with highly statistical significant difference ($P \leq 0.05$) and for the sFlt-1 showed that there is a higher value of sFlt-1 in patients which is have abortion than Control group with statistical significant difference ($P \leq 0.05$) and for the PlGF showed that there is a higher value of PlGF in Control group than patients which is have abortion with statistical significant difference ($P \leq 0.05$) figure (3.3) , (3.4) , (3.5) , (3.6).

Table 3.1 : Descriptive statistics of clinical and lab features of patients which have abortion and healthy control group

	Parameters	N	Mean ± Std. Deviation
1	**Age**	40	27.1250 ± 4.18292
	Control Age	45	27.2444 ± 4.11293
2	**Gest. age**	40	9.6000 ± 1.29694
	Control Gest. age	45	9.5333 ± 1.09959
3	**BetaHCG**	40	1024.7740 ± 45.939406
	Control BetaHCG	45	2309.9409 ± 300.711697
4	**Glycodein-A**	40	70.9447 ± 5.687327
	Control Glycodlin-A	45	193.7416 ± 20.056648
5	**sFlt-1**	40	118.2153 ± 7.642478
	Control sFlt-1	45	22.2696 ± 1.916359
6	**PIGF**	40	141.4350 ± 28.410843
	Control PIGF	45	340.2947 ± 38.701779

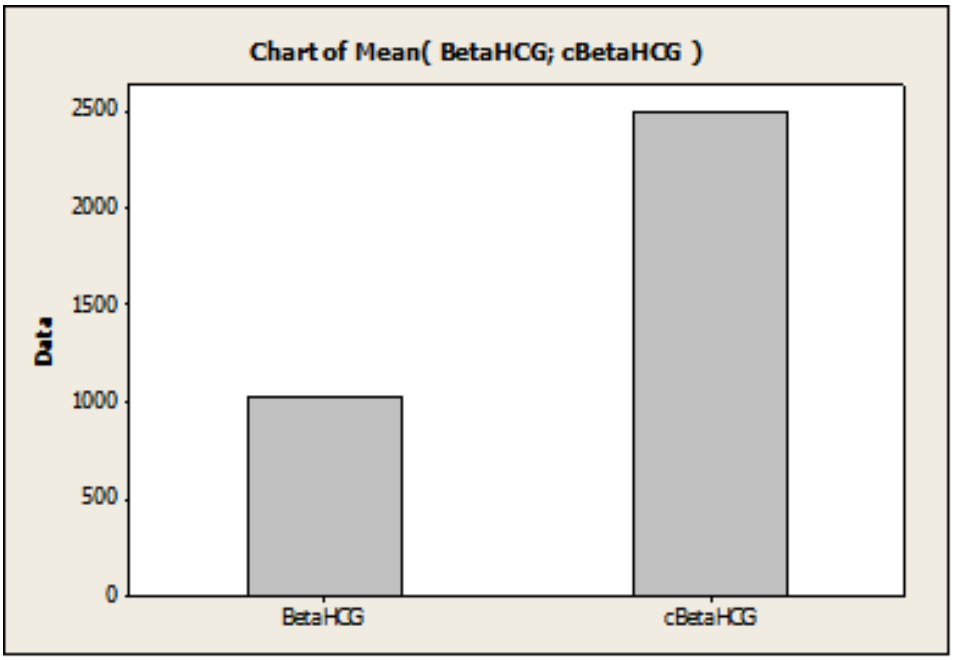

Figure 3.3:Distribution of patients Beta HCG and Healthy Control Beta HCG.

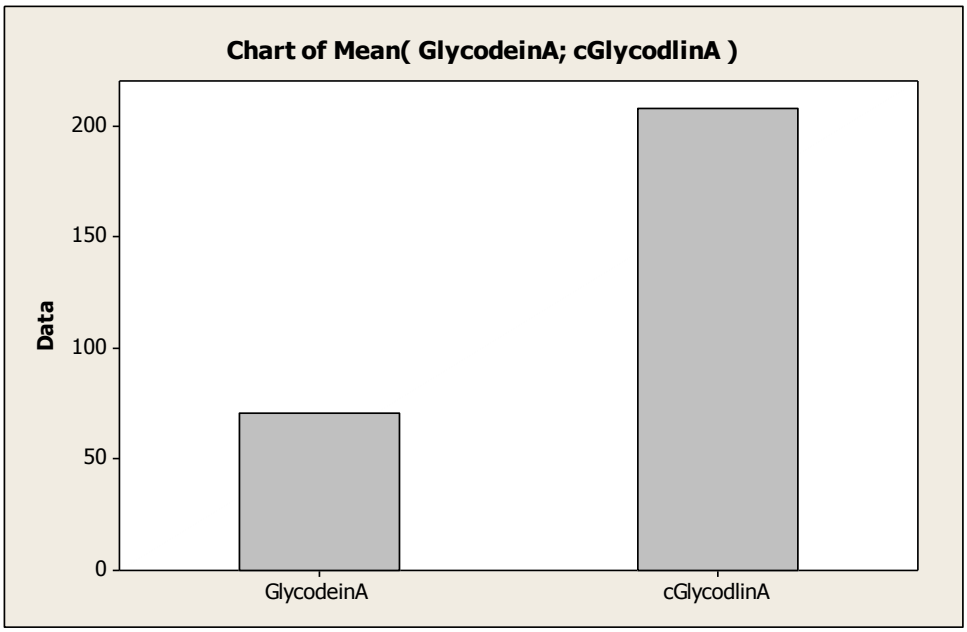

Figure 3.4:Distribution of patients Glycodeline-A and Healthy Control Glycodeline-A.

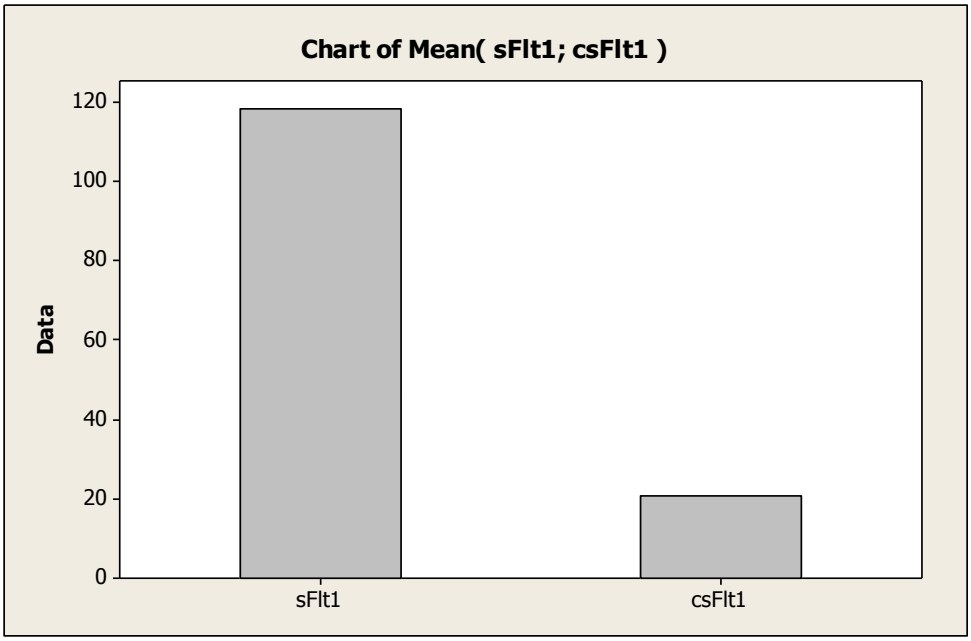

Figure 3.5:Distribution of patients sFlt-1 and Healthy Control sFlt-1

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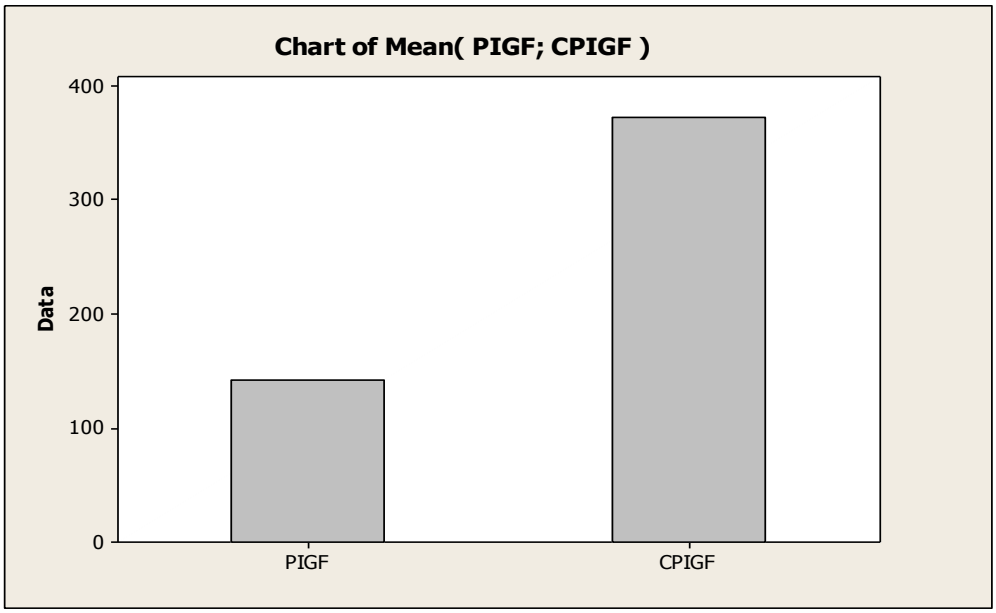


Figure 3.6:Distribution of patients PlGF and Healthy Control PlGF .

3.4 Diagnostic Utility of Biochemical Markers :

The receiver operator characterstics (ROC) of the validity of the studied biomarkers as predictor markers of first trimester pregnancy loss : beta HCG 0.625 , glycodeline-A 0.443 , sFlt-1 0.751, PlGF 0.684 , all of them have ability to differentiate patients have abortion from healthy controls by depend on other values of :

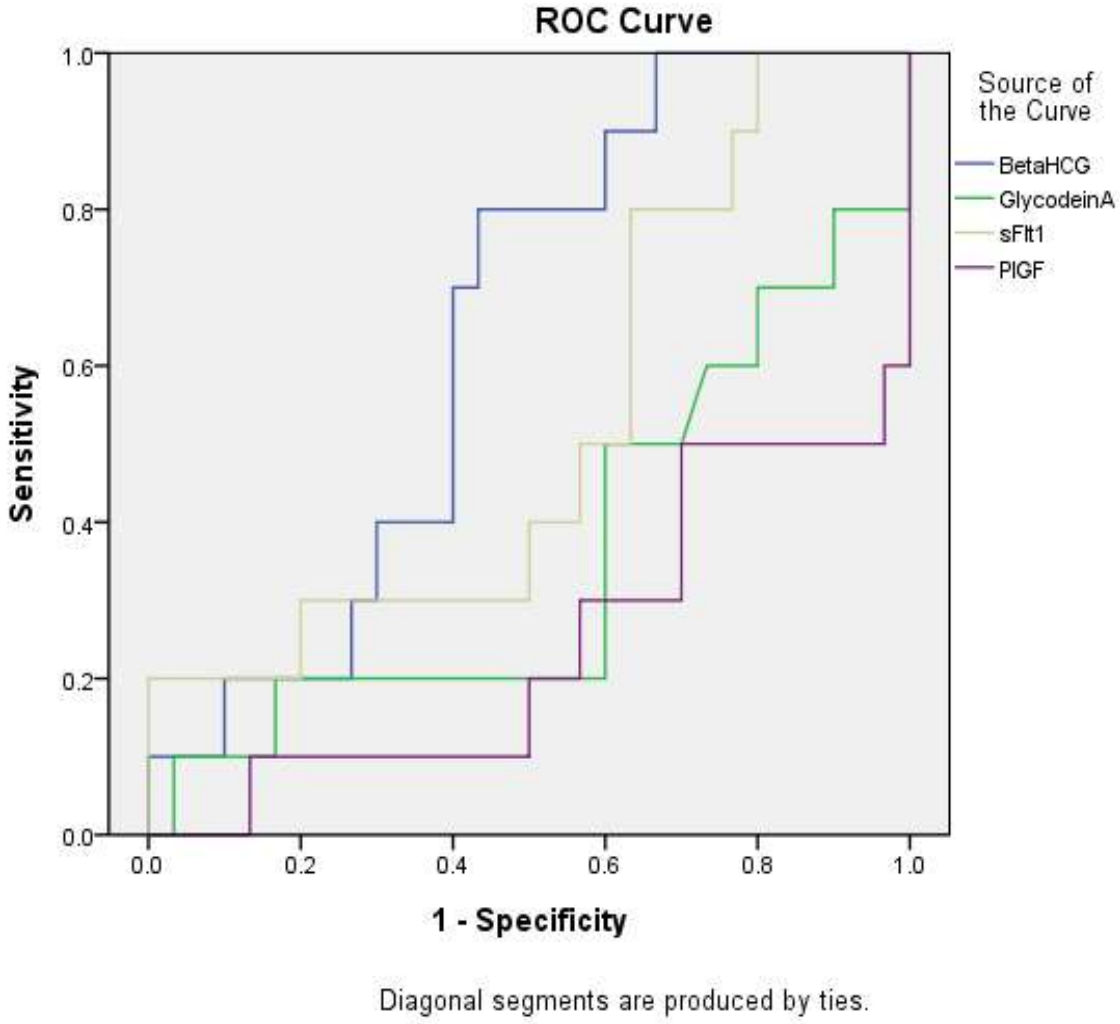
- P value beta HCG,Glycodeline-A,sFlt-1,PlGF ≤ 0.05 .
- Cut point beta HCG < 50.0 , Glycodeline-A > 1.0 , sFlt-1 < 5.0 , PlGF < 5.0 .
- Sensitivity beta HCG 86% , Glycodeline-A 77% , sFlt-1 75% , PlGF 77% .
- Specificity beta HCG 82% , Glycodeline-A 79% , sFlt-1 75% , PlGF 84% .
- Accuracy beta HCG 79% , Glycodeline-A 87% , sFlt-1 84% , PlGF 78% .
- Positive predictive value beta HCG 83% , Glycodeline-A 81% , sFlt-1 75% , PlGF 80% .
- Negative predictive beta HCG 78% , Glycodeline-A 74% , sFlt-1 77% , PlGF 75% , table (3.2) and figure (3.7) .

Table 3.2 : ROC analysis of the validity of the different markers as predictor of first trimester pregnancy loss .

Parameters	*ROC*	*Pvalue*	*Cut point*	*SN*	*SP*	*AC*	*PPV*	*NPV*
Beta HCG	0.625	0.03	< 50.0	86%	82%	79%	83%	78%
Glycodelin-A	0.443	0.01	> 1.0	77%	79%	87%	81%	74%
sFlt-1	0.751	0.05	< 5.0	86%	75%	84%	75%	77%
PlGF	0.684	0.05	< 8.0	77%	84%	78%	80%	75%

ROC:receiver operator characteristics , SN:sensitivity , SP:specificity

AC:accuracy , PPV:positive predictive value , NPV:negative predictive value



Figur 3.7 : ROC analysis of the validity of the different markers as predictor of first trimester pregnancy loss .

3.5 Correlation :

Table (3.2) reveales the correlation between different parameters studied in this work as seen as patients which have abortion , the present study showed :

- there is no significant correlation between each of age with other parameters ($P \geq 0.05$) :

- Beta HCG ($r = 0.024$)
- Glycodeline-A ($r = 0.013$)
- sFlt-1 ($r = 0.122$)
- PlGF ($r = 0.010$)

- and there is no significant correlation between gest.age with sFlt-1 ($r = -1.144$) ($P \geq 0.05$) :
- While there is significant positive correlation between ges.age with other parameters ($P \leq 0.05$) :

- Beta HCG ($r = 0.319$) .
- Glycodelin-A ($r = 0.324$) .
- PlGF ($r = 0.327$) .

- and there is significant negative correlation between sFlt-1 with other parameters ($P \leq 0.05$) :

- Beta HCG ($r = -0.399$) .
- Glycodelin-A ($r = -0.448$) .
- PlGF ($r = -0.385$) .
- Glycodelin-A have significant correlation with PlGF ($P \leq 0.05$) and ($r = 0.568$) and with Beta HCG ($P \leq 0.05$) ($r = 0.319$) .
- PlGF have significant correlation with Beta HCG ($P \leq 0.05$) ($r = 0.387$) .

Table 3.3 : Correlations coefficient among different parameters of patients which have abortion in the study.

		Age	GestAge	BetaHCG	GlycodeinA	sFlt1	PIGF
Age	*Pearson Correl.*	1	.265	.024	.013	-.122-	.010
	Sig. (2-tailed)		.099	.884	.934	.453	.953
	N	40	40	40	40	40	40
GestAge	*Pearson Correl.*	.265	1	.319	.324	-1.114	.327
	Sig. (2-tailed)	.099		.045	.447	.484	.039
	N	40	40	40	40	40	40
BetaHCG	*Pearson Correl.*	.024	.319	1	.319	-.399	.387
	Sig. (2-tailed)	.884	.045		.906	.011	.014
	N	40	40	40	40	40	40
GlycodeinA	*Pearson Correl.*	.013	.324	.319	1	-.448	.568
	Sig. (2-tailed)	.934	.447	.906		.362	.000
	N	40	40	40	40	40	40
sFlt1	*Pearson Correl.*	-.122-	-1.114	-.399	-.448	1	-.385
	Sig. (2-tailed)	.453	.484	.011	.362		.252
	N	40	40	40	40	40	40
PIGF	*Pearson Correl.*	.010	.327	.387	.568	-.385	1
	Sig. (2-tailed)	.953	.039	.014	.000	.252	
	N	40	40	40	40	40	40

Chapter Four

Discussion

Chapter Four

Discussion

4.1 Clinical Characteristics :

Early pregnancy loss is the most common complication of early pregnancy, affecting about 30 % of pregnancies following assisted reproduction and 10 % of spontaneously conceived pregnancies, the difference is explained by a later diagnosis of spontaneous pregnancy versus assisted reproduction pregnancy, and an early loss is easily overlooked, in fact, vaginal bleeding a common sign of early pregnancy loss can be confused with delayed menses and the loss remains unrecognized, the most common cause of a first trimester pregnancy loss is embryonal genetic abnormalities, which occurs in more than 50 % of the cases, with aneuploidy being the most frequent abnormality **(Lee SK et al, 2017),** multiple serologic and ultrasound markers have been investigated to identify pregnancies destined to be lost, however, serologic markers are unspecific and can help only after a pregnancy loss has already been diagnosed, transvaginal ultrasound (TVUS) provides high resolution images, low inter observer variability with high reliability, and is typically used to make diagnosis of intrauterine pregnancy and to follow up with its development, gestational sac (GS), yolk sac (YS), crown rump length (CRL), and heart rate (HR) are the parameters measured to evaluate early pregnancy, deviations in the ultrasound parameters have been alternatively investigated to predict first trimester pregnancy loss, the amniotic sac, which becomes visible at the beginning of the 7 week of gestation, is normally not contemplated in the prediction models, however it assists in dating a pregnancy correctly **(Calleja-Agius J et al, 2018) .**

Previous studies were cross sectional and provided estimates for pregnancy loss that were based on a combination of ultrasound, as well as serologic and demographic markers, the aim of this study was to estimate a role of Glycodelin-A, sFlt1, PlGF as predictor markers of first trimester pregnancy loss based on laboratory findings **(Kolte AM et al, 2015) ,** thus, we evaluated these markers and study the relation between them and first trimester pregnancy loss and known if there is a relationship between them, in addition, we wanted to identify which parameters were the most reliable to predict a pregnancy loss in first trimester, our hypothesis was that markers would sequentially become abnormal at different embryonal stages, when a pregnancy is destined to be lost according to previous studies, however, studies have found that the genetic factors are highly associated with reproductive loss, Studies have demonstrated that the successful pregnancy depends on immune balance, including immunotolerance, immune response, and relative cytokines levels, inflammatory immune responses play a key role in early pregnancy loss, the immune cells that reside at the interface between the placenta and the uterus were found to be a superimposed layer of regulation by maternal immune cells, which not only foster placental development and function but also reduce the possibility of the placental attacking the fetus, during implantation, natural killer (NK) cells migrate to the uterus and regulate the secretion of cytokines that limit the trophoblast invasion, in normal pregnancy, a systemic or local inflammation is necessary, however, this might be disrupted during miscarriage **(Esraa et al, 2018) .**

4.2 Biochemical parameters :

4.2.1 Beta HCG :

The present study shows the comparison of serum levels of beta HCG, Glycodelin-A, sFlt-1, PlGF between patients which have abortion in first trimester of pregnancy and healthy control which have not abortion at the same time .

Serum level of beta HCG in healthy control group (mean ± SD 2309.940 ±3007.116) in patients which have abortion (1024.774 ±4 59.394) which show significant correlation : P value ≤ 0.05, that mean the level of this marker in patients was lower than in healthy control, several recent studies have suggested that there is association between pregnancy complications and decreased serum level of β-hCG, however the only suggested association was found for higher risk of developing abortion in these patients at first trimester **(Hatav G et al, 2017) .**

previous research defined the natural decline in hCG presented for evaluation of pain and bleeding with an unconfirmed diagnosis at presentation and an ultimate diagnosis of miscarriage, in this population, a quadratic curve for log hCG was determined to best fit the pattern of hCG decline, the minimal rate of decline in patients with miscarriage was found to be more rapid for women who presented with higher initial hCG concentrations, and ranged from 21% to 35% 2 days after presentation or 6 0% to 84 % at 7 days of follow- up , a woman with a decline in hCG slower than this threshold was considered at risk for an ectopic pregnancy, therefore these measurements provide limited discrimination between a miscarriage and an ectopic pregnancy **(Samantha F et al, 2014),** researchers may be trends in quantitative β subunit of human chorionic gonadotropin (β-hCG) levels provide useful information when distinguishing normal from abnormal early pregnancy, the discriminatory level (1500 to 3000 µIU/ml) is the β-hCG level above which an intrauterine pregnancy should be visible on transvaginal

ultrasonography, failure to detect an intrauterine pregnancy, combined with β-hCG levels higher than the discriminatory level, should raise concern for early pregnancy loss or ectopic pregnancy, the incidence of ectopic pregnancy is 1% to 2% in the United States and accounts for 6% of all maternal deaths **(Erin H et al, 2019) .**

Som studies see that the serum hCG and have limited utility in the diagnostic evaluation of pregnant women suspected of having early pregnancy loss, hCG is useful if a sac is not seen on ultrasound to help determine if there is concern for ectopic pregnancy, there are no hCG levels or trends that are diagnostic for early pregnancy loss, though, a dramatic drop in hCG > 25 percent over 48 hours in the setting of uterine bleeding is highly suggestive of EPL and may be especially helpful if ultrasound is not easily available, by contrast, hCG testing is commonly performed as part of the assessment for pregnancy of unknown location and is often helpful in excluding ectopic pregnancy, in general, once an intrauterine gestational sac is seen on ultrasound **(Sara P et al, 2020) .**

In other studies about Beta HCG which ingratiated that the variation in beta hCG levels at any gestational age is rarely suggestive of pregnancy loss, and a single beta hCG value < 4000 milli-international units/mL should be interpreted with great caution, it is important to consider that the rate of hCG increase and trends may be unpredictable, particularly at low levels **(Espinoza J et al, 2017) .**

In the other hand previous studies identified PlGF as a member of the VEGF family, can promote placenta angiogenesis, increase vascular permeability, and enhance trophoblast cell activity, soluble fms-like tyrosine kinase-1 (sFlt-1) can decrease the serum concentration of PlGF, inhibit the biological function of PlGF, and impair the permeability and integrity of vascular wall, leading to angiogenesis disorders, edema, urine protein, and hemoconcentration, increasing evidences showed that there were elevated

serum concentration of sFlt-1 and declined serum concentration of PlGF in PE patients and that the degree of elevated or declined level was correlated with the severity of PE , because of this, sFlt-1 and PlGF were considered as the most promising serological indicators for PE diagnosis, Particularly, the sFlt-1/PlGF ratio could better reflect the antiangiogenic activity and could be used to predict the occurrence and prognosis of PE **(Fang J et al, 2019) .**

4.2.2 Glycodelin-A :

The present study shows that the serum level of glycodelin-A in healthy control group (mean ± SD 193.741 ± 20.056), in patients which have abortion (70.944 ± 5.687) which show significant correlation : P value ≤ 0.01, that mean the level of this marker in patients was lower than in healthy control, several recent studies have suggested that the glycodelin-A synthesis increases in first trimester of pregnancy, peaking at 10 weeks, the decline level of it in this period predict pregnancy complication may lead to abortion **(Sipra L et al, 2016) .**

Previous studies said that the female genital tract is mainly expressed in EECs (cultured endometrial epithelial cells) and secreted into the amniotic fluid, endometrium/decidua and maternal serum, Glycodelin-A has contraceptive and immunosuppressive functions, due to the fact that suppresses Natural Killer cells, achieving the prevention of the maternal rejection of the fetus at the fetomaternal interface, this lead to fact that the level of glycodeline-A at the beginning of pregnancy is necessary to successful implantation and successful pregnancy **(Brezina PR et al, 2017) .**

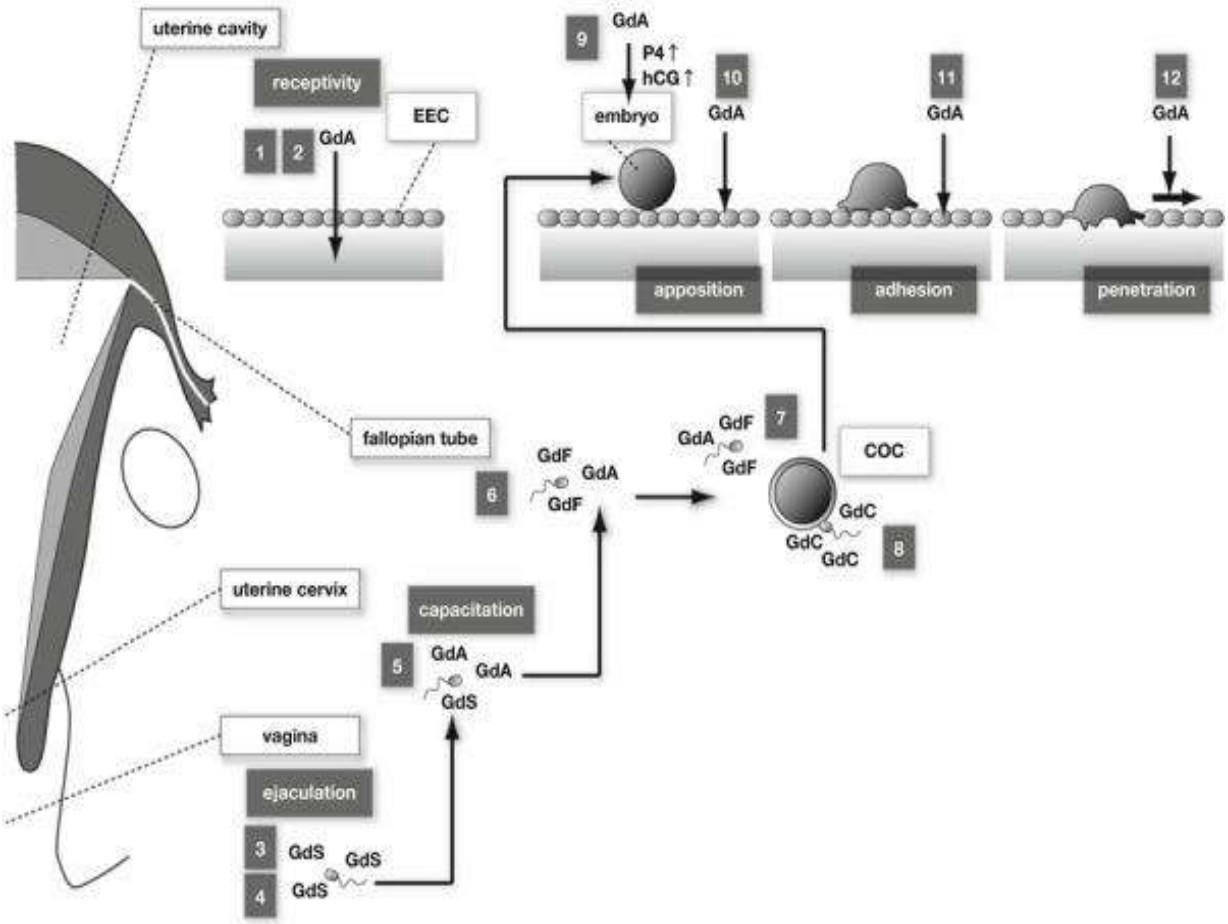
Glycodelin is a secretory glycoprotein that affects cell proliferation, differentiation, adhesion, and motility, glycodelin has four glycoforms (glycodelin- A, - S, - F and - C), differences in glycosylation affect each characteristic function, glycodelin has a unique temporospatial pattern of expression, primarily in the reproductive tract where glycodelin is mid- secretory phase- dominant, recent studies have demonstrated that glycodelin-A protein has the potential to regulate various processes, including immunosuppression, fertilization, and implantation **(Goldman CK et al, 2018) .**

Pregnancy is a type of semi- allograft implantation, therefore, suppression of maternal immune response is important to protect the combination of embryo and endometrial tissue for establishment of human

pregnancy, glycodelin- A suppresses the cytotoxicity of natural killer cells, Subsequently, numerous studies have clarified the role of glycodelin-A in regulating the immune system during pregnancy figure (4.1) **(Okamoto N et al, 2016) .**

In previous studies after glycosylation and dimerization, glycodelin protein acts as a multiple- regulator (immunosuppression, fertilization, and implantation) and a director to orchestrate the complex, step- by- step process of fertilization and implantation, there remain a number of unknown functions and mechanisms to be elucidated, however, collective evidence of glycodelin and glycodelin-A function and regulation should be applied for reproductive medicine, such as infertility, recurrent miscarriage, and anti- conception **(Lee CL et al, 2019) .**

Glycodelin-A (GdA) suppresses the cytotoxicity of natural killer cells, Th2 dominant Th1/Th2 balance is induced by GdA mediated apoptosis of Th1 cells, and increased secretion of Th2 cytokines, sperm morphology dependent glycodelin-S (GdS) binding to sperm, GdS prevents capacitation by inhibiting albumin induced cholesterol efflux, capacitation of sperm by replacement of GdS to GdA in the uterine cervix, GdF and GdA binds to sperm and inhibits the progesterone induced acrosome reaction, GdA suppresses the binding between sperm and oocyte, replacement of GdF/GdA to Glycodelin-C (GdC) derived from cumulus oocyte complex (COC) induces acrosome reaction, GdA induces secretion of progesterone and hCG from trophoblast, GdA transdifferentiates EECs, adhesion ability of EECs against embryo is up regulated by GdA, increased expression of GdA accelerates motility of EECs and thereby assists embryo penetration, in this study decrease level of GdA in patients in comparision with healthy control confirm the role of GdA in assists embryo penetration and growth of fetus which lead to successful pregnancy (figure 4.1) **(Laura Ditti et al 2020) .**



Figur 4.1 : Glycodelin map work (Laura Ditti et al 2020) .

4.2.3 sFlt-1 :

The present study shows that the serum level of sFlt-1 in healthy control group (mean ± SD 22.269 ± 1.916), in patients which have abortion (118.215 ± 7.642) which show significant correlation : P value ≤ 0.05, that mean the level of this marker in patients was higher than in healthy control, previous studies suggests that excess sFlt1 may be sufficient to produce generalized endothelial dysfunction and some of the clinical pregnancy complicated **(Sharon E et al, 2017) .**

Recurrent spontaneous abortion (RSA) is a health problem that affects hproximately 1% to 5% reproductive age woman, Yet, in around half of these patients, the mechanism for RSA is unexplained, recent studies have indicated that placental ischemia / hypoxia and endothelial dysfunction are important factors in miscarriage, other studies have indicated that the level and expression of soluble FMS-like tyrosine kinase-1 (sFlt1) is increased under a hypoxic environment, However, decreased sFlt-1 in the maternal circulation during the first trimester has recently (according to these studies) been proposed as a potential marker for identifying risk of pregnancy loss when clinical samples were obtained within a short time after the fetal death, protein expression and maternal serum levels of sFlt1 were assessed and compared to samples taken from those with normal pregnancies, the results indicate that levels of VEGF and sFlt-1 are both increased in women during early pregnancy compared women that are not pregnant (P ≤ 0.05) indicating that VEGF and sFlt-1 are both associated with pregnancy, and a significant (P ≤0.05) increase in sFlt1 and VEGF levels and expression in the RSA patients who suffered subsequent miscarriages compare to controls, these results demonstrate that there is likely a relationship between VEGF, sFlt-1 and RSA suggesting that the high levels and over expression of sFlt-1 and VEGF might be associated with the pathogenesis of RSA **(Lim JH et al, 2018) .**

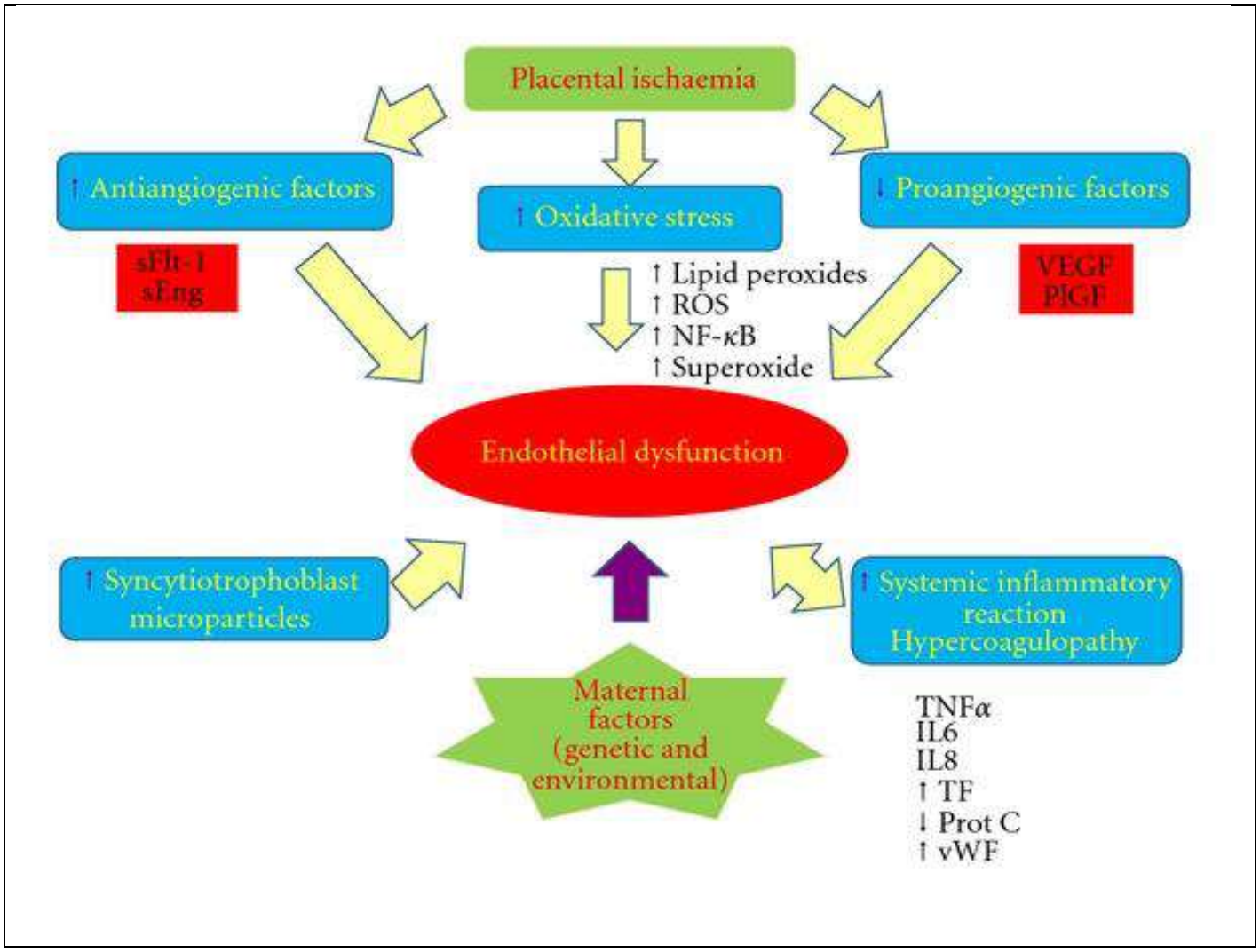
Vascular endothelial growth factor (VEGF) is amultifunctional cytokine that is produced by a variety of cell types, including the placenta. VEGF modulates physiological and pathophysiological vascular development , several studies have indicated that the biological activity of VEGF is regulated by a soluble portion of the fms-like tyrosine kinase (Flt-1) receptor (sFlt-1), an endogenous inhibitor of VEGF, which may play an important role in endothelial dysfunction.

Excess circulating sFlt-1 binds to VEGF with high affinity Vascular endothelial growth factor (VEGF) is a multifunctional cytokine that is produced by a variety of cell types, including the placenta. VEGF modulates physiological and pathophysiological vascular development .

Several studies have indicated that the biological activity of VEGF is regulated by a soluble portion of the fms-like tyrosine kinase (Flt-1) receptor (sFlt-1), an endogenous inhibitor of VEGF, which may play an important role in endothelial dysfunction , excess circulating sFlt-1 binds to VEGF with high affinity, thereby neutralizing it **(Hatav G et al, 2017)** , sFlt-1 dramatically increases over the course of gestation and then dramatically falls soon after delivery, It has been hypothesized that the placenta is the primary source of this circulating antiangiogenic factor **(Clark DE et al, 2015)** .

Two-thirds of pregnancies that are lost to miscarriage are believed to be due to defective placentation associated with an absence of physiological changes in maternal spiral arteries, some recent studies suggest that a dysregulation of the angiogenic factors, VEGF and its solute receptor sFlt-1, may be involved in the pathophysiology of miscarriage **(Muttukrishna S et al, 2017),** interestingly, other recent studies have shown that high levels of sFlt1 are associated with pre-eclampsia, in later pregnancy circulating sFlt-1 is increased in preeclampsia and also in other pregnancy complications hypoxia **(Maynard SE et al, 2016)** , these results indicate that circulating

sFlt-1 is elevated in preeclampsia during late gestation, The VEGF increase is much more dramatic, resulting in a high level of unbound or ‘‘free’’ VEGF in the system, a poorly vascularized (hypoxic) placenta stimulates excessive sFlt-1 production, the sFLt-1 is released into the maternal circulation throughout gestation, binding VEGF, which leads to endothelial dysfunction and hypertension, it has been well established that early embryo development in the human occurs in a hypoxic environment **(McKeeman GC et al, 2017)**, chronic hypoxia in early chick embryos resulted in increased sFlt-1 levels, however, the mechanisms by which VEGF and sFlt-1 induce miscarriage remain unclear, specifically, the exact time when the over expression of VEGF and sFlt-1 induces miscarriage and / or the significance of high levels of VEGF and sFlt-1 cannot be determined, but in this study when it is high level of sFlt-1 in patients lead to pregnancy loss in coparision with healthy control, in RSA patients, it is possible that a poorly vascularized (hypoxic) placenta stimulates excessive sFlt-1 and VEGF production throughout early gestation, stimulating additional sFlt-1 release, leading to endothelial dysfunction and miscarriage, it was conducted to test the hypothesis that under a hypoxic environment, sFlt-1 increases in the serum and chorionic villus during early pregnancy resulting in vascular-endothelial dysfunction and subsequent miscarriage (fig 4.2) **(Tintu A et al, 2019)** .



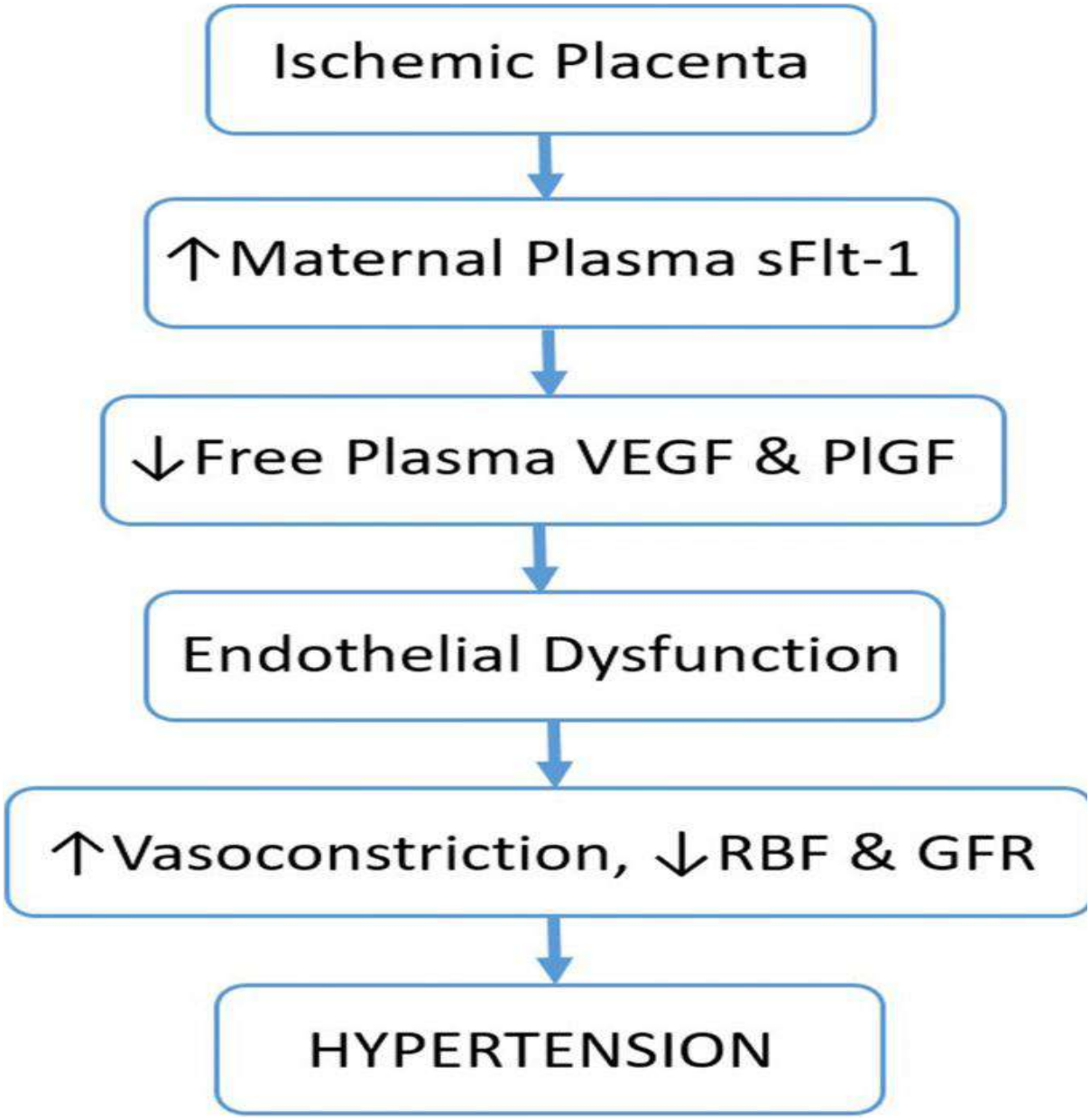
Figur 4.2 : sFlt-1 effect on endothelial dysfunction (Tintu A et al, 2019)

4.2.4 PlGF :

The present study shows that the serum level of PlGF in healthy control group (mean ± SD 340.294 ± 38.701), in patients which have abortion (141.453 ± 28.410) which show significant correlation : P value ≤ 0.05, that mean the level of this marker in patients was lower than in healthy control, several studies show that in both early and late onset preeclampsia, maternal serum levels of sFlt-1 are higher and PlGF lower in women presenting with preeclampsia, in addition, placental sFlt-1 levels were significantly increased and PlGF decreased in women with preeclampsia as compared to those with uncomplicated pregnancies, this suggests that placental concentrations of sFlt-1 and PGF mirror the maternal serum changes, this is consistent with the view that the placenta is the main source of sFlt-1 and PlGF during pregnancy **(Shibuya M et al, 2018) .**

The PlGF (placental growth factor) has been largely demonstrated to be associated with the diagnosis of the hypertensive disorders of pregnancy (HDPs), however, it is unclear how useful it is for the prognosis of the condition, previous studies like presnt study provide a summary of important findings of its prognostic ability by systematically reviewing studies that examined the ability of the PlGF, either independently or combined with other factors (Glycodeline-A, sFlt-1 like present study), to predict maternal and fetal complications resulting from the HDPs or first trimester pregnancy loss as in my study, Prognostic performance was evaluated by sensitivity, specificity, likelihood ratios, and area under the receiver operating characteristic curve ROC, PlGF may aid in the management of women with HDPs to avert fetal complications, Future studies should determine an optimum threshold for the marker to guide delivery and should examine whether its use for predicting adverse maternal outcomes in women with HDPs can be improved or Predictor marker of first trimester pregnancy loss **(Stephenson MD et al, 2017) .**

Definite pathogenesis of PE is not completely clear, but the imbalance between angiogenic factors like vascular endothelial growth factor or placental growth factor (PlGF) and anti-angiogenic factors like soluble fms-like tyrosine kinase 1 (sFlt-1) are known to be related to the disease pathogenesis as in most previous studies (figure 4.3) **(Louise W et al, 2017).**



Figur4.3:PlGF effect on endothelial dysfunction and hypertention (Louise W et al, 2017)

According to our knowledge, this is the important study which investigates the diagnostic accuracy of Glycodeline-A, sFlt1, PlGF in women with first trimester pregnancy loss, results of our study showed Glycodelin-A, sFlt1, PlGF have the highest accuracy for differentiating between patients which have first trimester pregnancy loss from normal pregnant women .

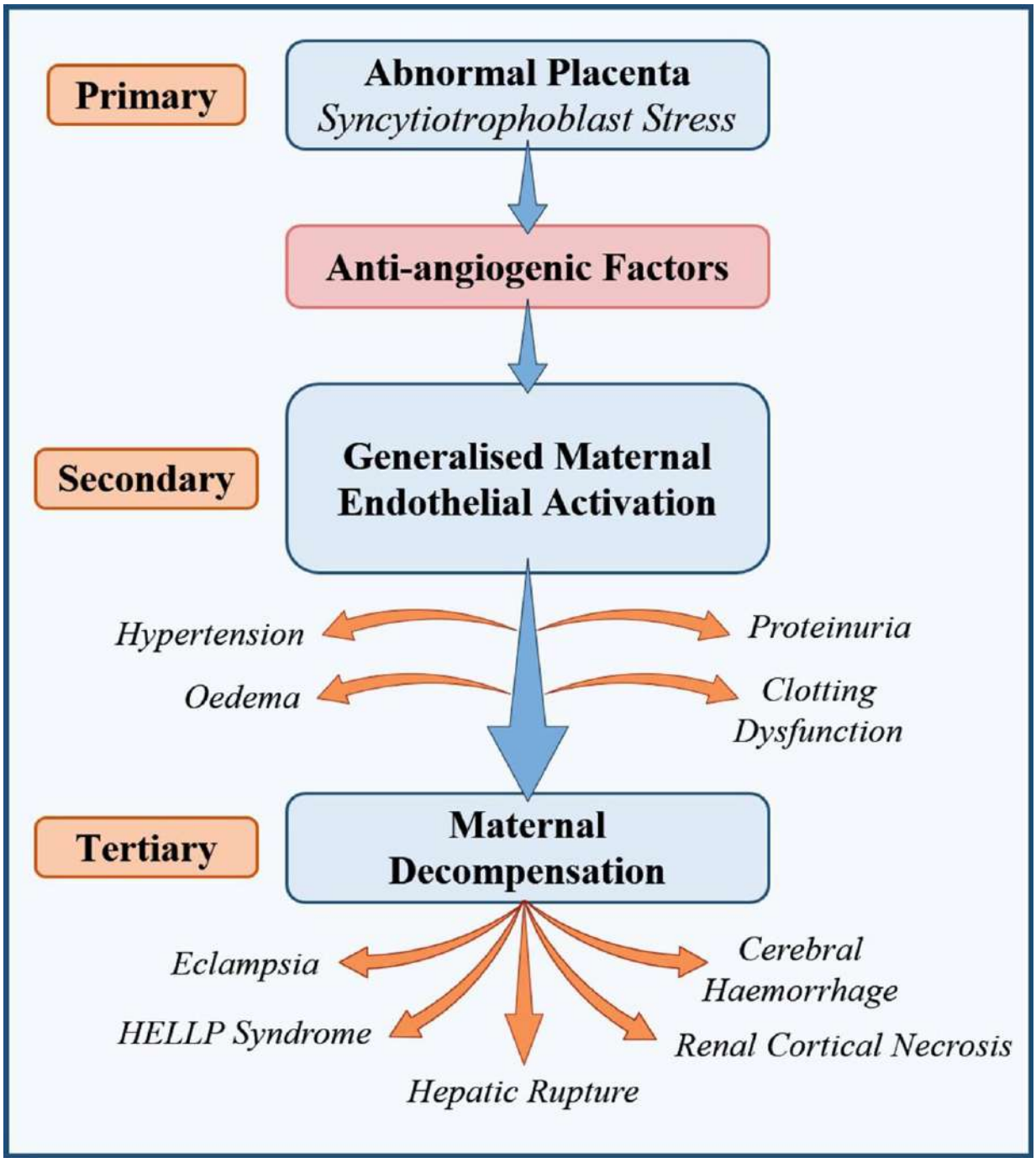
Previous studies have shown that women with very low levels of PlGF are more likely to develop severe pre-eclampsia, or have a stillborn baby, women with normal PlGF levels are at low risk, and can return to normal antenatal care, testing PlGF levels could mean that care is focused on the women that really need it, while those that don't avoid long stays in hospital **(Barnhart KT et al, 2018) .**

Normal early placental development after implantation of the blastocyst involves a rapid sequence of angiogenesis and vasculogenesis, synthesis of trophic substances and adaptation of placental and maternal vascular dynamics, by 8–12 weeks' gestation, these processes result in typical physiologic changes that can be observed in the mother, placenta and fetus, these include a significant decline in mean arterial blood pressure (MAP), decreasing blood flow impedance in the uterine and umbilical arteries, and exponential placental and fetal growth, current studies suggests that when early placental development is abnormal, maternal, placental and fetal adaptations are incomplete and may precede the development of maternal and fetal disease, since placental dysfunction appears to originate in the first trimester there is an increasing focus on identifying early markers of placental failure that can provide early prediction of patients who are at risk for clinical disease, the development of effective first-trimeste levels of PlGF have consistently been found throughout gestation in association with pre-eclampsia **(Tidwell SC et al, 2019),** other studies that show the PlGF levels have also been reported to be lowered as early as the first trimester in women who subsequently develop preeclampsia compared to normal pregnancy,

suggesting a role as a first-trimester predictor of pre-eclampsia, although a recent study using urinary PlGF levels in the first trimester failed to confirm this relationship, the predictive performance of PlGF is based on detecting a decrease from the expected normal distribution **(Ghosh SK et al, 2018).**

The results of the current study revealed the determining of beta HCG, Glycodeline-A, s-Flt-1, PlGF, for the prognosis of the disease has been investigated to help physicians identify patients at high risk of first trimester pregnancy loss who require close monitoring, interestingly, these biomarkers together show better performance than single markers in predicting the risk of first trimester pregnancy loss, several strategies have been investigated for first trimester screening of women at high risk of PE to introduce, if applicable, low-dose acetylsalicylic acid and during the second trimester or later, to predict or rule out the diagnosis of PE **(Green DN et al, 2016) .**

The objective of this study was to evaluate the routine use of beta HCG, Glycodeline-A, s-Flt-1, PlGF, in a specific population of high-risk patients which have abortion and healthy control to study the role of these markers in this situation, in clinical practice to improve care and prognosis for these patients, in this study the results demonstrate that the patients which have abortion at the first trimester pregnancy show decline level of Beta HCG, sFlt-1 and high level of Glycodeline-A, PlGF compared with healthy control group which have high level of Beta HCG, sFlt-1 and decline level of Glycodeline-A, PlGF (figure 4.4) .



Figur 4.4 : Phases of antiangiogenic factor effects

(Green DN et al, 2016).

Conclusion and Recommendations

Conclusion and Recommendations

Conclusion :

1. The serum Beta HCG , Glycodeline-A , and PlGF showed significant low level in patients compared with healthy control which help in the early diagnosis first trimester pregnancy loss .
2. The serum level of sFlt-1 showed significant elevation in patients which have abortion compared with healthy control in first trimester of pregnancy.
3. Glycodelin-A is more important to use in rotine work because its relationship to immunity and successful growth of placenta and fetus .

Recommendations :

1. Increase the sample size of subjects to emphasize the role of Glycodeline-A , sFlt-1 , PlGF as predictor markers of first trimester pregnancy loss .
2. Molecular technique or any advanced techniques (such as real time polymerase chain reaction) are recommended to detect the gene that is important in clear and specific predictor of first trimester pregnancy loss disease and to evaluate the monitoring processes (such as reliable measurement levels of cytokines as inflammation markers that effect on this problem) .
3. Further prospective studies are needed to confirm the combined utility of these markers in predicting the first trimester pregnancy loss and its complicated .

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Appendix

(١)

Case Sheet

Name :

Date :

No. :

Pssible way of communication :

- Mobile :

- Review date :

Age :

Gest.age :

The expected date of the end of the week 12 :

Any complication at the limited period :

Ensure that she is within the desired criteria :

Assay procedure for all parameters :

Ready sufficient plate units for all standards / controls and specimens .

1. Put 100 µl of standards , controls and specimens in the well .
2. Put all of these for 30 minutes at hall warmth .
3. **3.** Throw away the well contents and rinsing 3 times with 300 µl of rinse sol.
4. Put 100 µl of enzyme combine in every well .
5. Put all of these for 15 minutes at hall warmth .
6. Throw away thet well contents and rinse 3 times by 300 µl of rinse sol.
7. Put 100 µl of TMB subst. sol. in every well .
8. Put all of these for 15 minutes at hall warmth .
9. Put 100 µl of end sol. to every well of the units .
10. Put all of these 5 minutes at hall warmth .
11. The visual intensity is noticed and counting the outcomes , the advanced colour is remain for at least 30 minutes , noticed alteration during this time , standard curve which is obtained represent as the source for interpreating the result dependently.

جمهورية العراق
وزارة التعليم العالى
والبحث العلمى
جامعة بغداد
كلية الطب
فرع الكيمياء الحياتيه

دور المؤشرات البايوكيميائيه
Glycodeline-A , sFlt-1 , PlGF
كمؤشرات تنبؤيه عن خسارة الحمل في الثلث الأول

**أطروحة مقدمة الى
مجلس كلية الطب - جامعة بغداد
وهي جزء من متطلبات نيل شهادة الدكتوراه
فى الكيمياء الحياتية السريرية**

من قبل
محمد حنون داود
بكالوريوس علوم الكيمياء 1991
ماجستير كيمياء حياتية وسريريه 2013

بأشراف
أ.د. هالة غازى محمود
دكتوراه كيمياء حياتية وسريرية

1442 هـ 2020م

بسم الله الرحمن الرحيم

صدق الله العلي العظيم

سورة طه ايه 114

الخلاصـــــــة

خلفية الموضوع :

الثلث الأول من الحمل يبدأ من الأسبوع الأول للحمل الى الأسبوع الثاني عشر، الأجهاض هو خسارة الحمل خلال العشرين أسبوع الأولى من الحمل وطبقا للجمعية الأمريكيه للحمل فان كثير من النساء الحوامل ينتهي حملهن بالأجهاض واحيانا يحدث الأجهاض قبل أدراك المرأة بأنها حامل ، أن أغلب حالات الأجهاض أو خسارة الحمل لا يمكن الوقايه منها لأن كثير من هذه الحالات غير معروفة السبب وهي تحدث نتيجة لأسباب طبيه متعددة أغلبها ليست تحت سيطرة المريض نفسه .

الهدف من الدراسه :

الهدف من هذه الدراسه هو لمعرفة دور كل من المؤشرات البايوكيميائيه التاليه :

- Glycodeline-A
- sFlt-1
- PlGF

كمتنبئات بايوكيميائيه لخسارة الحمل في الثلث الأول .

المكان :

أجريت هذه الدراسه في مستشفى بغداد التعليمي في مدينه الطب / العراق للفتره من نيسان 2019 الى مارس 2020 وقد أختير الأشخاص المشاركين في الدراسة من المرضى القادمين الى العيادة الأستشاريه لأمراض النسائية والتوليد في هذه المستشفى وأجريت الفحوصات المختبريه في نفس المدينه الطبيه / المختبرات التعليميه .

الاشخاص :

من بين اكثر من 200 أمرأة حامل معرف اليوم الأول من اخر دورة شهريه لها والتي ثبت حملهم من خلال فحص الحمل الموجب في الدم تم اختيار 85 وسحبت عينات الدم منهم ، وأن الأعمار المختارة لهذه النساء هي 21 - 35 وأن فترة حملهم في هذه الدراسه هي 8 – 12 اسبوع وقد تم مواجهتهم بعد أكمال الأسبوع الثاني عشر من حملهم وبضوء المعلومات تم تقسيمهم الى مجموعتين :

- المجموعه الأولى 40 أمراه أعتبروا مرضى بعد أن ثبت خسارتهم لحملهم خلال هذه الفترة (12 أسبوع) الأولى من الحمل .
- المجموعة الثانيه 45 أمرأة الباقين أعتبروا أصحاء بعد أن أكملوا نفس الفترة بدون أي مشاكل في الحمل .

طرائق العمل :

بطريقة أيلايزا ELISA تم قياس مستويات :

Beta HCG , Glycodeline-A , sFlt-1 , PlGF

في مصل دم الأشخاص المختارين بعد أن أخذ 8 - 10 مل من دمهم وفصل المصل وخزنه في درجه حرارة - 20 درجة مئويه لحين أكمال جمع العينات واجراء الفحوصات تحت نفس ظروف الفحص والقياس .

النتائج :

أظهرت نتائج هذه الدراسه أن مستويات sFlt-1 أكبر بشكل مهم أحصائيا عند المرضى مقارنة لقيمهم عند الأصحاء المختارين بنفس معايير الأختيار في هذه الدراسه ، بينما أظهرت هده النتائج أن مستويات :

Glycodeline-A , PlGF , Beta HCG

أقل بشكل مهم أحصائيا عند المرضى مقارنة بالاصحاء في هذه الدراسه .