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The Role of Homocysteine , some Vitamins and Selenium in women with Recurrent Miscarriage in Thi-Qar Governorate

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إلا ما علمتنا إنك أنت
العليم الحكيم
صدق الله العلي العظيم
سورة البقرة
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Certificate

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Dedication

To ....

Who If I ask, he will give me, and if I thank him, he will increase me with blessings ... God Almighty

To.....

Humanity teacher Muhammad, peace be upon him and his family

To.....

Whoever I wished for My father to attend ..... Mercy of God

To....

The big heart for bright which clear the way of successful by pray and love ...to the one God made heaven under its feet . ...to the source of kindness for ever ...My mother

To....

Who walked with me together and shared me the successes' road .....and help me to make everything possible ..... My hasband

To....

Who taught me the meaning of gift ,and helped me to be strong ..... My brothers and sisters

To....

Everyone who wished me success and advancement

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List of Abbreviations

Abbreviation	Details
2nd	Second trimester
3nd	Third trimester
Ado Hcy	Adenosyl homocystiene
Ado met	Adenosyl methionine
ADP	Adenosine diphosphate
apL	Antiphospholipid
APLS	Antiphospholipid syndrome
ATP	Adenosine triphosphate
BHMT	Betaine – Hcy S-methyltransferase
˚C	Degree centigrade
CBS	Cystathionine β-synthase
CSE	Cystathionase
DHF	Dihydrofolate
DHFR	Dihydrofolate redutase
DMG	Dimethylglycine

DNA	Deoxyribonucleic acid
ELISA	Enzyme-Linked Immuno Sorbent Assay
FA	Folic acid
GPx	Glutathione peroxidase
GSH	Glutathione
HC	Hollow Cathode
Hcy	Homocystiene
HHcy	Hyperhomocysteinemia
HPR	Horse Radish Peroxidase
H_2S	Hydrogen sulphide
H_2Se	Selenide
LA	Lupus anticoagulant
IUGR	Intrauterine growth restriction
LPD	Luteal phase defect
LSD	Least Significant Difference
MAT	Methionine adenosyltransferase
Min	Minute
MMA	Methylmalonic Acid
MTHF	Methylenetetrahydrofolate

MTHFR	Methylenetetrahydrofolate reductase
MS	Methionine synthase
NTDs	Neural tube defects
OD	Optical density
PCOS	Poly cystic ovarian syndrome
PH	Power of Hydrogen
pg	Pictogram
P.value	Probability
r	Correlation coefficient
RM	Recurrent Miscarriage
RNA	Ribouncleic acid
RPL	Recurrent pregnancy loss
rpm	Rotor per minute
SA	Spontaneous abortion
SAH	S-adenosyl homocystiene
SAM	S-adenosyl-L-Methonine
SD	Standard Deviation
SeCys	Seienocysteine
SeMet	Selenomethionine

SHMT	Serine hydroxymethyltransferase
SPSS	Statistical package for social science
T_3	Triiodothyronine Hormone
tHcy	Totale homocystiene
THF	Tetrahydrofolate
TMB	3,3,5,5-tetramethylbenzidine
TORCH	Toxoplasmosis, Rubella virus, Cytomegalovirus , and Herpes
µL	Micro letter

Summary

Recurrent miscarriage: Researchers define recurrent miscarriage as two or more consecutive losses during the first or second trimester of pregnancy, which affecting 5% of couples, Its prevalence is less than sporadic miscarriage. The multiple causes of recurrent miscarriage including, chromosomal abnormalities, endocrine etiologies, karyotype abnormalities and others are known while 50 % of cases remain unknown. Therefore, this study was designed to investigate the possible causes of unexplained recurrent miscarriages.

This study was designed to determine the levels of Homocystiene ,Vitamin B_{12} , Folic acid and Selenium in women who suffer from recurrent miscarriage and healthy women.

The study was performed at Bint Al Huda Maternity and Children Hospital in Thi-Qar. It included (135) women aged between (18 - 40) ; (90) patients with recurrent miscarriage and (45) healthy women . The patients are divided into two groups, 45 pregnant women and 45 non-pregnant women, which were compared with 45 pregnant women (control group) who did not suffer from a previous miscarriage and had at least one previous birth. Miscarriages for known causes were excluded from our study.

The results showed a significant increase in the level of serum homocysteine in the group Recurrent pregnancy loss (RPL) (pregnant and non-pregnant) compared to the control group $P<0.05$ and a significant decrease in the level of serum vitamin B_{12} and Folic acid in group Recurrent

pregnancy loss(RPL) (pregnant and non-pregnant) compared to the control group. The results also showed a significant decrease in the concentration of serum Se in RPL pregnant groups in comparison with control group ($p<0.05$). It was also found no significant deference in the concentration of serum Se in RPL non pregnant and control groups ($p<0.05$).

The results showed negative correlation between Hcy and (vitamin B_{12}, Folic acid, Selenium)

Chapter One

Introduction

And

Literature Review

1. Introduction

Pregnancy is period of reproduction during which a women carries one or more live offspring from implantation of a fertilized zygote in the uterus throughout gestation. Childbirth usually occurs about 38 weeks after conception; in women who have a menstrual cycle length of four weeks, this is approximately 40 weeks from the start of the last normal menstrual period (Organization, 2006).

Pregnancy is a complex biological process where after conception a fetus formed as an embryo from a single cell. Pregnancy is a unique immunological challenge where the semi-allergenic fetus survives by escaping maternal immune recognition. Baby will be delivered after the completion of gestational period. The gestation period is typically divided into three trimesters of roughly three months each (Mustaqahamed et al., 2011).

Complications can arise in pregnancies for many reasons. Sometime a women's existing health conditions contribute to problems. New conditions arise because of hormonal and body changes that occur during pregnancy.some the most common complication include the following: anemia, hyperlipidemia, hypertension, diabetes and preeclampsia (Festus et al., 2011).

Miscarriage is one of the most common pregnancy complications. The term miscarriage is used to describe a pregnancy that fails to develop, resulting in the death and miscarriage of the fetus (NAZ et al., 2014).

Several complications in pregnancy have been attributed to hyperhomocysteinemia, such as neural tube defects , pre- eclampsia and recurrent pregnancy loss (Puri et al., 2013).

Recurrent miscarriage (RM) is the occurrence of three or more consecutive losses of pregnancy. According to this definition, it affects about 1% of couples trying to have a baby(Choi et al., 2014). The American Society Reproductive Medicine defines recurrent miscarriages as at least two successive miscarriages (Medicine, 2013). It is estimated that fewer than 5% of women will experience two consecutive miscarriages and only 1% experience three or more. At present, there exists a small number of accepted etiologies for recurrent pregnancy loss RPL. Most of the diagnosed etiologies include endocrine abnormalities, autoimmune disorders, uterine anomalies, and genetic factors. After evaluation for these causes, approximately half of all cases will still remain unexplained (Medicine, 2012).

1.1 Recurrent miscarriage

Recurrent pregnancy loss (RPL), defined as two or more consecutive pregnancy losses (Christiansen et al., 2018). Some experts consider two consecutive pregnancy losses is sufficient for the diagnosis of recurrent miscarriage (RM) because the recurrence rate and risk factors are similar to that after three losses(Jaslow et al., 2010). Recurrent miscarriage can occur at any stage of pregnancy, it could be early or late pregnancy period. During the first trimester of pregnancy if there is loss of embryo then it is called as early pregnancy loss. If there is loss of fetus after first trimester of pregnancy it is called as late pregnancy loss. A frequency of late pregnancy losses is less as compare to early losses and consist of only 1% of pregnancies (Meka & Reddy, 2006).

A miscarriage is a major problem that women suffer from all over the world. In a World Health Organization survey, it showed that approximately 25 million unsafe miscarriage occurred between 2010 and 2014, equivalent to 45% of the total miscarriages in the world (McGuinness & Montgomery, 2020).

1.1.1 Etiological factors for Recurrent miscarriage

The exact pathophysiological mechanism of miscarriage remain unrecognized in around 50% of remaining recurrent pregnancy loss RPL cases(Humadi, 2016). Some of the causes of recurrent miscarriage:

- **Chromosomal abnormalities** are the most common cause of first trimester miscarriage and are detected in 50-85% of pregnancy tissue specimens after miscarriage (van den Berg et al., 2012).

- **Endocrine Etiologies** Luteal phase defect (LPD), polycystic ovarian syndrome (PCOS), diabetes mellitus (As pregnancy progresses, these hormones weaken the pregnant woman's ability to tolerate glucose, which leads to high blood sugar, and the body tries to secrete the hormone insulin in more quantity so that body cells can consume blood sugar. Usually cells of the pancreas secrete insulin in large quantities, up to three times the amount of a normal person to overcome On the effect of pregnancy hormones on the sugar level, and in the event that the secreted insulin is not able to overcome this hormonal effect, the blood sugar level rises and results in gestational diabetes) (Sheikh & Sheikh, 2020) , thyroid disease (Uncorrected thyroid dysfunction in pregnancy has adverse effects on fetal and maternal health. The harmful effects of thyroid dysfunction can also extend beyond pregnancy and childbirth to affect neuropsychological development in a baby's early life. The necessary increase in thyroid hormone production is facilitated by high

concentrations of human chorionic heartwaves (hCG), which bind to the TSH receptor and stimulate the maternal thyroid gland to increase maternal thyroid hormone concentrations by approximately 50%)(Korevaar et al., 2017) , and hyperprolactinemia are among the endocrinologic disorders implicated in approximately 0.5% to 5% of Recurrent pregnancy loss RPL (Swadi & Swadi, 2014).

- **Thrombophilia** are common disordered, more than 15% of white population carrying an inherited thrombophilic mutation (Jauniaux et al., 2006). Thrombophilia defects that may correlated with recurrent pregnancy loss include: antithrombin deficiency, deficiency of protein C and protein S, factor V Leiden mutation, and hyperhomocysteinaemia (Pritchard et al., 2016).

- **karyotype abnormalities** in one of the parents is one of the most common of the known causes of recurrent miscarriages. They are most commonly found as balanced chromosome aberrations, i.e. abnormalities that cause no clinical symptoms in carriers but possibly induce the production of abnormal reproductive cells containing abnormal amounts of genetic material. Among couples with recurrent miscarriages, balanced translocations are confirmed in at least one of the partners in around 3% to 5% of cases (Pritchard et al., 2016) .Also, advanced maternal age is a strong risk factor for both spontaneous abortion (SA) and recurrent miscarriage (RM). The number of good quality oocytes in older mothers is fewer than younger mothers, which increase the frequency of chromosomal abnormalities leading to miscarriage (Andersen et al., 2000) The risk of miscarriage increases for women as they get older, so the incidence rate for those who do not exceed twenty years of age is about 10 %. As for women whose ages are estimated at the end of thirty,

about 18 %, and women whose ages are estimated at forty years and over about 50 % (Macías-Rioseco et al., 2020).

- **Antiphospholipid syndrome (APLS)** is one of the proven causes of recurrent miscarriage (Sugiura-Ogasawara et al., 2010). It is an autoimmune disease caused by the presence of circulating maternal antiphospholipid antibodies (aPL) such as anticardiolipin antibodies (IgG, IgM) and lupus anticoagulant (LA) that are directed against proteins that bind to phospholipids on plasma membranes (Rai & Regan, 2006). The antiphospholipid syndrome is associated both with vascular thrombosis (Garcia & Erkan, 2018). The reported incidence of Antiphospholipid syndrome APS is 5 - 15% (Branch & Heuser, 2010).

- **Anatomic abnormalities** account for 10% to 15% of cases of recurrent pregnancy loss RPL and are generally thought to cause miscarriage by interrupting the vasculature of the endometrium These include congenital uterine anomalies, intrauterine adhesions, and uterine fibroids or polyps (Lin, 2004).

- **Infection:** TORCH (toxoplasmosis, rubella, cytomegalovirus and herpes simplex virus) cause pregnancy loss. These infection causing organisms produce toxic metabolic by products. Pregnancy loss due to infectious agents includes direct infection of the uterus, fetus, or placenta, placental insufficiency, chronic endometritis. But there is a limited role for these infections as a main factor for recurrent miscarriage (Mei et al., 2019)

1.1.2 Diagnosis of recurrent miscarriage

Vaginal bleeding and pregnancy loss indicate a miscarriage. Miscarriage is classified into two types: complete miscarriage or incomplete miscarriage based on internal vaginal examination results or clinical history, and some studies have found that clinical diagnosis based on clinical symptoms and an internal vaginal examination are inaccurate in more than 50% when compared with ultrasound (Wieringa-de Waard et al., 2002) .

Ultrasound diagnosis of miscarriage is based on the morphological manifestations of pregnancy only and does not depend on the amount of vaginal bleeding or the results of detection of cervical dilatation (Tierney et al., 2006).

Complete miscarriage It is diagnosed when ultrasound fails to identify signs of intrauterine pregnancy tissue and is used in women who had clear evidence of pregnancy in previous ultrasound examinations (Newbatt et al., 2012).

Incomplete miscarriage is diagnosed by the presence of retained pregnancy remnants without a well-defined pregnancy sac. The ultrasound examination method for incomplete miscarriage is difficult. So the thickness of the endometrium was used to aid in the diagnosis ranging from 5 mm to 25 mm (Kurtz et al., 1991).

1.1.3 Treatment / Management of recurrent miscarriage

- **Metformin:** It is used to reduce hyperinsulinemia and normalize endocrine function, metabolism and reproduction. Small retrospective studies show that use of metformin during pregnancy is associated with a reduction in the miscarriage rate in women with polycystic ovarian syndrome (PCOS) (Glueck et al., 2002) .

- **Heparin:** Heparin and heparin sulfate are thromboprophylactic agents (Di Nisio et al., 2005). Its action is anticoagulation, antithrombosis, and protecting vascular endothelium , and its main function is to inhibit the activity of thrombin IIa and coagulation factor Xa (Tong & Wei, 2016). Heparin can bind to antiphospholipid antibodies, heparin helps to ameliorate the risk of placental fibrin deposition, thrombosis, and infarction (Di Nisio et al., 2005).

- **Progesterone:** Progesterone is secreted mainly by the corpus luteum, which is formed in the ovary after the rupture of the ovarian follicle at the time of ovulation. Because progesterone leads to secretory changes in the endometrium that are necessary for the implantation of the embryo, it has been suggested that some cases of miscarriage Due to insufficient secretion of progesterone at either in the postovulatory phase of the menstrual cycle or early pregnancy (Haas & Ramsey, 2013). a subgroup analysis of women with recurrent miscarriage suggests that progesterone use in the first trimester might be of benefit (Haas et al., 2019).

- **Aspirin:** It is a non-steroidal anti-inflammatory drug. It works by inhibiting platelet cyclooxygenase to reduce prostaglandin production and this reduces the tendency of thrombosis and is used in embryo

implantation and reduces the possibility of recurrent miscarriage (Tong & Wei, 2016).

• **Bromocriptene:** The hormone prolactin is important in ovulation and endometrial maturation. hyperprolactinemia causes recurrent miscarriage, so treatment with bromocriptine is used, which inhibits prolactin secretion from the anterior pituitary gland and significantly reduces the rate of recurrent miscarriage (Hirahara et al., 1998). Lack of expression of endometrial prolactin during the luteal phase of the menstrual cycle has also been associated with recurrent miscarriage (Garzia et al., 2004) .

There are three measures that can be taken in the case of miscarriage depending on the patient's condition and all of them are safe (Newbatt et al., 2012). Numerous studies indicate that expectant management and medical management are beneficial in the case of incomplete miscarriage (van den Berg et al., 2017).

1.1.3.1 Expectant management (that is, waiting for a period to see if the miscarriage will heal naturally without intervention from 7-14 days) is used as the primary procedure (Newbatt et al., 2012). Expectant management is usually chosen by women because of a desire to follow a natural approach. It has become an increasingly popular choice and in one observational study, 70% of women choose to wait until the pregnancy to resolve spontaneously (Luise et al., 2002). Placebo (expectant management) was successful in (55% - 85%) of women with incomplete miscarriage (Blohm et al., 2005). The chances of successful completion of the miscarriage automatically increase with the length of follow-up (Sairam et al., 2001).

1.1.3.2 Medical management is chosen as an option in (20-30%) of women (Shankar et al., 2007) . If bleeding continues to increase, then the vaginal or oral misoprostol is used according to the patient's preference (Lui & Ho, 2020). Sometimes Mifepristone which is an oral anti-progesterone drug (Kulier et al., 2004) given before misoprostol for miscarriage and despite this, a recent random experment showed that giving mifepristone does not increase the success rate compared to misoprostol alone (Stockheim et al., 2006).

The success of medical management depends on the type of miscarriage, the medical administration, and the dose of the drug (Lister et al., 2005). medical management is effective in women with incomplete miscarriage but success was not much better than expectant management (Bagratee et al., 2004) .

There are some side effects of medical treatment and include nausea (22-35%) of women, fever (15%), diarrhea (6-21.2%) and vomiting (7%) (Lister et al., 2005). Bleeding begins hours after taking the drug and continues for up to three weeks. Heavy bleeding rarely occurs requiring emergency surgery in only 1% of women treated with misoprostol (Grønlund et al., 2002). Women who bleed for more than three weeks should attend a clinical review to get rid of the remnants of pregnancy (Newbatt et al., 2012).

1.1.3.3 Surgical management Emergency surgery remains the primary treatment for women who have excessive or unstable bleeding or have signs of retained pregnancy products (Tierney et al., 2006) . Clinical trials have found that surgical management is more successful than medical treatment. Surgery is performed under general anesthesia in the operating room or local anesthesia in an outpatient setting (Newbatt et al., 2012).

1.2 Homocystiene

Homocysteine is an amino acid not supplied by the diet that can be converted into cysteine or recycled into methionine , with the aid of specific B vitamins. Homocysteine levels vary between men and women When homocysteine levels high , it signifies that there is a disruption in the metabolism of homocysteine (Veeranki et al., 2016) . Abnormally high levels of homocysteine in the serum, are a medical condition called hyperhomocysteinemia .This has been claimed to be a significant risk factor for the development of a wide range of diseases, including thrombosis (Cattaneo, 1999) . elevation of homocysteine concentration in plasma caused by genetic disorders in the enzymes involved in the metabolism, or because of deficiency of nutrition vitamin B_6, B_{12} or folic acid because these vitamins are major co-factors for enzymes that part in the metabolism of homocysteine, such as methionine synthase and 5,10-methylene tetrahydrofolic acid reductase (Silaste et al., 2001). It may be further metabolized to cystathionine and then cysteine (transsulphuration) which requires vitamin B_6 as a cofactor, or be remethylated to methionine, either by the vitamin B_{12} dependent enzyme methionine synthase, or by betaine-homocysteine methyltransferase (Weaving, 2010). and this explains the close relation between folate, vitamin B_{12} and Homocytiene Hcy. Deficiency of either vitamin leads to elevated total homoystiene (tHcy) level in plasma or serumreferred to as hyperhomocysteinemia (Allen et al., 1993) .

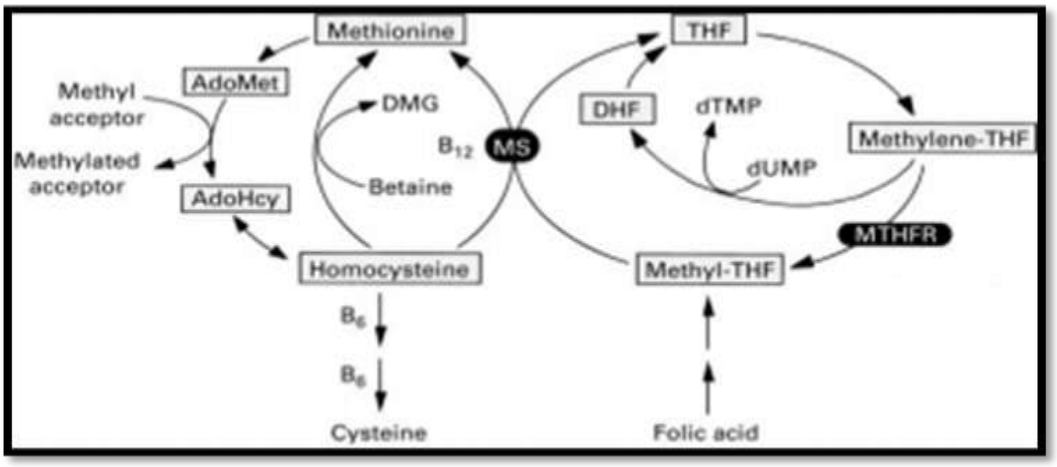

Figure (1-1) . Interrelation between folate, vitamin B_{12} and homocysteine metabolism. AdoHcy, adenosylhomocysteine; AdoMet, adenosylmethionine; DHF, dihydrofolate; DMG, dimethylglycine; MS, methionine synthase; MTHFR, methylenetetrahydrofolate reductase; THF, tetrahydrofolate. (Rozen, 1996).

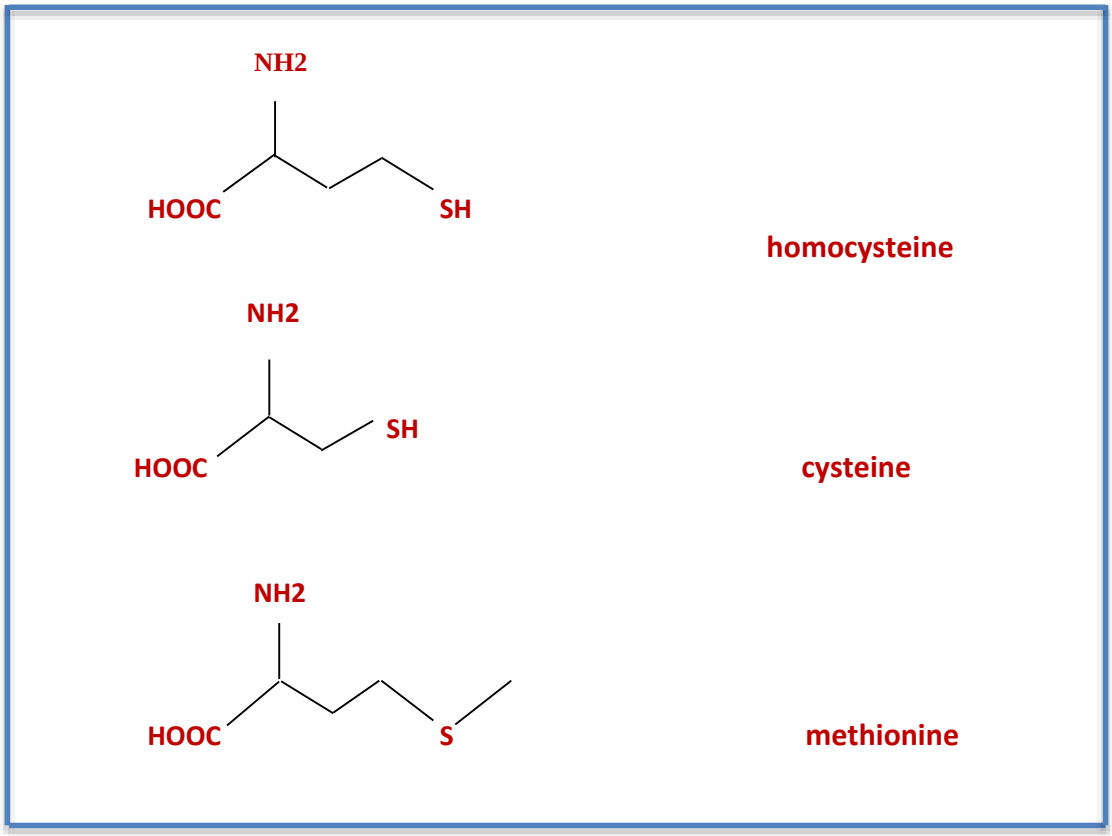

Figure (1-2) . The structures of homocysteine, cysteine and methionine

1.2.1 Forms of homocysteine in the blood

There are several forms of homocysteine present in human blood. The free sulfhydryl form, homocysteine , is present in low amounts in blood (1% – 2%) as a protein-bound mainly bound to albumin (about 70% to 85% in normal subjects) and soluble disulphides (30%–25%; e.g., homocystine or cysteine). The amount of total homocysteine is the sum of these three components. Values obtained could vary by laboratory and collection method (Škovierová et al., 2016).

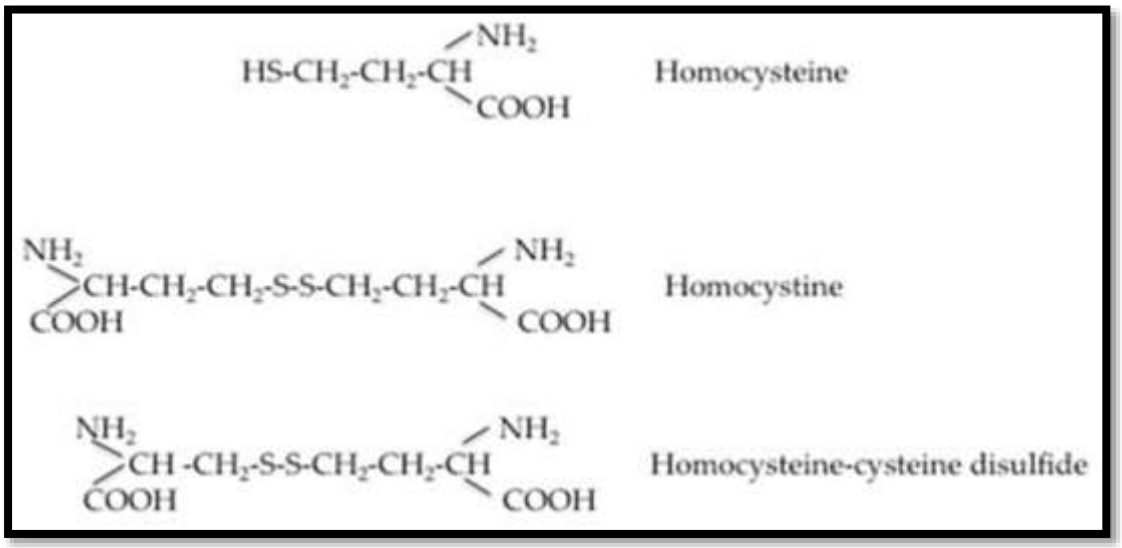

Figure (1-3) . Forms of homocystine Hcy present in human blood (Škovierová et al., 2016).

Even if correct functioning of the enzymes involved in the metabolic pathways of homocysteine Hcy has an essential role in determining the plasma and urine levels of homocysteine Hcy, several modifiable factors exert a strong effect opn its concentration, thus characterizing a wide range of pathologies. These factors include adequate dietary intake of folate and vitamins B_6 or B_{12}, intake of proteins rich in methionine that help to regulate Hcy biochemical pathways, and unhealthy lifestyle

choices such as alcohol abuse, high coffee intake, smoking habits, and use of drugs that may interfere with vitamin utilization and thus could increase the risk of hyperhomocysteinemia HHcy -related pathologies (Refsum et al., 2004). There are certain implications of having elevated homocysteine that are specifically relevant for women. Elevated homocysteine levels have been observed more frequently among women with certain pregnancy complications, including preeclampsia (elevated blood pressure that can lead to dangerous consequences), placental abruption (where the placenta detaches from the uterus), recurrent pregnancy loss, and giving birth to a small, low-birth-weight baby (called intrauterine growth restriction) (Ray & Laskin, 1999).

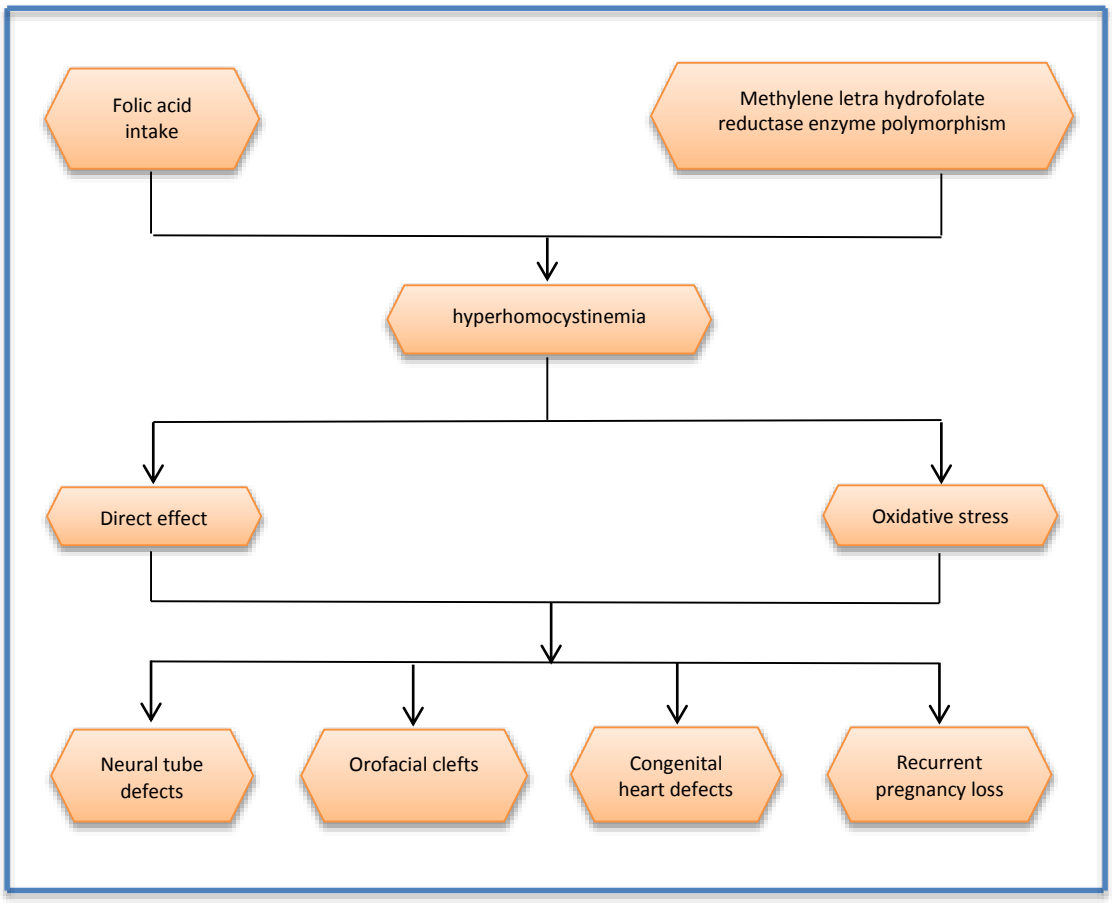

Figure (1 - 4) . .Hyperhomocystinemia and pregnancy loss (Nelen et al., 2000)

1.2.2 Homocysteine metabolism

Homocysteine Hcy is a sulfur-containing amino acid not present in dietary protein or used for its endogenous synthesis, but represents an intermediate in methionine metabolism. Methionine is an essential sulfur-containing amino acid present in various protein foods such as meat, eggs, dairy products and legumes. Figure (1 - 5) represents the pathways of homocysteine Hcy metabolism, which include a system of transmethylation, remethylation and transsulfuration paths. In most cells, by transmethylation route homocysteine Hcy and methionine cycle metabolically, the methyl group on activated methionine (S-adenosyl-methionine or SAM) may be added to methyl acceptors (Deoxyribonucleic acid DNA, Ribonucleic acid RNA, and protein) by methyltransferases, and the S-Adenosyhomocystein (SAH) is rapidly hydrolyzed to adenosine and Homocysteine , which could improve its concentration. Transmethylations, chemical reactions transferring a methyl group from one compound to another, are generally regulated by the intracellular concentration of involved compounds; thus, S-adenosyl-methionine SAM and S-Adenosyhomocystein SAH concentrations determine a cell's methylation balance. Once formed, Homocysteine can be recycled into methionine or converted into cysteine by remethylation and transsulfuration routes, respectively. Homocysteine Hcy is remethylated to methionine through two separate reactions catalyzed by three different enzymes. In all tissues, folic acid donates a methyl group across methylenetetrahydrofolate reductase (MTHFR) in a reaction catalyzed by methionine synthase, a vitamin-B_{12}-dependent enzyme (Esse et al., 2019).

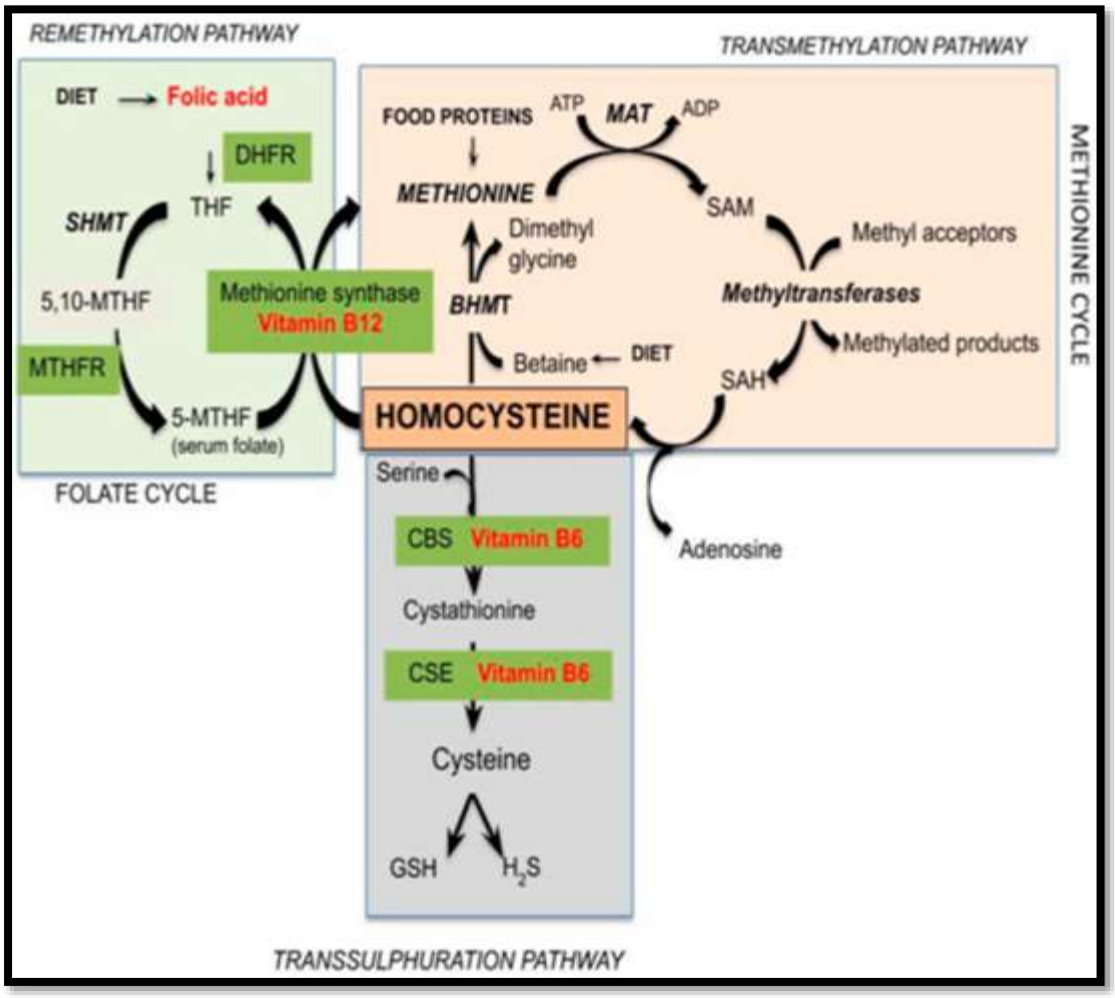

Figure (1-5) . Schematic representation of pathways of Homoystiene Hcy metabolism (DHFR = dihydrofolate reductase; THF = tetrahydrofolate; SHMT = serinehydroxymethyltransferase; MTHF = methylenetetrahydrofolate; MTHFR = 5,10-methylene-THF reductase; ATP = adenosine triphosphate; MAT = methionine adenosyltransferase; ADP = adenosine diphosphate; SAM = S-adenosylmethionine; SAH = S-adenosylHcy; BHMT = betaine-Hcy S-methyltransferase; CBS = cystathionine β-synthase; CSE = cystathionase; GSH = glutathione; H_2S = hydrogen sulphide) (Esse et al., 2019).

1.2.3 Homocysteine with recurrent miscarriage

Homocysteine, derives from the demethylation of methionine during Deoxyribonucleic acid (DNA) and Ribonucleic acid (RNA) methylation (Alan, 1996). Elevated total homocysteine has been associated with a variety of adverse outcomes linked to placental insufficiency, including preeclampsia, spontaneous abortion, abruptio placentae, intrauterine growth restriction, recurrent pregnancy loss (Mignini et al., 2005) and preterm birth (Ronnenberg et al., 2002). Hyperhomocysteinemia causes a three times higher risk of early pregnancy loss (Unfried et al., 2002). impairment of chorionic villous vascularisation is linked to hyperhomocysteinemia (van Oppenraaij et al., 2010). Exchange between foetus and mother is essential for normal foetal growth, but defects of chorionic villous vascularisation provoke pregnancy loss (Serapinas et al., 2017). Folic acid supplementation may further reduce the total homocysteine concentration during pregnancy (Murphy et al., 2002). Elevated plasma total homocysteine can arise from inadequate folate or vitamin B_{12} status, but also from a range of other nutritional, genetic, physiological, and pathological causes. There is substantial circumstantial evidence to suggest that high circulating total homocysteine may itself increase the risk of a wide range of abnormalities in vascular function, most likely through increased oxidative stress leading to endothelial cell dysfunction. In pregnancy this is seen primarily as affecting placental function (Van der Molen et al., 2000) .

These levels are linked to the effect of homocysiene Hcy on vascular endothelial function, elevated pro-oxidant and thromboembolic activities (Laskowska & Oleszczuk, 2011). However, medical research suggests

that elevated homocysteine levels may be a consequence of these complications, rather than the cause. Hyperhomocysteinemia is observed more commonly among women who have a child with a neural tube defect (an abnormality of the fetal spine or brain). Neural tube defects include spina bifida (an opening in the fetal spine) and anencephaly (a severe birth defect in which the brain and skull do not form properly). Approximately 20% of women who have a child with a neural tube defect have abnormal homocysteine metabolism (Botto & Yang, 2000).

1.2.4 Causes of hyperhomocystinemia HHcy

There are three main hyperhomocystinemia (HHcy) causes

1. Genetic defects in the enzymes of homocysteine Hcy metabolism – 5, 10-MTHFR 677C → T mutations that inhibit homocysteine conversion to methionine; T133C, p.I278T and p.T191M mutations of the CBS gene (Ilhan et al., 2008).

2. Nutritional disorder, resulting in a deficiency of folate or vitamin B_{12} Folate deficiency can result from malabsorption syndromes, alcoholism and liver diseases. Since the source of vitamin B_{12} is meat and dairy products, vegetarians are more prone to B_{12} deficiency (Blom & Smulders, 2011) .

3. Impaired renal function – although the exact mechanism of homocysteine accumulation in patients with renal insufficiency is not understood completely, possible cause may be a defective clearance and/or a decrease in extrarenal metabolism (Azzini et al., 2020)

1.3 Vitamins

Vitamins are the organic compounds required by the human body which are considered as vital nutrients needed in specific amounts. They cannot be synthesized in sufficient amount by the human body, and therefore must be obtained from the diet. Thirteen different types of vitamins are known that are classified by their biological and chemical activity; each one of them has a specific role in our body (Combs Jr & McClung, 2016).

Vitamins are classified as either water-soluble or fat-soluble. Of the 13 vitamins, 4 are fat soluble (A, D, E, and K) and the other 9 are water soluble (8 B vitamins and vitamin C). The water-soluble vitamins easily dissolve in water and are excreted from the body rapidly since they are not stored for a long time, except for vitamin B_{12} (Chatterjea & Shinde, 2011), some vitamins are vital for the body cell growth and development (e.g. folic acid and vitamin B_{12}). Folic acid is known as vitamin B_9 which has vital functions. Our body needs folic acid for the synthesis, repair, and methylation of (DNA) (Krebs et al., 2009). Folate has an important role in cell division and it is especially needed during infancy and pregnancy. Human body requires folate in order to produce healthy red blood cells and prevent anemia, while vitamin B_{12} plays an important role in supplying essential methyl groups for protein and (DNA) synthesis. Vitamin B_{12} is bound to the protein in food and hydrochloric acid in the stomach releases B_{12} from protein during digestion. Once released, B_{12} combines with a substance called intrinsic factor (Aghajanian & Marek, 2000).

1.3.1 Vitamin B_{12}

$C_{63}H_{88}CoN_{14}O_{14}P$

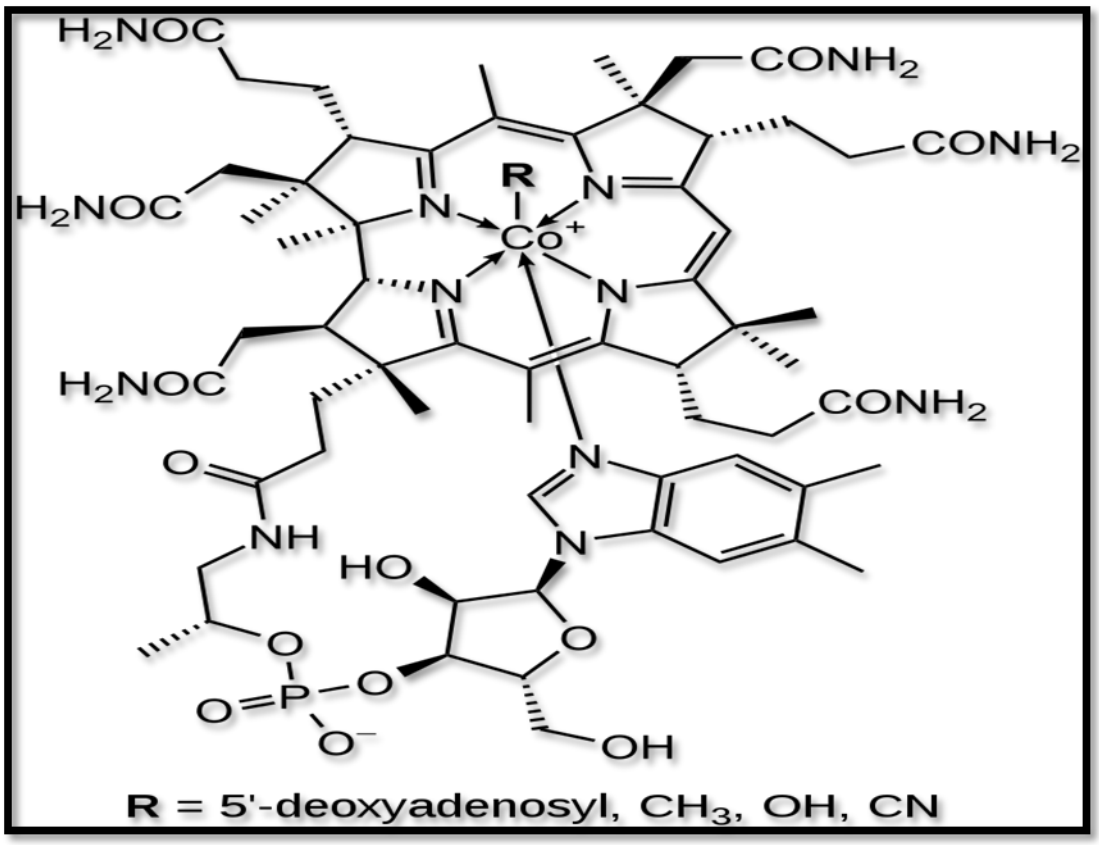

Figure (1-6). The structure of Vitamin B_{12}

Vitamin B_{12} (cobalamin) is a water-soluble vitamin obtained through the ingestion of fish, meat, and dairy products, as well as fortified cereals and supplements It is coabsorbed with intrinsic factor, a product of the stomach's parietal cells, in the terminal ileum after being extracted by gastric acid. Vitamin B_{12} is crucial for neurologic function, red blood cell production, and Deoxyribonucleic acid (DNA) synthesis, and is a cofactor for three major reactions: the conversion of methylmalonic acid to succinyl coenzyme A, the conversion of homocysteine to methionine; and the conversion of 5-methyltetrahydrofolate to tetrahydrofolate (Hunt et al., 2014). deficiency relates to a series of pathophysiological

mechanisms that can occur in infancy, childhood, adolescence and adult life, all of which can affect the vitamin B_{12} supply, the demand or both. Cellular deficiency in vitamin B_{12} is caused by inadequate intake, malabsorption, chemical inactivation, or inherited disruption of either B_{12} transport in the blood or intracellular metabolism (Green, 2017). Vitamin B_{12} maintains normal folate metabolism which is essential for cell multiplication, specifically in the rapidly dividing placental and fetal tissues. Together with folate and vitamin B_6, it also functions as a coenzyme in Deoxyribonucleic acid DNA synthesis and numerous methylation reactions which occur in developing embryos (Molloy et al., 2008). Also, Vitamin B12 regulates the energetic balance through its action as a co-factor of the enzyme methyl malonyl CoA mutase in the mitochondria (Kalhan, 2016).

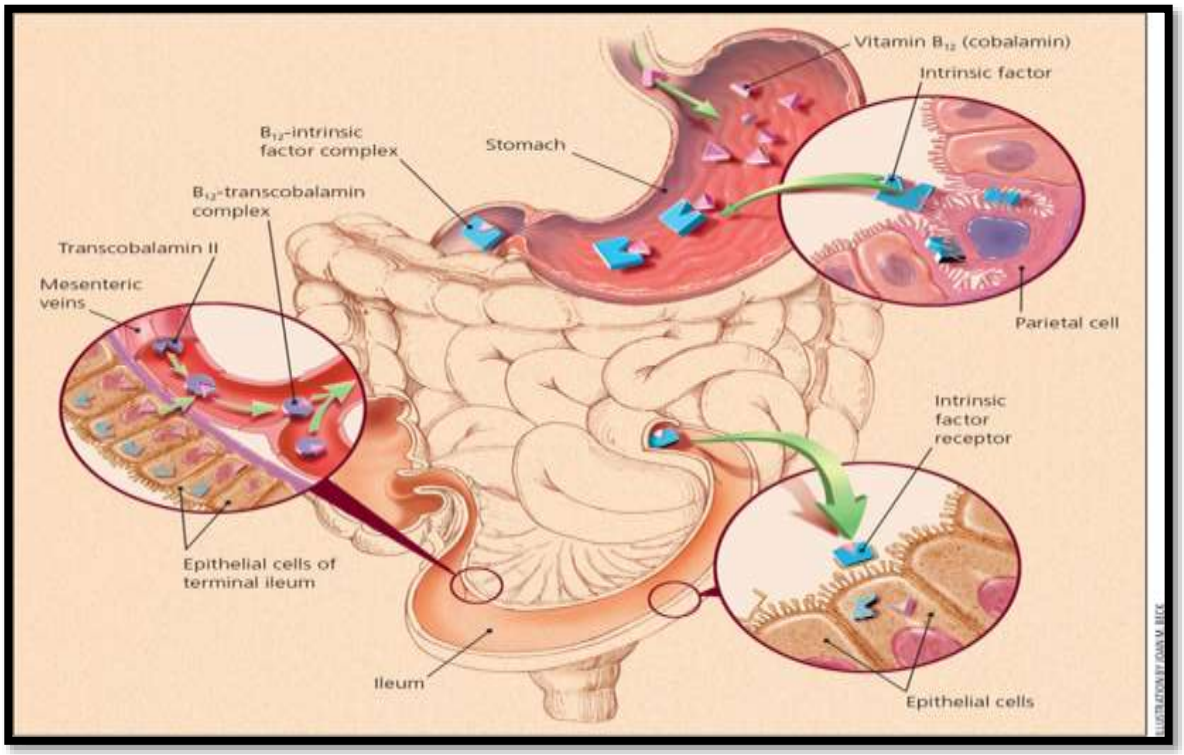

Figure (1-7) Vitamin B_{12} absorption and transport (Langan & Goodbred, 2017)

1.3.1.1 Vitamin B_{12} deficiency

Vitamin B_{12} deficiency is common during pregnancy and is associated with adverse outcomes for the mother and infant. Observational studies suggest that maternal vitamin B_{12} deficiency is associated with increased risk of pregnancy complications, such as Neural tube defects NTDs (Murray et al., 2012), spontaneous abortions and preeclampsia (Refsum et al., 2001).

Studies have also found an association between low vitamin B_{12} status in mothers and increased risk for birth defects when starting pregnancy with a deficient or inadequate vitamin B_{12} status (Krishnaveni et al., 2009). A vitamin B_{12} deficiency results in elevated concentrations of homocysteine and methylmalonic acid in serum (Refsum, 2001). The placenta synthesizes transcobalamin and contains transcobalamin receptors. In the intervilous space of the placenta, vitamin B_{12} and folate are sequestered so they can be distributed to the fetus. The fetus uses the available amount of vitamin B_{12} for biochemical reactions, but is not able to synthetize vitamin B_{12} (Molloy et al., 2008) .

1.3.1.2 Vitamin B_{12} metabolism

Vitamin B_{12} is used by the body in two forms, either as methylcobalamin or 5-deoxyadenosyl cobalamin. The enzyme methionine synthase needs methylcobalamin as a cofactor. This enzyme is normally involved in the conversion of the amino acid homocysteine into methionine, while methionine, in turn, is required for (DNA) methylation. 5-Deoxyadenosyl cobalamin is a cofactor needed by the enzyme that converts l-methylmalonyl CoA to succinyl CoA. This conversion is an important step in the extraction of energy from proteins

and fats. In addition, succinyl CoA is necessary for the production of hemoglobin which is the substance that carries oxygen in red blood cells (Mahmood, 2014). Vitamin B_{12} (cobalamin) plays a vital role in the conversion of homocysteine to methionine in methionine cycle, since it takes the methyl group from 5-methyl tetrahydrofolate (folic acid) and forms methyl cobalamin which then releases this methyl group in order to convert homocysteine into methionine (Blencowe et al., 2010), cobalamin is needed in the conversion of the methionine to homocysteine, where methionine is converted to S-Adenosyl-L-Methionine "SAM" product in the presence of Adenosine triphosphate (ATP) by methionine adenosyl transferase. In case of vitamin B_{12} deficiency, the body does not have the ability to produce methionine, which leads to many problems. Also, the body does not have the ability to produce S-adenosyl methionine which is known as S-Adenosyl -L-Methionine "SAM" product (Reynolds, 2002).

1.3.2 Folic acid B_9

$C_{19}H_{19}N_7O_6$

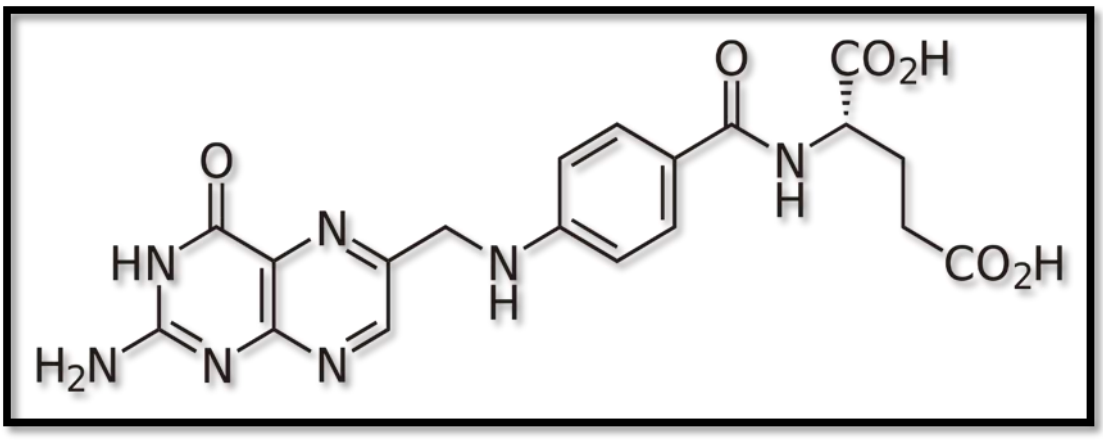

Figure (1-8). The structure of Folic acid

Folic acid (FA) is an oxidised synthetic form of the vitamin, which does not exist in nature, being only found in fortified foods, supplements and pharmaceuticals. Folic acid (FA) and dietary folate lack the ability to act as a substrate until they have been absorbed from the gastrointestinal tract and hepatically converted to the metabolically active 5-methyltetrahydrofolate (5-methylTHF) (Pietrzik et al., 2010). known as vitamin B_9 , folate, or folic acid. All B vitamins help the body to convert the food (carbohydrates) into fuel (glucose), which is used to produce energy. These B vitamins, often referred to as B complex vitamins, help the body use fats and protein. B complex vitamins are needed for healthy skin, hair, eyes, and liver. Also, they help the nervous system function properly (Bailey & Ayling, 2009). Folic acid is crucial for proper brain functioning and plays an important role in mental and emotional health. It helps in the production of (DNA) and (RNA), the body's genetic material, especially when cells and tissues are growing rapidly, such as during infancy, adolescence, and pregnancy. Folic acid works closely with vitamin B_{12} in making red blood cells and helps iron function properly in the body. Vitamin B_9 works with vitamins B_6 and B_{12} and other nutrients in controlling the blood levels of the amino acid homocysteine (Goh & Koren, 2008).

Rich sources of folate include spinach, dark leafy greens, legumes , nuts ,eggs , beef liver ,oranges and bananas (Abularrage et al., 2007).

1.3.2.1 Folic acid and Recurrent pregnancy loss

Folic acid is a B vitamin that helps the body make healthy new cells. Human body needs folic acid, especially pregnant women Getting enough folic acid before and during pregnancy may prevent major birth defects of baby's brain or spine (Bailey & Ayling, 2009). It is widely recognised that periconceptional folic acid (FA) supplementation reduces the risk of neural tube defects and other congenital malformations. Observational studies have suggested that reduced circulating folate in pregnancy is associated with increased risk for preterm birth, placental abruption, intrauterine growth restriction (IUGR) and pre-eclampsia (Czeizel et al., 2010). Due to the association of low plasma folate levels with a higher risk of early spontaneous abortion, the increased intake of folate supplements could make improvements in pregnancy outcomes (Gaskins et al., 2014).

1.3.2.2 Folic acid metabolism and mechanism

Folate metabolism is closely linked to homocysteine metabolism, and, as a result, increased plasma folate is associated with decreased plasma homocysteine (Mao et al., 2016). Folic acid is biochemically inactive. It was converted by reducing hydrofolate to tetrahyrofolic acid and methyl tetrahydrofolate . These folic acid congeners are transported by receptor-mediated endocytosis across cells where they are needed to maintain normal erythropoiesis, interconvert amino acids, methylate transfer Ribonucleic acid (tRNA) , synthesize purine and thymidylate nucleic acids. Using vitamin B_{12} as a cofactor, folic acid can normalize high homocysteine levels by remethylatiopn of homocysteine to methionine via methionine synthetase (Krebs et al., 2009) .

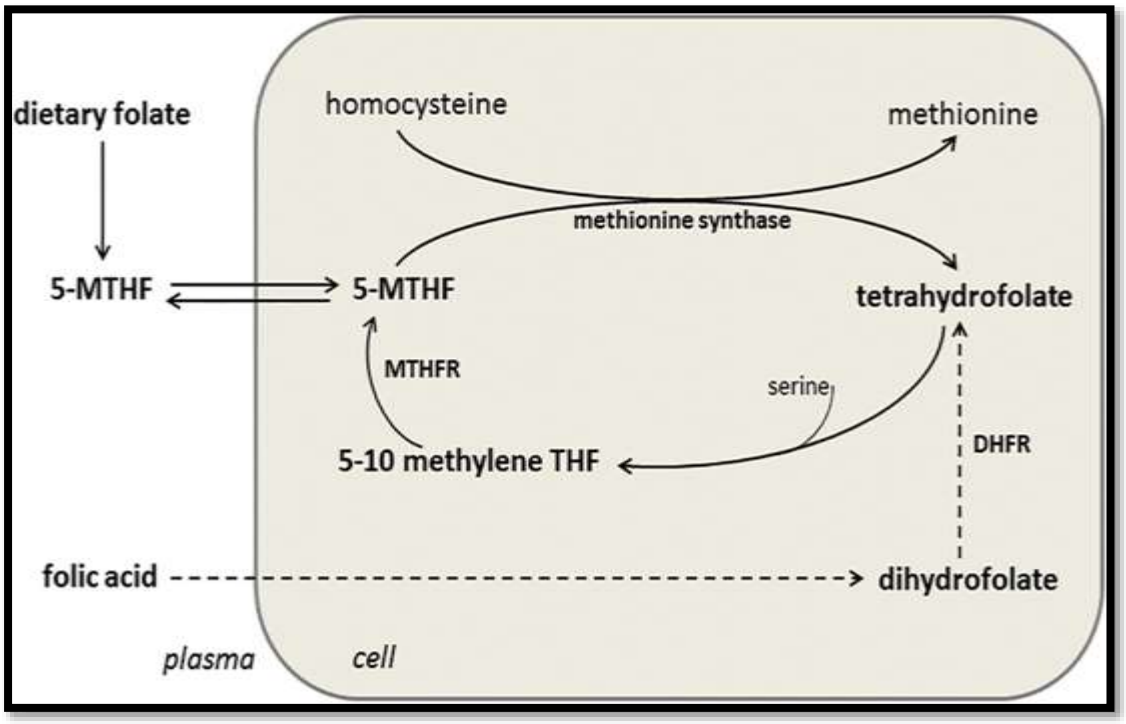

Figure (1-9) . Metabolism of folate .Abbreviations: 5-MTHF, 5-methyl tetrahydrofolate; MTHFR, 5,10-methylene tetrahydrofolate reductase; DHFR, dihydrofolate reductase; THF, tetrahydrofolate

Folate plays a vital role in the methionine cycle. It is involved as 5-methyl tetrahydrofolate methionine in the methylation process where the methyl group is transferred to homocysteine to form methionine in the presence of methionine synthase enzyme. Methionine synthase is one of the only two enzymes known to be B_{12} dependent enzymes. This process depends on both folic acid and vitamin B_{12} (Dietrich et al., 2005).

1.4 Selenium

Selenium is an essential trace element primarily obtained from bread, cereals, fish, poultry and meat. It plays a vital role in many metabolic functions (Kieliszek, 2019). The selenium reference nutrient intake is 70µg per day for adult (Stoffaneller & Morse, 2015) Their functions selenoproteins range from thyroid homeostasis, promoting fertility, optimising immune/antioxidant function (Williams, 2010).

Selenium is set aside in the form of selenomethionine and stored in the organs and tissues with variable density: 30% in liver, 30% in muscle, 15% in kidney, 10% in plasma, and 15% in other organs (Mistry et al., 2012). The selenium homeostasis is primarily achieved by the reserves of selenomethionine in the kidney and liver. The stored selenium is used when selenium food intake is too low for selenoproteins synthesis (Schrauzer, 2000). Selenium is integrated into proteins to make selenoproteins which include glutathione peroxidase, thioredoxin reductases, and selenoprotein-P (Tinkov et al., 2020). Reduced levels of maternal selenium concentrations have been shown to be associated with recurrent early pregnancy loss and preeclampsia (Maleki et al., 2011). It is also important to note that both inorganic and organic forms must first be converted to inorganic selenide (H_2Se) before the synthesis of selenocysteine (SeCyS), which crucially contributes to the formation of selenoproteins (Duntas, 2020)

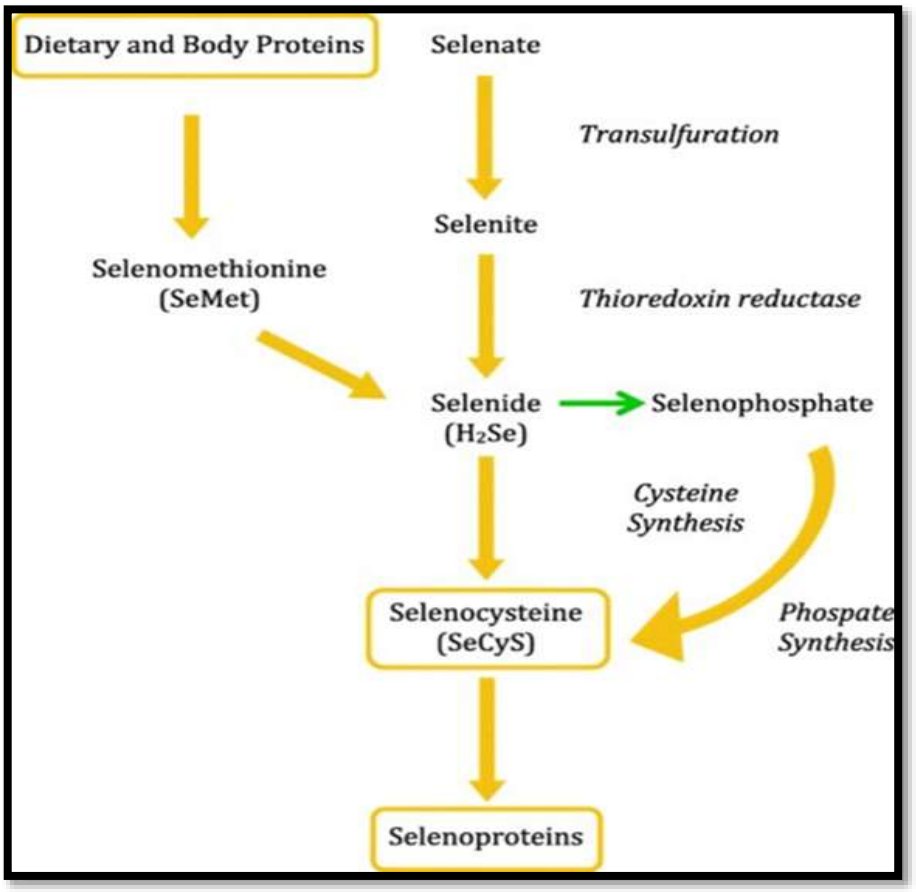

Figure (1-10) . The main metabolic steps of metabolism of inorganic and organic Se Both inorganic (selenite) and organic (selenomethionine, SeMet) are converted to selenide (H_2Se) before the formation of selenocysteine (SeCys) and its insertion in bioactive selenoproteins (Ip, 1998)

Selenium is the key component of a number of functional selenio-proteins required for normal health. The best known of these is the antioxidant glutathione peroxidase which removes hydrogen peroxide and other damaging lipid and phospholipid hydroxides generated by free radicals and other oxygen derived species (Bedwal & Bahuguna, 1994). If not removed, lipid hydroxyperoxides impair the structure and function of cell membranes, and cause coagulation disturbances by decreasing the production of prostacyclin while increasing the production of

thromboxane. Thus selenium deficiency can predispose to thromboembolic events (Taysi et al., 2019). Selenium deficiency in women results in infertility, miscarriage and retained placenta (Sattar, 2021). The newborn infants of mothers deficient in selenium may suffer from muscular weakness; but the concentration of selenium during the pregnancy does not have any effect on the weight of the baby or the length of the pregnancy. The selenium requirement of a pregnant or lactating mother increases as a result of selenium transport to the fetus via the placenta and to the infant via breast milk (Iqbal et al., 2020).

1.4.1 Selenium and recurrent miscarriage

Low Selenuim serum has been associated with 1st trimester miscarriages / recurrent miscarriages (Mistry et al., 2012) and increased risk of pre-eclampsia (Poston et al., 2011) Pregnancy stresses the thyroid T_3 is essential for brain development and function, especially during 2nd & 3rd trimesters. Selenium and iodine are both important for thyroid T_3 function. If T_3 autoimmune thyroiditis is suspected, confirm and supplement with 200µg Se Methionine (post-partum) to lower risk of post-partum thyroid disease and permanent hypothyroidism (Negro et al., 2007).

A miscarriage can occur due to many factors, including oxidative stress (Micle et al., 2012). Oxidative stress is a condition caused by an imbalance of free radical production and the body's antioxidant defense system. One enzyme that serves as a primary antioxidant defense factor in humans is glutathione peroxidase (GPx). The main role of glutathione peroxidase (GPx) is to maintain appropriately low levels of hydrogen peroxide within the cell, thus decreasing the potential for damage to membranes and other cell structures(Ighodaro & Akinloye, 2018).

Glutathione peroxidase GPx is a selenium (Se)-dependent enzyme, and selenium Se is incorporated into its active site (Liu et al., 2010). Thus, the concentration of Se in the body, which depends on dietary intake, influences the levels of glutathione peroxidase GPx and other Se-dependent antioxidant enzymes that function to protect the body against free radicals (Mistry et al., 2012). The nutritional benefits of Se are believed to be related to its role as an important component of several antioxidant enzymes that protect cells against the effects of free radicals (Abdulah et al., 2005). Se has also been reported as an essential nutrient in pregnancy, and maternal Se concentration has been reported to be associated with reproductive performance, including infertility, preeclampsia, early childhood wheezing symptoms, miscarriage, and retained placenta (Maleki et al., 2011). Some studies have been conducted regarding the effectiveness of selenium supplementation on pregnancy complications. A meta-analysis conducted by (Xu et al., 2016) elucidated that selenium supplementation to pregnant women significantly decreased the incidence of preeclampsia. Moreover, it was observed that low selenium status during pregnancy is associated with miscarriage and preterm delivery (Okunade et al., 2018).

1.5 Aims of Study

The main aim of this study is to shed light on women who suffer from two or more consecutive miscarriages for unknown reasons, through the following measurements:

1. The study of the effect of homocysteine levels on women with recurrent pregnancy loss

2. To investigate the role of vitamin B_{12} and folic acid in women suffering from recurrent miscarriages of unknown cause.

3. Measuring selenium levels and its effect on recurrent pregnancy loss

4. Showing possible correlations between homocysteine level and the studied criteria (vitamin B_{12} and folic acid)

5. The aim of dividing the study groups is to find out the proportion of homocysteine in miscarriaged women (pregnant and non-pregnant) compared to healthy women

Chapter Two

Materials

And

Methods

2. Materials and Methods

2.1. Design of Study:

This study was conducted at Bint Al-Huda Hospital in Thi-Qar and in the Biochemistry Laboratory in the College of Science (University of Thi-Qar) in the period between (May ,2020) to (October ,2020). The study included (135) women, 45 control subjects and 90 patients Their ages were between 18-40 years , controls and patients were divided into.

The first group : consists of 45 women who have had two consecutive miscarriages or more who are currently pregnant, the period between (8-20) weeks, in the first or second trimester pregnancy .

The second group : consists of 45 women who have had two or more consecutive miscarriages, who are not pregnant , in a period of one to three after miscarriage .

The third group : consists of 45 women who have not experienced a previous miscarriage and are currently pregnant at the first or second trimester pregnancy, and are considered as a control group.

Cases excluded from this study: Women who were exposed miscarriage for known reasons. All of the women in the groups had no chronic diseases and did not use any medications.

Table (2-1) : Data of the studied groups .

Age groups	Age (years)	No.	Item
A1	20 – 40	45	RPL Pregnant
A2	20 – 37	45	RPL non-pregnant
A3	18 – 40	45	Control

2.2. Collection of Blood Sample:-

About (5ml) of blood was collected from women by venous . Placed in a gel tube to separate the serum .The blood was allowed to clot at room temperature , and then the serum was separated by centrifugation (10 min at 3000 xg) the serum samples was removed and stored at (-20ºC) for later measurement biochemical parameters, unless used immediately. The clinical Criteria of this study were described in the following table.

Table (2-2): The clinical chart of the study

Patient profile
Name
Age
BMI Kg/m^2
Number of previous miscarriages
The total number of children

the period of miscarriage	First trimester
	Second trimester
Other causes of miscarriage	Virus
	Congealment
	The cause is unknown
Case	pregnant
	Non pregnant
Biochemical tests	
Homocystiene (Hcy)	
Vitamin B_{12}	
Folic acid (FA)	
Selenium (Se)	

2.3. Chemicals:

The chemicals which used in this study with their companies are showed in table (2-3).

Table (2-3): The chemicals and their suppliers

No.	Chemicals	Supplied Company
1.	Folic acid Kit	Roch-Germany
2.	Homocysteine Kit	CUSABIO-USA
3.	Hydrochloric acid	BDH, England
4.	Nitric acid	BDH, England
5.	Paraffin oil	BDH, England

6.	Perchloric acid	BDH, England
`7.	Vitamin B12	Roch-Germany

2.4. Instruments:

The instruments are available in clinical biochemistry laboratory in Maternity and children (Bint Al- Huda) Hospital , laboratories of chemistry department in the College of Science (University of Thi-Qar) which are shown in table (2-4).

Table (2- 4): The instruments and their manufacturer

No.	Instruments	Suppliers	Origin
1.	Atomic absorption spectrophotometer	Shimadzu	Japan
2.	Centrifuge	Steinberg systems	UK
3.	Cobas e411	Human	Germany
4.	Elisa ELx800 reader	Bio Kit	USA
5.	Incubator	Jard	Japan
6.	Oil bath	L513-0814	French
7.	Washer	Bio kit	USA
8.	Water bath	L513-0814	French

2.5. Biochemical Parameters:

Several considerable methods were used to measure the studied parameters .

2.5.1 Determination of Homocysteine by ELISA :

Principle:

This kit was based on Competitive-ELISA detection method. The microtiter plate provided in this kit has been pre-coated with Hcy. During the reaction, Hcy in the sample or standard competes with a fixed amount of Hcy on the solid phase supporter for sites on the Biotinylated Detection Antibody specific to Hcy. Excess conjugate and unbound sample or standard were washed from the plate, and HRP-Streptavidin (SABC) was added to each microplate well and incubated. Then TMB substrate solution was added to each well. The enzyme-substrate reaction was terminated by the addition of a sulphuric acid solution and the color change was measured spectrophotometrically at a wavelength of 450 nm. The concentration of Hcy in the samples was then determined by comparing the OD of the samples to the standard curve ((Malik et al., 2018; Skurikhin et al., 2015) .

Kit Components

Reagents supplied	**96 Tests**	
ELISA Microplate (Dismountable)	96	2-8°C/-20°C
Lyophilized Standard	2 vial	2-8°C/-20°C
Sample Dilution Buffer	20 ml	2-8°C
Biotin- labeled antibody (Concentrated)	60 ul	2-8°C (Avoid direct light)
Antibody dilution buffer	10 ml	2-8°C
HRP-Streptavidin Conjugate(SABC)	120 ul	2-8°C(Avoid direct light)
SABC dilution buffer	10 ml	2-8°C
TMB substrate	10 ml	2-8°C(Avoid direct light)
Stop solution	10 ml	2-8°C
Wash buffer (25X)	30 ml	2-8°C
Plate Sealer	5 Pieces	

Reagent Preparation

The kit was left at room temperature for 20 minutes before using.

1. Wash Buffer: 30mL of concentrated wash buffer was diluted into 750 mL of wash buffer with deionized or distilled water. Unused solution was Put back at 4°C. If crystals have formed in the concentrate, you could warm water bath 40°C (Heating temperature should not exceed 50°C) and mix it gently until the crystals have completely dissolved. The solution have been cooled to room temperature before use.

2. Standard:

a) One milliliter ml of sample dilution buffer was added into one standard tube , and then the tube was kept at room temperature for 10 minutes and mixed them thoroughly.

b) Seven EP tubes with 1/2, 1/4, 1/8, 1/32, 1/64 and blank respectively. 0.3 ml of the sample dilution buffer was added into each tube. 0.3 ml of the above standard solution (from zero tube) was added into 1st tube and mixed them thoroughly. 0.3ml from 1st tube was transferred to 2nd tube and mixed them thoroughly. 0.3ml from 2nd tube was transferred to 3rd tube and mixed thoroughly, and so on. Sample dilution buffer was used for the blank control.

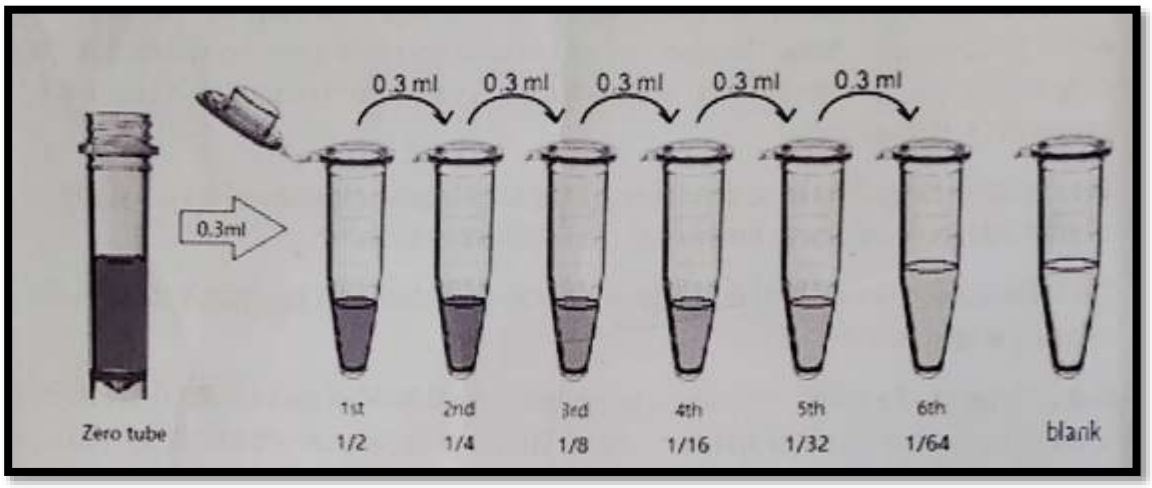

Figure (2-1). Prepare standard solutions

3. Preparation of biotin-labeled antibody working solution: It was prepared within 1 hour before experiment.

a) Required total volume of the working solution was calculated: 0.05 ml/well × quantity of wells. (Allow 0.1-0.2 ml more than the total volume)

b) The biotin-detection antibody with antibody dilution buffer was diluted at 1:100 and mixed them thoroughly. (i.e. Added 1µl of Biotin- labeled Antibody into 99µl of Antibody Dilution Buffer).

4. Preparation of HRP-Streptavidin conjugate (SABC) working solution: It was prepared within 30 minutes before experiment.

a) Required total volume of the working solution was calculated: 0.1 ml / well × quantity of wells. (Allow 0.1-0.2 ml more than the total volume)

b) The SABC with SABC dilution buffer was diluted at 1:100 and mixed thoroughly. (i.e. Added 1µl of SABC into 99µl of SABC dilution buffer).

Procedure

Before adding reagents into wells, equilibrated the TMB substrate for 30 min at 37 °C. When diluting samples and reagents, they must be mixed completely and evenly. It is recommended to plot a standard curve for each test.

1. Standard, test sample and control (blank) wells on the pre-coated plate respectively were set respectively, and then, their positions were recorded. It is recommended to measure each standard and sample in duplicate. The plate was washed 2 times before adding standard, sample and control (blank) wells!

2. Fifty Microliters of standard, blank, or sample was added per well. The blank well was added with sample/standard dilution buffer. Immediately, 50μL biotin-labeled antibody working solution was added into each well. With the Plate sealer was covered we provided. Gently the plate was tapped to ensure thorough mixing. It was incubated for 45 minutes at 37°C. (solutions are added to the bottom of microplate well, avoiding inside wall touching and foaming as much as you can).

3. The cover was removed, and plate was washed 3 times with wash buffer, and let the wash buffer stay in the wells for 1 minute each time. After the last wash, any remaining wash buffer was removed by aspirating or decanting.

4. Hundred Microliters SABC working solution was added into each well. It was covered with a new plate sealer. It was incubated for 30 minutes at 37°C.

5. The cover was removed and plate was washed 5 times with wash buffer, and let the wash buffer stay in the wells for 1-2 minute each time.

6. Ninety Microliters TMB substrate was added into each well, the plate was covered and incubated at 37°C in dark within 10-20 minutes. (The reaction time can be shortened or extended according to the actual color change, but not more than 30 minutes. You can terminate the reaction when apparent gradient appeared in standard wells).

7. Fifty Microliters stop solution was added into each well. The color turned yellow immediately. The adding order of stop solution should have been as the same as the TMB (3,3,5,5-tetramethylbenzidine) substrate solution.

8. The Optical Density O.D was read. Absorbance at 450 nm in microplate reader immediately after adding the stop solution.

Calculation of results

Average the duplicate readings for each standard and samples. A standard curve was created by plotting the mean OD Value for each standard on the y-axis or x-axis against the concentration on the x-axis or y-axis and drew a best fit curve through the points on the graph. It was recommended to use some professional software to do this calculation, such as curve expert 1.3 or 1.4. In the software interface, a best fitting equation of standard curve would be calculated using OD Value and concentrations of standard sample. The software would calculate the concentration of samples after entering the OD Value of samples. Also, I could enter the corresponding fitting equation and OD Value of samples into Excel to get the concentration of samples. If samples had been diluted, the concentration calculated from the standard curve must have been multiplied by the dilution factor. If the OD of the sample surpassed the upper limit of the standard curve, I should have re-tested it after appropriate dilution. The actual concentration is the calculated

concentration multiplied dilution factor. Recommended to use professional software curve expert to 1.3

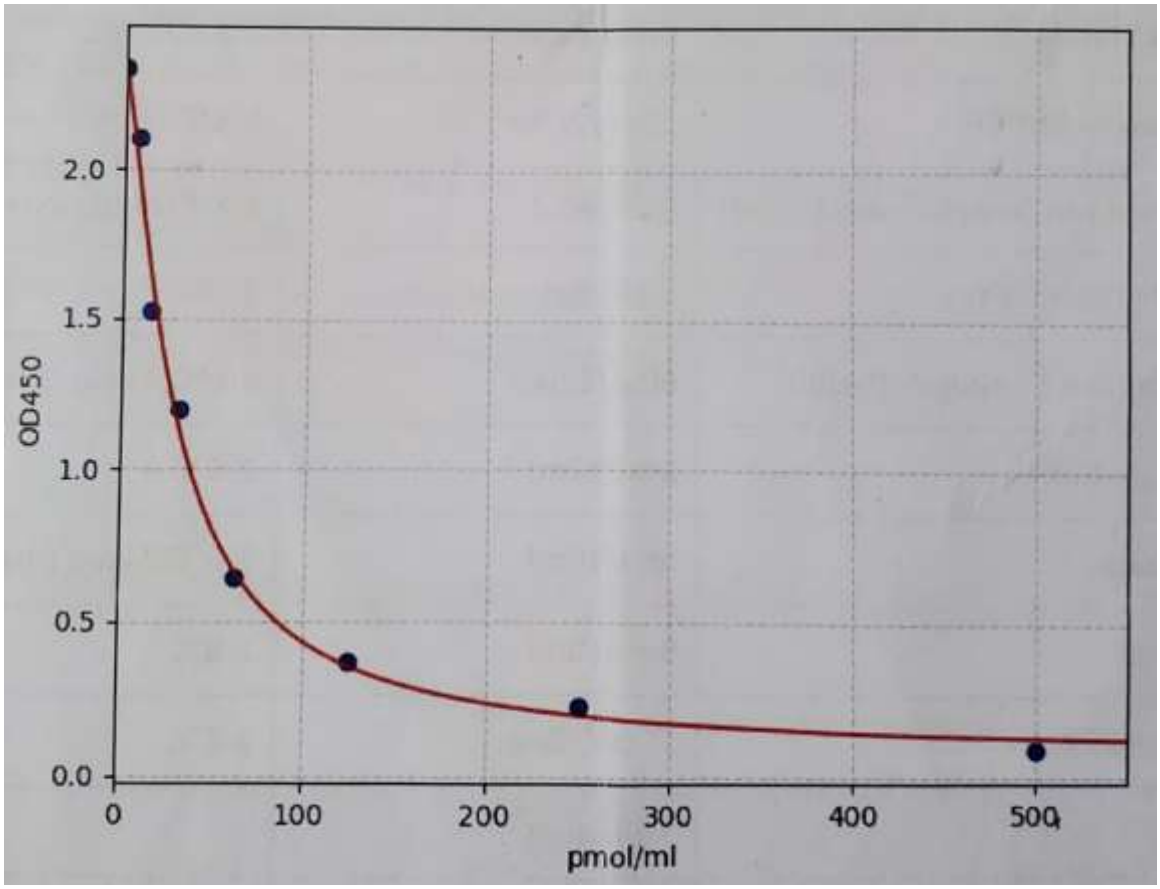

Figure (2-2). Standard curve of homocystiene

The X-coordinate : Homocysteine concentration

The Y-coordinate : Absorbency

2.5.2 Determination of vitamin B_{12} by Cobas E411:

Principle:

Competition principle. Total duration of assay: 27 minutes.

• **1st incubation :** By incubating the sample (15 µL) with the vitamin B_{12} pretreatment 1 and pretreatment 2, bound vitamin B_{12} is released.

• **2nd incubation :** By incubating the pretreated sample with the ruthenium labeled intrinsic factor, a vitamin B_{12} -binding protein complex is formed, the amount of which is dependent upon the analyte concentration in the sample.

• **3rd incubation :** After addition of streptavidin-coated microparticles and vitamin B_{12} labeled with biotin, the still-vacant sites of the ruthenium labeled intrinsic factor become occupied, with formation of a ruthenium labeled intrinsic factor vitamin B_{12} biotin complex. The entire complex becomes bound to the solid phase via interaction of biotin and streptavidin.

• The reaction mixture was aspirated into the measuring cell where the microparticles were magnetically captured onto the surface of the electrode. Unbound substances were then removed with ProCell M. Application of a voltage to the electrode then induces chemiluminescent emission which was measured by a photomultiplier. (Castillo, 2016; Mailankody & Landgren, 2016)

Reagents

The reagent rackpack (M, R_1, R_2) and the pretreatment reagents (PT_1, PT_2) are labeled as B_{12} II.

PT_1 Pretreatment reagent 1 (white cap), 1 bottle, 4 mL: Dithiothreitol 1.028 g/L; stabilizer, pH 5.5.

PT_2 pretreatment reagent 2 (gray cap), 1 bottle, 4 mL: Sodium hydroxide 40 g/L; sodium cyanide 2.205 g/L.

M streptavidin-coated microparticles (transparent cap), 1 bottle, 6.5 mL: streptavidin-coated microparticles 0.72 mg/mL; preservative.

R_1 Intrinsic factor~Ru(bpy) (gray cap), 1 bottle, 10 mL: Ruthenium labeled recombinant porcine intrinsic factor 4 µg/L; cobinamide dicyanide 15 µg/L; stabilizer; human serum albumin; phosphate buffer, pH 5.5; preservative.

R_2 vitamin B_{12}~biotin (black cap), 1 bottle, 8.5 mL: Biotinylated vitamin B_{12} 25 µg/L; biotin 3 µg/L; phosphate buffer, pH 7.0; preservative.

Procedure:

For optimal performance of the assay, it is important to follow the directions given for the analyzer used, and to check that the system's inventory of assay materials and other consumables is adequate. Re-suspension of the microparticles before use and the reading in of the test-specific parameters via the reagent bar code take place automatically. No manual input is necessary. If in exceptional cases the bar code cannot be read, enter the 15-digit sequence of numbers.

E411: The cooled reagent was brought to approximately 20°C and placed on the reagent disk of the analyzer. The formation of foam was avoided. The system automatically regulated the temperature of the reagents and the opening/closing of the bottles.

- **AMR (Analytical Measurement Range):** 150-2000 pg/mL ((defined by the lower detection limit and the maximum of the mater curve).

- Values below the detection limit were reported as <150 pg/ml. Values above the measuring range were reported as >2000 pg/ml.

Calculation

The analyzer automatically calculates the analyte concentration of each sample (either in pmol/L or pg/mL).

2.5.3 Determination of Folic acid by Cobas E411:

Principle

Competition principle. Total duration of assay: 27 minutes.

• **1st incubation:** By incubating 25 µL of sample with the folate pretreatment reagents 1 and 2, bound folate was released from endogenous folate binding proteins.

• **2nd incubation:** By incubating the pretreated sample with the ruthenium labeled folate binding protein, a folate complex was formed, the amount of which was dependent upon the analyte concentration in the sample.

• **3rd incubation:** After addition of streptavidin-coated microparticles and folate labeled with biotin, the unbound sites of the ruthenium labeled folate binding protein become occupied, with formation of a ruthenium labeled folate binding protein-folate biotin complex. The entire complex became bound to the solid phase via interaction of biotin and streptavidin.

• The reaction mixture was aspirated into the measuring cell where the microparticles were magnetically captured onto the surface of the electrode. Unbound substances were then removed with ProCell l M. Application of a voltage to the electrode then induces chemiluminescent emission which was measured by a photomultiplier (Filippatos et al., 2016; Gertz, 2016).

Reagents

The reagent rackpack (M, R_1, R_2) and the pretreatment reagents (PT_1, PT_2) are labeled as Fol III.

PT_1 Pretreatment reagent 1 (white cap), 1 bottle, 4 mL: Sodium 2 mercaptoethanesulfonate (MESNA) 40 g/L, pH 5.5.

PT_2 Pretreatment reagent 2 (gray cap), 1 bottle, 5 mL: Sodium hydroxide 25 g/L.

M Streptavidin-coated microparticles (transparent cap), 1 bottle, 6.5 mL: Streptavidin-coated microparticles 0.72 mg/mL; preservative.

R_1 Folate binding protein~Ru(bpy) (gray cap), 1 bottle, 9 mL: Ruthenium labeled folate binding protein 75 µg/L; human serum albumin (stabilizer); borate/phosphate/citrate buffer 70 mmol/L, pH 5.5; preservative.

R_2 Folate~biotin (black cap), 1 bottle, 8 mL: Biotinylated folate 17 µg/L; biotin 120 µg/L; human serum albumin (stabilizer); borate buffer 100 mmol/L, pH 9.0; preservative.

Procedure:

For optimal performance of the assay, it was important to follow the directions given for the analyzer used, and to check that the system's inventory of assay materials and other consumables was adequate. Re-suspension of the microparticles before using and the reading in of the test-specific parameters via the reagent bar code took place automatically. No manual input was necessary. If in exceptional cases the bar code could not be read, the 15-digit sequence of numbers was entered.

E411: The cooled reagent was brought to approximately 20°C and placed on the reagent disk of the analyzer. The formation of foam was avoided. The system automatically regulated the temperature of the reagents and the opening/closing of the bottles.

- **AMR (Analytical Measurement Range):** 150-2000 pg/mL ((defined by the lower detection limit and the maximum of the mater curve).

- Values below the detection limit were reported as <150 pg/ml. Values above the measuring range were reported as >2000 pg/ml.

Calculation

The analyzer automatically calculates the analyte concentration of each sample (either in nmol/L or ng/mL).

2.5.4 Determination of Serum Selenium Concentrations:

Principle

Concentration of element selenium in serum samples were measured by flame atomic absorption method. In this method, light of hollow cathode (HC) lamp is absorbed by ground state atoms in a lean air-acetylene flame. The amount of light absorbed is directly proportional to the concentration of atoms in the gaseous state in the light path and hence to the concentration of selenium in the solution.

Digestion of samples

The serum samples were digested by adding 2 mL of conc. Nitric acid and 1 mL of conc. Perchloric acid to 0.5 mL serum in Pyrex tube. Heated for 1 hour at 160 ˚C using oil bath, then samples were cooled, and the volume was completed to10 mL by 0.3 N hydrochloric acid.

2.6. Statistical Analysis :

All statistical analysis was performed using SPSS, Windows version 23.0 software and Microsoft Excel 2010. The results were expressed as mean ± standard deviations (mean ± SD) ,and Least Significant Difference (LSD). One - way analysis of variance (ANOVA) was used to compare parameters in different studied groups. P-values ($P< 0.05$) were considered statistically significant.

Person correlation coefficient (r) is used to measure the strength at a linear association between two variables (r=+1) ,(r=-1) . Person correlation coefficient (r) was used to test the correlation relationship among the different parameters in each patients group.

Chapter Three

Results and Discussion

3. Results and Discussion

3.1. Clinical and Characteristic Features of the Study Groups

There are (135) women included in the current study, the group of patients (90) divided into two groups (RPL pregnant and RPL non-pregnant) were compared with the control group of healthy subjects (45). Characteristic data for all studied groups shown in Table (3-1).

Table (3-1):- Characteristic data for studied groups

Group	No.	Age (years) mean±SD	No.of.children mean±SD	No.of.miscarriage mean±SD
RPL non-pregnant	45	30.6±6.65	1.68±1.39	2.82±1.02
RPL pregnant	45	32.6±6.45	2.22±1.62	3.2±1.32
Control	45	27.2±6.73	1.88±0.77	0

In this study, parameter levels were compared between women with recurrent miscarriages in the first and second trimesters of pregnancy and healthy women. There were statistically significant differences between the patients and the control group in all biochemical variables ($p<0.05$).

3.2. Biochemical Parameters:-

3.2.1 Serum Homocystiene Concentration

Table (3-2) and Figure (3-1) shows significant increase in the concentration of serum Hcy in RPL pregnant group in comparison with RPL non pregnant and control groups ($p<0.05$). Also it was found a significant increase in the concentration of serum Hcy in RPL non pregnant group in comparison with control group ($p<0.05$). There were also previous studies reporting an increase in the homocystiene concentration in women who suffer from recurrent miscarriage comparison with control group (D'Uva et al., 2007).

The results showed an increase in homocysteine in pregnant women with recurrent pregnancy loss high levels of homocysteine are associated with vitamin B_{12} deficiency (Herrmann & Obeid, 2008). It is also linked to nutritional influences , Malnutrition and malabsorption of folic acid or vitamin B_{12} or due to genetic factors such as mutation in the gene of methylene tetrahydrofolate reductase (MTHFR) enzyme ,The MTHFR gene mutation reduces enzyme activity and increases the concentration of homocysteine in the blood (Kumar et al., 2003).

Impairment of chorionic villous vascularisation is linked to hyperhomocysteinemia (Serapinas et al., 2017). Increased levels of homocysteine have been associated with increased pregnancy complications and negative pregnancy outcomes such as abnormalities, especially neural tube defects (Refsum et al., 2006).

The risk associated with hyperhomocysteinemia may be due to fetal toxicity (Govindaiah et al., 2009) or homocystiene can potentially interact with haemostatic genetic determinants lead to by increasing the

thrombogenic potential (Durand et al., 1997) due to the elevated ability of homocysteine to activate factor V and inactivate protein C, thrombomodulin, heparan sulfate, or due to cellular methylation that alters gene expression or due to misincorporation of uracil into the DNA leading to damage. DNA (Govindaiah et al., 2009).

Thrombomodulin is an endothelial cell surface glycoprotein that promotes activation of the anticoagulant protein C and inhibits the procoagulant activities of thrombin (Dittman & Majerus, 1990) The resulting decrease in protein C activation may contribute to the development of thrombosis and disseminated intravascular coagulation (Goldenberg & MANCO-JOHNSON, 2008) homocysteine does not decrease thrombomodulin synthesis but , directly inhibit the thrombomodulin cofactor activity and than Inhibition of thrombomodulin contribute to decreased protein C activation (Hayashi et al., 1992)

Table (3-2): Serum homocystiene levels of control and patients groups

Groups	No.	Hcy (p mol/ml) Mean ±SD
RPL non- pregnant	45	284.24±48.79 b
RPL pregnant	45	535.27±76.18 a
Control	45	229.40±42.94 c
LSD		20.23

Note :- Each value represents mean ±S.D values with non identical superscript (a , b or c…etc.) for vertical comparison, are considered significantly differences ($P < 0.05$).

- No. : Number of subjects .
- SD : Standard deviation .
- LSD : Least Significant Difference
- RPL : Recurrent pregnancy loss

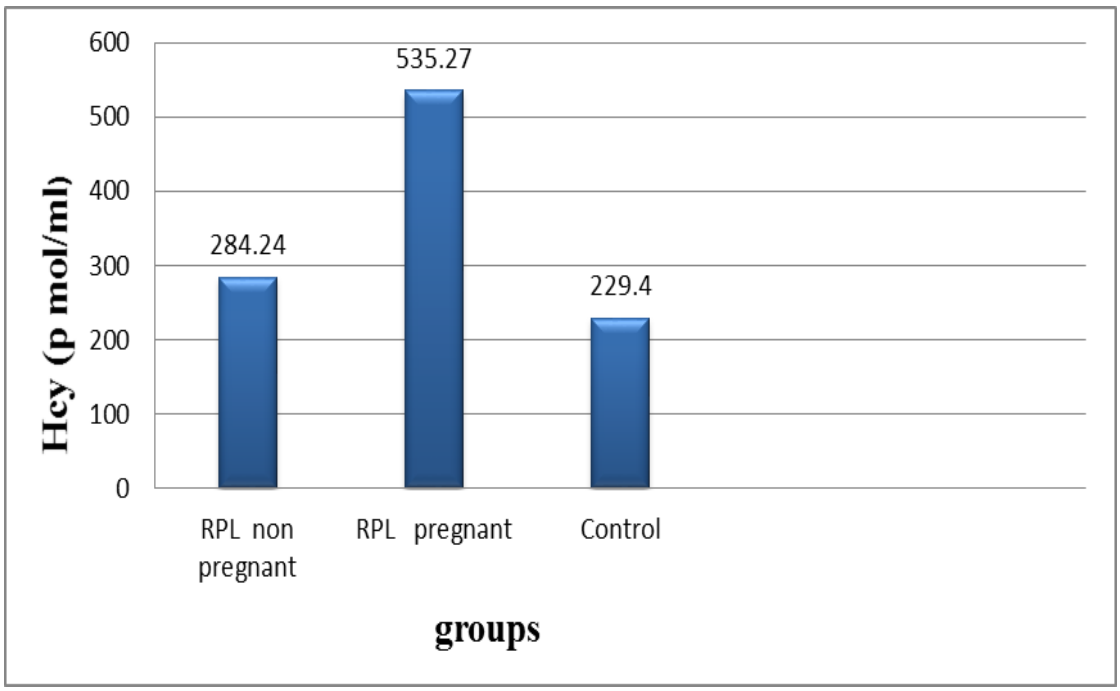

Figure (3-1): Serum Homocystiene levels of control and patient groups

3.2.2 Serum Vitamin B_{12} Concentration

Table **(3-3)** and Figure **(3-2)** shows significant decrease in the concentration of serum B_{12} in RPL pregnant group in comparison with RPL non pregnant and control groups ($p<0.05$). Also it was found a significant decrease in the concentration of serum B_{12} in RPL non pregnant group in comparison with control group ($p<0.05$).

Table (3-3) showed a decrease in vitamin B_{12} in pregnant women who suffer from recurrent miscarriage that the lack of vitamin B_{12} is due to the increased need of pregnant women for these vitamins for the growth of the fetus, mother's tissues and placenta, and that many women do not get enough nutrients even if they use the supplements or due to malabsorption . This result is consistent with previous studies (Abd-Ellatef et al., 2018; Sawant, 2015) While, There was a study contrast our

results where the mean vitamin B12 was not statistically higher than the control group (Sikora et al., 2007).

Vitamin B_{12} participates in methionine metabolism and its deficiency is related to hyperhomocysteinemia, and it has been shown that vitamin B_{12} plays a role in recurrent pregnancy loss, as vitamin B_{12} deficiency leads to faulty and sporadic ovulation producing a faulty oocyte and vitamin B_{12} deficiency leads to incomplete trophoblastic invasion of spiral arteries thereby leading to defective placentation (Sawant, 2015).

Vitamin B_{12} deficiency usually results from a lack of intake and may also occur as a result of malabsorption, some intestinal disorders, a decrease in the amount of binding proteins, and the use of some medications. Vitamin B_{12} is a rare vitamin that is found in plant sources, so vegetarians are more susceptible to vitamin B_{12} deficiency, as well as with regard to infants born to vegetarian mothers, they face a greater risk of vitamin B_{12} deficiency than others. Vitamin B_{12} deficiency may affect between 40% and 80% of vegetarians who do not consume supplements containing vitamin B_{12} (Pawlak et al., 2013).

Vitamin B_{12} is a cofactor for enzyme methionine synthase that play a role in the two reactions of converting homocysteine to methionine and converting 5-methyltetrahydrofolate 5-MTHF to tetrahydrofolate THF . When vitamin B_{12} is decrease , it leads to accumulation of 5-methyltetrahydrofolate and failure of formation of tetrahydrofolate, which leads to a defect in folate metabolism (folate concentrations decrease) because the folatepolyglutamate synthase enzyme uses only tetrahydrofolate (Sawant, 2015).

Table (3-3) Vitamin B_{12} levels of control and patients groups

Groups	No.	B_{12} (pg/ml) Mean ±SD
RPL non pregnant	45	260.49±46.66 b
RPL pregnant	45	149.01±30.91 c
Control	45	340.05±59.52 a
LSD		16.51

-Legend as table (3-2)

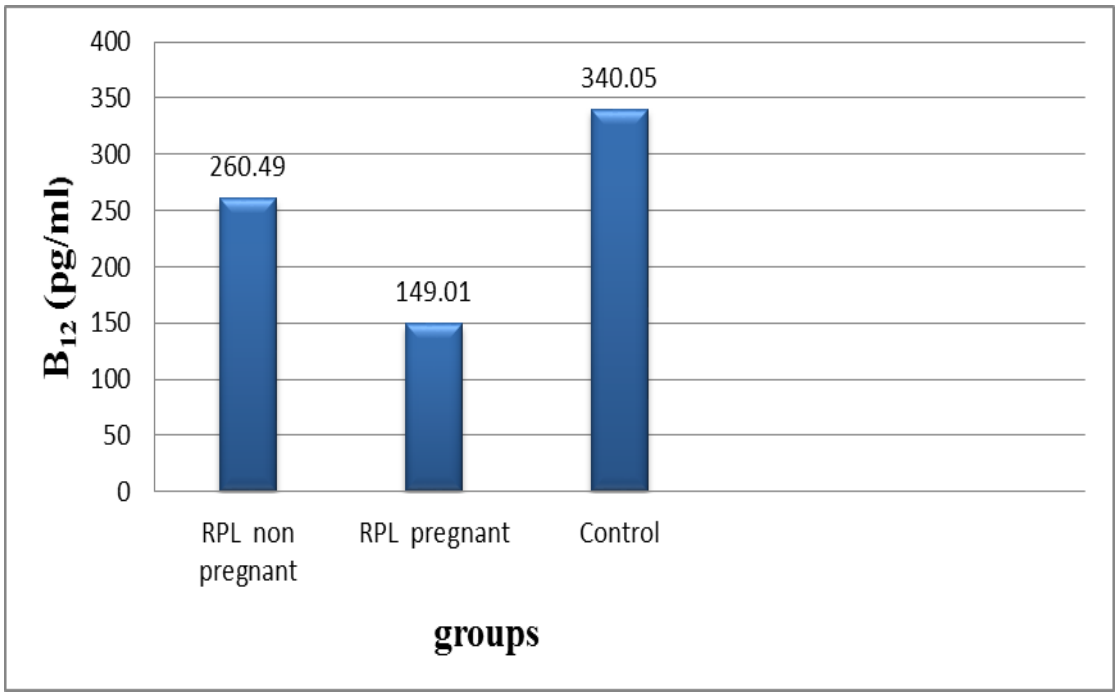

Figure (3-2): Serum Vitamin B_{12} levels of control and patient groups

Table **(3-4)** and Figures **(3-2 ,3-3)** shows the negative correlation between homocystiene and Vitamin B_{12} in RPL non-pregnant with correlation coefficient (r= -0.18 , P.value=0.244) and RPL pregnant with correlation coefficient (r= -0.08 , P.value= 0.619).and this result indicates the possibility of using vitamin supplements to prevent further miscarriage in women with high homocysteine levels, and this result is consistent with (Puri et al., 2013) .

Table (3-4) Correlation coefficient of Homocysteine and Vitamin B_{12}

Hcy with B12	r	p. value	Results
RPL non pregnant	- 0.18	0.244	negative correlation
RPL pregnant	- 0.08	0.619	negative correlation

r: Pearson correlation coefficient

P. value : Probability

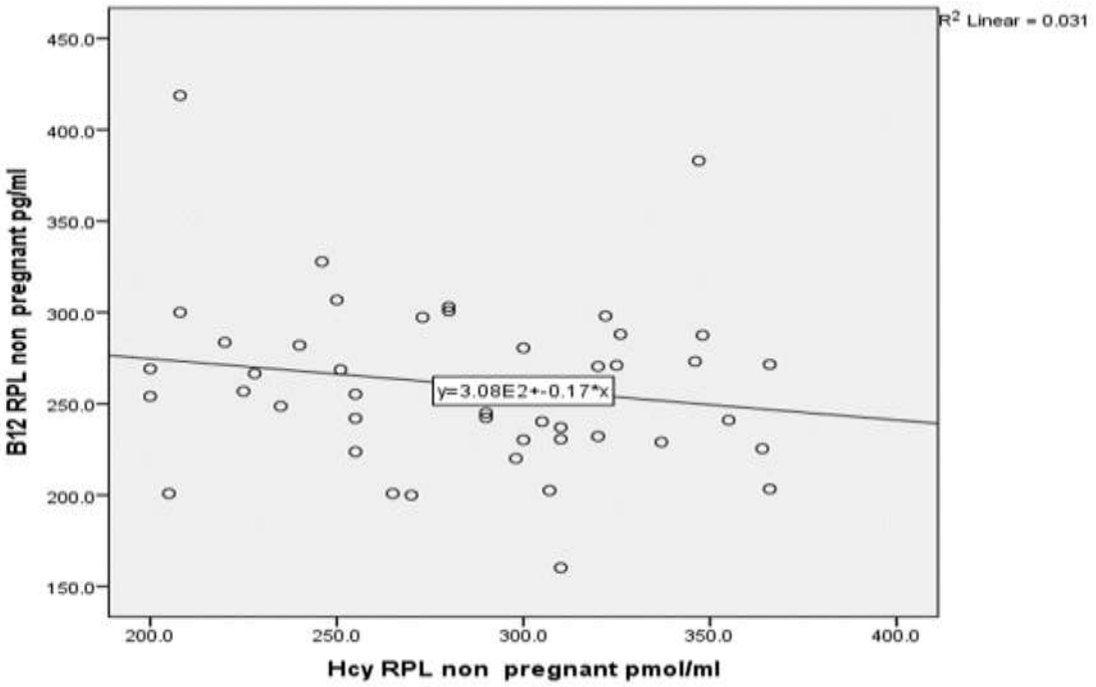

Figure (3-3): Correlation between serum Hcy and Vitamin B_{12} in RPL non pregnant group

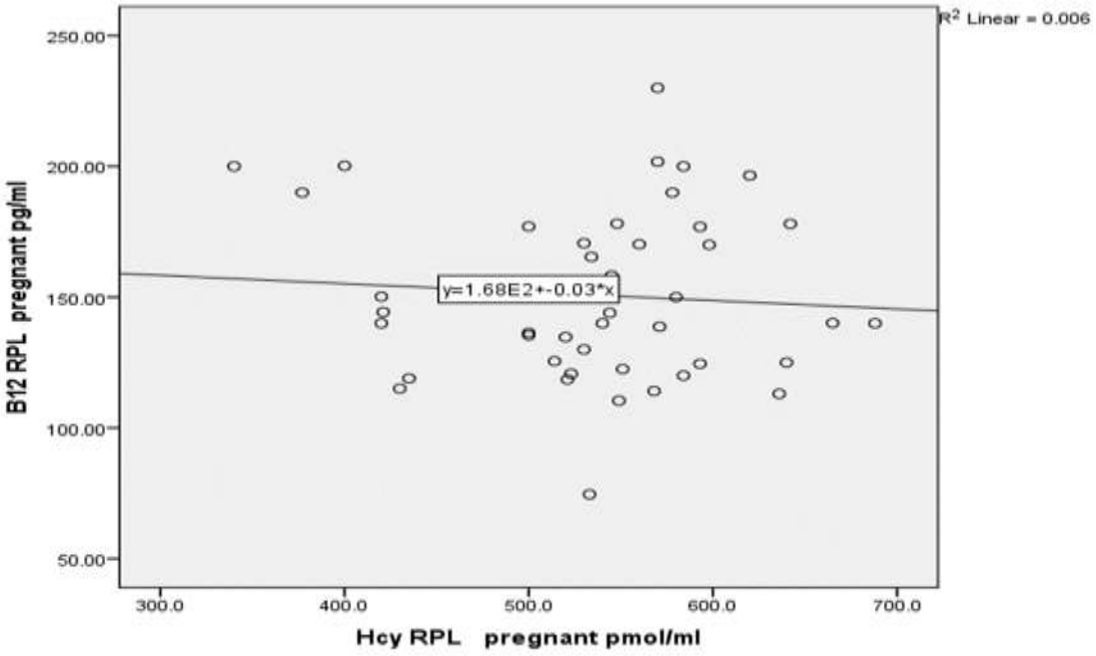

Figure (3-4): Correlation between serum Hcy and Vitamin B_{12} in RPL pregnant group

3.2.3 Serum Folic acid Concentration

Table **(3-5)** and Figure **(3-5)** show significant decrease in the concentration of serum FA in RPL pregnant group in comparison with RPL non pregnant and control groups ($p<0.05$). Also it was found a significant decrease in the concentration of serum FA in RPL non pregnant group in comparison with control group ($p<0.05$). and this result is consistent with a previous study, where reduced folate concentrations were found in RPL group compared than control group ($p <0.05$) (D'Uva et al., 2007).

Folate deficiency occurs in pregnant women due to the increased need of the body and not eating a diet rich in folic acid (Khan & Jialal, 2018) or because of a defect in methyltransferase homocysteine or a deficiency of vitamin B_{12} into a so-called (methyl trap) where it turns into methyltetrahydrofolate, which is an unusable storage form (Hoffbrand & Weir, 2001).

Observational studies have suggested that reduced circulating folate in pregnancy is associated with increasing risk for preterm birth, placental abruption, intrauterine growth restriction (IUGR) and pre-eclampsia (Czeizel et al., 2010). Folic acid (FA) is important for Deoxyribonucleic acid (DNA), Ribonucleic acid (RNA) and protein synthesis (Gluckman et al., 2015) also, it is substantial in energy production and normal cell division. Therefore, it plays an essential role in ensuring the quality of oocyte, its maturation, embryo implantation and subsequent normal pregnancy (Ebisch et al., 2007). Reduced folate intake is associated with decreased cell division, disruption of methylation reactions, increased inflammatory cytokine production, increased levels of oxidative stress, and programmed cell death, all of which could subsequently affect the

developing fetus (Forges et al., 2007). Low folate levels also increase neural tube defects (NTD) , and fetuses with NTD are more likely to miscarry (Kraljić, 2018)

Natural folic acid contains less Folic acid that is absorbed and available for metabolic reactions and storage. many luminal factor hinder the absorption of natural folic acid, so the higher rates of absorption of synthetic folic acid (McNulty & Pentieva, 2004). It has been observed that the degradation and leaching of folic acid occurs in cooking, which leads to the absorption of only 50% of the cooked folate. or, folic acid supplementation may not lead to active metabolic transformations and may lead to inactive forms of folic acid, which is called the folate trap. This is more evident when there is deficiency in Vitamin B_{12} (Mahajan et al., 2007).

Table (3-5) Folic acid levels of control and patients groups

Groups	No.	FA (ng/ml) Mean ±SD
RPL non pregnant	45	11.23±1.99 b
RPL pregnant	45	10.56±1.83 c
Control	45	12.16±1.91 a
LSD		0.65

-Legend as table (3-2)

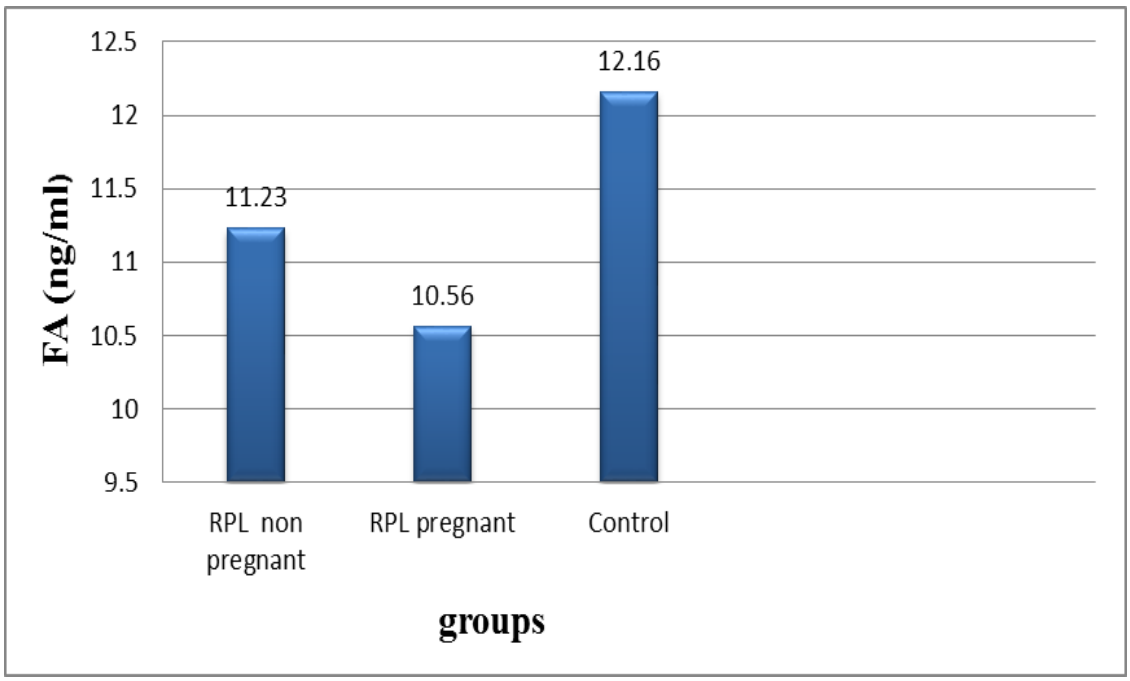

Figure (3-5): Serum Folic acid levels of control and patient groups

Table **(3-6)** and figures **(3-6 , 3-7)** shows the negative correlation between homocystiene and folic acid in RPL non-pregnant with correlation coefficient (r= - 0.05 , P.value=0.746) and RPL pregnant with correlation coefficient (r= - 0.10 , P.value= 0.535).

Table (3-6) Correlation coefficient of Homocysteine and Folic acid

Hcy with FA	R	p. value	Results
RPL non pregnant	- 0.05	0.746	negative correlation
RPL pregnant	- 0.10	0.535	negative correlation

-Legend as table (3-4)

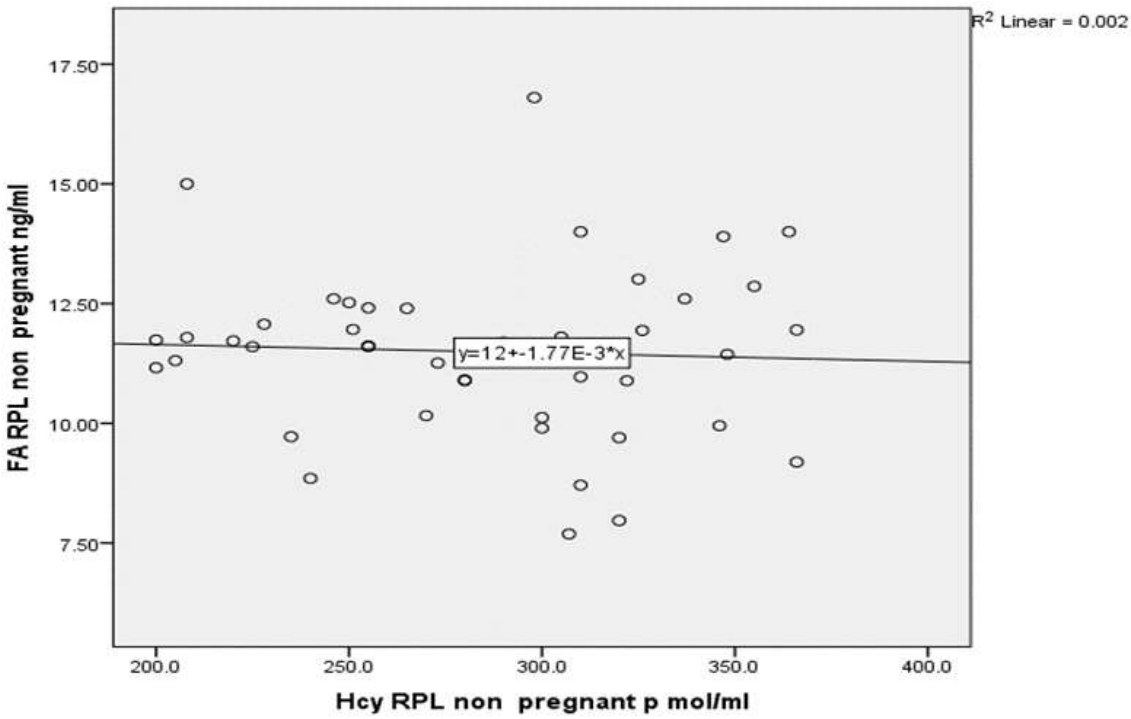

Figure (3-6): Correlation between serum Hcy and Folic acid in RPL non pregnant group

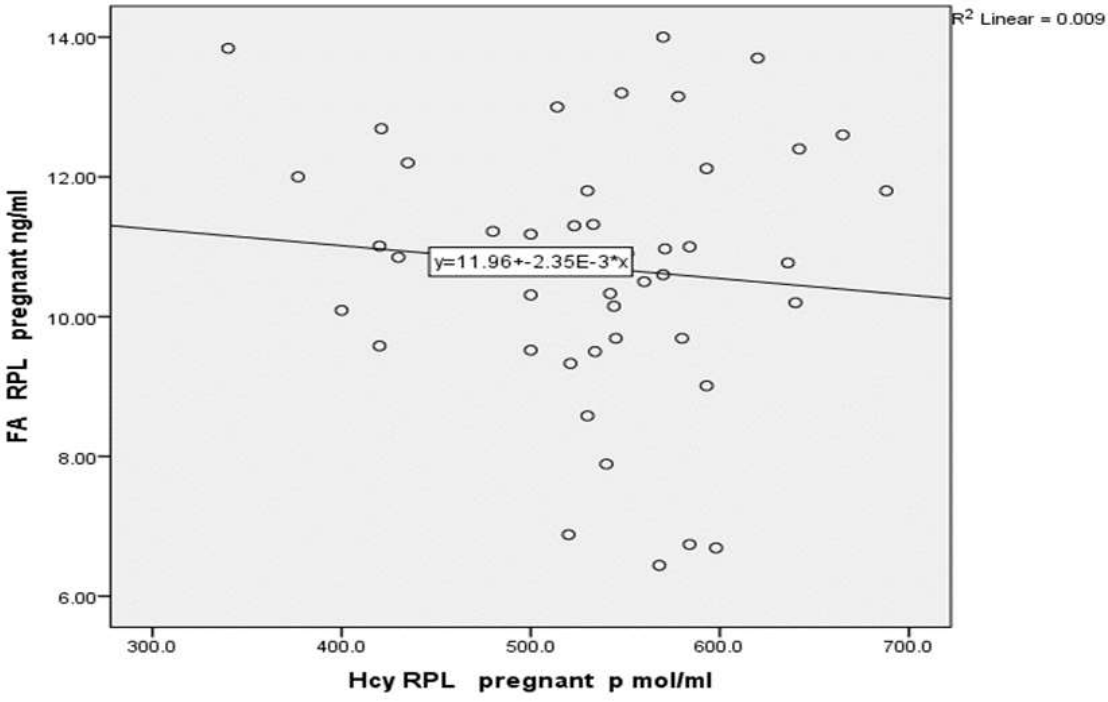

Figure (3-7): Correlation between serum Hcy and Folic acid in RPL pregnant group

3.2.4 **Serum Selenium Concentration**

Table (3-7) and Figure (3-8) show significant decrease in the concentration of serum Se in RPL pregnant groups in comparison with RPL non pregnant and control group ($p<0.05$). Also it was found no significant difference in the concentration of serum Se in RPL non pregnant and control groups ($p<0.05$). and this result is consistent with (Abdulah et al., 2013), The serum selenium concentrations in RPL pregnant group were significantly decrease compared than control group. and also, this study agree with (Al-Kunani et al., 2001). There was no difference in selenium concentrations between RPL non-pregnant group compared to control group.

The results of our study showed that women in the RPL pregnant group had lower concentrations of selenium because Selenium requirements increase during pregnancy as a result of transport to the growing fetus (Kumru et al., 2003) resulting in decreased maternal blood and tissue concentrations of selenium (Kupka et al., 2004) Decreased selenium in women with miscarriage may lead to an increase in free radicals and oxidative stress, as oxidative stress plays a role in early miscarriage (Duhig et al., 2016). In previous studies, there was an increase in signs of oxidative stress in placental tissues in women experiencing pregnancy loss (Burton & Jauniaux, 2004). Increased oxidative stress changes the blood vessels in the placenta, leading to early miscarriage(Sultana et al., 2017), or oxidative stress causes impair placental development at the beginning of pregnancy and thus affects the outcome of pregnancy, including miscarriage or preeclampsia (Burton et al., 2003). There mechanisms have been proposed the association between miscarriage and selenium :First, reduced antioxidant protection

leading to damage of biological membranes and DNA (Mistry et al., 2012). Second, Decreased activity of the seleno-enzymes (enzymes containing selenium), which reduce pro-inflammatory genes associated with negative pregnancy outcomes (Sultana et al., 2017) .

Studies indicate that a diet rich in selenium may reduce the risk of pregnancy loss (Derbyshire et al., 2009).

Table (3-7) Selenium levels of control and patients groups

Groups	No.	Se (mg/l) Mean ±SD
RPL non pregnant	45	6.23±0.98 a
RPL pregnant	45	5.12±1.21 b
Control	45	5.86±1.09 a
LSD		0.38

-Legend as table (3-2)

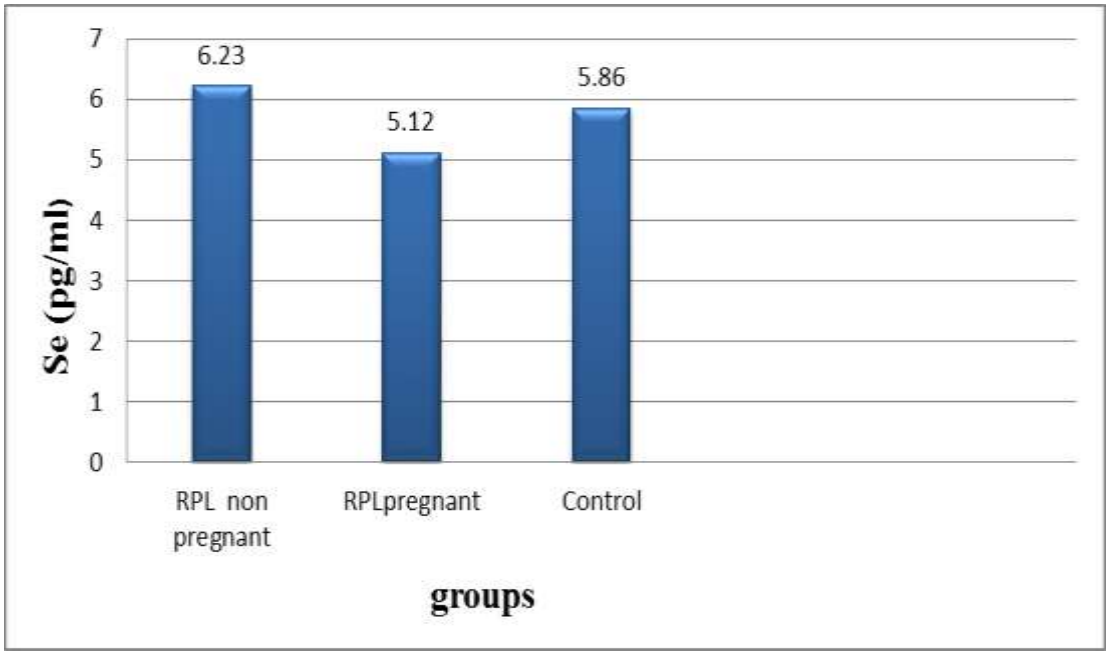

Figure (3-8): Serum Selenium levels of control and patient groups

Table **(3-8)** and Figures **(3-8 , 3-9)** shows the negative correlation between homocystiene and Selenium in RPL non-pregnant with correlation coefficient (r = -0.03 ,P.value= 0.866) and RPL pregnant with correlation coefficient (r=-0.09 , P.value= 0.558).

Table (3-8) Correlation coefficient of Homocysteine and Se

Hcy with Se	R	p. value	Results
RPL non pregnant	- 0.03	0.866	negative correlation
RPL pregnant	- 0.09	0.558	negative correlation

-Legend as table (3-4)

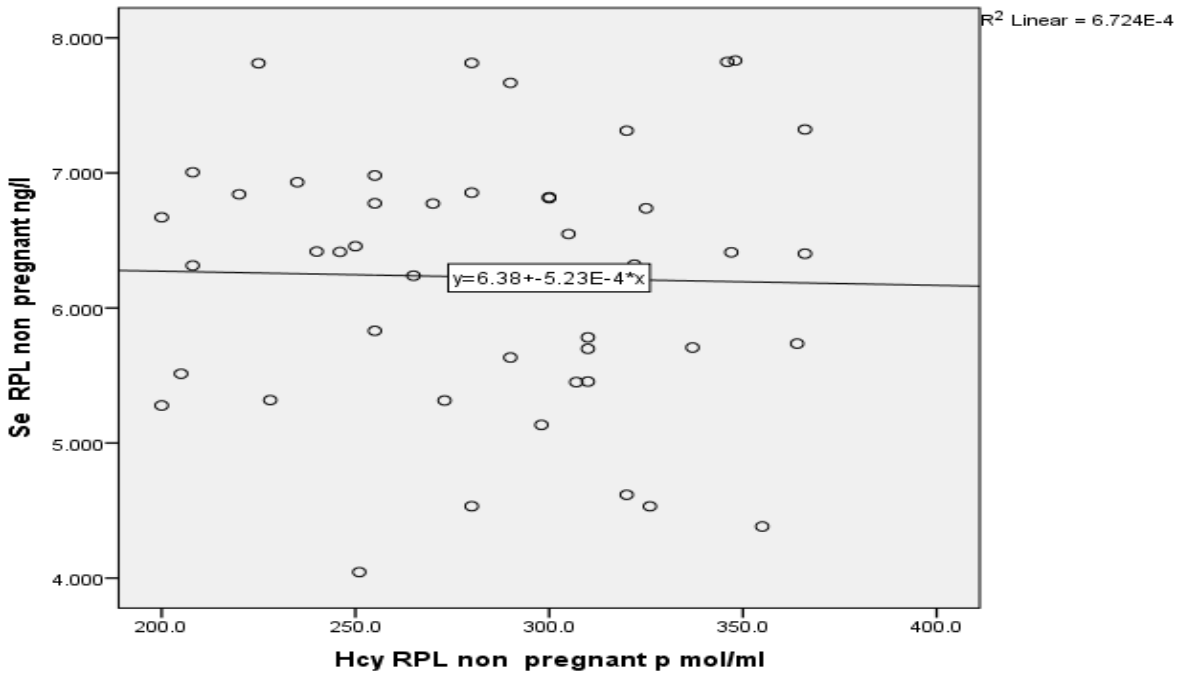

Figure (3-9): Correlation between serum Hcy and Se in RPL non pregnant group

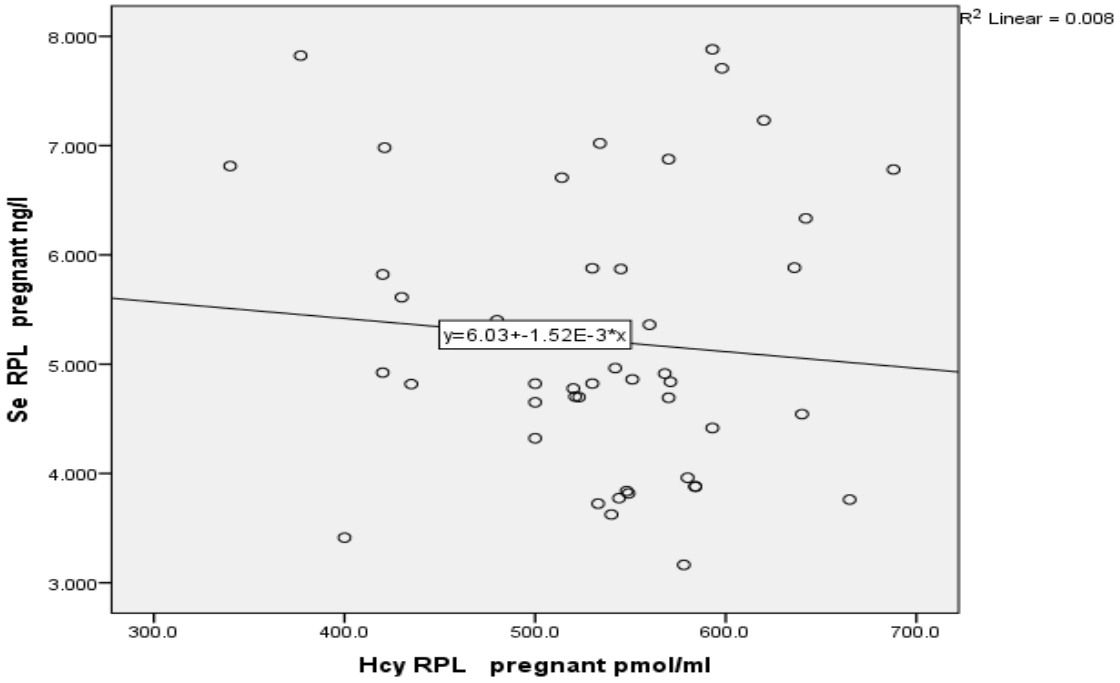

Figure (3-10): Correlation between serum Hcy and Se in RPL pregnant group

Conclusions

&

Recommendation

Conclusions

From the data of this study, the following points were concluded:

1- Homocysteine increased in women who have miscarriages (pregnant and non-pregnant). Therefore, it is considered a significant factor in recurrent miscarriage .

2- Low vitamin B_{12} and folic acid contributed to an increase in homocysteine.

3- Selenium decreased in women who have recurrent miscarriages pregnant , while we did not find a significant decrease in non-pregnant amiscarriages women compared to the control group.

4- Negative relationship between homocysteine and B vitamins (B_{12} and folic acid) were found

Recommendations

The Following points are suggested as Recommendations:

1- The homocysteine, vitamin B_{12}, and folic acid test are recommended for women who have had an idiopathic recurrent miscarriage before pregnancy, These steps might be beneficial to improve pregnancy outcome

2- Using of B vitamin (B_{12} and folic acid B_9) supplementation and a diet rich in vitamins was recommended at least two to three months before pregnancy

3- Antioxidant enzymes are essential to prevent miscarriage because they reduce free radical concentrations and oxidative stress (Antioxidants found in plant-based foods, carrots, spinach, green leafy vegetables and oranges), Selenium are found in whole grains, eggs and legumes

4- A clinical study of other genetics factors that affect homocysteine, such as mutationin the gene of methylene tetrahydrofolate reductase MTHFR enzyme.

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الخلاصة

الإجهاض المتكرر: يعرف الباحثون الإجهاض المتكرر على أنه خسارتين متتاليتين أو أكثر خلال الثلث الأول او الثاني من الحمل ، والتي تؤثر على 5٪ من الأزواج ، ومعدل إنتشاره أقل من الإجهاض المتقطع. هنالك أسباب متعددة للإجهاض المتكرر تتضمن تشوهات الكروموسومات ، مسببات الغدد الصماء ، تشوهات النمط النووي ، وغيرها. بينما 50٪ من الحالات لا تزال غير معروفة. لذلك تم تصميم هذه الدراسة للتحقيق في الأسباب المحتملة للإجهاض المتكرر الغير مبرر. صممت هذه الدراسة لتحديد مستويات الهوموسستايين ، فيتامين B_{12} ، حامض الفوليك والسيلينيوم في النساء المصابات بالإجهاض المتكرر والنساء الاصحاء.

أجريت هذه الدراسة في مستشفى بنت الهدى للولادة والاطفال في ذي قار. شملت الدراسة 135 امرأة تتراوح اعمارهن بين (18-40) عام ، 90 مريضة تعاني من الاجهاض المتكرر و45 نساء أصحاء. تم تقسيم المرضى الى مجموعتين 45 امرأة حامل و45 امرأة غير حامل وتم مقارنتهن مع 45 امرأة حامل (مجموعة الاصحاء) لم يعانين من اجهاض سابق ولديهن ولادة سابقة واحدة على الاقل وتم استبعاد حالات الاجهاض معروفة السبب من دراستنا.

أظهرت النتائج وجود زيادة معنوية في مصل الهوموسستايين في النساء اللاتي يعانين من فقدان الحمل المتكرر في المجوعتين (الحوامل وغير الحوامل) مقارنة بمجموعة الاصحاء ($P<0.05$) وانخفاض معنوي في مستوى فيتامين B_{12} وحامض الفوليك في مصل الدم في النساء التي تعاني من الاجهاض المتكرر في المجموعتين (الحوامل وغير الحوامل) مقارنة بمجموعة التحكم. كما أظهرت النتائج انخفاضاً معنوياً في تركيز مصل السيلينيوم في مجموعة المجهضات الحوامل مقارنة بمجموعة الاصحاء ($P<0.05$) كما لم يتم العثور على اختلاف معنوي في تركيز مصل السيلينيوم في مجموعة المجهضات غير الحوامل بالمقارنة مع مجموعة التحكم ($P<0.05$).

أظهرت النتائج وجود علاقة سلبية بين الهوموسستايين و (فيتامين B_{12} ، حامض الفوليك والسيلينيوم).

جمهورية العراق
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كلية العلوم

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الرسالة

مقدمة الى مجلس كلية العلوم / جامعة ذي قار

كجزء من متطلبات نيل شهادة الماجستير في الكيمياء الحياتية

من قبل

زينة نعيم مطيلي

بكالوريوس علوم كيمياء (جامعة ذي قار)

2007

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