



**Sudan University of Science and
Technology
College of Graduate Studies**



**Plasma Homocysteine Level and Red Blood Cells Parameters in
Third Trimester of Pregnancy among Sudanese Women in
Khartoum State**

مستوى الهيموسستين في البلازما و مؤشرات كريات الدم الحمراء في الثلث الأخير
من الحمل بين النساء السودانيات في ولاية الخرطوم

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الآية

بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

(لَا يُكَلِّفُ اللَّهُ نَفْسًا إِلَّا وُسْعَهَا لَهَا مَا كَسَبَتْ وَعَلَيْهَا مَا اكْتَسَبَتْ رَبَّنَا لَا تُؤَاخِذْنَا إِنْ نَسِينَا أَوْ أَخْطَأْنَا رَبَّنَا
وَلَا تَحْمِلْ عَلَيْنَا إصْرًا كَمَا حَمَلْتَهُ عَلَى الَّذِينَ مِنْ قَبْلِنَا رَبَّنَا وَلَا تُحَمِّلْنَا مَا لَا طَاقَةَ لَنَا بِهِ وَاعْفُ عَنَّا
وَاعْفِرْ لَنَا وَارْحَمْنَا أَنْتَ مَوْلَانَا فَانصُرْنَا عَلَى الْقَوْمِ الْكَافِرِينَ)

سورة البقرة الآية (286)

DEDICATION

To my beloved beautiful mother

To my father my solid rock

To my sister

To my brothers

To my teachers

To my colleagues

To my best friends

To everyone who supported me

I dedicate this work

Acknowledgment

All and first thanks to the almighty ALLAH.

Then I would like to extend my sincere gratitude to Prof. Babiker Ahmed Mohammed, for his close supervision, valuable advices, guidance and encouragement that helped me during this research.

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My sincere gratitude to my family for their support and encouragement,

A special thanks to my friends for their support.

Abstract

The case control study was conducted in Omdurman Maternity Hospital during the period from September to November 2018, to determine non fasting plasma homocysteine and red blood cells parameters in 35 pregnant Sudanese women (from age 18-43 years) attending Omdurman Maternity Hospital at their third trimester consistent with use of the folate supplement as a case group and 35 non pregnant healthy Sudanese women at matched age as a control group.

2.5ml of venous blood was collected in EDTA anticoagulant container. Automated hematological analyzer (Sysmex-xp300) was used to obtain red blood cells parameters (HB, RBCs, PCV, MCV, MCH, MCHC). An automated chemistry analyzer (DIRUI CS-T240) used to determine the plasma homocysteine level. results were analyzed using statistical package for social science (SPSS version19) computer program. Independent T test was used for data analysis and person's correlation test was used for correlation.

Results showed that there was significant decrease in mean of HCY($\mu\text{mol/L}$), RBCs($\text{c}/\mu\text{L}$), PCV(%) , MCV(ft) in case group when compared with control (3.70 ± 1.03 versus 11.03 ± 1.19 , P -value 0.000) ,(4.31 ± 0.39 versus 4.92 ± 0.72 , P -value 0.000), (33.88 ± 2.82 versus 41.11 ± 6.19 , P -value 0.000), (76.93 ± 9.94 versus 85.87 ± 6.26 , P -value 0.000) respectively , there was significant increase in mean of MCH(pg) and MCHC(%) in case group when compared with control group (27.03 ± 2.92 versus 25.49 ± 2.24 , p -value 0.015) ,(34.54 ± 1.59 versus 29.59 ± 0.83 , P -value 0.000).

While there was insignificant difference in mean concentration of Hb(g/dl) in case and control group (11.91 ± 1.4 versus 12.30 ± 1.75 , P -value 0.306).

Results also showed there was no correlation between values of homocysteine and the Red blood cells parameters: Hb, RBC, PCV, MCV, MCH, MCHC

(*R*-value-0.013, *P*-value 0.943), (*R*-value 0.188, *P*-value 0.280), (*R*-value -0.059, *P*-value 0.736), (*R*-value -0.035, *P*-value 0.840) (*R*-value 0.072, *P*-value 0.680), (*R*-value 0.084, *P*-value 0.631) respectively.

There was no correlation between plasma homocysteine and age of pregnant women at their third trimester (*R*-value=0.040, *P*-value= 0.820).

And there was no correlation between plasma homocysteine and number of pregnancies (*R*=0.090, *P*-value =0.605).

This study concluded that plasma homocysteine is significantly decreased in pregnant women at their third trimester, and that there is no relationship between plasma homocysteine and red blood cell parameters, age or number of pregnancies.

المستخلص

أجريت هذه الدراسة علي مجموعة الحالات والضوابط في مستشفى أم درمان للولادة خلال الفترة من سبتمبر إلى نوفمبر 2018 ، لتحديد مستوى الهيموسستين في البلازما ومؤشرات خلايا الدم الحمراء في 35 امرأة حامل سودانية (من سن 18-43 سنوات) حضور في مستشفى أم درمان التعليمي في الفصل الأخير من الحمل بما يتوافق مع استخدام مكملات الفولات كمجموعة حالة و 35 امرأة صحيه غير حامل سودانية في سن متطابقة كمجموعة ضبط. تم جمع 2.5 مل من الدم الوريدي في حاوية مضادة للتجلط تحتوي علي ماده(EDTA) ، تم استخدام جهاز محلل الدم الآلي (Sysmex-xp300)للحصول على مؤشرات خلايا الدم الحمراء وتم استخدام جهاز محلل الكيمياء الآلي(DIRUI CS-T240) لتحديد مستوى الهيموسستين في البلازما. تم تحليل النتائج باستخدام برنامج الحزم الإحصائية للعلوم الاجتماعية، أظهرت نتائج الدراسة انخفاض معنوي في الهيموسستين ، عدد خلايا الدم الحمراء ، حجم الخلايا المكده و متوسط حجم الخلايا ، في مجموعة الحالة عند مقارنتها بمجموعه الضبط (1.03 ± 3.70 مقابل 11.03 ± 1.19 ، قيمة الاحتمال 0.000)، (0.39 ± 4.31 مقابل 0.72 ± 4.92 ، قيمة الاحتمال 0.000) ، (2.82 ± 33.88 مقابل 6.19 ± 41.11 ، قيمة الاحتمال 0.000) ، (9.94 ± 76.93 مقابل 85.87 ± 6.26 ، قيمة الاحتمال 0.000) على التوالي.

في حين كانت هناك زيادة معنوية في متوسط الهيموجلوبين في الخلايا ومتوسط تركيز الهيموجلوبين في الخلايا في مجموعة الحالة عند مقارنتها بمجموعة الضبط (2.92 ± 27.03 مقابل 2.24 ± 25.49 ، قيمة الاحتمال 0.015) ، (1.59 ± 34.54 مقابل 0.83 ± 29.59 ، قيمة الاحتمال 0.000).

كما اظهرت النتائج وجود فروق ضئيلة في متوسط تركيز الهيموجلوبين في مجموعة الحالات ومجموعة الضبط (1.4 ± 11.91 مقابل 1.75 ± 12.30 ، قيمة الاحتمال 0.306).

أظهرت النتائج عدم وجود علاقة بين قيم الهيموسستين و مؤشرات خلايا الدم الحمراء ، الهيموجلوبين ، عدد خلايا الدم الحمراء ، حجم الخلايا المكده، متوسط حجم الخلايا، متوسط الهيموجلوبين في الخلايا ، ومتوسط تركيز الهيموجلوبين في الخلايا (قيمة الارتباط -0.013 ، قيمة الاحتمال 0.943) ، (قيمة الارتباط 0.188 ، قيمة الاحتمال 0.280) ، (قيمة الارتباط -0.059 ، قيمة الاحتمال 0.736) ، (قيمة الارتباط -0.035 ، قيمة الاحتمال 0.840) ، (قيمة الارتباط 0.072 ، قيمة الاحتمال 0.680) ، (قيمة الارتباط 0.084 ، قيمة الاحتمال 0.631) على التوالي.

ولم تكن هناك علاقة بين الهيموسستين في البلازما وعمر الحوامل في الفصل الأخير من الحمل (قيمة الارتباط = 0.040 ، قيمة الاحتمال = 0.820) ولم تكن هناك علاقة بين الهيموسستين وعدد حالات الحمل (قيمة الاحتمال = 0,605 ، قيمة الارتباط = 0,090).

استنتجت هذه الدراسة ان الهيموسستين في البلازما ينخفض بشكل كبير في النساء الحوامل في الفصل الأخير من الحمل، وانه لا توجد علاقة بين الهيموسستين في البلازما ومؤشرات خلايا الدم الحمراء، العمر أو عدد حالات الحمل.

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List of Abbreviations

AdoHCY	S-denosylhomocysteine
AdoMET	S-denosylmethionine
BHMT	Betaine-homocysteine methyltransferase
CB1	Cobalamine
CBC	complete blood count
CBS	Cystathionine β -synthase
CTH	Cystathionine λ -lyase
CYS	Cysteine
DHFR	Dihydrofolate reductase
DVT	Deep vein thrombosis
DNA	Deoxyribonucleic acid
EPO	Erythropoietin
GNMT	glycine N-methyltransferase
Hb	Hemoglobin
HCG	Human chorionic gonadotropin
HCY	Homocysteine
IDA	Iron deficiency anemia
L-DOPA	Levodopa
LMP	Last menstrual cycle
MAT	Methionine adenosyltransferase
MCH	Mean Corpuscular/cell Hemoglobin
MCHC	Mean Corpuscular/cell Hemoglobin Concentration
MCV	Mean cell volume
MET	Methionine
MTHFR	Methyltetrahydrofolate reductase
MTR	Methionine synthase
NTD	Neural tube defect
PCV	Packed cell volume
PE	Pulmonary embolism
RBC	Red blood cells
RM	Remethylation
RNA	Ribonucleic acid

SAHH
THF

S-adenosylhomocysteine hydrolase
Tetrahydrofolate

Chapter one

Introduction

Chapter one

Introduction

1.Introduction:

Homocysteine (Hcy) is a sulfur containing amino acid formed during the metabolism of methionine(Met) to cysteine (Cys), Homocysteine was first isolated from the urinary bladder stone(s) by Vincent du Vigneaud in 1933(Kumar *et al*,2017).

Investigations of the biological and pharmacological regulation of circulating homocysteine have received a considerable interest due to the evidence that associates elevated fasting total plasma homocysteine (tHcy) with increased morbidity and mortality from the earliest stages of life until old age(Makowski, 2011).

Pregnancy, known as gravidity or gestation, is the time during which one or more babies develop inside a woman, it usually lasts around 40 weeks from the Last Menstrual Period (LMP),An embryo is the developing offspring during the first eight weeks following conception, after which, the term fetus is used until birth(Stephanie *et al*,2016).

Anemia, defined as a decreased quantity of circulating red blood cells (RBCs), otherwise known as erythrocytes, is a major source of morbidity and mortality worldwide. Recent epidemiological studies suggest that one third of the world's population is anemic, with considerable resultant morbidity and mortality(Sankaran and weiss,2015).

Anemia in pregnancy is a common problem in most developing countries and a major cause of morbidity and mortality especially in malaria endemic areas. In pregnancy, anemia has a significant impact on the health of the fetus as well as that of the mother. 20% of maternal deaths in Africa have been attributed to

anaemia (Idowu *et al*, 2005).

1.2 Rationale:

Pregnancy is associated with several hematological changes, including decrease in plasma homocysteine level (homocysteine is an amino acid that is involved in several key metabolic processes including methylation and sulphuration pathways). Throughout life, mean tHcy increases by 3-5 $\mu\text{mol/L}$, at the age 40-42 there is a difference of about 2 $\mu\text{mol/L}$ between men and women with mean values of about 11 and 9 $\mu\text{mol/L}$, respectively.

The mechanisms responsible for this fall include the normal increase in the glomerular filtration rate that accompanies pregnancy, the increase in plasma volume and associated haemodilution, and a postulated increased uptake of homocysteine by the fetus.

disturbance of maternal or fetal homocysteine has been associated with various condition (pre-eclampsia, abruption and recurrent pregnancy loss).

It is also associated with anemia, Severe anemia is associated with poor outcome (incidence of pre-term labour, pre-eclampsia).

This study was conducted to estimate plasma homocysteine, hemoglobin and red blood cells parameters in third trimester of normal pregnancy.

To our knowledge there is no previous study that was conducted in Sudan on Sudanese pregnant women at their third trimester similar to this study.

1.3 objectives

1.3.1 general objectives:

To determine the changes in non-fasting total plasma homocysteine concentration that occur during normal pregnancy in Sudanese pregnant women.

1.3.2 specific objectives:

1- To estimate the total plasma homocysteine level and RBCs parameters (RBCs count, HB concentration, PCV and RBCs indices), in pregnant Sudanese women at their third trimester.

2-To compare means between plasma homocysteine level and RBCs parameters in case and control group.

3- To detect the relationship between the plasma homocysteine level and RBCs parameters (RBCs count, HB concentration, PCV, MCV, MCH, MCHC), age and number of pregnancies.

Chapter two

Literature Review

Chapter two

Literature Review

2.1 Homocysteine

Homocysteine is a sulphur-containing amino acid that is closely related to methionine and cysteine. There are no specific base-triplets for this amino acid and homocysteine is therefore not present in naturally occurring proteins. All homocysteine found in organisms is formed during the metabolism of the essential amino acid methionine, in the methylation cycle(Gouaille, 2002).

Hcy plays a pivotal role in folate metabolism and in the catabolism of choline(fowler, 2005).

The plasma levels of tHcy are influenced by age, gender, menopausal status and other physiological determinants. Renal excretion does not seem to be an important route of elimination, Only about 1% of the Hcy filtered by the glomeruli is normally found in the urine. The rest is reabsorbed and metabolized. Plasma tHcy increases throughout life in both sexes. Before puberty, children of both sexes have low and similar levels, or mean values of about 6 $\mu\text{mol/L}$. During puberty, levels markedly increase, more in boys than in girls(Gouaille, 2002).

2.1.1 Metabolism

Homocysteine is an amino acid not used in protein synthesis. Its role is to serve as an intermediate in methionine metabolism. Homocysteine itself is located at a branch-point of metabolic pathways: either it is irreversibly degraded via the transsulphuration pathway to cysteine or it is remethylated back to methionine(Blom and Smulders, 2011).

2.1.1.1 Transmethylation

Methionine adenosyltransferase (MAT) catalyzes the biosynthesis of S-adenosylmethionine (AdoMet) from methionine and ATP. MAT is encoded by two genes that display a tissue-specific expression pattern. *MAT1A* encodes MAT I/III and is only expressed in adult liver, whereas *MAT2A*-expressing MAT II is present in almost all tissues. AdoMet donates a methyl group to, for example, DNA, RNA, proteins and neurotransmitters. Over 100 different methyltransferases may exist. Each of these reactions produces S-adenosylhomocysteine (AdoHcy) which is a potent inhibitor of most methyltransferases. AdoHcy hydrolase (SAHH) hydrolyzes AdoHcy to adenosine and homocysteine. Because the equilibrium of SAHH favors AdoHcy formation, both homocysteine and adenosine need to be metabolized or transported out of the cell to prevent AdoHcy accumulation ((Blom and Smulders, 2011)).

2.1.1.2 Remethylation

Homocysteine remethylation to methionine is catalyzed by the methionine synthase (MTR) enzyme and links the folate cycle with homocysteine metabolism. MTR requires cobalamin (Cbl) as a cofactor, and the resulting complex, Cbl(I)MTR, binds the methyl group of 5-methylTHF to form methylcbl(III)MTR. Upon transfer of the methyl group to homocysteine, Cbl(I)MTR is reformed, which can accept another methyl group from 5-methyltetrahydrofolate (5-methylTHF). Cob(I)alamin can also be oxidized to cob(II)alamin, which results in an inactive Cbl(II)MTR complex. Methionine synthase reductase (MTRR) reactivates the Cbl(II)MTR complex by reductive methylation, using AdoMet as a methyl donor. Whereas the MTR enzyme is

ubiquitously expressed, another homocysteine remethylation system, the betaine-homocysteine methyltransferase (BHMT), is mainly expressed in the liver and kidneys (Blom and Smulders, 2011).

2.1.1.3 Transsulphuration

The homocysteine molecule is retained during remethylation and transmethylation reactions, but in the transsulphuration pathway homocysteine is irreversibly degraded to cysteine. Transsulphuration is facilitated by the action of two vitamin B6-dependent enzymes: cystathionine β -synthase (CBS) and cystathionine γ -lyase (CTH). CBS catalyzes the condensation of homocysteine and serine to cystathionine and CTH subsequently catalyzes the hydrolysis of cystathionine to cysteine and α -ketobutyrate. Human CBS is expressed in liver, kidneys, muscle, brain and ovary and also during early embryogenesis in the neural and cardiac systems. Apart from its role in protein synthesis, cysteine is a precursor of glutathione, a strong antioxidant and an essential compound in detoxification of many xenobiotics(Blom and Smulders, 2011).

2.1.1.4 Regulation

In most tissues, homocysteine is either remethylated via methionine synthase or exported out of the cell. The liver is the main organ of degradation of excess methionine and in maintaining homocysteine at adequate levels via a unique set of enzymes, including MAT I/III, CBS, CTH, BHMT, GNMT (glycine N-methyltransferase).This produces that in the liver high methionine results in increased AdoMet. One main regulatory mechanism is that high levels of AdoMet inhibit MTHFR and activate CBS activity, respectively. Methionine

excess thus results via the higher levels of AdoMet in homocysteine degradation via the transsulphuration pathway. Conversely, if methionine levels are low, for example during fasting, the low AdoMet levels will not activate CBS nor inhibit MTHFR, resulting in conservation of homocysteine via remethylation back to methionine(Blom and smulders, 2011).

2.1.1.4.1 Role of S-adenosylmethionine in the control of homocysteine metabolism

S-Adenosylmethionine is an allosteric inhibitor of methylenetetrahydrofolate reductase, an *in vitro* inhibitor of betaine-homocysteine methyltransferase and an activator of cystathionine β -synthase. The ability of S-adenosylmethionine to act as an enzymatic effector of homocysteine metabolism provides a means by which remethylation and transsulphuration pathways can be coordinated. When cellular S-adenosylmethionine concentration is low, the synthesis of 5-methyltetrahydrofolate will proceed uninhibited whereas cystathionine synthesis will be suppressed, resulting in the conservation of homocysteine for methionine synthesis. Conversely, when S-adenosylmethionine concentration is high, homocysteine is diverted through the transsulphuration pathway because of inhibition of 5-methyltetrahydrofolate synthesis and stimulation of cystathionine synthesis. Thus, although the primary effect of this coordinated control is the regulation of cellular S-adenosylmethionine concentrations, it also contributes to the maintenance of a homocysteine concentration compatible with the need for *de novo* methyl groups(Medina *et al*, 2001).

2.1.2 Inborn errors of homocysteine metabolism

Severe hyperhomocysteinemia (total homocysteine >50 μM) or homocystinuria is caused by defects in remethylation or transsulphuration. Disturbed remethylation can be caused by MTHFR as well as MTR deficiency due to mutations in their genes. MTR can also be dysfunctional due to defects in cobalamin metabolism. In MTR deficiency or dysfunction, 5-methylTHF cannot cycle through MTR resulting in 5-methylTHF accumulation at the expense of the other folates, hampering the synthesis of purines and thymidine. In particular, rapidly dividing cells, such as bone marrow, will be affected and result in megaloblastic anemia and pancytopenia, which are also observed in folate deficiency. MTHFR deficiency does not limit the availability of folates for purines and thymidine syntheses and therefore shows no abnormalities in blood cells. Remethylation defects result in elevated homocysteine and decreased methionine. In the transsulfuration pathway, CBS deficiency also results in accumulation of homocysteine, but in contrast to remethylation defects, methionine is increased. one typical characteristic of severe hyperhomocysteinemia is the wide array of different neurological presentations, ranging from schizophrenia and depression to severe mental retardation. Another common finding is arterial and venous occlusive disease in patients with severe hyperhomocysteinemia, irrespective of whether the defect is in remethylation or transsulphuration(Blom and smulders,2011)

2.1.3 Homocysteine and MTHFR

MTHFR is an important enzyme in Hcy metabolism, which catalyzes the conversion of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, the predominant circulating form of folate. The *MTHFR* gene has at least two

functional polymorphisms, 677C>T and 1298A>C. The *MTHFR* 677T allele is associated with reduced enzymatic activity, decreased concentrations of folate in serum, plasma, and red blood cells, and mildly increased plasma tHcy concentrations. The *MTHFR* polymorphism 1298A>C also affects MTHFR activity but is not associated with higher plasma Hcy or lower folate levels . Normal MTHFR activity is crucial to maintain the pool of circulating folate and methionine and to prevent the accumulation of Hcy. Double heterozygosity for *MTHFR* 677C>T and 1298A>C polymorphisms results in lower MTHFR activity as compared to heterozygosity for either *MTHFR* variant separately. Individuals with the 677TT genotype have approximately 30% the MTHFR enzyme activity of those with the 677CC genotype, whereas 677CT heterozygotes have about 65% enzymatic activity(Brustolin *et al*,2010).

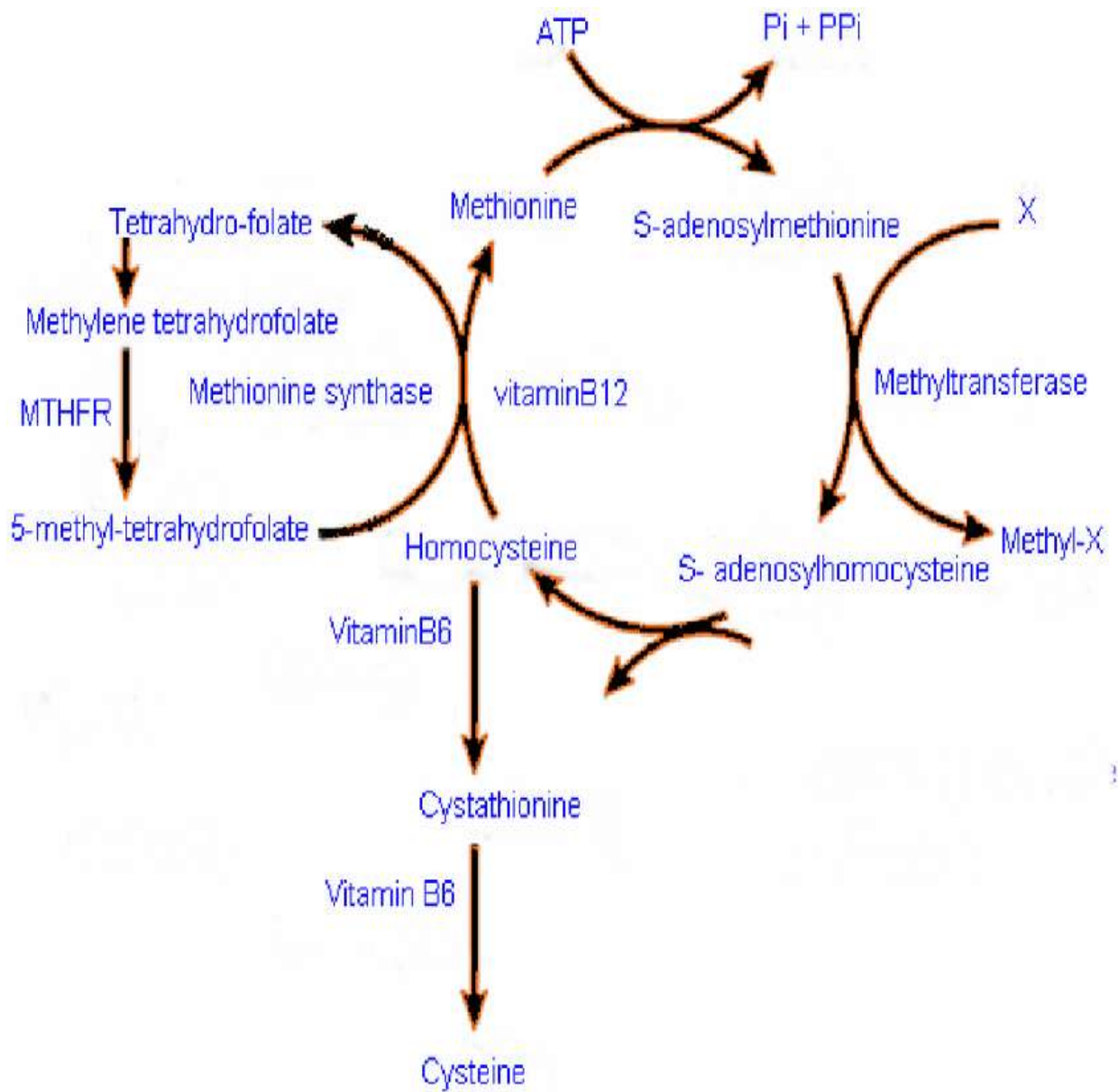


Figure (2-1) diagram demonstrates homocysteine metabolism

2.1.4 Homocysteine in plasma

Human plasma contains both reduced and oxidized species of homocysteine. The thiol group of homocysteine allows it to form a disulfur bond with other homocysteine molecules (leading to formation of homocystine), with free cysteine, or with thiol groups of plasma proteins, such as albumin. It is remarkable that the oxidized forms are overwhelmingly in the majority (up to 99%) and reduced homocysteine represents no more than 1% of total plasma homocysteine. The sum of all the forms of homocysteine existing in plasma is usually called total homocysteine. Protein-bound homocysteine represents up to 75% of total homocysteine. Both experimental and clinical studies demonstrate the presence in plasma of binding sites for aminothiols, which interact preferentially with homocysteine. A rapid equilibrium exists between free and protein-bound homocysteine fractions *in vivo*. Thus, transiently increased free homocysteine, induced by an increase in homocysteine export or by methionine loading, becomes progressively bound to plasma protein in a redistribution which takes place in less than 24h. It should be kept in mind that most homocysteine in stored plasma is present as a protein-bound fraction (Medina *et al*, 2001)

Factors influencing plasma homocysteine levels include demographic, genetic and physiological factors, as well as acquired determinants, including habits, nutrition, diseases, transplants and medication. Plasma homocysteine levels increase with age and are higher in men than in women. High tHcy levels are associated with impaired renal function, high plasma creatinine, smoking, coffee consumption, alcoholism, and certain drugs, including folate antagonists, nitrous oxide, and L-DOPA (Medina *et al*, 2001)

Normal concentrations of total homocysteine in plasma are in the range of 5–16 $\mu\text{mol}\cdot\text{L}^{-1}$, although perhaps 10 $\mu\text{mol}\cdot\text{L}^{-1}$ should be considered the desirable upper limit, because it is possible to achieve this level by optimal nutrition with respect to folic acid and both vitamins B₆ and B₁₂. Over 16 $\mu\text{mol}\cdot\text{L}^{-1}$, three ranges of hyperhomocysteinemia are defined: moderate (16–30 $\mu\text{mol}\cdot\text{L}^{-1}$), medium (30–100 $\mu\text{mol}\cdot\text{L}^{-1}$) and severe (>100 $\mu\text{mol}\cdot\text{L}^{-1}$)hyperhomocysteinemia, reaching values as high as 500 $\mu\text{mol}\cdot\text{L}^{-1}$ in patients with homocystinuria(Medina *et al*, 2001).

2.1.5 Methionine-loading

Methionine loading test was originally introduced to identify subjects with defects in transsulfuration due to congenital cystathionine beta synthase defects or due to pyridoxine phosphate deficiency. Since then it has been used to identify hyperhomocysteinemia in a number of clinical disorders, and in micronutrient deficiency states. It has been suggested that the concentrations of total homocysteine (tHcy) in the plasma during fasting are very sensitive to the abnormality of the remethylation (Rm) of homocysteine while the post methionine load increase in plasma total homocysteine are not as sensitive a measure of the remethylation.(Bhat,2018).

2.1.6 hyperhomocysteinemia

2.1.6.1 Risks That Have Been Associated With Elevated Homocysteine Levels

Coronary artery disease (atherosclerosis), heart attack, stroke, peripheral arterial disease, venous thrombosis, deep vein thrombosis (DVT), pulmonary embolism (PE), having a child with a neural tube defect (ie, spina bifida),

pregnancy complication (preeclampsia , placental abruption, pregnancy loss), Role of homocysteine has been investigated in many other diseases(Moll and Varga, 2015).

2.1.6.2 Treatment and Management

Folic acid (also referred to as folate), vitamin B6, and vitamin B12 can decrease homocysteine in the blood. A good source for folic acid is fruits and vegetables (especially green leafy vegetables), and fortified breads and cereals, lentils, chickpeas, asparagus, spinach, and most beans, as well. Daily intake of pills containing folic acid, vitamin B6, vitamin B12, or a combination of the 3 can lower homocysteine levels. Although taking a daily supplement of folic acid, vitamin B6, or vitamin B12 can effectively lower blood homocysteine levels, such lowering does not lead to a decreased risk of cardiovascular disease, DVT, or PE. Therefore, at the present time, such supplementation with folic acid, vitamin B6, or vitamin B12 for primary prevention of cardiovascular disease is not recommended. Similarly, treating patients with elevated homocysteine and cardiovascular disease or DVT or PE is also not recommended(Moll and Varga, 2015).

2.1.7 Hypohomocysteinemia

2.1.7.1 Significance of Low Plasma Homocysteine

Homocysteine is not obtained from the diet. Rather, it is synthesized from methionine via a multistep process. The first clue that homocysteine plays an important role in the body is that this synthesis requires energy. Homocysteine is a non-protein α -amino acid homologue of cysteine, differs from cysteine by an additional methylene bridge (-CH₂-). It is biosynthesized from methionine by the removal of a terminal methyl group, Stating the question differently: Is

hypohomocysteinemia associated with metabolic dysfunction or disease? Turns out the answer is yes: Low homocysteine levels do indeed have disease correlations. For example, low homocysteine has been shown to have a strong association with peripheral neuropathy. A surprising 41% of patients with idiopathic peripheral neuropathy have hypohomocysteinemia, homocysteine can be considered a storage molecule for sulfur and a transfer molecule for methyl metabolism. For example, homocysteine is clearly a storage molecule for cysteine, the rate limiting amino acid for glutathione production, Homocysteine enables single carbon units to be shuttled from the reduced folate pool to the principal methyl donor in the cell. Hypohomocysteinemia—defined as less than 6.0 mmol/L, is not common, occurring in only 0.5% to 1.0% of the population. Low homocysteine may also be indicative of excessive conversion to cystathione for use in the transsulfuration pathways for production of glutathione, taurine, and sulfate. Low homocysteine would suggest impaired ability for de novo production of glutathione and thus increased susceptibility to oxidative stress(pizzorno,2014).

2.1.7.2 Clinical associations

Hypohomocysteinemia causes reduced availability of cysteine.

Cysteine restriction causes limitation in production of sulfate, taurine and glutathione. The limited production ability is exacerbated in conditions that cause increased demand for any of the sulfur compounds produced from homocysteine. Alcohol intake greatly increases the production of taurine, and many drugs and xenobiotics increase sulfate requirement for conjugation and elimination. One of the body's main uses of sulfate and taurine is in Phase II liver detoxification. Taurine is involved in the formation of bile acids whereas the sulfation pathway is required for removal of steroid hormones, phenolic

compounds and numerous drugs. Glutathione metabolic activities include Phase II conjugation reactions, prostaglandin synthesis and reduction/oxidation reactions. Any condition that increases oxidative stress tends to increase the demand for hepatic glutathione production. Thus oxidative stress draws homocysteine into glutathione synthesis, potentially causing a drop of plasma homocysteine to levels where total body glutathione status is critical(Lord and Bralley, 2008).

2.1.7.3 Treatment and Management

The most common treatment for low homocysteine is administration of sulfur-containing amino acids such as methionine, N-acetylcysteine and taurine. Preformed glutathione and inorganic sulfate salts (potassium sulfate) may also be employed to support hepatic and renal demands for toxin removal through sulfation and mercaptan formation. Plasma methionine and urinary sulfate, pyroglutamate or alpha-hydroxybutyrate may be performed for confirmation of significant cysteine deficit(Lord and Bralley, 2008).

2.2 Anemia

Anaemia is a global public health problem affecting both developing and developed countries with major consequences for human health as well as social and economic development. It occurs at all stages of the life cycle, but is more prevalent in pregnant women and young children(Benoist *et al*,2008).Anemia is a condition of low hemoglobin (Hb). It can be subdivided several different ways: symptomatic versus non-symptomatic, or more frequently, by laboratory findings such as macrocytic versus microcytic or normochromic versus hypochromic(Turner and Bhimji, 2018).

Normal Hb-specific laboratory cut-offs will differ slightly, but in general, the normal ranges are as follows: 135 to 180 g/L in men, 120 to 150 g/L in women, 110 to 160 g/L in children, varied in pregnancy depending on the trimester, but generally greater than 100 g/L (Turner and Bhimji, 2018).

2.2.1 Red blood cell parameters :

Red blood cell parameters include Red Blood Cell (RBC) count, Hematocrit (Hct), Hemoglobin (Hb or Hgb), Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), and Mean Corpuscular Hemoglobin Concentration (MCHC). RBCs, Hgb and Hct can be measured directly in the lab, the rest are calculated based on previous values.

MCV, MCH and MCHC give evidence to support pathologies in RBCs. The red corpuscular indices give an overview of anemias and polycythemias while in parallel differentiating alcohol use, liver problems, thalassemia, kidney problems and sickle cell disease (Mutua *et al*, 2018).

2.2.1.1 Hemoglobin (Hgb) is an iron-containing protein complex which binds oxygen and carbon dioxide for transport by RBCs. It forms more than 95% of dry weight of RBCs. Hgb has a quaternary structure with four globular proteins (2 α and 2 β globulins) and a heme group (Fe²⁺ which binds oxygen). In pregnancy, the normal Hgb reference range is 11-12 g/dL. Critical values for Hgb include: Hgb < 5 g/dL and Hgb > 20 g/dl which can cause heart failure and hemoconcentration-clotting, respectively. Hgb level begins to decline from the 16th week of gestation as a result of increased plasma volume. Similar trends are seen in RBC count and Hct (Mutua *et al*, 2018).

2.2.1.2 Red blood cells (RBCs) are the most abundant cells in blood, The average number per μ L is 5.2 million (range: 4.4-6.0 million). RBCs are enucleated-biconcave corpuscles filled with hemoglobin, They transport oxygen

from lungs to tissues and CO₂ from tissues to lungs, Erythrocytes have life span of 120 days, after which their amino acids and iron are recycled. Reduced oxygen (hypoxia) triggers the kidney to release erythropoietin (EPO), a hormone responsible for erythropoiesis (Mutua *et al*,2018).

2.2.1.3 Hematocrit (Hct), also known as Packed Cell Volume (PCV), is the percentage of RBCs in (centrifuged) whole blood, Normal range is 37-54%. PCV has a wide coefficient of variation hence should be viewed together with other red cell indices (Mutua *et al*,2018).

2.2.1.4 Mean Cell Volume (MCV) is the average volume of single RBC. It is calculated as Hct/RBC and measured in femtolitres (fL) (Mutua *et al*,2018).

2.2.1.5 Mean Cell Hemoglobin (MCH) is the average mass (amount) of Hgb/RBC (Mutua *et al*,2018).

2.2.1.6 Mean Cell Hemoglobin Concentration (MCHC) is the average concentration (amount) of Hgb in one RBC. It is calculated as Hgb/Hct (Mutua *et al*,2018).

2.2.2 Signs and symptoms

Symptoms of anemia include fatigue, weakness, lightheadedness, headache, pallor/jaundice, tachycardia/palpitations, chest pain, dyspnea, and cold distal extremities, and claudication. These symptoms can be quite limiting and are primary negative consequences of anemia. The cause of the anemia modulates the presence and magnitude of these symptoms. The greater the lethal underlying pathology, the more dramatic the symptomatology(Freeman and morando,2018).

2.2.3 Etiology

Anemia is the result of a wide variety of causes that can be isolated, but more often coexist. Globally, the most significant contributor to the onset of anemia

is iron deficiency so that IDA and anemia are often used synonymously, and the prevalence of anemia has often been used as a proxy for IDA. It is generally assumed that 50% of the cases of anemia are due to iron deficiency, but the proportion may vary among population groups and in different areas according to the local conditions. The main risk factors for IDA include a low intake of iron, poor absorption of iron from diets high in phytate or phenolic compounds, and period of life when iron requirements are especially high (i.e.growth and pregnancy), Among the other causes of anemia, heavy blood loss as a result of menstruation, or parasite infections such as hook-worms, ascaris, and schistosomiasis can lower blood haemoglobin (Hb) concentrations. Acute and chronic infections, including malaria, cancer, tuberculosis, and HIV can also lower blood Hb concentrations. The presence of other micro-nutrient deficiencies, including vitamins A and B12, folate, riboflavin, and copper can increase the risk of anemia. Furthermore, the impact of haemoglobinopathies on anemia prevalence needs to be considered within some populations(Benoist *et al*,2008).

2.2.4 Evaluation

The main investigations required are laboratory blood work-up. This includes a complete blood count (CBC) including differentials to look for microcytic or macrocytic anemia and hypochromic or normochromic anemia, transferrin saturation, and transferrin levels. These will allow clinicians diagnose pancytopenia, microcytic anemia, or macrocytic anemia, look for compensation such as reticulocyte production, and define, at least in part, whether iron deficiency causes it.A peripheral blood smear can also be useful in identifying

other causes of anemia, such as sickle cell disease, Heinz body anemia or other, other hemoglobinopathies(Turner and Bhimji,2018).

Anemia in neonates requires a slightly different evaluation and should include assessment of conjugated bilirubin levels because neonates are at risk of kernicterus and other issues secondary to the breakdown products of heme, and anemia may be an initial finding of hemolysis(Turner and Bhimji, 2018).

2.2.5 Health consequences

Anemia is an indicator of both poor nutrition and poor health. The most dramatic health effects of anemia, i.e., increased risk of maternal and child mortality due to severe anemia, in addition the negative consequences of IDA on cognitive and physical development of children, and on physical performance – particularly work productivity in adults – are of major concern(Benoist *et al*,2008).

2.2.6 Treatment and Management

Management depends on the cause of the anemia. If due to dietary deficiency, oral supplementation is preferred (iron, B12, and folate), although intravenous (IV) iron can also be given if a rapid resolution is needed. If a patient is unstable and severely anemic, consider a blood transfusion as well as supplementation. If due to blood loss, minimize the blood loss; if menorrhagia, give mefenamic acid and tranexamic acid to reduce blood loss. If due to other factors, treat those as appropriate, and consider supplementation with iron, B12, and folate to help repopulate the red blood cells(Turner and Bhimji, 2018).

2.3 Pregnancy

During pregnancy, anatomical and physiological changes occur to meet the increased metabolic needs, to permit appropriate development of fetus and to prepare the body for childbirth. The changes begin to occur early in the first trimester, peaking at the term or labour and revert to pre-pregnancy levels by a few weeks into the postpartum. These changes are well tolerated in healthy females but may aggravate or unmask a pre-existing disease or a pregnancy-related pathophysiology(Bhatia and Chhabra,2018).

During the first 2 weeks after ovulation, development phases include: (1) fertilization, (2) blastocyst formation, and (3) blastocyst implantation. Primitive chorionic villi are formed soon after implantation. With the development of chorionic villi, it is conventional to refer to the products of conception as an embryo(Cunningham *et al*,2009).

2.3.1 Phases of conceptus development

2.3.1.1 The ovum: the products of conception in the first 2 weeks after fertilization.

2.3.1.2 The embryo: from 3 to 5 weeks.

2.3.1.3 The foetus: the developing infant (6-40 wks) (El-mowafi,2002)

2.3.2 Fertilization and Implantation

Ovulation frees the secondary oocyte and adherent cells of the cumulus-oocyte complex from the ovary. Although technically this mass of cells is released into the peritoneal cavity, the oocyte is quickly engulfed by the infundibulum of the

fallopian tube. Further transport through the oviduct is accomplished by directional movement of cilia and tubal peristalsis. Fertilization normally occurs in the oviduct, and it is generally agreed that it must take place within a few hours, and no more than a day after ovulation(Cunningham *et al*,2009).

2.3.3 Cleavage and blastocyst formation

On its way to the uterine cavity, the fertilized ovum (zygote) divides into 2,4,8 then 16 cells (blastomeres). This division (cleavage) starts within 24 hours of fertilization and occurs nearly every 12 hours repeatedly the resultant 16 cells mass is called morula which reaches the uterine cavity after about 4 days from A cavity appears within the morula converting it into a cystic structure called blastocyst. In which the cells become arranged into an inner mass (embryoblast) which will form all the tissues of the embryo, and an outer layer called trophoblast which invade the uterine wall fertilization. The blastocyst remains free in the uterine cavity for 3-4 days, during which it is nourished by the secretion of the endometrium (uterine milk)(Elmowafi,2002).

2.3.4 Embryonic Period

The embryonic period commences at the beginning of the third week after ovulation and fertilization, which coincides in time with the expected day that the next menstruation would have started. The embryonic period lasts 8 weeks and is when organogenesis takes place. The embryonic disc is well defined, and most pregnancy tests that measure human chorionic gonadotropin (hCG) become positive by this time (Cunningham *et al*,2009).

2.3.5 Fetal Period

The end of the embryonic period and the beginning of the fetal period is arbitrarily designated by most embryologists to begin 8 weeks after

fertilization—or 10 weeks after onset of last menses, at this time, the embryofetus is nearly 4 cm long, development during the fetal period consists of growth and maturation of structures that were formed during the embryonic period. Because of the variability in the length of the legs and the difficulty of maintaining them in extension, crown-to-rump measurements, which correspond to the sitting height, are more accurate than those corresponding to the standing height(Cunningham *et al*,2009).

2.3.6 Placental formation

Human placental development is as uniquely intriguing as fetal embryology. During its brief intrauterine passage, the fetus is dependent on the placenta for pulmonary, hepatic, and renal functions. These are accomplished through the unique placental anatomical association with the maternal interface.

The placenta links mother and fetus by indirect interaction with maternal blood that spurts into the intervillous space from uteroplacental vessels. Maternal blood bathes the outer syncytiotrophoblast to allow exchange of gases and nutrients with fetal capillary blood within connective tissue at the villous core. Fetal and maternal blood are not normally mixed in this hemochorial placenta(Cunningham *et al*, 2009).

2.3.7 Sex Determination

The mature ovum carries 22 autosomes and one X chromosome, while the mature sperm carries 22 autosomes and either an X or Y chromosome. If the fertilizing sperm is carrying X chromosome the baby will be a female (46 XX), if it is carrying Y chromosome the baby will be a male (46 XY) (Elmowafi,2002).

2.3.8 Stages of pregnancy:

2.3.8.1 First trimester development of embryo/fetus (0-12 weeks):

In the 12 Gestational Weeks the uterus usually is just palpable above the symphysis pubis, and the fetal crown-rump length is 6 to 7 cm. Centers of ossification have appeared in most of the fetal bones, and the fingers and toes have become differentiated. Skin and nails have developed and scattered rudiments of hair appear. The external genitalia are beginning to show definitive signs of male or female gender. The fetus begins to make spontaneous movements(Cunningham *et al*, 2009).

2.3.8.2 Second trimester development of the fetus (13-28weeks):

In the 16 Gestational weeks the fetal crown-rump length is 12 cm, and the weight is 110 g. Gender can be determined by experienced observers by inspection of the external genitalia by 14 weeks.

The 20 Gestational weeks this is the midpoint of pregnancy as estimated from the beginning of the last menses. The fetus now weighs somewhat more than 300 g, and weight begins to increase in a linear manner.

From this point onward, the fetus moves about every minute and is active 10 to 30 percent of the time.

In the 24 Gestational weeks the fetus now weighs about 630 g. the head is still comparatively large.

The canalicular period of lung development, during which the bronchi and bronchioles enlarge and alveolar ducts develop, is nearly completed. A fetus born at this time will attempt to breathe, but many will die because the terminal sacs, required for gas exchange, have not yet formed(Cunningham *et al*, 2009).

2.3.8.3 Third trimester development of the fetus (29-40 weeks):

In the 28 Gestational weeks the crown-rump length is approximately 25 cm, and the fetus weighs about 1100 g. The thin skin is red and covered with vernix caseosa. The otherwise normal neonate born at this age has a 90-percent chance of survival without physical or neurological impairment.

In the 32 Gestational weeks the fetus has attained a crown-rump length of about 28 cm and a weight of approximately 1800 g. The skin surface is still red and wrinkled.

In the 36 Gestational weeks the average crown-rump length of the fetus is about 32 cm, and the weight is approximately 2500 g. Because of the deposition of subcutaneous fat, the body has become more rotund, and the previous wrinkled appearance of the face has been lost.

In the 40 Gestational weeks this is considered term from the onset of the last menstrual period. The fetus is now fully developed, The average crown-rump length is about 36 cm, and the weight is approximately 3400g (Cunningham *et al*, 2009).

2.3.9 Anemia in pregnancy

During pregnancy, anemia is defined as a haemoglobin concentration (Hb) <110 g/L at sea level, which is two standard deviations below the mean Hb expected. Consequent to the physiological haemodilution which is maximal during 20–24 weeks of gestation, the Hb varies with the period of gestation. The Hb increases with high altitudes and in those who smoke, in those who smoke, the decrease in plasma volume and increase of Hb, both of which adversely affects fetal growth, are adaptations to increased carboxyhaemoglobin which has no oxygen carrying capacity. Quitting smoking can reduce the Hb to

its original levels within five years. Genetic differences may also affect the Hb. A haematocrit of $< 33\%$ could also be considered for the diagnosis of anemia in pregnancy, severe anemia in pregnancy (Hb < 70 g/L) requires urgent medical treatment and Hb < 40 g/L is an emergency carrying a risk of congestive cardiac failure, sepsis and death(Goonewardene *et al*, 2011).

2.3.9.1 Prevalence of anemia in pregnancy

According to world Health Organization estimates, up to 56% of all women living in developing countries are anemic.

According to WHO, hemoglobin level below 11gm/dl in pregnant women constitutes anemia and hemoglobin below 7gm/dl is severe anemia. The Center for Disease Control and Prevention (1990) defines anemia as less than 11gm/dl in the first and third trimester and less than 10.5gm/dl in second trimester. Serum Ferritin of 15 microgm/L is associated with iron deficiency anemia(Sharma and Shankar, 2010).

2.3.9.2 Physiological anemia

During pregnancy there is a disproportionate increase in plasma volume, RBC volume and haemoglobin mass. As plasma volume increase more than the RBC mass hemodilution occurs called asphysiological anemia of pregnancy,

Criteria are: RBC 3.2 million/cumm, Hemoglobin 10 gm%, RBC morphology on peripheral smear is normal i.e. normocytic, normochromic, PCV 30% (Sharma and Shankar,2010).

2.3.9.3 Effect of anemia on pregnancy

2.3.9.3.1 Maternal effects

Mild, anemia may not have any effect on pregnancy and labour except that the mother will have low iron stores and may become moderately-to-severely anemic in subsequent pregnancies. Moderate anemia may cause increased weakness, lack of energy, fatigue and poor work performance. Severe anemia, however, is associated with poor outcome. The woman may have palpitations, tachycardia, breathlessness, increased cardiac output leading on to cardiac stress which can cause decompensation and cardiac failure which may be fatal.

Increased incidence of pre-term labour (28.2%), pre-eclampsia(31.2%) and sepsis have been associated with anemia(Sharma and Shankar, 2010).

2.3.9.3.2 Fetal effects

Irrespective of maternal iron stores, the fetus still obtains iron from maternal transferrin, which is trapped in the placenta and which, in turn, removes, and actively transports iron to the fetus. Gradually, however, such fetuses tend to have decreased iron stores due to depletion of maternal stores. Adverse perinatal outcome in the form of pre-term and small-for-gestational-age babies and increased perinatal mortality rates have been observed in the neonates of anemic mothers(Sharma and shanagr,2010).

2.3.9.4 Benefits of Folic Acid Supplementation

In addition to the prevention of NTD, periconceptional supplementation with folic acid also appears to have other beneficial effects, including the prevention of congenital heart disease and oral clefts and possibly preterm birth. The mechanism by which folic acid prevents structural anomalies in the fetus is not

known, but may involve the regulation of homocysteine metabolism(Greenberg *et al*,2011).

Several investigators have suggested that folic acid supplementation may have additional benefits on pregnancy outcome. This line of investigation began because of epidemiologic studies showing that pregnancies exposed to folic acid antagonists have significantly higher rates of placenta-related pregnancy complications. Folic acid antagonists encompass a broad spectrum of drugs used for a variety of clinical indications, including the treatment of seizure disorders, mood disorders, and urinary tract infections. Folic acid antagonists can be divided into two groups:

(a) DHFR inhibitors (eg, sulfamethoxazole-trimethoprim), which block the conversion of folate to its more active metabolites , and

(b) other folic acid antagonists, a group consisting primarily of anticonvulsant medications (phenobarbital, phenytoin, primidone, and carbamazepine) but also including Spasmophen (an antispasmodic drug that contains low doses of phenobarbital) and cholestyramine, it is biologically plausible that folate deficiency may interfere with the early stages of placental development leading to complications later in gestation(Greenberg *et al*,2011).

2.3.9.5 Risks of High-Dose Folate Supplementation

Although folic acid supplementation to supraphysiologic levels has demonstrated many of the benefits to pregnant women and fetuses noted above, the potential risk of high-dose folate supplementation must also be considered. First, folate supplementation can mask vitamin B12 deficiency (pernicious anemia) and care must be taken with susceptible individuals to avoid missing this diagnosis. Also, concerns have been raised about the potentially untoward effects of unmetabolized synthetic folic acid with regard to cancer, depression,

and cognitive impairment. With all these concerns, early data suggest supplementation with l-methylfolate rather than folic acid may mitigate these risks(Greenberg *et al*, 2011).

2.3.9.6 homocysteine and Pregnancy

plasma homocysteine concentrations fall in normal human pregnancy. The mechanisms responsible for this fall include the normal increase in the glomerular filtration rate that accompanies pregnancy, the increase in plasma volume and associated haemodilution, and a postulated increased uptake of homocysteine by the fetus. Although in one study homocysteine concentrations in amniotic fluid in early pregnancy were very low compared with those found in maternal serum, by contrast methionine concentrations were high, suggesting the possibility of active remethylation of homocysteine in the fetal tissues to support its growth and development.

A small maternal– fetal gradient of homocysteine has also been demonstrated in late pregnancy(Hague, 2003).

2.3.9.7 Homocysteine and Pregnancy complications.

The oxidative stress by which homocysteine can produce endothelial cell damage has also been considered as a mechanism in the pathophysiology of pre-eclampsia, Antithrombin III, protein C, and protein S deficiencies, resistance to activated protein C, and mutations of factor II are thrombophilias, that is congenital hypercoagulable states . Hyperhomocysteinemia has also been recognized as a thrombophilia, Pregnancy is itself a thrombotic risk factor. Thus, the combination of hyperhomocysteinemia and pregnancy constitutes an important risk for thromboembolic events(Medina *et al*, 2001).

Elevated homocysteine levels have been observed more frequently among women with certain pregnancy complications, including preeclampsia (dangerously elevated blood pressure in pregnancy), placental abruption (where the placenta detaches from the uterus), and recurrent pregnancy loss. However, it appears that elevated homocysteine levels may be a consequence of these complications, rather than the cause. Elevated homocysteine levels are 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. It is recommended that all women of childbearing age take a multivitamin containing 0.4 mg of folic acid per day to reduce the chance of neural tube defects in their children. This recommendation is independent of a person's homocysteine level. A higher dosage of folic acid, usually 4 mg, may be recommended if the woman has had a previous child with a neural tube defect(Moll and Varga,2015).

Chapter three

Materials and methods

Chapter three

Materials and methods

3.1 Materials

3.1.1 study design

This is analytical case control study conducted in Omdurman maternal hospital from September to November 2018, aimed to measure non-fasting homocysteine plasma level in pregnant woman at third trimester (case) and non-pregnant at child bearing age women as healthy individuals(control).

3.1.2 study area and population

This study was conducted in Omdurman Maternity Hospital, sample size of 35 venous blood samples was collected from pregnant women at their third trimester and 35 samples were collected from healthy woman at child bearing age as control.

3.1.3 sampling

Pregnant women at their third trimester were selected and data collected using self-administrated per-coded questionnaire which was specifically designed to obtain information that helped in this study.

3.1.4 Inclusion Criteria

- Case group were pregnant women at third trimester ranging from 18-43 years
- Control group were healthy non-pregnant women at child bearing age

3.1.5 Exclusion Criteria

-Pregnant women at first or second trimesters

-Pregnant women with chronic illnesses such as diabetes or hypertension

3.1.6 Ethical considerations

selected individuals were informed with all detailed objectives of the study. The specimens and information that collected from the participating were under privacy and confidentially.

3.1.7 Data collection

Data was collected using structured interview questionnaire, which was designed to collect and maintain all valuable information concerning each case examined.

3.2 methods

3.2.1 Blood sample collection and preparation

Pregnant women was either sat or lid down right on an examination table, the arm was positioned on the armrest so that the vein is identified, the skin was cleaned up using 70% alcohol, and allowed to dry, the personal details were checked up on the form and blood vials, Tourniquet was applied to the arm, 2.5 ml of blood was collected from the vein in the forearm using a syringe, blood was collected in EDTA anticoagulant container, EDTA blood samples were analyzed by sysmex-xp300, blood samples were then separated using centrifuge

at 3500 rpm and the plasma obtained were collected in plain containers, obtained plasma were stored at -20C, then they were thawed and well mixed before they were analyzed using DIRUI CS-T240.

3.2.2 Requirement of the test

- 1-Automated chemistry analyzer (DIRUI CS-T240)
- 2-Automated hematological analyzer (Sysmex-xp300)
- 3-Centrifuge
- 4-homocysteine enzymatic reagents
- 5-EDTA container
- 6-plain container
- 7-alcohol swab 70%
- 8-cotton, tourniquet and syringes

3.2.3 Estimation of red blood cells parameters:

3.2.3.1 principle of red blood cells parameters

The traditional method for counting cells is electrical impedance, described by Wallace Coulter, Hemoglobin concentration; A non-ionic detergent is included to ensure rapid cell lysis and reduce turbidity caused by cell membranes and plasma lipids, blood is highly diluted in a buffered electrolyte solution, the blood cell is carried through the aperture, it displaces some of the conducting fluid and increases the electrical resistance, this produces a corresponding change in potential between the electrodes, which last as long as the red cell takes to pass through the aperture, the height of the pulses produced indicates the volume of the cells passing through(Bain *et al*,2001).

3.2.3.2 procedure:

Fully automated multichannel instruments (sysmex xp-300) require only that an appropriate blood sample is presented to the analyzer and usually measure from 8 to 20 components for the basic FBC and white blood cell differential, Impedance counting systems depends on the fact that red cells are poor conductors of electricity, where as certain diluents are good conductors, this difference form the basis of the counting system(Bain *et al*, 2011).

3.2.4 Estimation of plasma homocysteine level:

3.2.4.1 Principle of plasma homocysteine level

This assay is based on an assay principle that asses the co-substrate conversion product (a molecule that is not a substrate of the Hcy conversion enzyme,and does not contain any element from sample Hcy) instead of assessing co-substrate or Hcy conversion product of Hcy. (See appendix II)

3.2.4.2 Procedure: provided by fortress diagnostics limited (see Appendix II).

3.2.5 Quality control

the precision and accuracy of all methods used in this study were checked by commercially prepared control sample before it's application for the measurements of test and control samples.

3.2.6 Statistical analysis

Data obtained from this study was analyzed using statistical package for the social science (SPSS version 19). Independent T test was used for data analysis and person's correlation was used for correlation.

Chapter four

Results

Chapter four

Results

The results of the enzymatic determination of plasma homocysteine level in the third trimester of pregnancy consistent with folate supplement (case group) and non-pregnant healthy individuals (control group) are given in figures and tables: Figure(4-1):show correlation between plasma homocysteine and age of pregnant women at their third trimester ($R= 0.040$, $P\text{-value}= 0.820$), there were no correlation.

Figure(4-2): show correlation between plasma homocysteine and number of pregnancies ($R=0.090$, $P\text{-value}=0.605$), there were no correlation.

Table(4-1):represent the comparison mean \pm SD of plasma homocysteine, Hemoglobin, Red blood cells, packed cell volume, mean cell hemoglobin, mean cell hemoglobin concentration, mean cell volume in case group versus control group, the result showed there was significant decrease in homocysteine HCY (3.70 ± 1.03 versus 11.03 ± 1.19 , $P\text{-value} 0.000$), RBCS (4.31 ± 0.39 versus 4.92 ± 0.72 , $P\text{-value} 0.000$), PCV (33.88 ± 2.82 versus 41.11 ± 6.19 , $P\text{-value} 0.000$), and MCV (76.93 ± 9.94 versus 85.87 ± 6.26 , $P\text{-value} 0.000$), there was significant increase in MCH (27.03 ± 2.92 versus 25.49 ± 2.24 , $P\text{-value} 0.015$), MCHC (34.54 ± 1.59 versus 29.59 ± 0.83 , $P\text{-value} 0.000$), and there was insignificant difference in mean concentration of Hb (11.91 ± 1.4 versus 12.30 ± 1.75 , $P\text{-value} 0.306$).

Table(4-2):represent the values of homocysteine and the Red blood cells parameters, Hb ($R\text{-value}-0.013$, $P\text{-value} 0.943$), RBCS ($R\text{-value} 0.188$, $P\text{-value} 0.280$), PCV ($R\text{ value} -0.059$, $P\text{-value} 0.736$), MCV ($R\text{-value} -0.035$, $P\text{-value} 0.840$), MCH ($R\text{-value} 0.072$, $P\text{-value} 0.680$) MCHC ($R\text{-value} 0.084$, $P\text{-value} 0.631$), results showed no correlation between homocysteine and Red blood cells parameters.

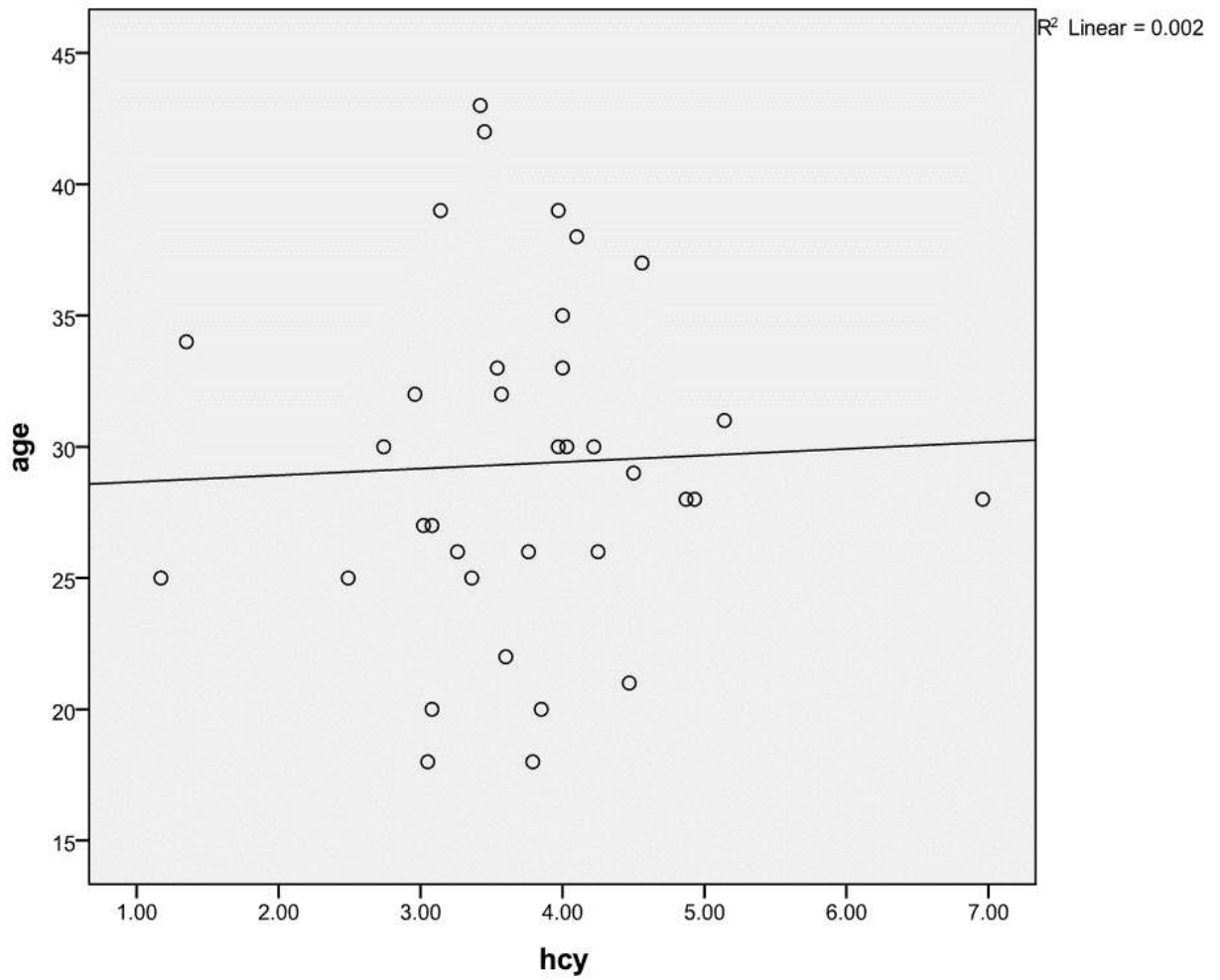


Figure (4-1): Correlation between plasma homocysteine level and age of pregnant women group: (R-value=0.040, P-value =0.820).

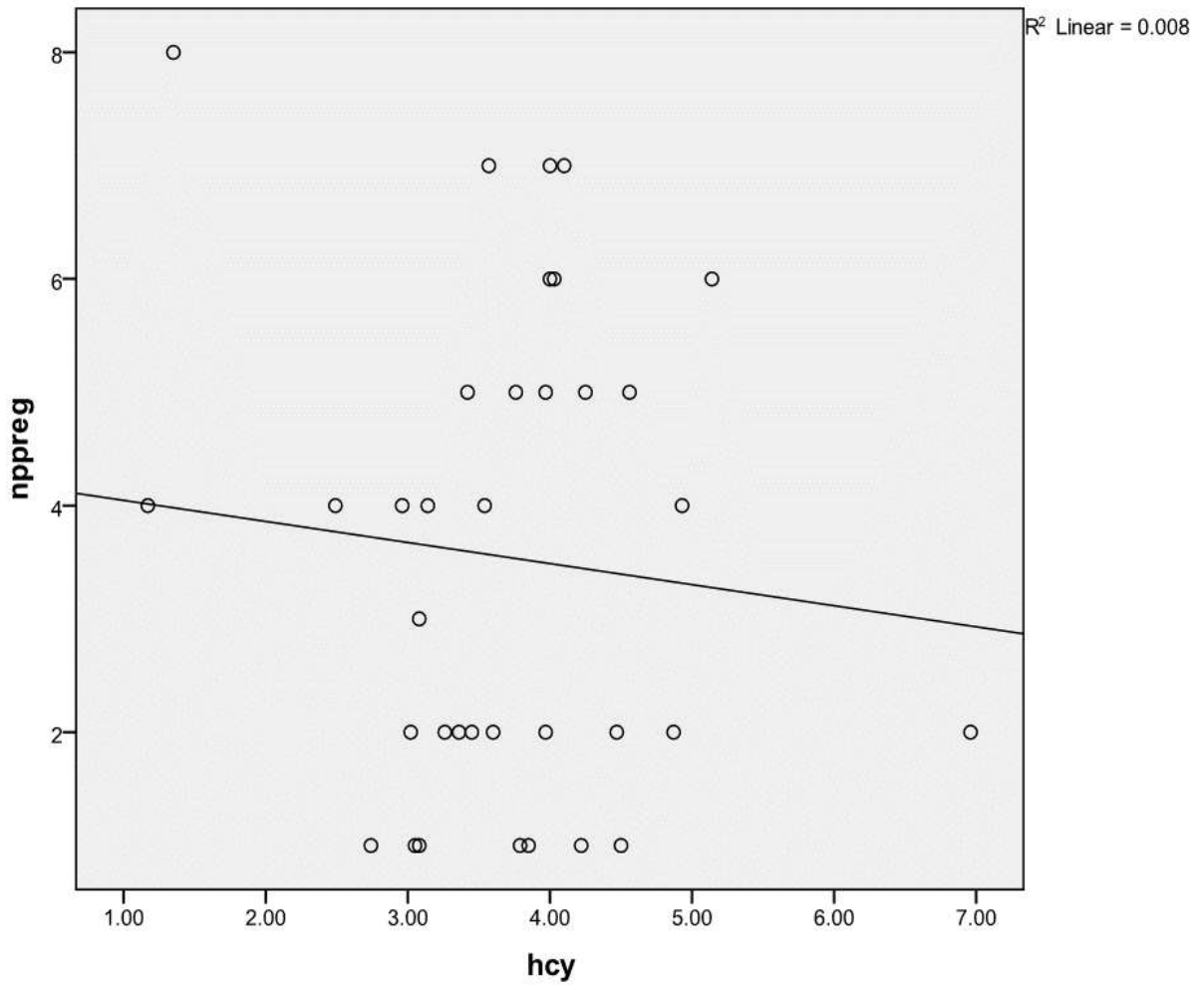


Figure (4-2): Correlation between plasma homocysteine level and number of pregnancies: (R=0.090, P-value =0.605).

Table (4-1): Mean concentration and values of Hemoglobin, Red blood cells, packed cell volume, mean cell hemoglobin, mean cell hemoglobin concentration, mean cell volume and homocysteine in case and control groups

Parameters	Case (Mean±SD)	Control (Mean±SD)	<i>P-value</i>
Hb	11.91±1.46	12.30±1.75	0.306
RBCS	4.31±0.39	4.92±0.72	0.000
PCV	33.88±2.82	41.11±6.19	0.000
MCH	27.03±2.92	25.49±2.24	0.015
MCHC	34.54±1.59	29.59±0.83	0.000
MCV	76.93±9.94	85.87±6.26	0.000
HCY	3.70±1.03	11.03±1.19	0.000

Result given in mean ±SD, P-value ≤ 0.05 consider significant.

Independent T test was used for data analysis and presentation.

Table (4-2): Values of HCY with RBCs parameters

Parameters	<i>R-value</i>	<i>P-value</i>
Hb	-0.013	0.943
RBCS	0.188	0.280
PCV	-0.059	0.736
MCH	0.072	0.680
MCHC	0.084	0.631
MCV	-0.035	0.840

Result given in R-value and P-value, $P\text{-value} \leq 0.05$ consider significant (no correlation).

Person's correlation was used for correlation.

Chapter five

Discussion, conclusions and recommendations

Chapter five

Discussion, conclusions and recommendations

5.1 Discussion:

The study aimed to estimate non-fasting plasma homocysteine level in pregnant women ranging from 18-43 years at their third trimester with consistent folate supplement, the results of this study showed that there was significant decrease in Homocysteine(HCY) in pregnant women group compared to control group, the result agree with (Holmes *et al*, 2005) ($p < 0.05$), the decrease in homocysteine is thought to be as a result of hemodilution, decreased albumin concentrations during pregnancy, or a relationship with maternal folic acid supplementation during pregnancy, but there were another explain by (Murphy *et al*, 2002) suggesting the cause to be endocrine based.

significant decrease in red blood cells (RBCs) agree with (Chaudhari and Bodat, 2015) ($p < 0.05$), and packed cell volume (PCV) suggested to be due to increase in plasma volume during pregnancy this result agree with (Arora *et al*, 2017; Chaudhari and Bodat, 2015) ($p < 0.05$), mean cell volume (MCV) also showed a significant decrease, this result agree with (Chaudhari and Bodat, 2015) ($p < 0.05$), and disagree with (Arora *et al*, 2017) ($p < 0.05$).

results showed a significant increase in mean cell hemoglobin(MCH), mean cell hemoglobin concentration (MCHC), this agree with (Chaudhari and Bodat, 2015) ($p < 0.05$), and disagree with (Arora *et al*, 2017) ($p < 0.05$).

there was insignificant difference in mean concentration of hemoglobin (Hb) in pregnant group compared to control group , this result disagrees with (Arora *et al*, 2017) ($p < 0.05$).

Results showed values of homocysteine and the Red blood cells parameters Hb, RBCs, PCV, MCV, MCH, MCHC, the results showed no correlation between homocysteine and Red blood cells parameters, agree with (Rosa *et al*, 2004) ($p > 0.05$).

And there was no correlation between plasma homocysteine and the age of pregnant women at their third trimester, And showed no correlation between plasma homocysteine and number of pregnancies, this result agree with study done by(Rosa *et al*,2004) ($p > 0.05$).

5.2 conclusion:

According to results of this study it's concluded that: plasma homocysteine in third trimester of pregnancy is decreased compared to control, also there is no correlation between homocysteine and red blood cells parameters, number of pregnancies and age.

5.3 recommendations:

1. Follow up of general health of pregnant women, should be done regularly during pregnancy
2. folic supplement must be taken during pregnancy to avoid pregnancy complication.
3. Further studies with large sample size should be carried out in all trimesters.

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Appendices

**Sudan University for Sciences and Technology
College of Graduate Studies**

Appendix (I)

Pregnancy Questionnaire No. ()

**Research title: plasma homocysteine level and red blood cells parameters
in third trimester of pregnancy among Sudanese women in Khartoum state**

1-Name

2-Age

3-Address

4-Education.....

5-Occupation/ husband's occupation

6-Is this your first pregnancy?

7-How far along is your pregnancy?

8-do you have any chronic illnesses (hypertension, diabetes...etc)?

9-Are you taking oral folic acid supplementation?

10-cbc

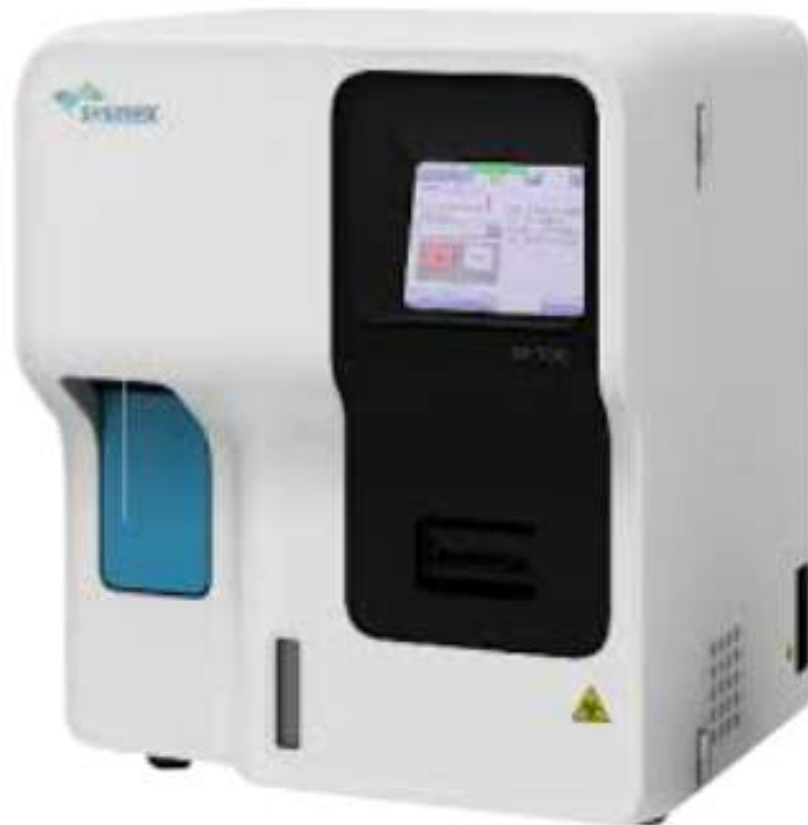
HCY $\mu\text{mol/L}$ Hb g/dl RBCs c/ μl PCV%

MCV.... ft MCH pg MCHC %

Date: / /2018

Appendix II

Appendix III



Automated Hematology analyzer: Sysmex-xp300

Appendix IV



Automatic chemistry analyzer: DIRUI CS-T240