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Study the Correlation Between Biochemical Characters and Oxidant-Antioxidant Status of Hypertensive and Preeclampsia Pregnant Patients

A Thesis

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بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

﴿وَالضُّحَىٰ ① وَاللَّيْلِ إِذَا سَجَىٰ ② مَا وَدَّعَكَ رَبُّكَ وَمَا قَلَىٰ ③
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⑩ وَأَمَّا بِنِعْمَةِ رَبِّكَ فَحَدِّثْ ⑪﴾

صدق الله العظيم

Supervision certification

We certify that this thesis, entitled " **Study the Correlation Between Biochemical Characters and Oxidant-Antioxidant Status of Hypertensive and Preeclampsia Pregnant Patients**" has been prepared under our supervision by " **Mustafa Hamzah Ghazi** " at the College of Science/Al-Muthanna University, in partial fulfillment of the requirements for the Master's degree in Chemistry.

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Dedication

To my parents

To my brothers Ali and Murtdha

My dear sister

Zahraa

For the endless love Support and encouragment

Mustafa hamzah

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Abstract

Pregnancy-induced hypertension (PIH) is a major pregnancy complication that leads to maternal mortality. Oxidative stress (OS) occurred during PIH because of an excessive increase in free radical and a decrease in antioxidant capacity.

The present study aimed to evaluate lipid peroxidation products as serum MDA and antioxidant status in hypertensive complications of pregnancy by measuring the activity of Cp and estimate the serum concentration of Vit C, Ald, Alb, urine ACR, as well as to highlight the possible correlations of each Hb and MAP with those parameters in the patient groups. This study was conducted at Al Samawa maternity and children teaching hospital in collaboration between maternity and biochemistry departments from October 2020 to February 2021. Forty healthy women with normal, uncomplicated pregnancy, divided into 20 subjects at 2nd trimester (2nd C) and 20 subjects at 3rd trimester (3rd C); 40 women with PIH (20 subjects as 2nd H and 20 subjects as 3rd H) and 40 women with pre-eclampsia (20 subjects for each 2nd PE and 3rd PE). The results revealed a weak positive correlation in Hb levels with serum MDA, Cp, Vit.C and no correlation with serum Alb. The results also revealed to weak negative correlation with Ald, serum ACR,urine ACR, along with 2nd and 3rd trimesters. The results also revealed strong positive correlation in MAP with serum MDA, Cp, serum Alb, serum ACR , urine ACR and weak positive correlation with Hb. The results showed to weak negative correlation in MAP with serum Ald and Vit.C along with 2nd and 3rd trimesters. The results also displayed a positive correlation between urine ACR and serum ACR.The present study demonstrated that Hb levels have significant increasing in the 2nd PE compared with each 2nd H and 2nd C group. This parameter showed nonsignificant difference among all groups at the 3rd trimester. Each serum MDA, Cp and urine ACR levels has a significant increasing in PE compared with H and healthy groups, contrary to Ald and Vit.C revealed

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nonsignificant differences among each groups at each trimester. The results Implied a significant increase of serum Alb levels in PE and H patient groups compared with the healthy controls at each trimester. The study has clarified that each serum ACR and MAP levels have significant increasing in PE compared with H and significant increase in H compared with healthy control at 2nd trimester, whereas, at the 3rd trimester MAP levels revealed significant increasing in H and PE groups compared with the healthy control.

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List of Abbreviations

Abbreviations	Meaning
11- betaHSD-2	11- β -hydroxysteroid dehydrogenase type 2
ACR	Albumin/Creatinine Ratio
Alb	Albumin
ALD	serum Aldoserone
AsA	ascorbic acid
BMI	Body mass index
BP	Blood pressure
Cp	Ceruloplasmin
DBP	Diastolic Blood Pressure
DMC	demethylcreatinin
DNA	Deoxyribonucleic acid
DW	Distilled Water
GFR	glomerular filtration rate
GST	glutathione S-transferases
H ₂ O ₂	Hydrogen peroxide
HAS	Human Albumin Serum
Hb	Haemoglobin
HELLP	Haemolysis, Elevated liver enzymes and Low Platelets
HOCl	Hypochlorous acid
HRP	Horse Radish Peroxidase
LPO	Lipid Peroxidation
MAP	Mean Arterial Pressure
MDA	Malondialdehyde
μ mol	Micromole
NaN ₃	Sodium azide

List of Abbreviations

NO [•]	Nitric oxide radical
NO ₂ [•]	Nitrogen dioxide
ONOO ⁻	Peroxynitrite anion
OS	Oxidative stress
PCOS	polycystic ovary syndrome
PE	Preeclampsia
PIH	Pregnancy induced hypertension
PPD	Para-phenylene diamine
PUFAs	polyunsaturated fatty acids
RCS	Reactive chlorine species
RNS	Reactive nitrogen species
RO	Alkoxyl
ROS	Reactive oxygen species
RPL	recurrent pregnancy loss
rpm	Rotor per minute
SBP	Systolic Blood Pressure
SOD	Superoxide dismutase
TBA	Thiobarbituric acid
TBARS	Thiobarbituric acid reactive substance
TCA	Trichloroacetic acid
WHO	World Health Organization

Chapter One
Introduction
&
Literature Review

1. Introduction and literature review

1.1 Introduction

Pregnancy is a physiological state characterised by an increased oxygen requirement and a high energy demand. Numerous compensatory adaptive alterations, including amplified ventilation to improve oxygen demand, followed with progressing pregnancy to encounter the accumulative supplies for appropriate mother biological functions to accomplish fetus requirements. This circumstance may be responsible for oxidative stress elevating during pregnancy[1]. Hypertensive disorders of pregnancy are considered to be major worldwide health problem that successively amplified the risk of perinatal and maternal mortality[2].

Pregnancy-induced hypertension (PIH) is elevated in systolic blood pressure (SBP) level of more or equal 140 mmHg and/or diastolic blood pressure (DBP) level of more or equal 90 mmHg. PIH is one of the primary causes of death for pregnant mothers, PIH is defined as a new onset proteinuria (≥ 300 mg/24 h) in hypertensive women exhibiting no proteinuria before 20 weeks of gestation[3]. Oxidative stress is central pathogenic in pregnancy-induced hypertension. Oxidative stress usually results from either excessive reactive oxygen species (ROS), mitochondrial dysfunction, impaired antioxidant system, or a combination of these factors. The pro-oxidative/anti-oxidative cellular imbalance between the ROS production and the ability of the biological systems' defense mechanisms to eliminate the cellular stress disturbances lead to a vicious circle since the oxidative stress reciprocally aggravates ROS production. ROS can be generated at elevated rates under normal aging, as well as in acute or chronic pathophysiological conditions[4].

Oxidative stress (OS) destruction occurs when reactive oxygen species (ROS) or free radicals overwhelm the antioxidant defenses of the host[5]. Throughout oxidative metabolism, the consuming of oxygen occurs in mitochondria by consecutive stages

and result in water. A small amount of this oxygen is transformed to highly reactive and toxic products identified as reactive oxygen species (ROS), including superoxide anions, hydrogen peroxide, lipid peroxides, and hydroxyl radicals. Endothelial dysfunction is considered a central factor in the pathogenesis of pregnancy-induced hypertension, which results from an imbalance between oxidative damage and antioxidant defenses during pregnancy-induced hypertension. Antioxidants are substances or enzymes present in tissues with the capacity to balance or neutralize these free radicals[6].

1.2 Literature Review

1.2.1 Definition of Pregnancy

Pregnancy is a period of intensive physical and physiological changes in the maternal organism, from fertilization to childbirth, during which the embryo and then the fetus develops in the maternal uterus[7]. The duration of Average pregnancy 266 days (38 weeks) after ovulation that equals 9 months. For many women, pregnancy is a natural and cheerful event[8].

1.2.2 Renal changes during Pregnancy

The kidneys are displaced in a cephalad orientation by the expanding uterus. They increase approximately 1 cm in size[9] due to increased vasculature, interstitial volume, and dead space[10]. The renal collecting system dilates of the first trimester, a result of a combination of the effects of progesterone, and the compression of the ureters at the pelvic brim[11]. Consequently, there is an increase in glomerular filtration rate (GFR) and effective renal plasma flow (RPF). Creatinine clearance increases 25% by four weeks' gestation, increasing to 45% at 9 weeks gestation[12]. At the same time, there is increased urinary excretion of protein and albumin mediated by the increase in GFR and changes in charge selectivity of the glomerular membrane.

This makes the diagnosis and monitoring of renal diseases during pregnancy complicated. An average of 200 mg to a maximum of 300 mg of protein is excreted daily in normal pregnancy[13].

1.2.3 Hypertension in Pregnancy

Hypertension is associated in most of the complications of pregnancy and is mostly accountable for maternal mortality, as well as other dangerous effects on pregnancy outcomes[14]. Worldwide about 50000 mothers die due to PIH every year and are responsible for approximately 25% of all cases of fetal growth restriction and 15% of preterm birth in developed countries. The first widespread epidemiologic work on PIH was done in 1978 by Chesley, and then numerous types of research have been done[15]. The term eclampsia was first used in 1739 to distinguish acute convulsion from epilepsy. Proteinuria was noted for the first time in eclamptic women in the mid-19th century, and sphygmographic tracings were utilized to mentioning a high blood pressure later. Pregnant women presenting with high blood pressure and proteinuria were defined as preeclampsia[16].

1.2.3.1 Classification of Hypertension in Pregnancy

Pregnant women who have hypertension fall into one of three categories: chronic hypertension, nonproteinuric gestational hypertension(often called gestational hypertension), or preeclampsia. It is vital to differentiate them since treatment and diagnosis are two separate concepts. Gestational hypertension is a term used by the International Society for the Study of Hypertension in Pregnancy (ISSHP) for women who were previously normotensive[17].

1.2.3.1.A Chronic Hypertension

Chronic hypertension is described by pre-gestational high blood pressure, or elevated BP observed for the first time prior the second trimester of gestation, which

continues after delivery. Chronic hypertension complicates between 1% and 5% of pregnancies[18]. It is a relatively common comorbidity in pregnancy and a well-established danger factor for number of reverse perinatal results, including perinatal death, intrauterine growth restriction, and preterm birth, as well as adverse maternal outcomes, including preeclampsia, stroke, acute renal failure, pulmonary edema, and maternal death. Although the majority of chronic hypertension among pregnant women is due to primary hypertension, about 10% of cases occur secondary to other medical conditions, such as chronic renal disease, collagen vascular disease, diabetes mellitus, and thyroid disease[19].

1.2.3.1.B Gestational Hypertension

Hypertension emerges for the first time after the twentieth week of gestation in the absence of proteinuria (< 300 mg in a 24-hour urine collecting), has neither important maternal nor fetal consequences. Blood pressure return to normal by 6 weeks postpartum or prior this time[20]. Gestational hypertension or transient hypertension happens in the second half of gestation, where there is nonproteinuric elevated BP in women who were having normal blood pressure previous this period. An abnormal high in BP for about a quarter of an hour is seen in the worse form of gestational hypertension, which then resolves within a week after birth. The incidence of gestational hypertension is approximately 6–7%, and the high BP normalizes after delivery [21]. There is about 15-26% tendency of gestational hypertension evolving into superimposed preeclampsia, and this risk is depend on the time of gestation at which hypertension evolved, and this danger is decreased to 10% when it is diagnosed in the last part of pregnancy[22]. Another studies observed that about half of women presenting with nonproteinuric rise BP would advance to evolve proteinuric high BP and this might be as a outcome of a history of loss of pregnancy, PIH, and rise BP[23].

The high in BP with this kind of PIH resolves or regularizes one and a half months postbirth[24]. Also, it is noted that pregnant women presenting with non proteinuric rise BP have a high tendency of advancing to proteinuric rise BP even through puerperium. They also noticed that the accurate diagnosis of gestational hypertension is generally made after hypertension resolves after delivery and the laboratory tests remain natural[23].

1.2.3.1.C Preeclampsia

Preeclampsia is described as a maternal syndrome, which is distinguished by high blood pressure, tissue edema, proteinuria, abnormal clotting, abnormal liver, and renal functions as a result of placental involvement[25]. Cunningham et al. (2001) clarified that placental substances might cause malfunctioning of the blood vessels of pregnant women, thus leading to rise in blood pressure. The progression of an high blood pressure repeatedly measured four to six hours apart and the existence of protein in urine in the second half of gestation are the attributes of preeclampsia[26]. In most cases, these symptoms evolve in the third trimester of pregnancy and generally disappear within a little weeks after delivery.

Preeclampsia forms around 5 to 10% of the complications of pregnancy and may lead to maternal and fetal morbidity[27]. Preeclampsia generally occurs in nulliparous women than in multipara ones with the maternal blood pressure normalizing after birth but still has the possibility to evolve essential hypertension in the future[28]. The pathophysiology of preeclampsia is an enigma, but maternal, immunological, genetic factors and placental participation have been referenced as possible causes[29]. The etiology of proteinuric rise BP can be attributed to an amplified inflammatory response in the pregnant woman, oxidative stress and unusual produce of vascular substances (vasodilators and vasoconstrictors). Previously, 24 h

urine collection was considered the gold standard for confirming proteinuria, but it has numerous drawbacks: it is time-consuming, requires freezing, samples are sometimes incomplete, and is seldom used in hospitals[26]. Spot urine albumin to creatinine (A: Cr) or protein to creatinine (P: Cr) ratios are currently advised to assess proteinuria after a positive dipstick test (one or more proteins). Proteinuria, as measured by 24 hour urine, has a strong correlation with both P: Cr and A: Cr tests[30]. Diagnostic thresholds of 30 mg/mmol and 8 mg/mmol have been determined to provide high sensitivity and specificity, respectively[30].

1.2.3.1.D Pre-eclampsia Superimposed on Chronic Hypertension

Chronic hypertension in pregnancy might be complicated by preeclampsia. This situation will be determined when there is a new onset of proteinuria or unexpected deterioration of either hypertension or proteinuria, or progression of other signs and symptoms of preeclampsia after the twentieth week of gestation[31]. Pregnant subjects who have pre-gestational hypertension might present with preeclampsia which may appear symptoms that are very acute than if it had happened independently[32]. The border between superimposed preeclampsia and deteriorating chronic hypertension is narrow. This is due to the difficulty in distinguishing between the symptoms of no proteinuria in the first half of pregnancy and the unexpected rise in liver enzymes, proteinuria, BP, and a reduction in platelets of this pregnant woman[32]. In 2005, Sibai et al. reported that quarter of pregnant women with chronic hypertension had superimposed preeclampsia, which is a substantially greater risk than is generally seen.

1.2.3.1.E Eclampsia

When tonic-clonic convulsion happens in a woman with established preeclampsia in the absence of any other neurological or metabolic cause, it is deemed

a severe life-threatening complication of preeclampsia. Eclampsia is described as a convulsive attack in preeclamptic women, which cannot be connected to any other clinical causes. A majority of eclamptic patients suffer their first seizure after birth, although pregnancy-induced hypertension is unique to a pregnancy and is assumed to resolve after birth[33]. This worse form of PIH may cause irreparable destruction of the brain, liver, and kidneys. When this situation is ill-managed, it can result in coma, cerebral harm, liver rupture, renal failure, cerebral hemorrhage, pulmonary edema, cortical blindness, maternal death, cerebral edema, cerebrovascular incident, and hypertensive encephalopathy. Early detection through gynecological care and proper treatment will prevent progression to eclampsia[34]

1.2.4 Risk Factors for Pre-eclampsia

- First pregnancy.
- Multiparous with:
 - ❖ preeclampsia in any previous pregnancy,
 - ❖ ten years or more since last pregnancy.
- Age 40 years or more.
- Body mass index(BMI) of 35 or more.
- Family history of preeclampsia (in mother or sister).
- Booking diastolic blood pressure(DBP) of 80 mmHg or more
- Booking proteinuria (of $\geq 1+$ on more than one opportunity or quantify at ≥ 0.3 g/24 hours).
- Multiple pregnancies.
- Certain underlying medical conditions:
 - ❖ preexisting hypertension.
 - ❖ Pre-existing renal disease.

- ❖ pre-existing diabetes.
- ❖ antiphospholipid antibodies[35]

1.2.4.1 Symptoms and Signs of Preeclampsia

- Increased blood pressure,protein in the urine,edema (swelling),sudden weight gain,visual changes such as blurred or double vision,nausea, vomiting,right-sided upper abdominal pain or pain around the stomach,urinating small amounts, Breathing problems, changes in liver or kidney function tests[36].

1.2.4.2 Pathogenesis of Preeclampsia

Pregnancy-induced hypertension, especially preeclampsia, is a multisystemic disorder due to the fact that it affects essential organs such as the liver, kidney, brain, and blood systems and is also associated with generalized vasospasm[37]. The placenta plays an essential role in PIH. The changed theory of the "two-stage model" proposes that abnormal placental implantation and vascularization or function together with the influence factors of pregnant women and result in PE[38]. Many factors that have been concerned in PE. pathophysiology are cardiovascular vasoconstriction, genetic and immunologic factors of motherly tissue, vascular endothelial dysfunction, and activation of platelet[39]. Moreover, simultaneous metabolic features can associate with endothelial dysfunction, hyperlipidemia, and insulin resistance which have been related to preeclampsia[40]. Genetic and immune influences lead to reduced uteroplacental perfusion pressure and low placentation throughout late pregnancy and activate the release of circulating bioactive factors. Bioactive factors affect the blood vessels and cause vasoconstriction, hypertension, and increased vascular resistance, or cause edema and increased permeability of vassal. The bioactive factors also could harm the kidney, which leads to increased plasma volume and severe hypertension, as well as glomerular endothelins and proteinuria. Amplified edema and cerebral

vascular permeability lead to seizures and eclampsia. Endotheliosis of the hepatic vessels could lead to activation of coagulation pathways and manifest as HELLP syndrome (hemolysis, elevated liver function enzymes, and low count of platelets) [41].

1.2.4.3 Genetic Factors of Pregnancy Induced Hypertension

In 2005, Roberts and Gammill suggested that the pathophysiology of PIH might be attributed to the interaction of placental blood insufficiency and intrinsic factors that predisposes pregnant women to PIH[42]. This is in accordance with PIH as a result of repeated malplacentation and consequent delayed presence of intrinsic or ecological vulnerability for endothelial damage. It has been suggested that the etiology of PIH might be genetic, an example being the gene for thrombophilia[43]. Endothelial dysfunction gene: endothelin-1 is responsible for how a mother responds to weakness in the function of the placenta. Since hypertension has genetic pre-dispositioning, popular danger factors such advanced maternal age and diabetes mellitus can trigger the genetic expression of endothelin-1, which could lead to PIH.(Lie et.al.) reported that there is a paternal risk of reoccurrence of preeclamptic pregnancies in different women impregnated by the same man, thus proposing paternal genetic factors in proteinuric rise BP[44]. However, a father whose genetic profile predisposes him to cause PIH could only do so through the fetus or the placenta.

1.2.4.4 Maternal Role of Pregnancy-Induced Hypertension

The associating of normal pregnancy with preeclampsia, vascular resistance is raised in the fetoplacental circulation, and the vessels are constricted, plasma volume is reduced, renin expression is increased, and plasma renin activity is also lowered in preeclampsia which leads to increase blood pressure[45]. The contents of placental

microparticles released into the maternal circulation during PIH also contributes to the elevated level of systemic oxidative stress.

Circulating antiangiogenic proteins have been concerned with the pathogenesis of many of the maternal features of Preeclampsia[46]. In normal pregnancy, the vasoconstrictive effect of angiotensin II on the vasculature is not felt despite its high circulating concentrations; in PIH, however, this protection against the effects of angiotensin II is impaired, resulting in arterial vasoconstriction and an increase in blood pressure[47]. Angiotensin II is one reason for renal inflammation and oxidative stress by stimulating increasing chemokine release and superoxide formation[48].

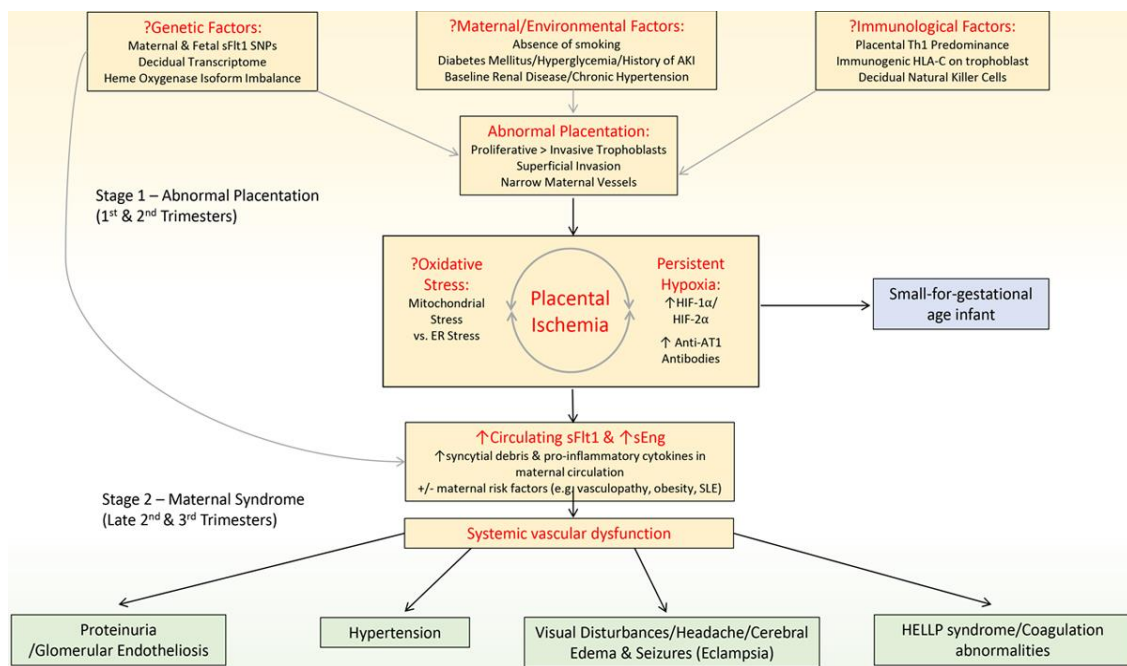


Figure 1.1: Schematic of the pathogenesis of preeclampsia[49]

1.2.4.5 Role of the Placenta in Pregnancy Induced Hypertension

The placenta is a vital organ of pregnancy that serves as a maternal-fetal connection through which oxygen, hormone, and nutrient exchanges occur. It also offers defense and protection to the fetus. In humans, normal placentation instigates with proper trophoblastic invasion of the maternal spiral arteries and is the key event that initiates the onset of these placental[50]. Placental ischemia-hypoxia plays a

central role in the initiation of oxidative stress, which can result in systemic vasoconstriction and endothelial cell dysfunction[51]. Preeclampsia represents a much greater state of oxidative stress than normal pregnancy.

During the first half of a normal pregnancy, the shape of spiral arteries was modified in response to the invasion of extravillous trophoblasts, which are made from trophoblast of the anchoring villi, which resulted in the formation of dilated, inelastic vessels with no maternal vasocontrol, thus improving placental and fetal blood supply, and this could extend through the myometrium (Figure1-2). In the condition of this maternal syndrome, this mechanism is altered, and there is impaired placentation which affects the lower of the strength of trophoblast attack and the amount of vessels attacked, consequently leading to uteroplacental blood insufficiency, which initiates the symptoms of this syndrome in the last half of gestation[52].

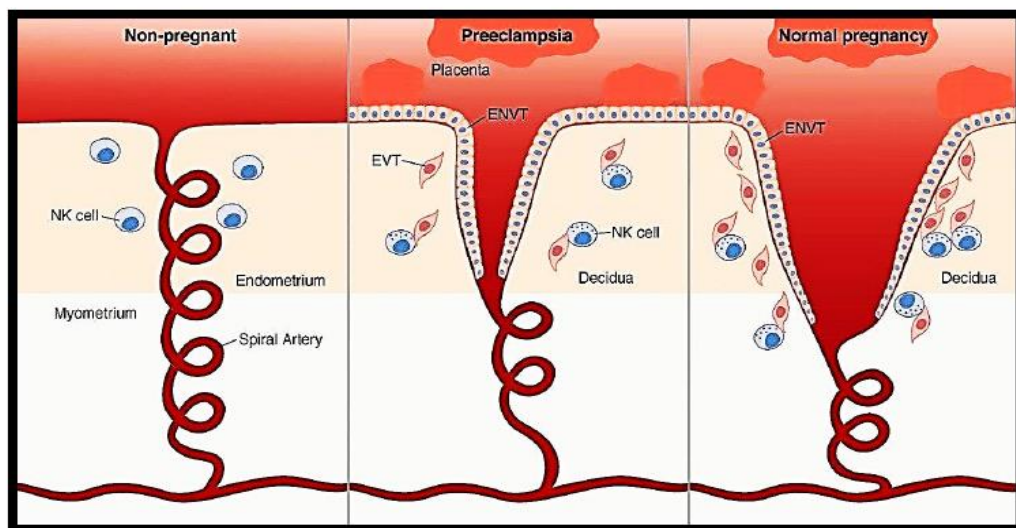


Figure 1.2: Remodelling of spiral arteries[53]

1.2.5 Oxidative Stress

The imbalance between prooxidants and antioxidants causes oxidative stress (OS)[54]. This ratio can be altered either by increased levels of reactive oxygen species (ROS) and/or reactive nitrogen species (RNS) or a reduction of antioxidant defense mechanisms[55]. The role of ROS is important for progressing normal cell functions, provided that upon oxidation, and then every molecule returns to its reduced state. However, the excessive production of ROS may suppress the body's natural antioxidant defense system and creating an inappropriate environment for normal female physiological reactions[56]. This in turn, can lead to several reproductive diseases involving polycystic ovary syndrome (PCOS), unexplained infertility, and endometriosis. Also, it can also cause different obstacles through pregnancy, such as recurrent pregnancy loss (RPL), spontaneous abortion, and preeclampsia, figure (1-3)[51]. Oxidative damage belongs to biological substances, for instance, proteins, lipids, and DNA[57] in addition to the aging as well as the pathogenesis of several diseases like hypoxia, hyperoxia, ischemia-reperfusion injury, intoxication, and iron excess. This process generates variety of metabolic products, involving lipid peroxides, oxidized thiols, and isoprostane, which have been laboring as an indicator for increased oxidative stress[58]. Currently, there is a great interest in the role of placental oxidative stress in the pathophysiology complications of human pregnancy. The cause of the oxidative stress is not certain, but the major complications of pregnancy have long been related to deficient alteration of uterine spiral arteries, suggesting that impaired perfusion of the placenta is the initiating insult[59]. The reason for maternal oxidative stress increase during pregnancy is unknown; however, much evidence proposes that the placenta plays an essential role in production of oxidative stress through pregnancy[60].

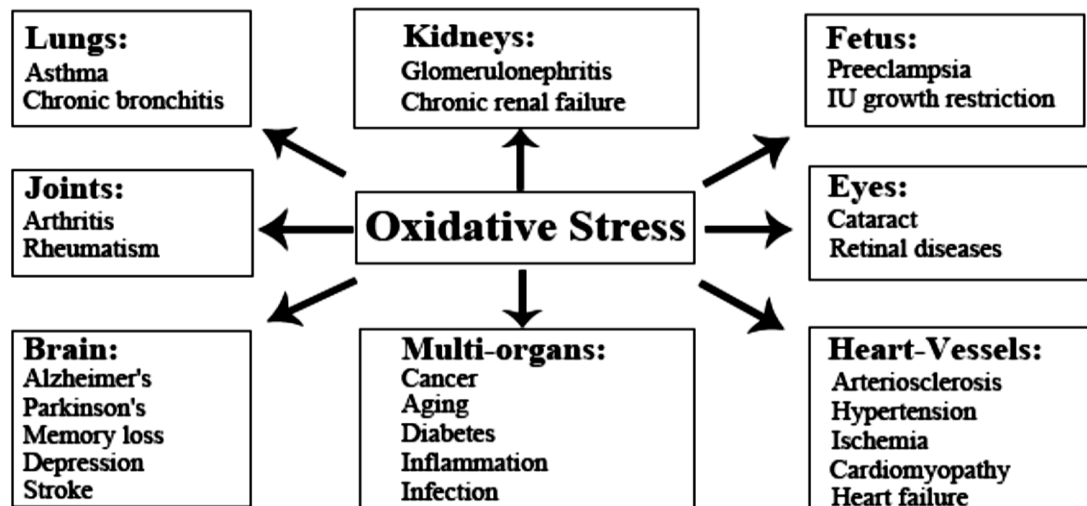


Figure 1.3: Oxidative stress-induced diseases in humans.

1.2.5.1 Lipid Peroxidation (LPO)

Because of the presence of oxygen and the effect of free radicals, peroxidation is Known as damage to unsaturated fatty acids contained in cell membranes[61]. Lipid peroxidation oxidizes polyunsaturated fatty acids (PUFAs), which are an important cellular molecules that enter the membranes of the cells[61]. Unsaturated fatty acids have two or more double bonds which are the main target of free radical reactions. The presence of double bonds in polyunsaturated fatty acids (PUFA) weakens the bond the carbon atom content and the dual bond between (carbon-hydrogen) and makes hydrogen removal easier. Free radicals and ROS in these membranes disrupt cellular membranes by over-oxidating unsaturated fatty acids[62], The process of lipid peroxidation is shown in figure (1-4).

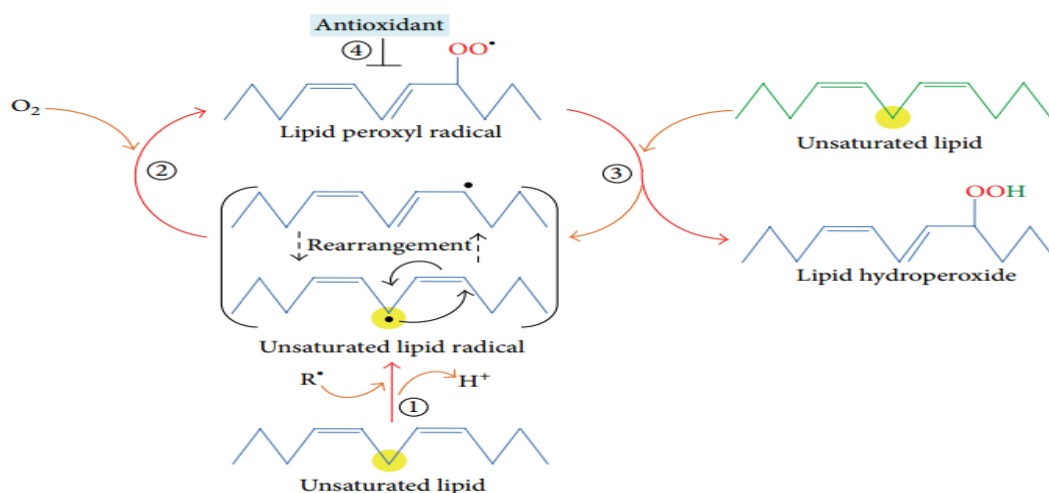


Figure 1.4: The process of lipid peroxidation[63]

Malondialdehyde (MDA) is one of the most common and reliable markers in clinical situations which determines oxidative stress. MDA has been commonly used as a convenient biomarker for lipid peroxidation of omega-3 and omega-6 fatty acids for many years due to its simple reaction with thiobarbituric acid.

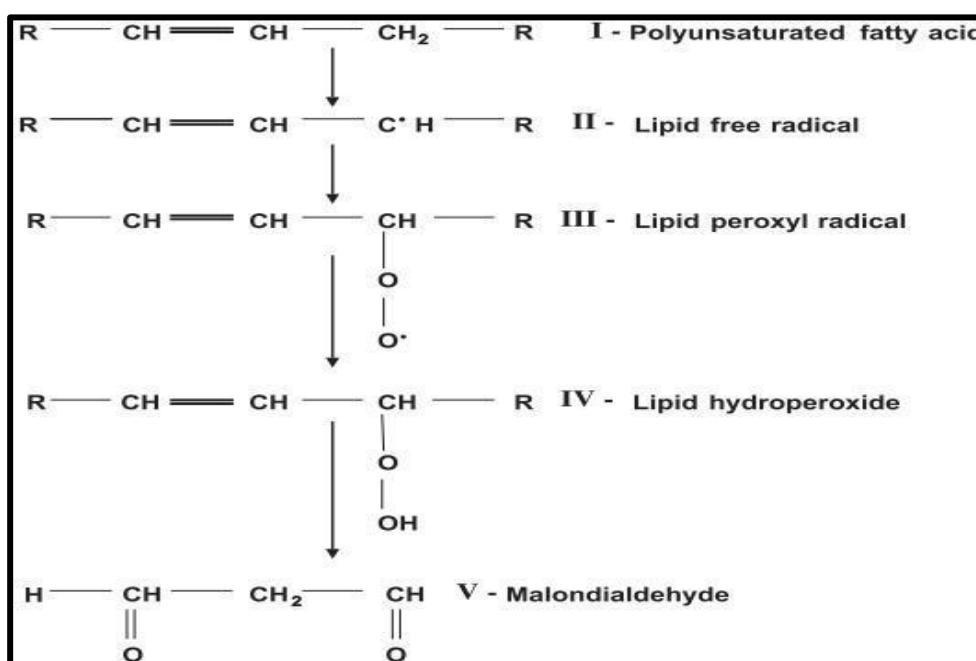


Figure 1.5: Schematic steps of the production of MDA from polyunsaturated fatty acids[64].

A route to shape an MDA is defined in Figure(1-5). The carbon-carbon double bond of polyunsaturated fatty acids (I) is the target of reactive species. This double bond

weakens the carbon-hydrogen bond, which allows a free radical to easily abstract the hydrogen. The hydrogen atom can then be abstracted by a free radical, and a lipid-free radical is formed (II).

1.2.5.2 Free Radicals

Free radicals and other reactive species are formed constantly in the body and have important functions for the immune system, as our white blood cells produce oxidants such as hydrogen peroxide and superoxide to kill bacteria[65]. ROS and RNS have important biological functions necessary for normal Physiology (Tables 1-1 and 1-2). Overproduction or insufficiency of ROS and RNS may result in impaired homeostasis and associated pathology. In healthy organisms, oxidants are balanced by reducing agents (antioxidants). According to recent reports, it is presumed that almost all pathogenetic mechanisms include disequilibrium in the redox balance as the "final common pathway" [66]. Free radical-induced damage in oxidative stress has been confirmed as a contributing to the pathogenesis and pathophysiology. When free radicals of an oxygen species attack a cell, they damage its membrane, its proteins, and even its DNA. The cell is then oxidized: it cannot function properly and dies. When its DNA is affected, the disrupted cell can even become a "mutant" cell, therefore potentially a cancerous cell[67].

Table 1.1 : Main reactive oxygen species (ROS) [68]

Free radicals	Non-radicals
Superoxide $O_2^{\cdot -}$	Hydrogen peroxide H_2O_2
Hydroxyl $\cdot OH$	Singlet oxygen $^1O_2$
Hydroperoxyl HO_2	Ozone O_3
Peroxyl $ROO^{\cdot}$	Hypochlorous acid $HOCl$
Alkoxyl $RO^{\cdot}$	

Table 1.2: Main reactive nitrogen species (RNS).

Free radicals	Non-radicals
Nitric oxide $\text{NO}\cdot$	Nitrous acid HNO_2
Nitric dioxide $\text{NO}_2\cdot$	Nitrosonium cation NO^+
Nitrate radical $\text{NO}_3\cdot$	Nitroxyl anion NO^-
	Peroxynitrite ONOO^-
	Dinitrogen tetroxide N_2O_4
	Dinitrogen trioxide N_2O_3

The production of reactive oxygen species (ROS) is an unavoidable consequence of aerobic life. ROS is a collective term that includes both oxygen radicals, like superoxide ($\text{O}_2\cdot^-$), hydroxyl ($\cdot\text{OH}$), peroxy ($\text{ROO}\cdot$), etc. and other non-radicals such as hydrogen peroxide (H_2O_2), singlet oxygen ($^1\text{O}_2$) and ozone (O_3) Table 1.1. Reactive nitrogen species (RNS) is a similar collective term that includes radicals like nitric oxide ($\text{NO}\cdot$) and nitric dioxide ($\text{NO}_2\cdot$), as well as non-radicals such as nitrous acid (HNO_2) and dinitrogen tetroxide (N_2O_4)

among to Table 1.2[68]. Several free radicals can enter the body continually from exogenous sources[69]:

- Cigarette smoke, Drugs, Air pollution, Pesticides, Heavy metal exposure, including mercury, cadmium and lead, Exposure to ionizing radiation, Exposure to a variety of environmental chemicals, Alcohol consumption.

Additional sources are endogenous sources, such as inflammation and respiratory burst. The main source of free radical production is the placenta, but the maternal white blood cell and endothelium are also likely contributors[70]. Latest indication proposes that neutrophils, through their capacity in ROS production could act in mediating endothelial damage of preeclamptic women

1.2.6 Antioxidants

Antioxidants are described as any substance that, when existent at low concentrations to compete with those of an oxidizable substrate, significantly retards or prevents the oxidation of that substrate. An oxidizable substrate might be a lipid, DNA, protein, or any molecule found in vivo[71]. The antioxidant can also be defined as any substance that delays, prevents, or removes oxidative damage to a target molecule. However, it defined antioxidant as any substance that directly scavenges ROS or indirectly acts to up-regulate antioxidant defenses or inhibit ROS production. An antioxidant may have the ability to convert free radicals to a new stable radical through intramolecular hydrogen bonding on further oxidation[72]. Antioxidant functions imply lowering oxidative stress, DNA mutations, malignant transformations , as well as other parameters of cell damage. Epidemiological studies proved antioxidants' ability to contain the effects of reactive oxygen species activity and diminish the incidence of cancer and other degenerative diseases[73]. Antioxidants (either endogenously generated or externally supplied) are capable of scavenging ROS and reducing the oxidation of cellular molecules, thus alleviating oxidative stress[74]. The possible mechanisms by which antioxidants may project against ROS toxicity are:

- Prevention of ROS formation
- Interception of ROS attacks by scavenging the reactive metabolites and converting them to less reaction molecules and/or by enhancing the resistivity of sensitive biological targets to ROS attacks.
- Facilitating the repair of damage caused by ROS.
- Providing (e.g., a cofactor or by acting to maintain a suitable redox status) a favorable environment for the effective functioning of other antioxidants[65]

Classification of Antioxidants

The antioxidant defense systems of the human body are complex, and various classification systems exist. An antioxidant defense can be parted into two groups: the enzymatic and non-enzymatic the first one include SOD, peroxidases such as catalase and glutathione peroxidase, glutaredoxins, thioredoxins and glutathione S-transferases (GST)[75]. The second group typically consist of small molecules which are soluble in an aqueous environment or in a lipid environment, which include glutathione, ascorbate (vitamin C), tocopherols (vitamin E), phytochelatins, polyamines, metallothioneins, and other products[75]

Antioxidants can be a category by their mode of function (Fig. 1-6):

Preventive antioxidants: preventive antioxidants act by binding to and sequestering oxidation-enhancers and transition metal ions, such as iron and copper, which contain unpaired electrons and strongly accelerate free radical formation such superoxide dismutase enzymes, catalase, glutathione peroxidase, and DNA repair enzymes.

Scavenging antioxidants: scavenging or chain-breaking antioxidants. These antioxidants act by presenting themselves for oxidation at an early stage in the free radical chain reaction, producing low-energy molecules that are unable to propagate the chain further. Scavenging antioxidants are included ascorbate, carotenoids, uric acid, α -tocopherol, polyphenols, bilirubin, albumin, ubiquinone, reduced glutathione, and other thiols[76].

Exogenous antioxidants: carotenoids, ascorbic acid, Vitamin E (tocopherols, tocotrienols) ,polyphenols, folic acid cysteine .

Endogenous antioxidants: catalase, superoxide dismutase, , reduce glutathione ,glutathione peroxidase, ceruloplasmin ,glutathione-S-transferase, transferrin, ferritin, glycosylases[76] .

Antioxidants are synthesized inside the body and can also be gained from the food when humans eat it, such as vegetables, fruits, nuts, seeds, oil meats and. There is a balance between antioxidant production and free radicals in the body. The ability of antioxidants to counteract placental lipid peroxides in pregnancy induced hypertension may be a pathologic state in oxidative stress development[78].

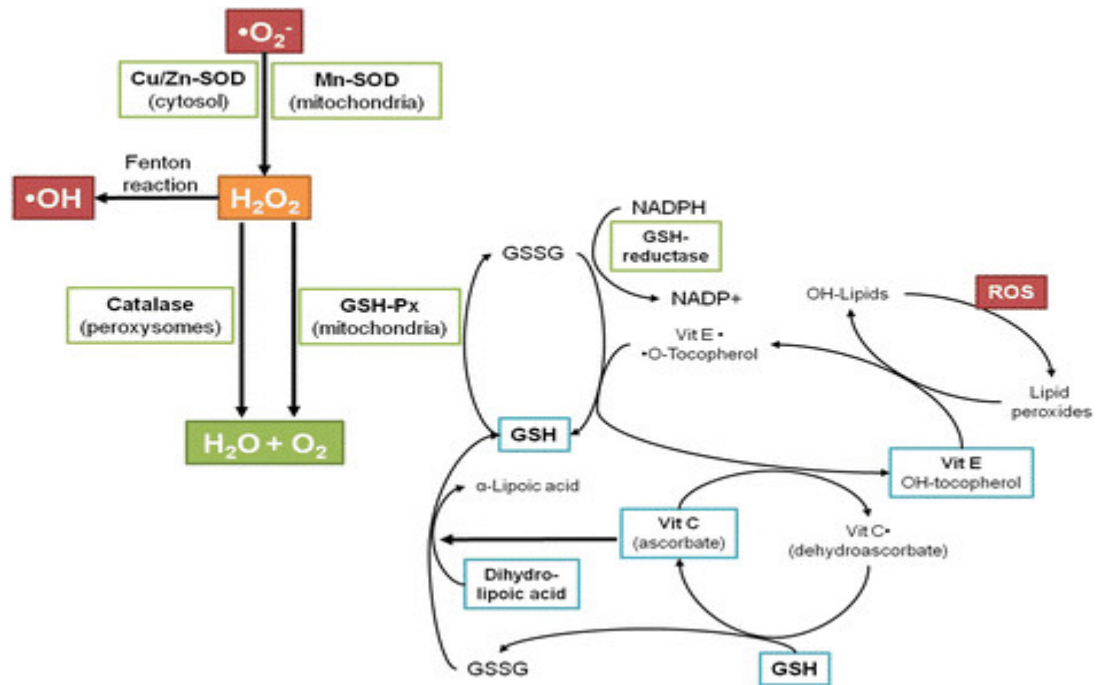


Figure 1.6: Antioxidants defenses in the organism.

1.2.6.1 Ceruloplasmin (Cp)

Ceruloplasmin is a blue multi-copper oxidase used as a large carrier of copper ions in the plasma. The protein is primarily synthesized as a single-chain polypeptide in the liver and secreted as $\alpha 2$ -glycoprotein in the plasma[79]. It is a metal-associated protein. This consists of a single sequence of peptides containing 1046 amino acids and three amino-glucose molecules together with a side chain of several sugars and the molecular weight of the 132 KD peptide sequence, and each Cp molecule has 6 to 8 copper atoms, The effectiveness of Cp as an antioxidant depends on the amount of Cu^{+2} in the protein, since Cp acts to eliminate the radical $\text{O}_2^{\cdot -}$ superoxide by reducing the protein's copper atom[80]. Substantial inactivation of Cp may occur during

exposure to oxidative stress, and free copper ions may be released. Therefore, when exposed to oxidative stress, damaged Cp can cause increased free radical-mediated damage to other macromolecules. Cp is, therefore, a very important component of the cellular defense mechanism against toxicity[81]. Cp is responsible for the control of levels of free copper ions, thus preventing the start of lipid peroxidation[82]. In addition, Ceruloplasmin possesses ferroxidase activity in that it oxidises the more toxic ferrous ions to the less toxic ferric form, The oxidation of Fe^{+2} to Fe^{+3} by Cp is believed to decrease oxidative stress by inhibiting the fenton reaction which uses Fe^{+2} to produce ROS[83]. As shown in (Fig1-7).

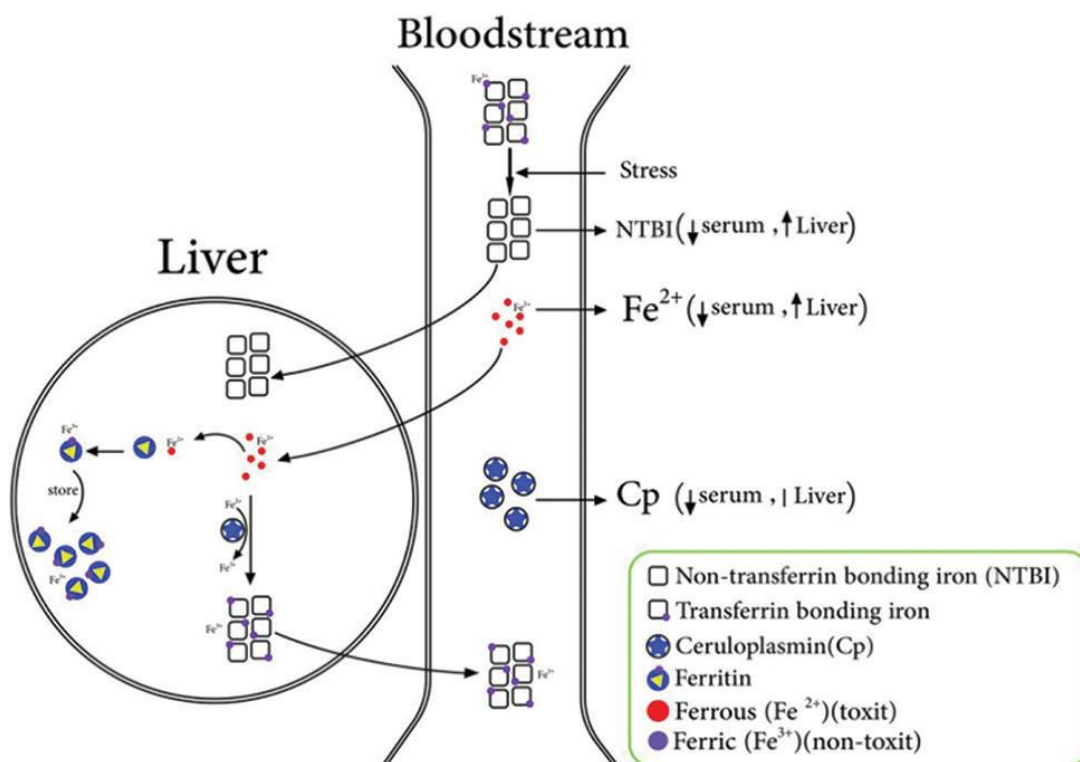


Figure 1.7: The suggested mechanism for ceruloplasmin function as a ferroxidase[84].

1.2.6.2 Albumin (Alb)

Albumin (Alb) is a single polypeptide consisting of 585 amino acids with MW of approximately 66248 Dalton (Figure 1-8), synthesized by the liver. It is most abundant in human plasma. It usually constitutes about 55–60% of all plasma proteins and has a serum half-life of about 20 days[85]. Albumin is not stored by the liver, but it is secreted into the portal circulation as soon as it is manufactured. Albumin may have significant antioxidant potential additions to its property as a carrier protein for copper ions[86]. It is involved in the scavenging of oxygen free radicals. Albumin has several important physiological functions. Regulates colloidal osmotic pressure and transports many endogenous compounds such as fatty acids, hormones, bile acids, amino acids, metals, and toxic metabolites. Additionally, there are wide diversity of drugs that are delivered to their target organs / tissues by binding to HAS.

It also functions as antioxidant from its ability to bind bilirubin and homocysteine because it contains one high-affinity site (Lys240) for bilirubin[85]. The resulting HAS bound bilirubin acts as an inhibitor of lipid peroxidation and thus represents an indirect antioxidant property of HAS[85] . Several studies have revealed that albumin may suppress hydroxyl radicals and peroxy radicals as a strong oxidant scavenger in human plasma[87].

Hypoalbuminemia is a state of low-level albumin in serum that occurs in women with the disease state. Previous researches in biochemical proven that serum albumin has a role as an antioxidant, scavenging carbon-centered free radicals. However, the correlation between levels of human serum albumin, oxidative stress, and endothelial function in pregnant women, especially those with PIH, remains unknown[88]

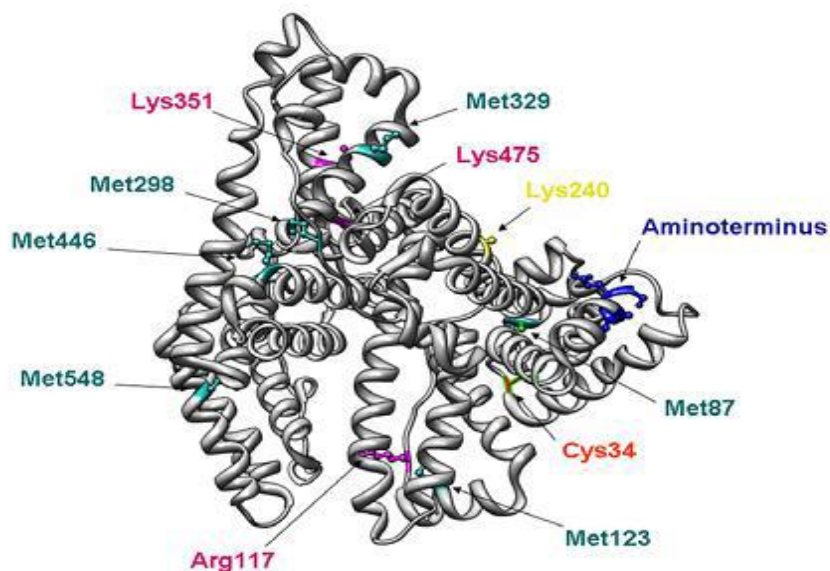


Figure 1.8: Structure of Albumin

1.2.7 Creatinine

Creatinine is the cyclic anhydride of creatine, which is an end product of the dissociation of phosphocreatine[89]. The kidneys, liver, and pancreas are sites of production of creatine, which are enzymatically modulated. The initial reaction involves the transamidation of arginine and glycine to form guanidine acetic acid. This is then followed with methylation of acid occurs with s-anosylmethionine as the methyl donor[90]. Creatinine is then transported to other organs such as the brain and muscles, where it is phosphorylated to phosphocreatine, a high-energy compound. The interconversion of phosphocreatine to creatinine is a particular feature of the metabolic process of muscle contraction[91]. In human subjects, creatinine reacts non-enzymatically with $\cdot\text{OH}$ to form creatol (CTL:5-hydroxycreatinine) and demethylcreatinin (DMC) in a one-to-one ratio, and CTL partially decomposes to methyl guanidine (MG) as shown in Figure (1-9):

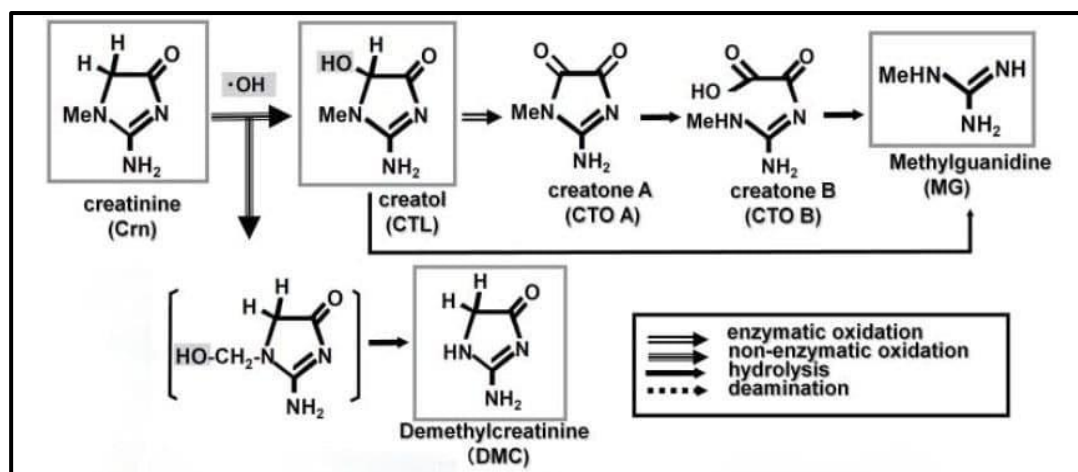


Figure 1.9: Metabolic pathway of Creatinine[91]

1.2.8 Aldosterone

Aldosterone, a mineralocorticoid synthesized from cholesterol in the zona glomerulosa of the adrenal cortex, has appeared in recent years as a key cardiovascular hormone. In addition to the regulation of liquids and electrolyte equilibrium and blood pressure, it is now known to exert its effects on number of various non-adrenal sites, including the heart and the central nervous system. Up to 15% of patients with essential hypertension have inadequate regulation of aldosterone[92], and the hormone is believed to play an crucial role in determining cardiovascular risk. Aldosterone concentrations increase in the luteal phase of the menstrual period, and if conception happens, its levels raise significantly (around 20-fold) throughout pregnancy towards term[93]. Normal pregnancy is distinguished by marked vasodilatation and by raise in the extracellular and intracellular volume of around 6-7 liters[94], the risen aldosterone concentrations are believed to facilitate this volume expansion, ensuring adequate uteroplacental perfusion. Levels of glucocorticoid cortisol are also markedly elevated through normal pregnancy. The developing placenta is protected from high levels of cortisol, however, by the enzyme 11- β -hydroxysteroid dehydrogenase type 2 (11- β HSD-2), which converts cortisol into the inactive cortisone. As a result, cortisol is

practically absent in normal placentas. In preeclampsia, the balance of cortisol and aldosterone regulation is changed; 11- β HSD-2 activity is lowered, and as a result, trophoblasts are exposed to increased concentrations of cortisol. Aldosterone levels are also substantially lowered in preeclampsia, and this reduction is paralleled by a decrease in plasma volume expansion and placental growth[95].

Given the important role that cortisol and aldosterone play in blood pressure regulation in the non-pregnant population, these findings have led to the proposal that genetic differences in their production can contribute to the progression of preeclampsia. (Shojaati et al) reported that some women with preeclampsia carry genetic mutations that lead to reduced activity of aldosterone synthase, resulting in increased levels of aldosterone precursors but lowered and inefficient production of aldosterone itself[96]. Supporting this theory, elevated aldosterone concentrations, measured as urine tetra-aldosterone excretion, are linked with decrease maternal blood pressure in pregnancy and with larger, healthier neonates[97]. Reduced aldosterone production, due to genetic differences in the CYP11B2 gene, or for other reasons, in the face of elevated progesterone availability, may therefore decrease the compensatory potential for shifts in circulating volume (Figure 1-11). The practical consequences of these findings are unclear. Given its role in essential hypertension, aldosterone may also help to clarify the elevated cardiovascular risk seen in women with a history of preeclampsia. The aldosterone synthase pathway includes three enzymatic reactions (Figure 1-10). First, the hydroxylation of deoxycorticosterone to corticosterone by 11 β -hydroxylase activity, second the hydroxylation of corticosterone to 18-hydroxycorticosterone by 18 β -hydroxylase activity, and third, the methylation of 18-hydroxycorticosterone by 18-methyloxydase to yield aldosterone. To assess the different enzyme activities, urinary steroids may be quantified (Figure 1-10). Thus,

single components of aldosterone synthase can be quantified separately. TH-aldosterone increases during pregnancy, but in preeclampsia, it is almost as decrease as in non-pregnant women. Its immediate precursor, 18-OH-TH-corticosterone, is increased in preeclampsia when compared to normal pregnancy. Because of a change in 18-methyloxidase activity, aldosterone production is lowered, favouring thus extracellular volume depletion[98].

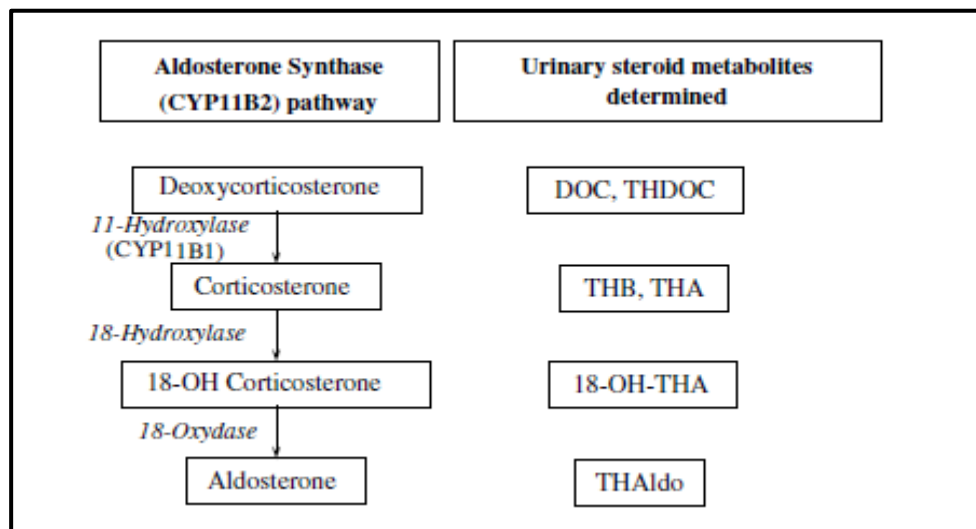


Figure 1.10: Enzymatic pathway of 11-hydroxylase and aldosterone synthase and their corresponding urinary metabolites[98]

As high aldosterone levels are required during normal pregnancy to increase the circulating plasma volume as an adaptative mechanism to support placental perfusion and fetal substrate delivery, a diminished aldosterone production attributed to genetic predisposition or exogenous factors would abrogate the pregnancy-associated expansion of circulating fluid volume in preeclampsia. As a consequence, the reduced intravascular volume depletion would reduce placental perfusion, causing placental ischemia and maternal hypertension (Figure 1-11)

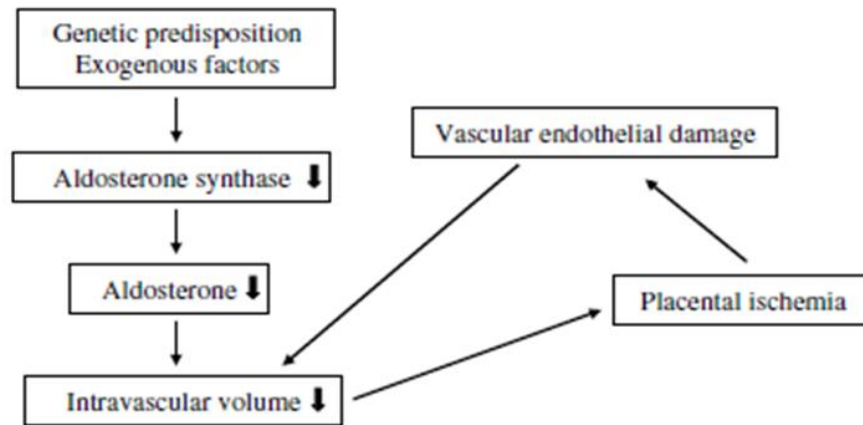


Figure 1.11: Possible mechanism of hypertension development in preeclampsia[99]

1.2.9 Role of Vitamin C in Lipid Peroxidation

The chemical and biological properties of the ascorbic acid proposition that it can serve as an antioxidant in vivo[100]. Vitamin C is the most important antioxidant because it directly neutralizes free radicals. It is not very reactive with common cellular oxidants like hydrogen peroxide and may preferentially react with hydrogen peroxide breakdown products[101]. Vitamin C has the capacity to protect against lipid peroxidation as in (Figure 1-12), by acting as a scavenger of ROS and by one-electron reduction of lipid hydroperoxyl radicals via the vitamin E redox cycle[102].

LPO[•] radical may damage the macromolecules (DNA, RNA, and proteins) and can initiate cytotoxic, genotoxic, and inflammatory reactions. Vitamin C converts lipid peroxidation products into unreactive vitamin C-LPO products. This supports to prevention of the interaction of macromolecules (DNA, RNA, and proteins) with LPO[•] radicals.

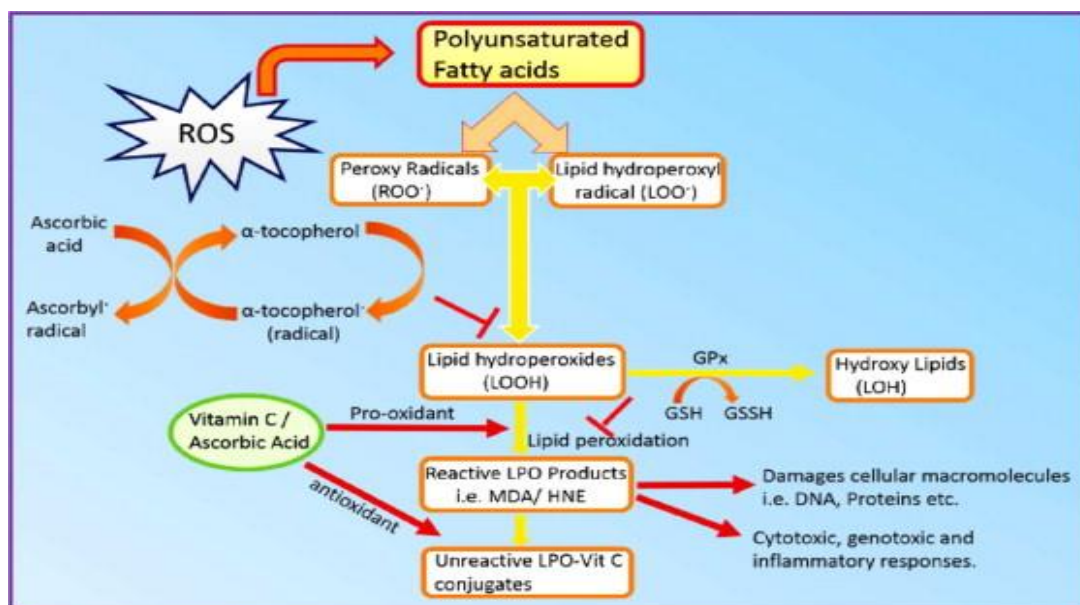


Figure 1.12: Schematic presentation of ROS-mediated lipid peroxidation chain reaction. Vitamin C serves a dual role of a prooxidant and an antioxidant[102]

1.3 Aim of Study

The aims of the present study are;

1. Evaluation of lipid peroxidation products as serum malondialdehyde and antioxidant status in hypertensive and preeclampsia patients in the 2nd and 3rd trimesters of pregnancy.
2. Highlighting the possible correlations of hemoglobin level with some biochemical parameters in hypertensive and preeclampsia patient's groups.
3. Estimation of the possible correlations between urine and serum albumin creatinine ratio in these patient's groups.
4. To quantify the effects of maternal characteristics, maternal blood parameter and some biochemical factors on MAP levels of PIH, PE and normotensive pregnant groups

Chapter Two

Materials & Methods

2. Materials and Methods

2.1 Design of Study

This study was conducted at Al Samawah maternity and children teaching hospital in collaboration between maternity and biochemistry departments from October 2020 to February 2021.

The study includes (120) subjects. 40 with healthy women, uncomplicated pregnancy, divided to 20 subjects at 2nd trimester (2nd C) and 20 subjects at 3rd trimester (3rd C); 40 women with pregnancy-induced hypertension (20 subjects as 2nd H and 20 subjects as 3rd H) and 40 women with pre-eclampsia (20 subjects for each 2nd PE and 3rd PE).

Table 2.1:Data of patients(Pregnant women with hypertension) and controls(healthy pregnant women) groups

Groups	N
Patients	80
Controls	40

Table 2.2: Data of the studied groups

Group	No.
Group (2 nd C)	20
Group (3 rd C)	20
Group (2 nd H)	20
Group (3 rd H)	20
Group (2 nd PE)	20
Group (3 rd PE)	20

2.1.1 Inclusion Criteria

1. Hypertensive patient (without proteinuria) and normotensive pregnant.
2. Preeclamptic patients(> 140/90mmHg, proteinuria and oedema)
3. All the pregnant women were in their second and third trimester of pregnancy.
4. Gestational age of >14 weeks with Age range 18-40 years.

2.1.2 Exclusion Criteria

Gestational Diabetes, Chronic hypertension, Twin pregnancy, Liver failure, Renal failure, Thyroid disorders, First trimester of pregnancy, Pregnant women with asthma or cardiovascular disease

2.2 Collection of Blood Samples

Blood samples are collected. About (5 mL) of blood samples of Pregnant women with hypertension and controls are taken and allowed to clot at room temperature in empty disposable tubes and centrifuged to separate it at 3000 rotor per minute (rpm) for 10 min , the serum samples are separated and stored at (-20°C) for later measurement of biochemical parameters , unless used immediately.

The clinical chart and biochemical tests of this study describe experimental parameters as in Tables (2-3) and (2-4).

Table 2.3: The clinical chart of the study

Date:
Name :
Weight in Kg:
Height in cm:
Age in years:
Gestational age in weeks:
Systolic blood pressure in mm Hg:
Diastolic blood pressure in mm Hg:
Accompanid diseases:
Others:

Table 2.4:Biochemical tests of the study

Haematological Parameters	Hb%
Lipid peroxidation	Serum Malondialdehyde (MDA)
Antioxidants	Serum ceruloplasmin (Cp) , Vitamin C, Albumin
Hormones	Aldosterone
Albumin/Creatinine ratio	Urine Albumin
	Urine Creatinine
	Albumin, creatinine in blood serum

2.3 Chemicals

The chemicals used in this study are shown with suppliers in Table (2-4)

Table 2.5:Chemicals and their suppliers

Chemicals	Suppliers
Glacial acetic acid	BDH, England
Para phenylenediamine (PPD)	BDH , England
Sodium acetate	BDH, England
Albumin Kit	Biolabo (France)
Creatinine Kit	Roche (Germany)
Thiobarbituric acid (TBA)	BDH , England
Aldosterone kit	Human, China
Vitamin C Kit	Human, China
Sodium azide	Reidledehain, Germany
Trichloroacetic acid	BDH, England
Urine albumin kit	Alere Abbott, USA

2.4 Instruments

The instruments are available in Al Samawa maternity and children teaching hospital in collaboration between maternity and biochemistry departments (Al-Muthanna, Iraq), which are shown in Table (2-6).

Table 2.6:The instruments and their manufacturers

Instruments	Manufacturers
Cobas C 111	Human , Germany
Centrifuge	Universal 16A , Germany
Incubator	Memmert , Germany
Sensitive balance	Kern ABS/ABJ-BA-e-1019, Germany
Shacking water bath	Lab Companion BS-11, Korea
UV/VIS spectrophotometer	Perkin Elmer Lambda 35
Water bath	L513-0814 , French
ELISA	Biotek Elx800 , USA

2.5 Blood parameter

Estimation of Hemoglobin from Packed Cell Volume (Microhematocrit method)

Principle

Anticoagulated whole blood is centrifuged in a capillary tube of uniform a bore to pack the red cells. Centrifugation is done in a particular microhematocrit centrifuge until packing of red cells is as complete as possible. The reading (length of packed red cells and total length of the column) is taken utilizing a microhematocrit reader, a ruler or an arithmetic graph paper[103].

Procedure

1. Fill the capillary tube by applying its tip to the blood (obtained from skin puncture or anticoagulated venous blood, depending on the type of tube used). About 2/3rds to 3/4ths length of the capillary tube should be filled with blood.
2. Seal the other end of the capillary tube (which was not in contact with blood) with a plastic sealant. If it is not available, heat-seal the tube using a spirit lamp.
3. The filled tubes were placed in the radial grooves of the centrifuge with the sealed ends toward the outer rim gasket. Counterbalance by placing the tubes in the grooves opposite to each other.
4. Centrifuge at relative centrifugal force 12000 g for 5 minutes to completely pack the red cells.
5. Immediately remove the tubes from the centrifuge and stand them upright. The tube will show three layers from top to bottom: column of plasma, thin layer of buffy coat, and column of red cells.
6. With the microhematocrit reader, hematocrit was directly read from the scale. If hematocrit reader is not available, the tube was held against a ruler and the hematocrit was obtained by the following formula

Length of red cell column in mm
Length of total column in mm

The above result is multiplied by 100 to obtain PCV.

Calculation

Hemoglobin (gm/dl)= $\frac{\text{PCV} - 1}{3}$

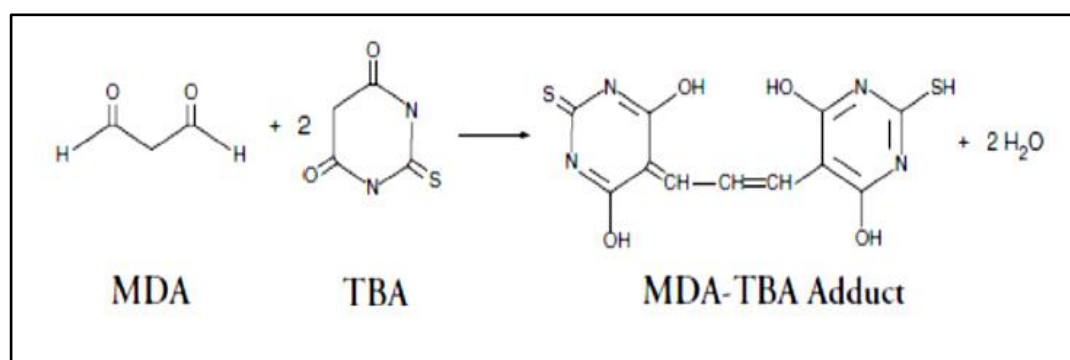
2.6 Biochemical Parameters

Several considerable methods were utilized to measure the studied parameters. It was notable that all measurements are duplicated for each sample.

2.6.1 Determination of Serum Malondialdehyde ($\mu\text{mol/L}$) Concentration

Principle

MDA formed from the breakdown of polyunsaturated fatty acids are described as the product of LPO that react with thiobarbituric acid (TBA) in coexisting trichloroacetic acid (TCA) to give a pink chromophore absorbing at 532 nm. MDA concentration is calculated by using the molar extinction coefficient of MDA equal to $1.56 \times 10^5 \text{ mol}^{-1} \cdot \text{cm}^{-1}$ [104]. MDA formed from the breakdown of polyunsaturated fatty acid serves as a convenient index of peroxidation reaction.



Procedure

The level of serum MDA was determined by spectrophotometrically with thiobarbituric acid (TBA) solution. In brief, to 150 μl serum sample, the following were added: 1ml (17.5%) trichloroacetic acid (TCA) and 1ml of 0.66 % TBA, mixed, incubated in boiling water for 15 minutes, and then allowed to cool. One ml of 70 % TCA was added and the mixture allowed to stand at room temperature for 20 minutes, centrifuged at 2000 rpm for 15 minutes, the supernatant was taken out for measuring spectrophotometrically at (532nm)[105]. The following equation was used to calculate the concentration of MDA:

$$MDA \left(\frac{\mu\text{mol}}{L} \right) = \frac{\text{absorbance at } 532\text{nm}}{L \times E_0} \times D \times 10^6$$

L : light path (1cm)

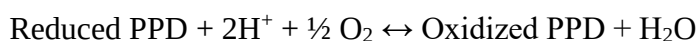
E₀ : Extinction coefficient 1.56×10⁵ M⁻¹.cm⁻¹

D : Dilution factor = 1 ml Vol. Used in ref./0.15 = 6.7

2.6.2 Determination of Ceruloplasmin (mg/dl) Concentration

Principle

Plasma Cp concentration was measured by the method of (Menden et al) [106]. It is based on the Cp-catalyzed oxidation of colourless paraphenylene diamine (PPD) to blue-violet oxidize form. The reaction was followed photometrically, and the blank value was determined after inhibition of the enzyme with sodium azide at ice.



(Colorless)

(Blue)

A mixture of serum, substrate and acetate buffer at pH = 6.0 was incubated at 37°C for 15 min. The reaction was stopped by the addition of sodium azide, and the absorbance of the purple color formed (Oxidized PPD) in the diluted test mixture is read at 525 nm against blank solution. The corrected absorbance is directly linked to the concentration of Cp [107].

Reagents

Substrate solution

Fifty mg of PPD was dissolved in 5 mL (4 mL of distilled water and 1 mL of glacial acetic acid). In another container 8.15gm sodium acetate trihydrate was dissolved in 30 mL of DW then added to the first solution, mixed and completed the volume to 50 mL with DW This solution is stable for three hours. It was kept refrigerated in a dark bottle [106]

Inactivating solution

One hundred mg of sodium azide was dissolved in (500 mL) of Dw then is kept cold in a refrigerator prior to use. This solution must be prepared every week.

Procedure

- One ml of the substrate was pipetted into test tubes, incubated at 37 °C for 1 min.
- One hundred µL of plasma was added, incubated at 37 °C for 15 min. Then tubes were removed and placed in iced-bath for 30 min.
- Five ml of cold inactivating solution was added,mixed and the temperature is brought to 25 °C in a water bath.
- Blank was prepared by combining the substrate,inactivating solution,and 0.1 ml of Dw, then the mixture is incubated as above.
- The absorbance of test and blank was read in a spectrophotometer at 525nm
- The concentration of Cp is calculated using the extinction coefficient of Cp (ξ_{Cp}) equal to (0.68)

$\text{Serum Cp (mg/dl)} = \frac{A_{\text{test}} - A_{\text{blank}}}{\xi_{Cp}} \times 10 = \frac{\Delta A \times 10}{0.68}$

2.6.3 Determination of Serum Aldosterone Concentration (ng/L)

Principle

This kit was an enzyme-linked immunosorbent assay (ELISA) type sandwich elisa. The plate was pre-coated with human ALD antibody. ALD present in the sample was added and bound to antibodies coated on the wells. And then biotinylated human ALD Antibody is added and bound to ALD in the sample. Then streptavidinHRP was added and binds to the biotinylated ALD antibody. After incubation, the unbound HRP streptavidin was washed away during a washing step. Substrate solution(TMB) was

then added and color develops in proportion to the amount of human ALD. The reaction was terminated by the addition of an acid stopping solution and the absorbance is measured at 450 nm.

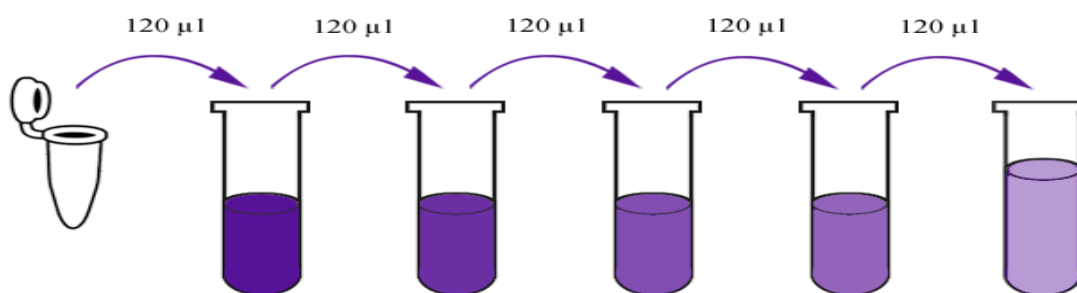
Reagents

Components	Quantity
Standard Solution (2400ng/L)	0.5 ml x1
Pre-coated ELISA Plate	12 * 8 well strips x1
Standard Diluent	3 ml x1
Streptavidin-HRP	6 ml x1
Stop Solution	6 ml x1
Substrate Solution A	6 ml x1
Substrate Solution B	6 ml x1
Wash Buffer Concentrate(25x)	20 ml x1
Biotinylated human ALD Antibody	1 ml x1
User Instruction	1
Plate Sealer	2 pics
Zipper bag	One pic

Reagents Preparation

- All reagents should be brought to room temperature before utilize.
- Standard Reconstitute the 120 μ l of the standard (2400ng/L) with 120 μ l of standard diluent to generate a 1200ng/L standard stock solution. Permit the standard to sit for 15 minutes with gentle agitation before making the dilutions. Prepare duplicate standard points by serially diluting the standard stock solution (1200ng/L) 1:2 with standard diluent to yield 600ng/l, 300ng/L, 150ng/L and 75ng/L solutions. The Standard diluent acts as the zero standard(0 ng/L). Any remaining solution should be frozen at -20 and utilized within one month. The suggested dilution of the standard solutions is as follows:

1200ng/L	Standard No.5	120 µl Original Standard + 120 µl Standard Diluent
600ng/L	Standard No.4	120 µl Standard No.5 + 120 µl Standard Diluent
300ng/L	Standard No.3	120 µl Standard No.4 + 120 µl Standard Diluent
150ng/L	Standard No.2	120 µl Standard No.3 + 120 µl Standard Diluent
75ng/L	Standard No.1	120 µl Standard No.2 + 120µl Standard Diluent



Standard Concentration	Standard No.5	Standard No.4	Standard No.3	Standard No.2	Standard No.1
2400ng/L	1200ng/L	600ng/L	300ng/L	150ng/L	75ng/L

- **Wash Buffer** Dilute 20ml of Wash Buffer Concentrate 25 times into deionized or distilled water to produce 500 ml of 1x Wash Buffer. If crystals have formed in the concentrate, mix gently till the crystals have wholly dissolved.

Assay Procedure

1. Prepare all reagents, standard solutions and samples according to the instructions. Bring all reagents to room temperature before utilize. The assay was done at room temperature.
2. Determine the number of strips needed for the assay. Insert the strips in the frames for utilize. Unused strips should be stored at 2-8°C.

3. Add 50 μ l of standard to the standard well. Note: Don't add the antibody to the standard well because the standard solution contains biotinylated antibody.
4. Add 40 μ l of sample to the sample wells and then add 10 μ l anti-ALD antibody to sample wells, then add 50 μ l streptavidin-HRP to sample wells and standard wells (Not blank control well). Mix well. Cover the plate with a sealer. Incubate 60 minutes at 37°C.
5. Remove the sealant and wash the plate 5 times with wash buffer. Soak the wells with at least 0.35 ml wash buffer for 30 seconds to 1 minute for each wash. For automated washing, aspirate all wells and wash 5 times with wash buffer, overfilling wells with wash buffer. Blot the plate onto paper towels or other absorbent material.
6. Add 50 μ l substrate solution A to each well and then add 50 μ l substrate solution B to each well. Incubate plate covered with a new sealer for 10 minutes at 37°C in the dark.
7. Add 50 μ l of Stop Solution to each well, the blue color will immediately change to yellow.
8. Immediately determine the optical density (OD value) of each well using a microplate reader set at 450 nm within 10 minutes of adding the Stop Solution.

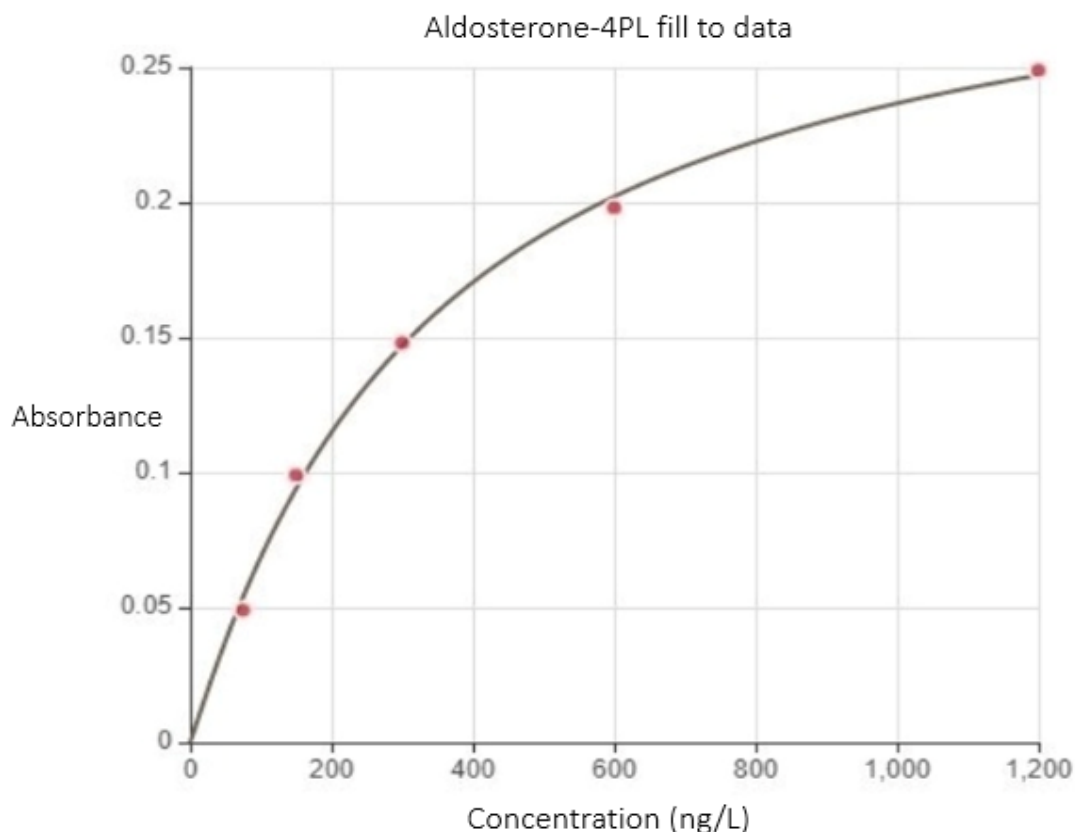


Figure 2.1: Standard curve of Aldosterone

2.6.4 Determination of Serum Vitamin-C Concentration (ng/ml)

Assay Principle

This kit was Enzyme-Linked Immunosorbent Assay (ELISA) type sandwich elisa. The plate was precoated with human VC antibody. The VC present in the sample was added, and the antibodies coated on the wells bind to the VC. And then a biotinylated human VC antibody is added which binds to the VC in the sample. Then streptavidinHRP is added and binds to the biotinylated VC antibody. After incubation unbound StreptavidinHRP was washed away during a washing step. Substrate solution (TMB) was then added and the color develops in proportion to the amount of human VC. The reaction was terminated by the addition of an acid stopping solution and the absorbance is measured at 450 nm.

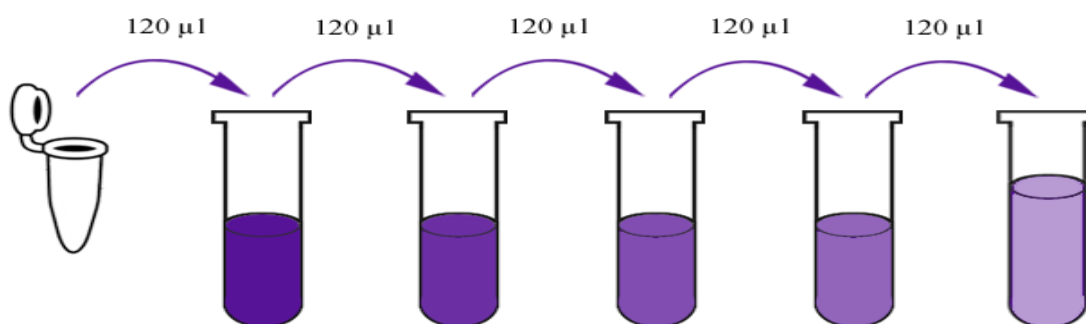
Reagents

Components	Quantity
Standard Solution (320ng/ml)	0.5ml x1
Pre-coated ELISA Plate	12 * 8 well strips x1
Standard Diluent	3ml x1
Streptavidin-HRP	6ml x1
Stop Solution	6ml x1
Substrate Solution A	6ml x1
Substrate Solution B	6ml x1
Wash Buffer Concentrate (25x)	20ml x1
Biotinylated human VC Antibody	1ml x1
User Instruction	1
Plate Sealer	2 pics
Zipper bag	1 pic

Reagents Preparation

- All reagents should be brought to room temperature before utilize.
- Standard Reconstitute the 120 μ l of the standard (320ng/ml) with 120 μ l of standard diluent to generate a 160ng/ml standard stock solution. Allow the standard to sit for 15 minutes with gentle agitation before making the dilutions. Prepare duplicate standard points by serially diluting the standard stock solution (160ng/ml) 1:2 with standard diluent to produce 80ng/ml, 40ng/ml, 20ng/ml and 10ng/ml solutions. The standard diluent acts as the zero standard(0 ng/ml). Any remaining solution should be frozen at -20 and utilized within one month. The suggested dilution of the standard solutions is as follows:

160ng/ml	Standard No.5	120 µl Original Standard + 120 µl Standard Diluent
80ng/ml	Standard No.4	120µl Standard No.5 + 120µl Standard Diluent
40ng/ml	Standard No.3	120µl Standard No.4 + 120µl Standard Diluent
20ng/ml	Standard No.2	120µl Standard No.3 + 120µl Standard Diluent
10ng/ml	Standard No.1	120µl Standard No.2 + 120µl Standard Diluent



Standard Concentration	Standard No.5	Standard No.4	Standard No.3	Standard No.2	Standard No.1
320ng/ml	160ng/ml	80ng/ml	40ng/ml	20ng/ml	10ng/ml

- **Wash Buffer** Dilute 20ml of Wash Buffer Concentrate 25 times into deionized or distilled water to produce 500 ml of 1x Wash Buffer. If crystals have formed in the concentrate, mix gently till the crystals have wholly dissolved.

Assay Procedure

1. Prepare all reagents, standard solutions and samples according to the instructions. Bring all reagents to room temperature before utilize. The assay was done at room temperature.
2. Determine the number of strips needed for the assay. Insert the strips into the frames for use. Unused strips should be stored at 2-8°C.

3. Add 50 μ l of standard to the standard well. Note: Don't add the antibody to the standard well because the standard solution contains biotinylated antibody.
4. Add 40 μ l of sample to the sample wells and then add 10 μ l anti-VC antibody to sample wells, then add 50 μ l streptavidin-HRP to sample wells and standard wells (Not blank control well). Mix well. Cover the plate with a sealer. Incubate 60 minutes at 37°C.
5. Remove the sealant and wash the plate 5 times with wash buffer. Soak the wells with at least 0.35 ml wash buffer for 30 seconds to 1 minute for each wash. For automated washing, aspirate all wells and wash 5 times with wash buffer, overfilling wells with wash buffer. Blot the plate onto paper towels or other absorbent material.
6. Add 50 μ l substrate solution A to each well and then add 50 μ l substrate solution B to each well. Incubate plate covered with a new sealer for 10 minutes at 37°C in the dark.
7. Add 50 μ l of Stop Solution to each well; the blue color will immediately change to yellow.
8. Immediately determine the optical density (OD value) of each well using a microplate reader set at 450 nm within 10 minutes of adding the Stop Solution.

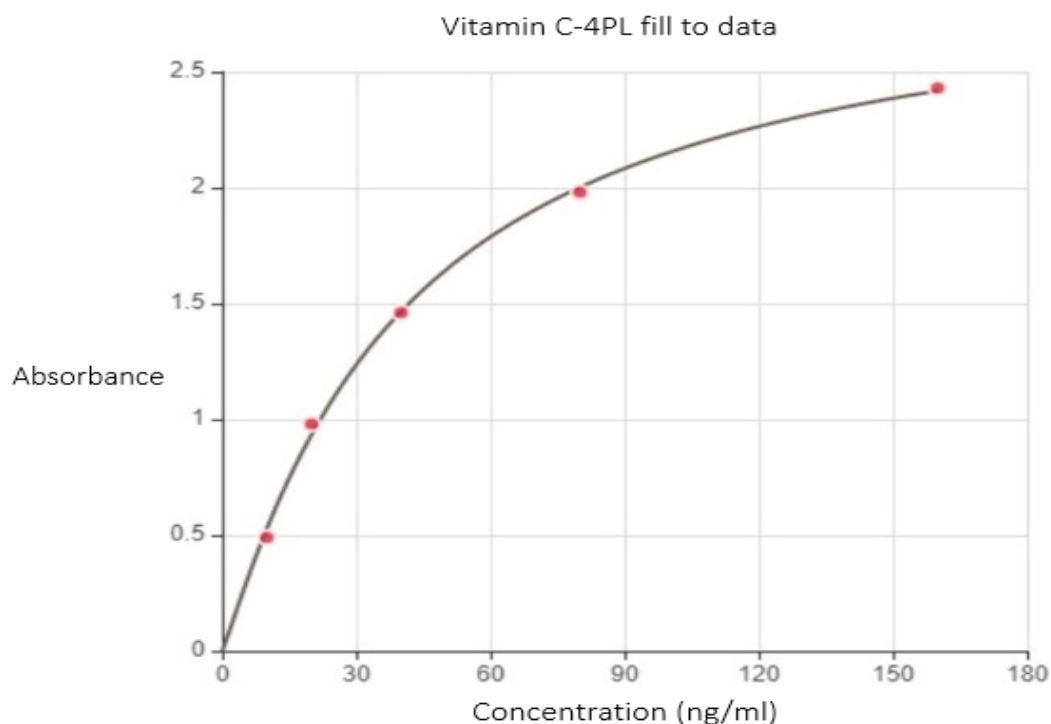


Figure 2.2: Standard curve of Vitamin C

2.6.5 Determination of Serum Albumin (Alb) Concentration (g/dL)

Principle

The simplest technique is the bromocresol green (BCG) method, a colorimetric method (spectrophotometric). The principle of measurement is based on the relatively high affinity of BCG towards Alb. Albumin in the existence of BCG at a slightly acid pH = 4.2, produces a color alter of the indicator from yellow-green to green-blue. The color intensity is proportionate to the concentration of Alb in the sample[108].

Reagents composition

No	Contents	Concentrations of Solutions
R1 (Bromocresol Green)	Succinic acid	83mmol/L
	Bromocresol green	167μmol/L
	Sodium hydroxide	50mmol/L
	Polyoxyethylene monolaurylether	1gm/L
R2(Standard)	Bovine albumin	5g/dL (725μmol/L)

Procedure

Left stand reagents and specimen at room temperature .

Pipette into well identified test tubes	Blank	Standard	Assay
Reagent	2mL	2mL	2mL
Demineralized water	10μL		
Sample			10μL
Standard		10μL	
Mixed well. Absorbance were recorded at 630nm against blank.			
Mixed well. Absorbance (A) were recorded at 630 nm (620-640 nm) within 3 minutes against reagent blank			

Calculation

The Concentration of serum Alb was calculated according to the equation below:

$$\text{Serum Alb (g / L)} = \frac{A_{\text{sample}}}{A_{\text{standard}}} \times \text{Standard concentration}$$

Standard concentration = 5 g/dL (725 µmol/L)

2.6.6 Determination Concentration of Creatinine in Blood Serum (mg/dl)

Principle

This kinetic colorimetric assay is based on the Jaffé method[109].In alkaline solution, creatinine forms a yellow-red complex with picrate. The rate of dye formation is proportional to the creatinine concentration in the specimen. The assay uses “rate-blanking” to minimize interference by bilirubin. To correct for non-specific reaction caused by serum/plasma pseudo-creatinine chromogens, including proteins and ketones, the results for serum or plasma are corrected by -18 µmol/L (-0.2 mg/dL). Serum and Creatinine was measured using an automatic analyzer (Cobas c111, Roche®)



Reagents - working solutions

Reagent	Composition
R1	Potassium hydroxide: 900 mmol/L; phosphate: 135 mmol/L;pH ≥ 13.5; preservative; stabilizer
SR	Picric acid: 38 mmol/L; pH 6.5; non reactive buffer

Pipetting parameters

Serum/plasma	Sample volume or Reagent	Diluent (H ₂ O)
R1	13µL	71 µL
Sample	10 µL	20 µL
SR	17 µL	16 µL

Calculation

Automatically calculates the serum Creatinine concentration of each sample. The results are given in mg/dl.

To convert serum creatinine mg/dl to µmol/l, multiply by 88.4

2.6.7 Determination of urine Albumin Concentration (Microalbumin Urine) (mg/dL)

Test principle

Urine Albumin is a solid phase,sandwich format, immunometric assay. The test device involves a membrane coated with immobilised albumin special monoclonal antibodies. When the diluted sample is applied to the test device, the sample flows through the membrane, and immobilised antibodies on the membrane capture albumin molecules. Albumin trapped on the membrane will bind the gold-antibody conjugate then added, in a sandwich-type reaction. Unbound conjugate removed from the membrane by the washing solution. The paper layer under the membrane absorbs excess liquid. Due to the bound gold particles the membrane seems purple with colour intensity proportionate to the albumin concentration of the sample.The colour intensity is measured quantitatively by utilizing the color densitometer NycoCard READER II.

Reagents Composition

Reagent	Composition
R1/Dilution liquid (1 x 24 x 1.0 mL)	Phosphate buffer(pH=5.6), organic solvent (<10%) and a small amount of a yellow pigment.
R2/Conjugate (1 x 2.0 mL)	Borate buffered solution involving monoclonal anti-albumin antibodies labelled with ultra-small gold particles.
R3/Washing Solution (1 x 2.0ml)	Phosphate buffered NaCl solution (pH 7.4).

Test procedure

1. Sample dilution:

Add 50 μ L urine sample or control to a test tube with R1/Dilution Liquid. blend well. Note: R1/Dilution Liquid must have room temperature (15-25°C) when used.

2. Application of sample:

Apply 50 μ L diluted urine or diluted control to a Test Device. Allow the diluted sample to soak completely into the membrane (approximately 50 sec.). Note: Avoid air bubbles.

3. Application of R2/Conjugate:

Apply 50 μ L of R2/Conjugate to the test device. Allow the conjugate to soak completely into the membrane (approximately 50 sec.). Note: Avoid air bubbles.

4. Application of R3/Washing Solution:

Immediately apply 50 μ L of R3/Washing Solution to the test device. Allow the washing solution to soak completely into the membrane (approximately 50 sec.). Note: Avoid air bubbles.

5. Read the results

Read the results within 5 minutes using the NycoCard READER II. Follow the instructions given in the NycoCard READER II Instruction Manual.



2.7.8 Determination of Urinary Creatinine Concentration (g/dL):

Reagents - working solutions

Reagent	Composition
R1	Potassium hydroxide: 900 mmol/L; phosphate: 135 mmol/L; pH $\geq$ 13.5; preservative; stabilizer
SR	Picric acid: 38 mmol/L; pH 6.5; non reactive buffer

Pipetting parameters

Urine	Sample volume or Reagent	Diluent (H ₂ O)
R1	13 μ L	71 μ L
Sample	10 μ L	20 μ L
SR	17 μ L	16 μ L

Calculation

Automatically calculates the urine Creatinine concentration of each sample. The results are given in mg/dl.

$$\text{Conversion factor} = \frac{C_{\text{creatinine}} (\text{mg/dl}) \times 50}{1000} = \text{g/dl}$$

2.7 Statistical Analysis

The statistical analysis was done by using Microsoft Excel 2010, the results were expressed as mean $\pm$ standard deviation (mean $\pm$ SD) with LSD test. One way ANOVA-test is used to compare parameters in different studied groups. Pearson's correlation was applied to determine the relationship among the present study parameters . P-values ($p \leq 0.05$) are considered statistically significant.

Chapter Three

Results & Discussion

3.1 Demographic parameters

3.1.1 Age Distribution

All groups have age range between (18-40) year. There were nonsignificant differences among all groups at the 2nd trimester. The present study showed that there was significant different increasing ($p<0.05$) in the 3rd trimester of 3H and 3PE when compared with 3C group. Mean value of ages in the 2nd trimester of 2C, 2H, 2PE group was 28.6 ± 5.71 , 26 ± 4.29 and 27.5 ± 4.47 year. Mean value of 3C, 3H and 3PE group in the 3rd trimester was 22.7 ± 2.97 , 26.7 ± 5.14 and 28.7 ± 5.62 year respectively (Table 3.12). Age has an important effect on the occurrence of hypertensive disorders of pregnancy. women under 20 year or over 30 year have an increased chance of hypertension[110]. Many different previous studies showed that nonsignificant variances in age[111].

3.1.2 Gestational Age Distribution

The gestational age was calculated from first day of last menstrual period. Mean value of gestational age in the 2nd trimester of 2C, 2H, 2PE group was 20.2 ± 3.28 , 20.1 ± 4.22 and 23.2 ± 2.39 week respectively. Mean value in the 3rd trimester of 3C, 3H, 3PE group was 34 ± 3.19 , 34.2 ± 2.91 and 34.1 ± 3.01 week respectively (Table 3.12). The present study showed that there were significant differences ($p<0.05$) in 2PE when compared with 2H and control groups. Gestational age showed nonsignificant difference among all these groups in the 3rd trimesters. This result has agree with [112].

3.1.3 Mean Arterial Pressure(MAP)

Pregnancy may be complicated by elevated blood pressure. Women with PIH have a more pronounced response to the potent vasoconstrictor angiotensin II (ANG II), which is responsible for the resulting PIH. In the normal pregnant state, there is an increase in the levels of ANG II and aldosterone, at the same time vascular ANG II sensitivity is greatly reduced, as early as 14 weeks of gestation. Several studies have documented significantly lower ANG II and aldosterone levels in women with PIH, but this reduction is lost after 22 weeks of gestation and predates the development of vascular ANG II sensitivity, then elevating of PIH[113].

The present study revealed that MAP levels in 2PE are significantly higher than 2H group, and each is higher than 2C, contrary to the 3rd trimester, in which 3PE is nonsignificantly differed with 3H, but these two groups are significantly higher than 3C. Mean arterial pressure in the 2nd trimester of 2C, 2H, 2PE group was 88.6 ± 5.24 , 108.9 ± 2.96 and 113 ± 3.42 mmHg. Mean of 3C, 3H, 3PE group in the 3rd trimester was 91.72 ± 4.61 , 111.4 ± 4.49 and 114.1 ± 4.12 mmHg respectively (Table 3.1&Fig 3.1).

Our results are in agreement with previous study which found that MAP in PIH was significantly higher than in normotensive [114]. Many studies confirmed that the development of hypertension in the 2nd trimester of pregnant women who develop preeclampsia, and reported MAP increased from 12 weeks onwards in women who develop preeclampsia[115].The explain of the rises of BP in PIH women is reliable with histological studies of impaired trophoblast invasion for myometrial arterial segments and thereby a necessity to increase placental perfusion, then BP increasing. The reduced circulating volume circumscribes placental blood supply is finally inducing an elevated blood pressure during preeclampsia compared with normal pregnancies[116]. Cnossen et al documented that MAP was a better predictor of pre-

eclampsia than systolic BP and diastolic BP and an increase in BP in first and second trimester [117]. Our study has corresponded with [118] in which systolic and diastolic levels were found significantly increasing in women with PIH and PE as compared to normal pregnant women (Table 3.11).

Table 3.1: MAP levels of pregnant groups at the 2nd and 3rd trimesters

Group	No.	MAP(mmHg) Mean±SD
2nd Trimester		
2nd C	20	88.6±5.24 ^c
2nd H	20	108.9±2.96 ^b
2nd PE	20	113±3.42 ^a
L.S.D		2.53
3rd Trimester		
3rd C	20	91.72±4.61 ^b
3rd H	20	111.4±4.49 ^a
3rd PE	20	114.1±4.12 ^a
L.S.D		2.79

* Each value represents mean ± SD values with non-identical superscript (a, b or c ... etc) were considered significantly different ($p \leq 0.05$).

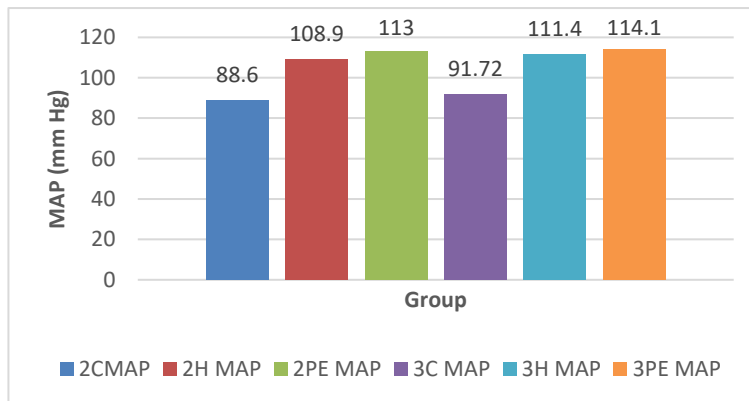


Figure 3.1: MAP levels of pregnant groups at the 2nd and 3rd trimesters

3.2 Serum Haemoglobin (Hb) Concentration(g/dL)

The present study demonstrated that Hb levels have significant increasing in 2PE comparison with 2H and nonsignificant difference in 2H comparing with 2C group. Hb levels showed nonsignificant difference among all these groups in the 3rd trimester. Mean value of Hb level in the 2nd trimester of 2C, 2H, 2PE group was 11.08 ± 1.00 , 10.93 ± 1.05 and 11.68 ± 0.83 g/dL. Mean value of 3C, 3H, 3PE group in the 3rd trimester was 11.13 ± 1.00 , 10.89 ± 1.11 and 10.83 ± 1.00 g/dL respectively (Table 3.2 & Fig 3.2). Hemoglobin concentration, hematocrit and red cell count fall during pregnancy because the expansion of the plasma volume is greater than that of the red cell mass. However, there is a rise in total circulating hemoglobin directly related to the increase in red cell mass. This, in turn, depends partly on the iron status of the individual.

The physiological changes that take place in the cardiovascular and haematological systems during pregnancy have been extensively researched, the increase in plasma volume from the first to the third trimester is between 1100 – 1250 ml, which is an increase in the total blood volume of between 40 to 50% [119]. Other previous studies show during pregnancy, a decrease in hemoglobin concentration occurs, Plasma volume continues to rise until the third trimester when it plateaus at 32 to 34 week. The average pregnancy has nearly 50% increase in blood volume[120]. A small decrease in Hb level is a normal physiological consequence of the increase in blood plasma volume during pregnancy. Normally, after an initial increase (due to the cessation of menstruation), Hb levels decrease by around 20 g/L and reach their lowest level during the second trimester, returning to pre-pregnancy levels as the pregnancy advances toward[121]. Patra et al. concluded that low hemoglobin levels in early gestation are related to preeclampsia and eclampsia and concluded that low periods

between pregnancies and low nutritional supports lead to low hemoglobin levels and adverse complications of pregnancy, particularly in multiparous women[122]. Our result has agreed with the above studies which demonstrated a decrease in mean Hb value in 3PE when compared to 3C, but it is statistically nonsignificant. However, the study found that Hb level of 2PE was significantly higher than of 2H and 2C, which is one limiting of our study.

Table 3.2: Hb levels of pregnant groups at the 2nd and 3rd trimesters

Group	N	Hb (g/dL) Mean±SD
2nd Trimester		
2nd C	20	11.08±1.00 ^b
2nd H	20	10.93±1.05 ^b
2nd PE	20	11.68±0.83 ^a
L.S.D		0.59
3rd Trimester		
3rd C	20	11.13±1.00 ^a
3rd H	20	10.89±1.11 ^a
3rd PE	20	10.83±1.00 ^a
L.S.D		0.66

* Each value represents mean ± SD values with non-identical superscript (a, b or c ... etc) were considered significantly different ($p \leq 0.05$).

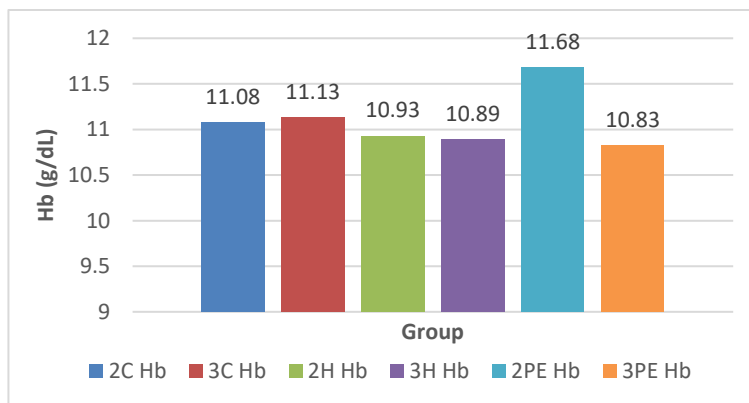


Figure 3.2: Hb levels of pregnant groups at the 2nd and 3rd trimesters

3.3 Serum Malondialdehyde Concentration ($\mu\text{mol/L}$)

The present study showed that serum MDA levels have significant increasing in PE compared with PIH and significant increasing in PIH compared with healthy groups for each 2nd and 3rd trimester. Mean of serum MDA level in the 2nd trimester of 2C, 2H, 2PE group was 1.85 ± 0.46 , 5.82 ± 0.56 and 9.18 ± 1.54 $\mu\text{mol/L}$. Mean value of 3C, 3H, 3PE group in the 3rd trimester were 2.18 ± 0.60 , 6.67 ± 0.99 and 10.16 ± 1.37 $\mu\text{mol/L}$ respectively (Table 3.3 & Fig 3.3). The obtained result was agreement with others studies which found that serum lipid peroxidation product assessed by MDA level is significantly higher in patients with preeclampsia than in normal pregnant women[123]. The increasing of MDA level in PIH is known to be due to increasing of generation of reactive oxygen species and reduction in anti-oxidants activity. Reactive oxygen species (ROS) thus produced can cause enhanced lipid peroxidation, which play a significant role in the pathophysiology of PIH[70]. Preeclampsia is associated with an imbalance between the oxidant and antioxidant status. Preeclamptic patients are exposed to increased oxidative stress due to either placental hyper secretion of lipid peroxides or decreased placental antioxidant enzyme activity leading to endothelial dysfunction. Insufficient antioxidant capacity leads to oxidative stress, and subsequently, oxidative injury may occur in both the maternal and placental compartment[124]. The increase in MDA levels found in this study is in agreement with the results of other study[125] which support the concept that lipid peroxidation is an important factor in the pathogenesis of PIH. Furthermore, serum MDA levels were highest in the PE than PIH and healthy control groups. The increase in MDA is strongly linked to lipid peroxidation caused by oxidative stress and is predicted to affect different tissues and organ systems including vascular endothelium. When oxidative stress reaches a certain level, cellular damage happens, including

structural damage in cellular membranes, in mitochondrial and nuclear DNA, and impairment of the enzymatic functions at various levels. In pre-eclamptic pregnancy observed higher level of MDA in the 3rd trimester than in the 2nd trimester, plasma malonaldehyde has been found to be progressively increasing in the course of pregnancy and may be utilized to predict occurrence of pre-eclampsia in the third trimester of pregnancy[126]. The obtained result also found weak positive correlation($R=0.06$) between Hb levels and serum MDA along with the 2nd and 3rd trimesters (Fig 3.4). Another study found that correlation between Hb and MDA level revealed that PIH patients had a higher MDA level than normotensive women. Pandey et al. found a concomitant decrease in hemoglobin in response to oxidative stress. Thus, higher levels of the lipid peroxidation product MDA and lower levels of Hb and antioxidants indicate oxidative stress in PIH[127].

Table 3.3: Serum MDA levels of pregnant groups at the 2nd and 3rd trimesters

Group	N	MDA(μmol/L) Mean$\pm$SD
2nd Trimester		
2nd C	20	1.85 $\pm$ 0.46 ^c
2nd H	20	5.82 $\pm$ 0.56 ^b
2nd PE	20	9.18 $\pm$ 1.54 ^a
L.S.D		0.62
3rd Trimester		
3rd C	20	2.18 $\pm$ 0.60 ^c
3rd H	20	6.67 $\pm$ 0.99 ^b
3rd PE	20	10.16 $\pm$ 1.37 ^a
L.S.D		0.66

* Each value represents mean $\pm$ SD values with non-identical superscript (a, b or c ... etc) were considered significantly different ($p \leq 0.05$).

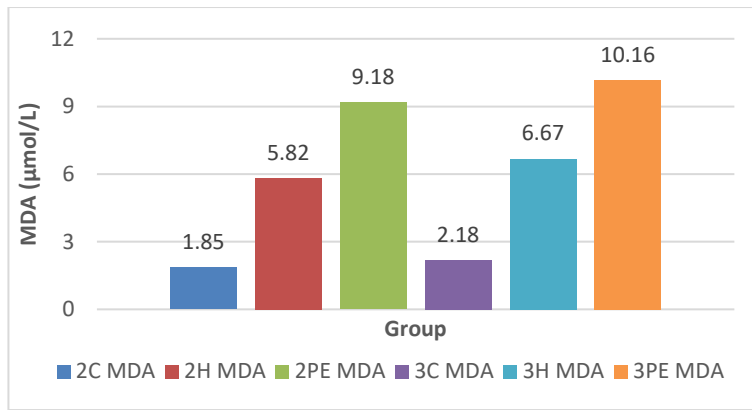


Figure 3.3: Serum MDA levels of pregnant groups at the 2nd and 3rd trimesters

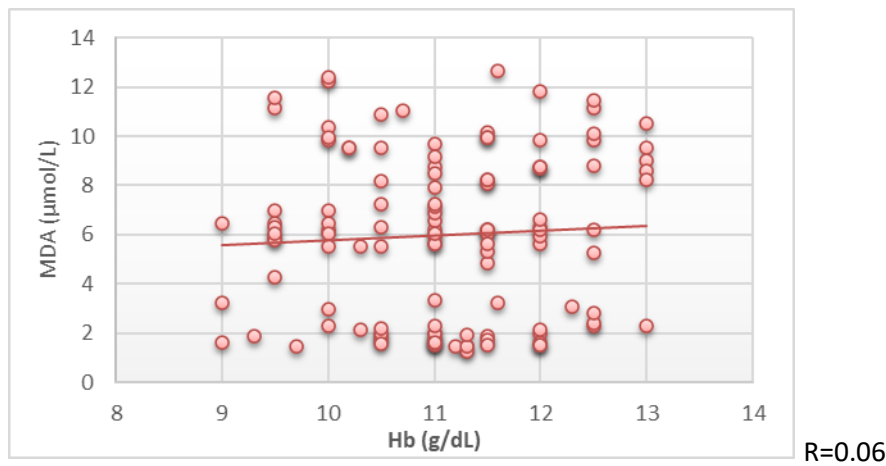


Figure 3.4: Correlation between Hb and MDA levels of pregnant patient groups at the 2nd and 3rd trimesters

3.4 Serum Ceruloplasmin Concentrations(mg/dl)

The present study showed that serum Cp has significant increasing in PE compared with PIH and significant increasing in PIH compared with healthy groups at each 2nd and 3rd trimester. Mean serum Cp level of 2C, 2H, 2PE group in the 2nd trimester was 2.48 ± 0.69 , 7.14 ± 1.11 and 9.18 ± 1.40 mg/L. Mean value of 3C, 3H, 3PE group in the 3rd trimester was 3.16 ± 0.99 , 7.13 ± 0.94 and 9.58 ± 1.36 mg/L respectively (Table 3.4 & Fig 3.5). Our results have agreed with previous study, which observed a significant difference of Cp levels between the control and preeclampsia cases during the middle of second trimester in patients who destined to develop preeclampsia in 18-20 weeks[128]. Aksoy et al noticed that the plasma antioxidant

potential in pre-eclampsia patients was decreased and the MDA with Cp levels were raised as compared to the healthy pregnant women [129]. Shamsi AZ reported that serum level of Cp significantly lower in normal pregnancy than mild and severe pre-eclampsia[130]. Cp is a ferroxidase and its ferroxidatic activity is an important function of this enzyme because even trace amounts of iron can produce hydroxyl radicals through the Fenton reaction which can destroy cellular architecture. Ferroxidatic activity of Cp is known to convert toxic ferrous iron to less toxic ferric iron, which reduces oxidative damage to lipids, proteins and DNA. Aleksandra et al found that Cp levels were significantly higher in pregnant women with preeclampsia compared to those with normal pregnancy[131]. Antioxidative effects of Cp are believed to be due to prevention of metal-ion catalyzed oxidation of cellular lipids. Our results have demonstrated that there is weak positive correlation between Hb levels and serum Cp along with the 2nd and 3rd trimester (R=0.03) (Fig 3.6).

Table 3.4: Serum Cp levels of pregnant groups at the 2nd and 3rd trimesters

Group	N	Cp (mg/dl) Mean±SD
2nd Trimester		
2nd C	20	2.48±0.69 ^c
2nd H	20	7.14±1.11 ^b
2nd PE	20	9.18±1.40 ^a
L.S.D		0.70
3rd Trimester		
3rd C	20	3.16±0.99 ^c
3rd H	20	7.13±0.94 ^b
3rd PE	20	9.58±1.36 ^a
L.S.D		0.71

* Each value represents mean ± SD values with non-identical superscript (a, b or c ... etc) were considered significantly different ($p \leq 0.05$).

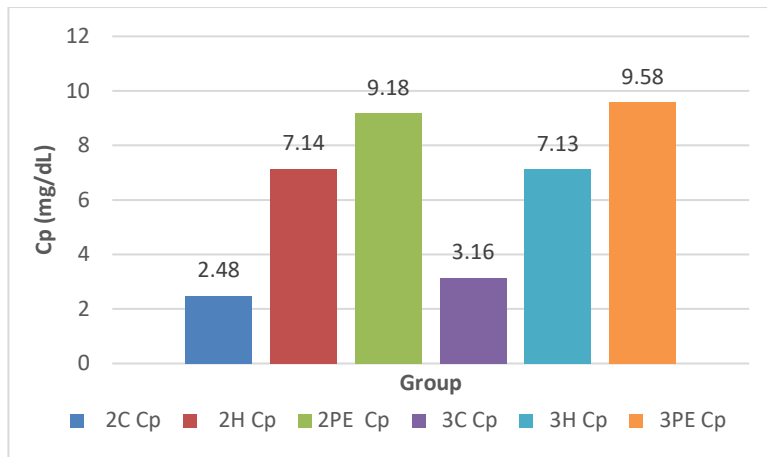


Figure 3.5: Serum Cp levels of pregnant groups at the 2nd and 3rd trimesters

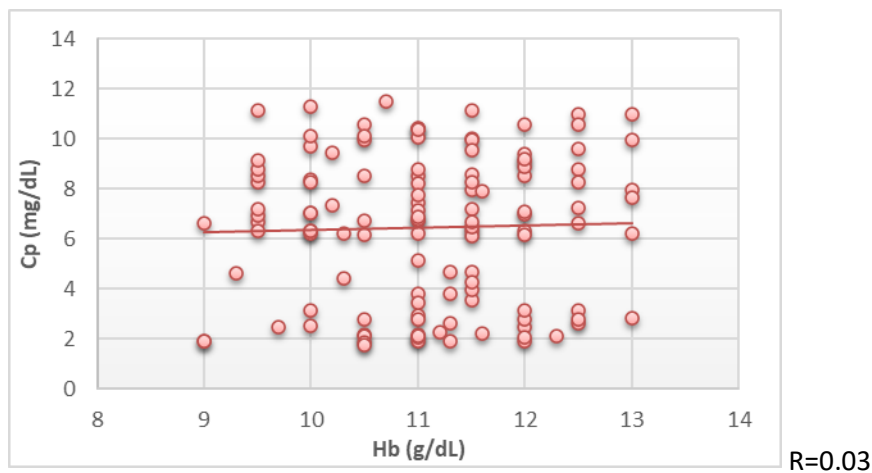


Figure 3.6: Correlation between Hb and Ceruloplasmin levels of pregnant patient groups at the 2nd and 3rd trimesters

3.5 Serum Aldosterone Concentration (ng/L)

The present study showed nonsignificant differences of serum Ald levels among the groups in each trimester. Mean serum Ald level of 2C, 2H, 2PE group in the 2nd trimester was 38.13 ± 25.80 , 32.28 ± 21.28 and 39.65 ± 15.66 ng/L. Mean value of 3C, 3H, 3PE group in the 3rd trimester were 40.99 ± 21.26 , 50.79 ± 31.93 and 24.38 ± 18.74 ng/L respectively. Previous study showed that the Ald level has decreased significantly in preeclampsia group compared with uncomplicated pregnancy[132] . Preeclamptic women are proven to experience much lower levels of circulating levels Ald[133]. The intravascular volume depletion would reduce placental perfusion with

subsequent placental ischemia and systemic maternal hypertensive response, leading to the clinical phenotype of preeclampsia. The hypothesis that reduced aldosterone synthesis account for preeclampsia at least in some patients is supported by repeatedly reported diminished aldosterone productions in preeclampsia. So far, it is unknown whether these reduced Aldosterone secretion rates are the cause or the consequence of preeclampsia[133]. Plasma aldosterone level increase gradually begins from the early gestational period. Some studies referred that in 16th gestational week, it increases up to 40 ng/dL, and in 32 gestational week it rises up to 60 ng/dL, and it is responsible for the increase in plasma volume[134]. This increase is partially due to increases in renin activity, and partly reduces due to increases in progesterone level. In different studies, maternal serum aldosterone levels were observed to be greater in all three trimesters in pregnant when compared with non-pregnants, while aldosterone levels were lower in all three trimesters in pregnant who developed preeclampsia compared with normotensive pregnant. Our study showed that maternal serum aldosterone levels were not statistically significantly different between hypertensive, and normotensive pregnant in each 2nd and 3rd trimester, and this status might lead to our different results from the literature, that is an important limitation of our study. The role of aldosterone in preeclampsia has been investigated in several studies, and contradictory results have been reported. Increased, unchanged or even decreased levels of aldosterone have been reported in cases with preeclampsia. These differences in the literature may stem from differences in methods or timing (1st, 2nd or 3rd trimester) measurements. In our prospective study, we aimed to evaluate the correlation between aldosterone levels measured through the 2nd and 3rd trimesters with Hb in PIH and preeclampsia. Our results demonstrated that there is weak negative correlation

between Hb levels and serum Ald along with the 2nd and 3rd trimester (R= - 0.05) (Fig 3.8).

Table 3.5: Serum Ald levels of pregnant groups at the 2nd and 3rd trimesters

Group	N	Ald (ng/L) Mean±SD
2nd Trimester		
2nd C	13	38.13±25.80 ^a
2nd H	13	32.28±21.28 ^a
2nd PE	13	39.65±15.66 ^a
L.S.D		16.96
3rd Trimester		
3rd C	15	40.99±21.26 ^a
3rd H	15	50.79±31.93 ^a
3rd PE	15	24.38±18.74 ^a
L.S.D		19.61

* Each value represents mean ± SD values with non-identical superscript (a, b or c ... etc) were considered significantly different ($p \leq 0.05$).

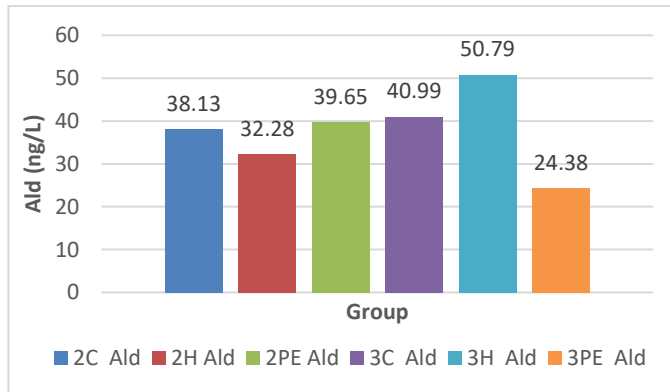


Figure 3.7: Serum Ald levels of pregnant groups at the 2nd and 3rd trimesters

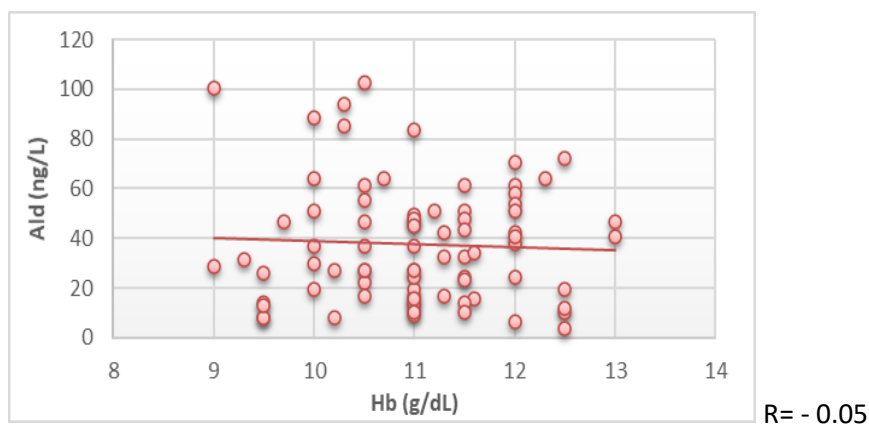


Figure 3.8: Correlation between Hb and serum aldosterone levels of pregnant patient groups at the 2nd and 3rd trimesters

3.6 Serum Vitamin-C Concentration (ng/ml)

Vitamin C is a quencher of free radicals as well as singlet oxygen, also acts as a potential antioxidant. Antioxidant vitamins have been reported to have an essential function in regulating blood pressure[135]. There is little information concerning the functions of antioxidant vitamins particularly plasma concentrations of Vit C and Aldosteron in preeclampsia especially in Iraqi populations. Thus, we have intended to estimate these two factors in preeclampsia, PIH and normotensive pregnant women in Almuthanna governorate / Iraq.

The present study revealed an increase of serum Vit. C levels in healthy pregnant as compared in patient with preeclampsia in the 2nd trimester, but statistically nonsignificant. Our study has also showed nonsignificant differences of serum Vit. C levels among the groups in the 3rd trimester. Mean value of serum Vit. C in the 2nd trimester of 2C, 2H, 2PE group were 35.20 ± 13.06 , 36.13 ± 8.19 and 31.48 ± 12.69 ng/mL respectively. Mean value of 3C, 3H, 3PE group in the 3rd trimester was 33.89 ± 11.25 , 35.08 ± 8.06 and 34.63 ± 9.01 ng/mL respectively (Table 3.6 , Fig 3.9). Our results in the 2nd trimester are approximately in agreement with previous study which found significant increase of Vit. C levels in healthy pregnant as compared to preeclamptic women [136] The antioxidant ascorbic acid, have essential role in the

defense mechanisms against lipid peroxidation, as a low molecular weight water soluble anti-oxidant, ascorbic acid traps most of the free radicals present in the aqueous phase of the plasma and functions as a first line defense mechanism against free oxygen radicals[137]. In preeclampsia, antioxidant activity is usually low but not uniform. Plasma ascorbate level reduces progressively throughout normal pregnancy. Chappell et al compared antioxidant concentrations in high and low risk women, base line concentrations of particular antioxidants were lower ascorbic acid. This propose, that the measurement of a single antioxidant in a especial biological fluid or tissue at a given point in pregnancy cannot adequately reflect the balance between pro-oxidant and antioxidant forces [138].

Agarwal had reported higher Vit C levels in normal pregnancy than in preeclamptic pregnant women[139]. Another study also revealed significant decrease in plasma levels of ascorbic acid in the preeclamptic patients, which is the first antioxidant consumed by oxidative stress [138]. Our results revealed weak positive correlation between Hb levels with serum Vit. C along with 2nd and 3rd trimesters (R=0.04) (Fig 3.10).

Table 3.6: Serum Vit. C levels of pregnant groups at the 2nd and 3rd trimesters

Group	N	Vit. C (ng/mL) Mean±SD
2nd Trimester		
2C	14	35.20±13.06 ^a
2H	14	36.13±8.19 ^a
2PE	14	31.48±12.69 ^a
L.S.D		8.81
3rd Trimester		
3C	14	33.89±11.25 ^a
3H	14	35.08±8.06 ^a
3PE	14	34.63±9.01 ^a
L.S.D		7.29

* Each value represents mean ± SD values with non-identical superscript (a, b or c ... etc) were considered significantly different ($p \leq 0.05$).

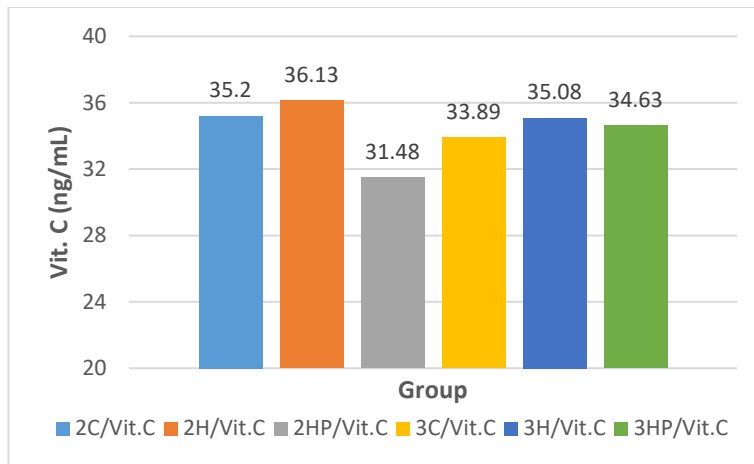


Figure 3.9: Serum Vit. C levels of pregnant groups at the 2nd and 3rd trimesters

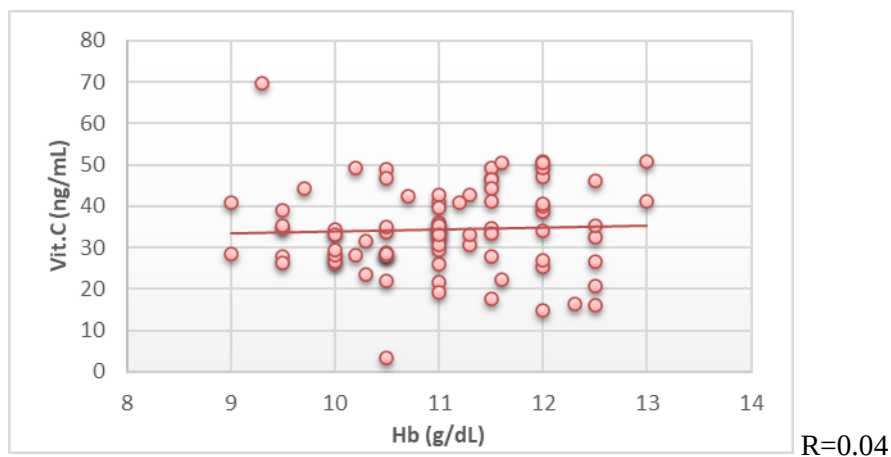


Figure 3.10: Correlation between Hb and serum Vit. C levels of pregnant patient groups at the 2nd and 3rd trimesters

3.7 Serum Albumin (Alb) Concentration (g/dL)

The present study clarified nonsignificant differences between serum Alb levels of PE and PIH patient groups, whereas these two groups have significant increasing ($p < 0.05$) in Alb levels comparing with the healthy controls in each 2nd and 3rd trimester. The mean of serum Alb in the 2nd trimester of 2C, 2H, 2PE group was 2.39 ± 0.20 , 3.60 ± 0.18 and 3.7 ± 0.20 g/dL. Mean value of 3C, 3H, 3PE group was 2.53 ± 0.22 , 3.76 ± 0.19 and 3.66 ± 0.24 g/dL respectively (Table 3.7, Fig 3.11). The current study has agreed with many previous studies, which demonstrated smeller results [140]. Serum levels of human albumin may be a dangerous factor of vascular oxidative stress in some human diseases. Serum albumin level may suppress vascular

oxidative stress and stop pregnant endothelial dysfunction so it is associated with oxidative stress inversely, but to the endothelial function positively in pregnant women including those with preeclampsia [88], which have been fully consistent with our results. Serum albumin levels were correlated with severity of preeclampsia and all patients with severe preeclampsia had values <3.0 g/dL, authors believed that hypoalbuminemia can be identified as an early sign in developing preeclampsia[141]. However, our present results indicate that patients with severe preeclampsia had serum Alb levels little higher than 3.0 g/dL. The current results have indicated the absence correlations between Hb levels and serum Alb along with the 2nd and 3rd trimesters (R=0.00, Fig 3.12).

Table 3.7: Serum Alb levels of pregnant groups at the 2nd and 3rd trimesters

Group	N	Serum Albumin (g/dL) Mean$\pm$SD
2nd Trimester		
2nd C	20	2.39 $\pm$ 0.20 ^b
2nd H	20	3.60 $\pm$ 0.18 ^a
2nd PE	20	3.7 $\pm$ 0.20 ^a
L.S.D		0.12
3rd Trimester		
3rd C	20	2.53 $\pm$ 0.22 ^b
3rd H	20	3.76 $\pm$ 0.19 ^a
3rd PE	20	3.66 $\pm$ 0.24 ^a
L.S.D		0.14

* Each value represents mean $\pm$ SD values with non-identical superscript (a, b or c ... etc) were considered significantly different ($p \leq 0.05$).

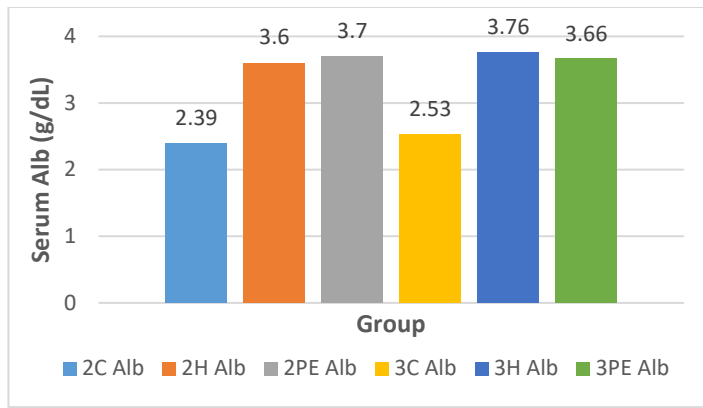


Figure 3.11: Serum Alb levels of pregnant groups at the 2nd and 3rd trimesters

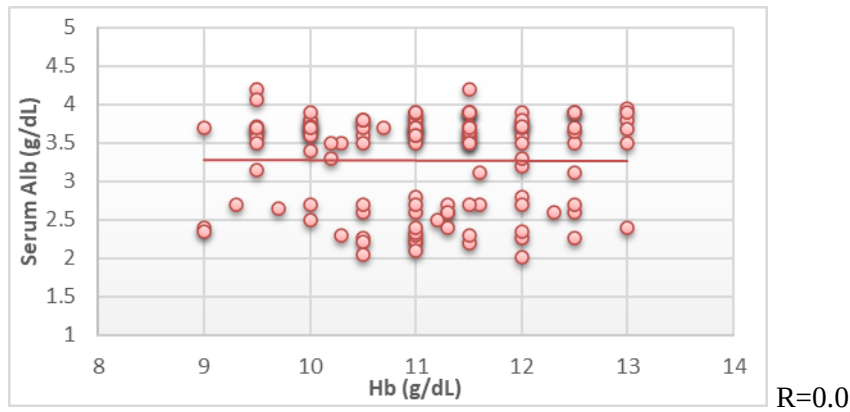


Figure 3.12: Correlation between Hb and serum albumin levels of pregnant patient groups at the 2nd and 3rd trimesters

3.8 Serum Albumin/Creatinine Ratio (g/ μ mol)

The current study demonstrated that serum ACR levels have significant increase ($p < 0.05$) in PE and PIH compared with healthy control groups along with each 2nd and 3rd trimesters. Mean value of serum ACR in 2nd trimester of 2C, 2H, 2PE group was 0.67 ± 0.10 , 0.97 ± 0.11 and 1.02 ± 0.18 g/ μ mol. Mean value of 3C, 3H, 3PE group in the 3rd trimester were 0.67 ± 0.08 , 0.99 ± 0.15 and 0.98 ± 0.18 g/ μ mol respectively (Table 3.8 ,Figure 3.13). Our results have demonstrated weak negative correlation between Hb and serum ACR along with 2nd and 3rd trimester ($R = -0.03$, Figure 3.14).

Table 3.8: Serum ACR levels of pregnant groups at the 2nd and 3rd trimesters

Group	N	Serum ACR (g/μmol) Mean±SD
2nd Trimester		
2C	20	0.67±0.10 ^b
2H	20	0.97±0.11 ^a
2PE	20	1.02±0.18 ^a
L.S.D		0.09
3rd Trimester		
3C	20	0.67±0.08 ^b
3H	20	0.99±0.15 ^a
3PE	20	0.98±0.18 ^a
L.S.D		0.08

* Each value represents mean ± SD values with non-identical superscript (a, b or c ... etc) were considered significantly different ($p \leq 0.05$).

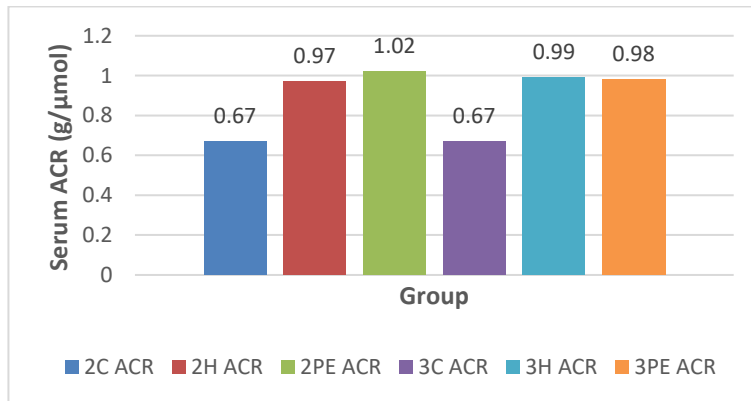


Figure 3.13: Serum ACR levels of pregnant groups at the 2nd and 3rd trimesters

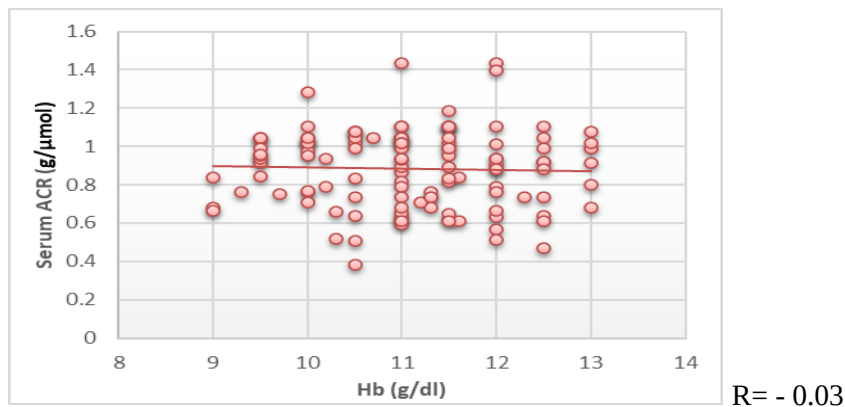


Figure 3.14: Correlation between Hb and serum ACR levels of pregnant patient groups at the 2nd and 3rd trimester

3.9 Urine Albumin/Creatinine Ratio (mg/mmol)

The albumin concentration in urine in itself is not a good measure of albumin leakage as its concentration is directly affected by the degree to which the urine is concentrated by the kidneys. The ratio between the urine concentration of albumin and creatinine will be independent of renal fluid handling. Urinary ACR is an independent prognostic factor for maternal and neonatal adverse outcomes in suspected preeclampsia[142]. Urine ACR may be a reliable method for prediction and assessment of severity of preeclampsia[143]. Our results indicated that urine ACR levels have significant increase in PE compared with PIH and C groups. Each PIH and C groups have not significant differences regarding this parameter in each trimester. Mean value of urine ACR in the 2nd trimester for 2C, 2H, 2PE were 2.59 ± 1.81 , 3.02 ± 1.38 and 17.55 ± 10.03 mg/mmol. Mean value of 3C, 3H, 3PE in the 3rd trimester were 3.11 ± 2.22 , 5.38 ± 2.22 and 24.29 ± 15.14 mg/mmol respectively (Table 3.9, Figure 3.15). This result is consistent with those reported by previous studies, which found urinary ACR of controls was significantly lower in healthy as compared to preeclampsia[144]. Urine collection through 24 hours is regarded the conventional comparator for quantization of proteinuria in pregnancy, when important proteinuria is described as proteinuria of 0.3 g/day or more[145]. Thus, urine needs refrigeration and its collection is difficult, time consuming (for women and ward staff), and possibly deceptive if collected inaccurately. Also, it may not be potential to complete the urine collection when delivery occurs, due to undetermined proteinuria status and an uncorroborated diagnosis of preeclampsia, less than half of women admitted with pre-eclampsia have a 24 hour urine collection sent for analysis[146]

Because of the difficulties of 24 hour urine collection, alternatives for the diagnosis of proteinuria in pregnancy have been termed, this involves urinary dipsticks, urine collections through a shorter time, the urinary spot protein/ creatinine ratio (PCR), and urinary albumin /creatinine ratio (ACR). The dipstick is cheap, simple to utilize, and gives a rapid outcome but has been shown to have low sensitivity and specificity for urinary protein secretion over 24 hours[146].

National Kidney Foundation presently suggests these tests (instead of 24-h urine collection) are used to diagnose proteinuria in most cases, without special mention of pregnancy [147]. The Australasian Society for the Study of Pregnancy Hypertension and the International Society for the Study of Pregnancy Hypertension have suggested using the urinary spot protein:creatinine ratio instead of 24-hour urine collection[148]. Our results also revealed a weak negative correlation between Hb levels and urine ACR along with 2nd and 3rd trimesters ($R = -0.04$, Figure 3.16). The results also clarified the presence of positive correlation between serum ACR and urine ACR along with the 2nd and 3rd trimesters ($R=0.32$, Figure 3.27). This strong positive correlation indicates the possibility of depending on analyzing serum ACR in diagnosing the preeclampsia or at least to confirm the results of urinary ACR in the pregnant.

Table 3.9: Urine ACR levels of pregnant groups at the 2nd and 3rd trimesters

Group	N	Urine ACR (mg/mmol) Mean±SD
2nd Trimester		
2 nd C	20	2.59±1.81 ^b
2 nd H	20	3.02±1.38 ^b
2 nd PE	20	17.55±10.03 ^a
L.S.D		3.76
3rd Trimester		
3 rd C	20	3.11±2.22 ^b
3 rd H	20	5.38±2.22 ^b
3 rd PE	20	24.29±15.14 ^a
L.S.D		5.65

* Each value represents mean ± SD values with non-identical superscript (a, b or c ... etc) were considered significantly different ($p \leq 0.05$).

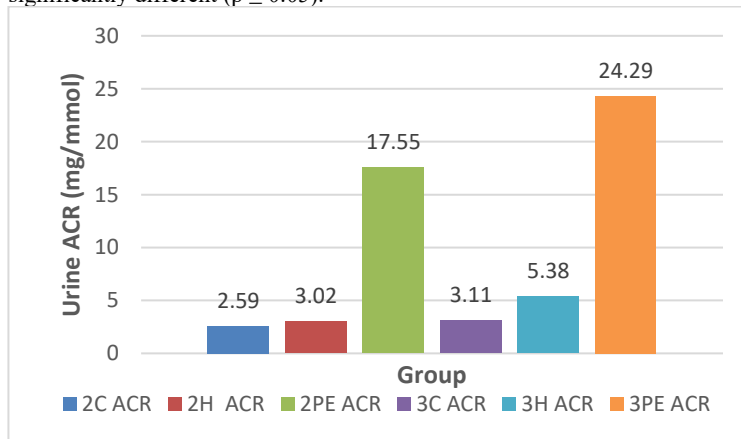


Figure 3.15: Urine ACR levels of pregnant groups at the 2nd and 3rd trimesters

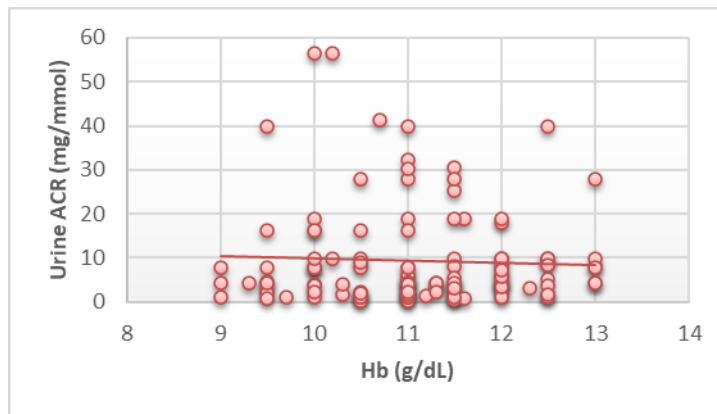


Figure 3.16: Correlation between Hb and urine ACR levels of pregnant patient groups at the 2nd and 3rd trimesters

3.10 Correlation of MAP with Characteristics, Blood Parameter and biochemical factors in Pregnant Patient groups along with the 2nd and 3rd trimester.

Although no efficient medication has been shown to prevent preeclampsia, much attention in recent years has focused on improving the ability to predict this common complication. A useful biophysical marker in screening for PE is mean arterial pressure (MAP)[149]. One of objectives of this study was to quantify the effects of maternal characteristics, maternal blood parameter and some biochemical factors on MAP levels of PIH, PE and normotensive pregnant groups. Baseline characteristics of the study population are shown in Table 3.10

Table 3.10: Descriptive Characteristics of pregnant groups at the 2nd and 3rd trimesters

Group	Age(years)	Gestational age (weeks)	SBP Mean±SD	DBP Mean±SD	MAP (SBP+2*DBP)/3
2 nd C	18-28	15-25	115.8±10.44	75±4.68	88.6±5.24
2 nd H	19-34	15-28	144.6±3.52	91.05±3.03	108.9±2.96
2 nd PE	21-37	20-28	148.5±4.30	95.25±3.64	113±3.2
3 rd C	18-27	28-39	123.15±7.81	76.00±4.11	91.72±4.61
3 rd H	18-36	28-38	147.3±5.63	93.45±4.48	111.4±4.49
3 rd PE	20-38	28-38	149.5±4.68	96.4±4.17	114.1±4.12

The results of this study demonstrate that, during pregnancy, significant independent contributions to the measurement MAP are provided by maternal characteristics, blood and biochemical parameters variable from medical history.

The present study demonstrated that MAP has smooth positive correlation with gestational age ($R=0.14$) (Figure 3.17). It is similar with another study, which showed that women with early-onset preeclampsia had higher mean arterial blood pressure

levels at 20 weeks of gestation, compared to the normotensive group, and women with late-onset preeclampsia had higher mean arterial blood pressure levels at 37 weeks of gestation, than the normotensive groups[150]. Another study has also revealed the same results[151]. However, some studies indicated that MAP has clearly decreased with gestational age in the case of unaffected group[152].

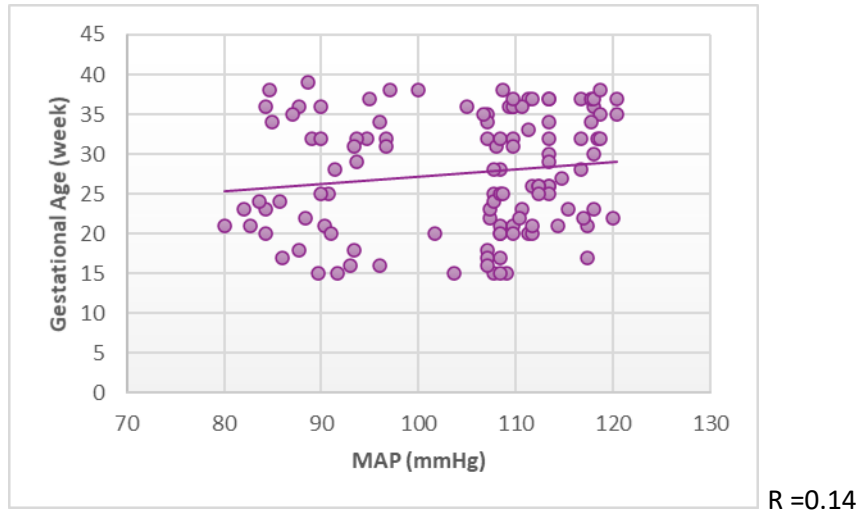


Figure 3.17: Correlation of MAP with gestational age of pregnant groups in the 2nd and 3rd trimesters
In addition, our study showed positively correlated of MAP with maternal age ($r=0.17$) (Figure 3.18). A previous study by Richardson et al[153] has shown a protective MAP increases with maternal age that is perfectly noticed in the present study.

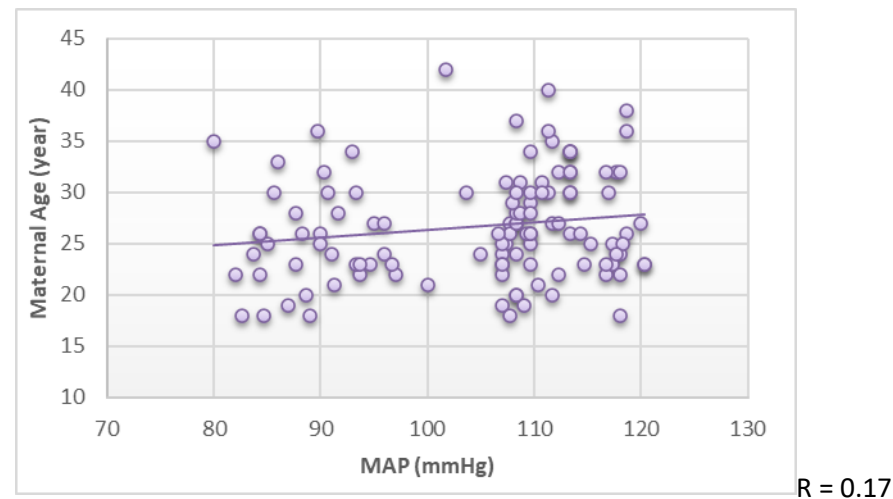


Figure 3.18 : Correlation of MAP with maternal age of pregnant groups in the 2nd and 3rd trimesters

Notably, the present study revealed different positive or negative correlation values of blood or biochemical parameters and MAP along with 2nd and 3rd trimesters, which are summarized as follow:

1. Weak positive correlation($R=0.05$) of MAP with Hb levels (Figure 3.19), agree with many studies, which also found a positive correlation of these two factors. The results are in agreement with a study regarding the correlation of these two factors in patients, which is well established in many recent studies[154].
2. Strong positive correlation($R= 0.84$) of MAP levels with serum MDA(Figure 3.20) Our study has shown similar results concerning the correlation of these two factors in patients which are well recognized in many of some recent studying[155].
3. Strong positive correlation($R=0.86$) of MAP levels with serum Cp (Figure 3.21). The present findings are also in good agreement with a study that reveal high positive relationship of MAP with serum Cp levels in the pregnant groups[156].In previous studies, serum Cp levels (antioxidant markers) were found to be higher in preeclamptic pregnant women than in healthy pregnant women[157].
4. Weak negative correlation ($R= -0.05$) of MAP levels with serum Ald (Figure 3.22) . These result agree with previous study which found that MAP was negatively correlated with the plasma aldosterone concentration in all pregnant women[158].
5. Weak negative correlation ($R= -0.06$) of MAP levels with serum vit.C(Figure 3.23) . Other similar studies that found the same correlation of these two factors have been reported by J. Islam et al. [159].
6. Strong positive correlation ($R=0.87$) of MAP levels with serum Alb (Figure 3.24)
7. Strong positive correlation ($R=0.63$) of MAP levels with serum ACR (Figure 3.25)
8. Positive correlation ($R=0.47$) of MAP levels with urinary ACR (Figure 3.26).

Most of the articles published, with some exceptions, showed microalbuminuric hypertensive patients to have higher levels of blood pressure (BP) compared to normoalbuminuric subjects[160].

9. Positive correlation($R=0.32$) of serum ACR with urinary ACR levels (Figure 3.27)

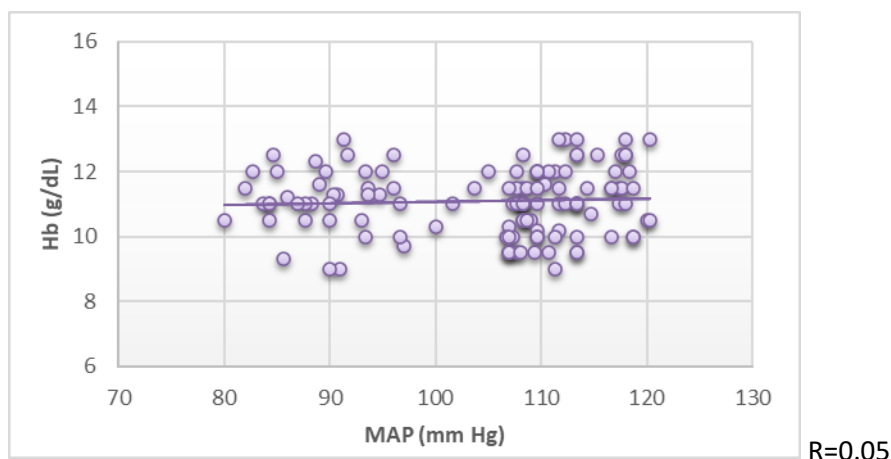


Figure 3.19: Correlation between MAP and Hb levels of pregnant groups at the 2nd and 3rd trimesters

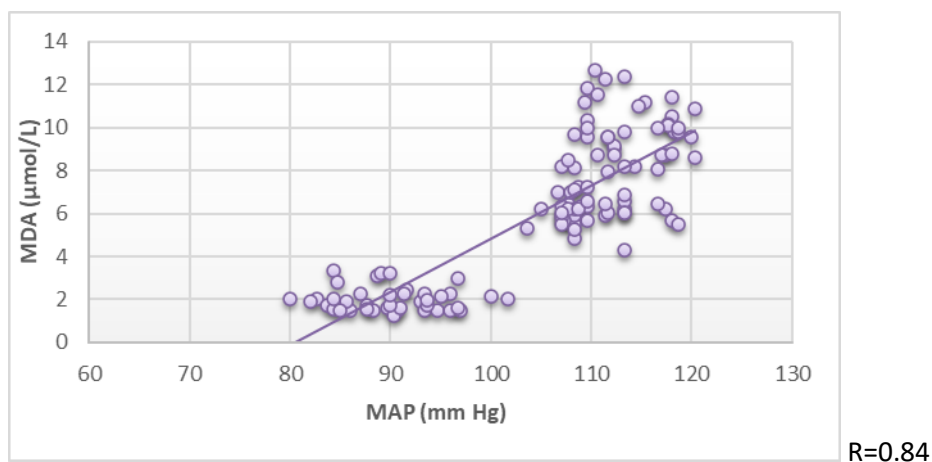


Figure 3.20: Correlation between MAP and serum MDA levels of pregnant groups at the 2nd and 3rd trimesters

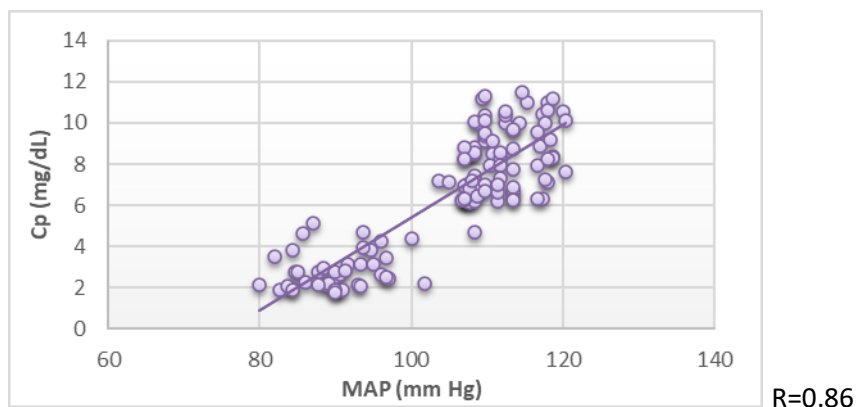


Figure 3.21: Correlation between MAP and serum Cp levels of pregnant groups at the 2nd and 3rd trimesters

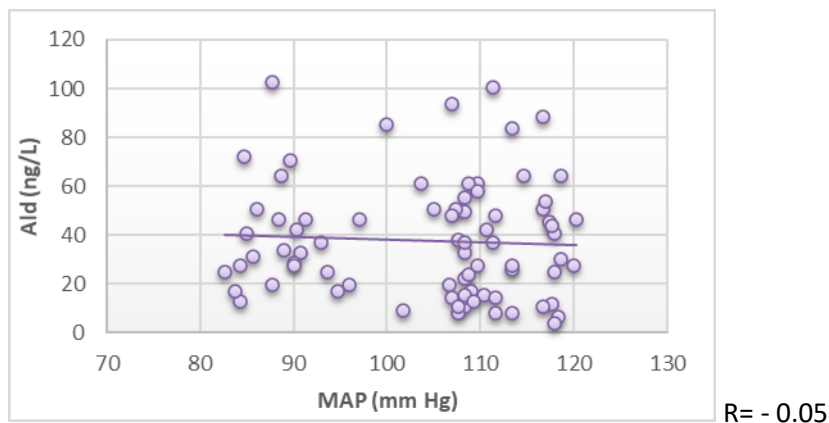


Figure 3.22: Correlation between MAP and serum Ald levels of pregnant groups at the 2nd and 3rd trimesters

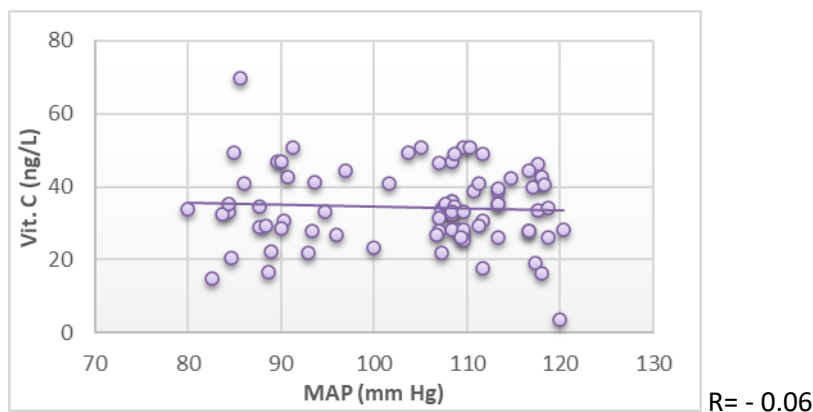


Figure 3.23: Correlation between MAP and serum Vit.C levels of pregnant groups at the 2nd and 3rd trimesters

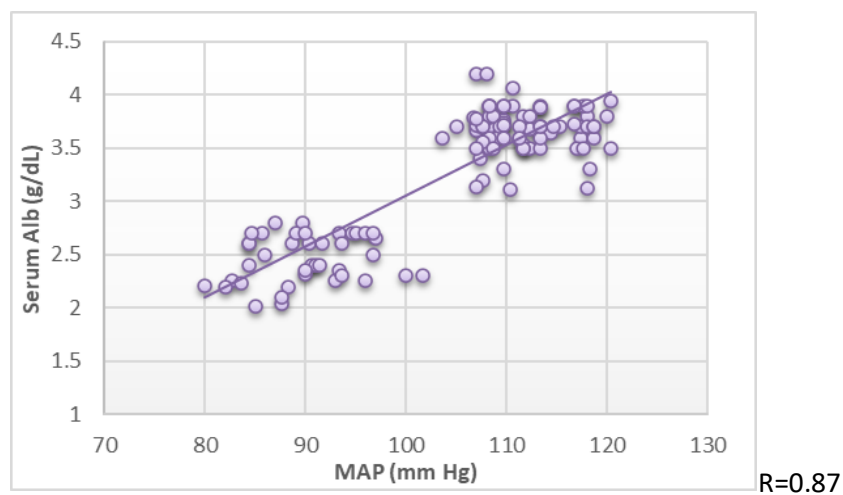


Figure 3.24: Correlation between MAP and serum Alb levels of pregnant groups at the 2nd and 3rd trimesters

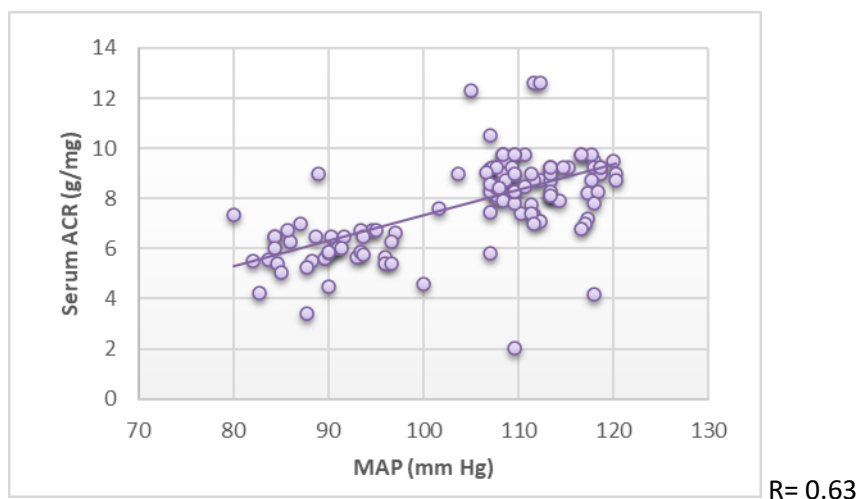


Figure 3.25: Correlation between MAP and serum ACR levels of pregnant groups at the 2nd and 3rd trimester

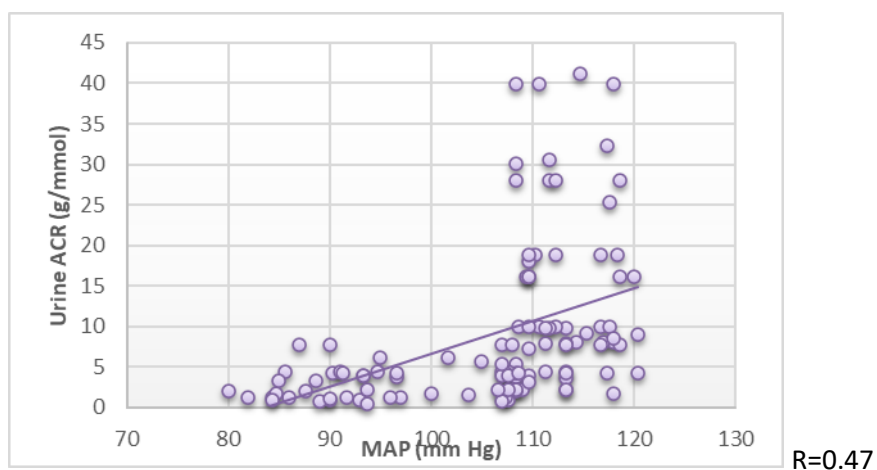


Figure 3.26: Correlation between MAP and urine ACR levels of pregnant groups at the 2nd and 3rd trimesters

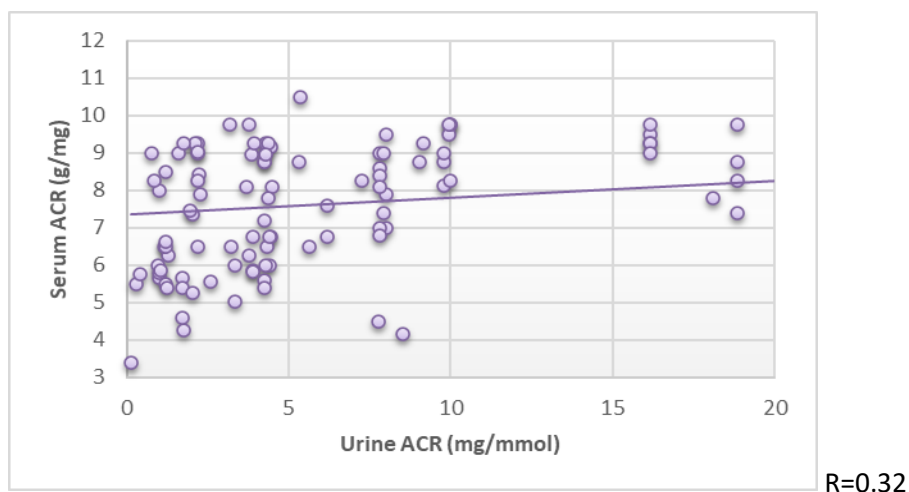


Figure 3.27: Correlation of serum ACR with urine ACR levels of pregnant groups in the 2nd and 3rd trimester

In the present study, we have investigated the association of mean arterial pressure (MAP) with some characteristic, blood and biochemical parameters of pregnant groups in Al-Muthanna population. It is the first study investigating the association of blood pressure with those parameters in this province.

According to the results shown in Figures: 3.20, 3.21, 3.24, 3.25 and 3.26, it is possible to predict the elevation of serum MDA, Cp, Alb, ACR and urine ACR levels respectively, alongside with the elevation of pregnant MAP levels. Conversely, Figures: 3.19, 3.22 and 3.23 have indicated the absence of this relationship with Hb, serum Ald and vit C, respectively, thus their levels become steady with respect to rising of MAP levels. Correspondingly, an interest result has been found in Figure 3.27 which indicated the presence of proportional relationship between the levels of serum and urine ACR.

Chapter Four
Conclusions
&
Recommendation

4. Conclusion and Recommendation

4.1 Conclusion

This study comes with the following concepts:

1. The imbalance between antioxidant levels and ROS generation (OS), which could be responsible for the start and progression of pathological processes related to PIH and may be critical determinant of diseases.
2. Hemoglobin levels have statistically weak correlation with the blood and biochemical parameters in each patient and normotensive pregnant. Thus, it cannot be regarded as appropriate predictor to estimate the levels of these parameters in the pregnant.
3. The high level of mean arterial pressure (MAP) is regarded a clear indicator of elevation of serum MDA, Cp, Alb, ACR and urine ACR in the pregnant.
4. The high level of serum ACR is considered an additional sign in the diagnosis of preeclampsia.

4.2 Recommendation

The current study suggests the following recommendation:

1. Further studies should be carried out include estimating the levels of other enzymes and blood and biochemical parameters to enrich and confirmed our results.
2. Testing the oxidative stress factors in early pregnancy is very important, that could help detect patients at risk and consequently prevent future hypertension in pregnancy.
3. More studies are needed to find out the role of the renin-angiotensin pathway in the pathogenesis of hypertensive pregnancy.
4. Further studies are recommended include supplementation of different metals (copper, chromium, cobalt, magnesium, manganese, selenium, and zinc) and vitamins as a preventive method in patients at risk of preeclampsia.
5. Estimating serum ACR along with urine ACR levels might give an additional knowledge to confirm the diagnosis of preeclampsia.

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Appendix

Normal Values for Lab Tests

Test	Normal value
Hemoglobin Concentration	11.5-14 gm/dL
Serum Albumin	3.5-5 gm/dL
Serum Creatinine	44-80 μ mol/L (0.50-0.90 mg/dL)
Urinary Creatinine	Urine in random (2.47-19.2 mmole/L),(0.028-0.217 g/dl) 24-h urine (7-14 mmole/24 h),(740-1570 mg/24 h)
urine Albumin	< 20 mg/L
Serum Vitamin-C	1ng/ml - 300ng/ml
Serum Aldosterone	10ng/L - 2000ng/L
Urine ACR	< 8 mg/mmol

Table 3.11: Blood and Biochemical parameters of pregnant groups at the 2nd and 3rd trimesters

Parameter	mean $\pm$ SD (2 nd trimester)	mean $\pm$ SD (3 rd trimester)	p
Hb	11.23 $\pm$ 0.98	10.95 $\pm$ 1.03	>0.05 (0.13)
MDA	5.62 $\pm$ 3.17	6.33 $\pm$ 3.45	>0.05 (0.24)
Cp	6.27 $\pm$ 3.03	6.62 $\pm$ 2.89	>0.05 (0.51)
Aldosterone	36.69 $\pm$ 21.00	38.72 $\pm$ 26.41	>0.05 (0.71)
Vit. C	34.22 $\pm$ 11.40	34.63 $\pm$ 9.06	>0.05 (0.85)
Serum Alb	3.23 $\pm$ 0.63	3.32 $\pm$ 0.60	>0.05 (0.45)
SBP	136.3 $\pm$ 16.16	139.98 $\pm$ 13.48	>0.05 (0.18)
DBP	87.1 $\pm$ 9.58	88.62 $\pm$ 10.0	>0.05 (0.40)
Mean arterial pressure (MAP)	(SBP +2DBP)/3 103.5 $\pm$ 11.4	105.74 $\pm$ 10.95	>0.05 (0.28)
Urine ACR	7.72 $\pm$ 9.12	10.93 $\pm$ 12.99	>0.05 (0.1)
Serum ACR	7.83 $\pm$ 1.75	7.72 $\pm$ 1.88	>0.05 (0.74)

Appendix

Table 3.12: comparative between demographic characteristics of pregnant groups along with the 2nd and 3rd trimesters

Characteristic	C (Mean±S D)	H (Mean±S D)	PE (Mean±S D)	P value	LSD
Gestational/Age (weeks) / 2 nd	20.2 ^b	20.1 ^b	23.2 ^a	< 0.05	2.144
Gestational /Age (weeks) / 3 rd	34	34.2	34.1	> 0.05	1.925
Age / 2 nd (year)	28.6	26	27.5	> 0.05	3.198
Age / 3 rd (year)	22.7 ^b	26.7 ^a	28.7 ^a	< 0.05	3.055
SBP / 2 nd (mmHg)	115.8 ^b	144.6 ^a	148.5 ^a	< 0.05	4.323
SBP / 3 rd (mmHg)	123.2 ^b	147.3 ^a	149.5 ^a	< 0.05	3.914
DBP / 2 nd (mmHg)	75 ^c	91.1 ^b	95.3 ^a	< 0.05	2.435
DBP / 3 rd (mmHg)	76 ^c	93.5 ^b	96.4 ^a	< 0.05	2.697
Hb / 2 nd (g/dl)	11.08 ^b	10.93 ^b	11.68 ^a	< 0.05	0.59
Hb / 3 rd (g/dl)	11.13 ^a	10.89 ^a	10.83 ^a	> 0.05	0.66

Table 3.13: correlation between Hb and biochemical parameters of pregnant patient groups at 2nd and 3rd trimester

Parameters	r
Mean Arterial pressure(MAP)	0.05
Serum MDA	0.06
Serum Cp	0.03
Serum Aldosterone	-0.05
Serum Vitamin.C	0.04
Serum Albumin	0.0
Serum ACR	-0.03
Urine ACR	-0.04

يعد ارتفاع الضغط الدم الحلمي (PIH) هو أحد مضاعفات الحمل الرئيسية التي تؤدي الى وفاة الأمهات . ان نسبة حدوث المرض تتراوح ما بين 5 الى 15 بالمئة من الإجمالي الكلي للنساء الحوامل عامة . يحدث الاجهاد التأكسدي (OS) خلال ارتفاع الضغط الحلمي نتيجة للزيادة المفرطة في الجذور الحرة وقلة مضادات الأكسدة . تهدف الدراسة الحالية إلى تقييم منتجات بيروكسيد الدهون مثل مصل المالمونديهايد وحالة مضادات الأكسدة في مضاعفات ارتفاع ضغط الدم الحلمي عن طريق قياس نشاط السيرولوبلازمين وتقدير تركيز فيتامين C , واللدوستيرون , والالبومين . ونسبة الالبومين:الكرياتينين في الأدرار , فضلا عن تسليط الضوء على العلاقات المحتملة للهيموجلوبين ومتوسط ضغط الدم مع تلك المعلمات لمجموعات المرضى. تضمنت الدراسة 40 امرأة تتمتع بصحة جيدة وبحمل طبيعي غير معقد , مقسمة الى 20 امرأة في الثلث الثاني من الحمل و20 امرأة في الثلث الثالث من الحمل , و40 امرأة مصابة بارتفاع ضغط الدم الحلمي (20 امرأة في الثلث الثاني و20 في الثلث الثالث من الحمل) و40 امرأة مصابات بتسمم الحمل (20 امرأة في الثلث الثاني و20 امرأة في الثلث الثالث من الحمل مصابات بتسمم الحمل) تم جمع العينات من مستشفى النسائية والتوليد التعليمي في محافظة المثنى خلال الفترة من أكتوبر 2020 الى فبراير 2021. تم قياس المعلمات في مختبر الكيمياء الحياتية المتقدم في جامعة المثنى . أظهرت النتائج وجود ارتباط ايجابي ضعيف جداً بين مستويات الهيموكلوبين ومستويات كل من المعلمات الآتية في مصل الدم: Vit C , Cp, MDA وعدم وجود ارتباط مع مستويات الالبومين كما أشارت النتائج الى وجود ارتباط سلبي ضعيف جداً مع كل من Ald , serum ACR و urine ACR في الثلثين الثاني والثالث من الحمل. أظهرت النتائج أيضاً وجود ارتباط ايجابي قوي بين متوسط ضغط الدم (MAP) مع كل من MDA , Cp, Alb , serum ACR, urine ACR , وارتباط ايجابي ضعيف مع الهيموكلوبين Hb . كما أظهرت النتائج الى وجود ارتباط سلبي ضعيف مع مستويات كل من serum Ald , serum Vit.C في الثلثين الثاني والثالث من الحمل. كما أشارت النتائج إلى وجود ارتباط ايجابي بين ACR في الادرار و ACR في الدم في الفصلين الأخيرين من الحمل. أظهرت الدراسة الحالية أن مستويات الهيموغلوبين قد ارتفعت ارتفاعاً معنوياً في مجموعة 2PE مقارنة مع مجموعتي 2H و 2C بينما كان الارتفاع غير معنوياً في الثلث الثالث من الحمل. أشارت الدراسة الى وجود ارتفاع معنوي لكل من مستويات MDA و Cp و ACR في الادرار في مجموعة تسمم الحمل مقارنة مع مجموعة ارتفاع الضغط الدم الحلمي ومجموعة السيطرة، على عكس مستويات Ald و Vit C التي أظهرت اختلافاً غير معنوي في كل المجاميع وفي كلا ثلثي الحمل. تضمنت نتائج الدراسة وجود زيادة معنوية في مستوى الالبومين بالمصل في مجموعتي تسمم الحمل ومجموعة ارتفاع ضغط الدم الحلمي مقارنة مع مجموعة السيطرة في كل فصل، كما اوضحت النتائج وجود ارتفاع معنوي في مستوى ACR في مصل الدم وكذلك في متوسط ضغط الدم في مجموعة تسمم الحمل مقارنة مع مجموعة ارتفاع الضغط الحلمي ومجموعة السيطرة في الثلث الثاني من الحمل، بينما كانت زيادة مستوى متوسط ضغط الدم في الثلث الثالث من الحمل غير معنوية في مجموعة تسمم الحمل مقارنة مع مجموعة ارتفاع الضغط الحلمي.



جمهورية العراق

وزارة التعليم العالي والبحث العلمي

جامعة المثنى / كلية العلوم

قسم الكيمياء

دراسة الارتباط بين المؤشرات البايوكيميائية وحالات الأكسدة-مضادات الأكسدة عند الحوامل المصابين بارتفاع الضغط وتسمم الحمل

رسالة مقدمة الى مجلس كلية العلوم / جامعة المثنى كجزء من متطلبات نيل
درجة الماجستير في علوم الكيمياء
من قبل

مصطفى حمزه غازي

بكلوريوس علوم كيمياء 2015/2014

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2021 م

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