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دراسة مقارنة للأنزيم المحلل للدهون , البروتين Apo C2 والبروتين ApoE في النساء الحوامل الاصحاء والمصابات بمرض ما قبل الارتعاج

رسالة

مقدمة الى مجلس كلية الطب في جامعة بابل

كجزء من متطلبات نيل شهادة الماجستير

في الكيمياء الحياتية السريرية

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علي محمد كاظم

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اشراف

الاستاذ الدكتور

مها فاضل سميسم

الاستاذ الدكتور

نادية مضر الحلي

Republic of Iraq
Ministry of Higher Education and Scientific Research
University of Babylon/ Collage of Medicine
Department of Chemistry and Biochemistry

Comparative Study of Lipoprotein Lipase Enzyme , ApoE and ApoC2 , in Pregnant women with and without preeclampsia

A thesis
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By
Ali Mohammed Kadhim

B.Sc. Medical laboratory technique \ Alkafeel University (2016)

Supervised By

Professor
Dr. Maha F. AL-Smaism

Professor
Dr. Nadia M. Al-Hilli

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بسم الله الرحمن الرحيم

﴿يُؤْتِي الْحِكْمَةَ مَنْ يَشَاءُ وَمَنْ يُؤْتَ الْحِكْمَةَ فَقَدْ أُوتِيَ خَيْرًا كَثِيرًا وَمَا يَذَّكَّرُ إِلَّا أُولُوا الْأَلْبَابِ﴾

صدق الله العلي العظيم

سورة البقرة

(الآية 269)

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ALI MOHAMMED KADHIM

DEDICATION

This work dedicates to my father and My mother for their kindness and support.

My Sister have been a pillar of strong. My lovely sons. My family for their blessings.

Everyone gave me support and help, especially Dr.Maha F. AL-Smaism and Dr. Nadia M. Al-hilli .

ALI MOHAMMED KADHIM

الخلاصة

مرض ما قبل الارتعاج هو احد الاسباب الرئيسية للوفيات عند النساء الحوامل والجنين وحديثي الولادة. يعتبر الارتعاج من الامراض المعقدة حيث ان النشوء المرضي غير مفهوم بالكامل الى هذه اللحظة. النشوء المرضي الأكثر شيوعًا هو حدوث ضعف في غزو الارومة الخلوية وفشل في إعادة تشكيل الشريان الحلزوني. اضطراب الدهون في الدم اصبح في الوقت الحاضر أحد أهم العوامل الخطرة للإصابة بمقدمات الارتعاج حيث تشير الدلائل الناشئة إلى أن خلل التنظيم في التمثيل الغذائي للدهون لدى الأم والمشيمة هو المتسبب في الحالة.

تهدف هذه الدراسة إلى تقدير مستوى الانزيم المحلل للدهون في الدم ، و البروتين Apo C2 والبروتينApoE في النساء الحوامل المصابات بمرض ماقبل الارتعاج بالمقارنة مع النساء الحوامل الطبيعيات ، وإيجاد علاقة بين العلامات البيوكيميائية للدراسة بين هؤلاء المرضى.

اجريت هذه الدراسة بالتعاون مع مستشفى بابل التعليمي لأمراض النساء والأطفال في محافظة بابل حيث تم جمع العينات في الفترة من أكتوبر 2021 حتى فبراير 2022. وشملت الدراسة 90 امرأة حامل ، حيث قسمت الدراسة الى مجموعتين , المجموعة الاولى تشمل خمسة وأربعون مصابات بمرض ما قبل الارتعاج ،وكان هنالك (9) نساء مصابات بمرض ما قبل الارتعاج من النوع الحاد و(36) امرأة مصابات بمرض الارتعاج من النوع المعتدل وشملت المجموعة الثانية خمسة وأربعون من النساء الحوامل الأصحاء اللائي تم أخذهن كعنصر تحكم في نفس فترة الحمل. تم استبعاد النساء اللواتي تزيد أعمارهن عن 40 عامًا ، ومؤشر كتلة الجسم أكبر من 30 كجم / م 2 ، ومرض السكري من النوع 1 أو 2 ، وارتفاع ضغط الدم الموجود مسبقًا أو أمراض الكلى والتدخين من هذه الدراسة.

تم جمع العينات وفصل مصل الدم لقياس مستويات الانزيم المحلل للدهون في الدم و البروتينApo C2 والبروتين Apo E بتقنية مقايسة الامتصاص المناعي المتصل بالأنزيمات . تم حساب متوسط العمر والانحراف المعياري للمشاركين في مجموعة الاولى (مصابات بمرض ماقبل الارتعاج) والمجموعة الثانية (النساء الحوامل الاصحاء) وأظهرت فروقًا ذات دلالة إحصائية (قيمة P. >0.05) بين المجموعات. أظهرت نتائج الدراسة الحالية زيادة غير معنوية (قيمة P. >0.05) في مؤشر كتلة الجسم للمرضى الذين يعانون من تسمم الحمل بالمقارنة مع مجموعة الاصحاء. واظهرت الدراسة عدم وجود فرق إحصائي (قيمة P. >0.05) بين متوسطات عمر الحمل في مجموعة المرضى والمجموعة الضابطة. كذلك بينت الدراسة النسبة المئوية لعدد مرات الانجاب للمشاركين في مجموعة المرضى حيث قسمت إلى ولادة لأول مرة (75.5٪) ، ولادة اكثر من مرة (24.5٪) ومجموعة التحكم مقسمة إلى ولادة لاول مرة (46.6٪) ، و ولادة اكثر من مرة (53.4٪) على التوالي تم حساب النتائج وأظهرت فروق ذات دلالة إحصائية عالية عند (قيمة الاحتمال <0.001) بين المجموعات المتطابقة.

اظهرت الدراسة ان مجموعة المرضى كانت لديهم نسبة عالية من البيليروبين الكلي (TSB) ، و AST (الانزيم الناقل لمجموعة الامين-اسبارتيت) ، و ALT (الانزيم الناقل لمجموعة الامين-اللانين) ، والفوسفاتيز القلوي مقارنةً بمجموعة التحكم ذات الأهمية الإحصائية العالية (قيمة p <0.001). وبينت

الدراسة متوسط مستوى اليوريا والجلوكوز في مرضى ما قبل الارتعاج ليس معنويا إحصائيا مع مجموعة السيطرة عند (p.value > 0.05).

اظهرت نتائج الدراسة ان مستويات الانزيم المحلل للدهون في الدم كانت أعلى بشكل ملحوظ عند (P.<0.001) في مرضى تسمم الحمل مقارنة بالضوابط ، بمعدل 200.10 مقابل 134.80، على التوالي. في حين أن متوسط مستويات البروتين Apo C2 في مرضى ما قبل الارتعاج (186.08) والضوابط (121.7) لها أهمية احصائية عالية حيث (P <0.001). كذلك البروتين Apo E كان له أهمية عالية عند (p. <0.001) في مرضى ما قبل الارتعاج مع مجموعة الضوابط ، بمعدل 793.2 مقابل 512.4 ، على التوالي. ،.

كما اظهرت الدراسة هناك ارتباطًا إيجابيًا معنويًا (P <0.001، r = 0.5) بين LPL و ApoC2 كما أن هناك ارتباطًا إيجابيًا معنويًا بين (LPL وApo E) و(ApoC2 و Apo E) مع (P.<0.001 ، r =0.35) (P.<0.001, r =0.319) على التوالي بين مرضى ما قبل الارتعاج كما اظهرت الدراسة هنالك علاقة طردية (موجبة) بين الانزيم المحلل للدهون ومؤشر كتلة الجسم (P.<0.001,r=0.442).

في الختام الاستنتاج من الدراسة هو زيادة مستويات الانزيم المحلل للدهون و البروتين Apo C2، وبين البروتين Apo E عند هؤلاء المرضى بالمقارنة مع الضوابط قد تعمل كمؤشر لتشخيص مرض ما قبل الارتعاج.

Summary

Pre-eclampsia is a multisystem progressive disorder characterized by the new onset of hypertension and proteinuria or the new onset of hypertension and significant end-organ dysfunction with or without proteinuria in the last half of pregnancy or postpartum. Preeclampsia is a complex disorder and the pathogenesis of it is still not fully understood . The most commonly accepted theory of pathogenesis assumes that there is impaired trophoblastic invasion and failure in spiral artery remodeling. Nowadays, dyslipidemia becomes one of the most important, modifiable risk factors for the development of preeclampsia. Emerging evidence suggests that dysregulation of maternal and placental lipid metabolism are involved in the pathogenesis of the condition.

This study aimed to estimate serum level of lipoprotein lipase, Apolipoprotein C2 and Apolipoprotein E in preeclamptic pregnant women in comparison with normal pregnant women and to assess the severity among those patients by studing some biochemical markers.

A case control study was carried out in Babylon Teaching Hospital for Maternity and Pediatrics, Babylon Province. The studied patients attended the outpatient clinic and Labour room. All samples were collected from October 2021 till February 2022. The study included (90) pregnant women , (45) of them were with preeclampsia and (45) healthy pregnant women taken as control group with matching gestational age . In patient group (9) women with sever preeclampsia and (36) woman without sever feature .All participant were with no preexisting hypertension ,renal disease , diabetes mellitus nor liver disease. Women with age

over 40 years, BMI ≥30Kg/m^2, Diabetes type 1 or 2,Pre-existing hypertension or renal disease and smoking were excluded from this study.

Information was collected from patient such as age , parity , gestational age and some sign and symptom then venous blood was withdrawn, and serum collected for biochemistry to determine the levels of LPL, ApoC2 and APoE by ELISA technique.

The means of age and gestational age of participants of preeclampsia group and control group were calculated and showed no significant differences (P. value >0.05) between the matched groups. The percentage of parity for participants of preeclampsia group included primigrivida (75.5%), multigravida (24.5%) and control group also included primigravida (46.6%), multigrivida (53.4%). The results were calculated and showed highly significant difference at (p. value <0.001) between the matched groups.

Preeclampsia patients had a significantly higher total bilirubin (TSB), AST(aspartate aminotransferase), ALT(alanine aminotransferase) and alkaline phosphatase than the control with highly statistical significance (p.value <0.001) . The average serum level of urea and glucose in preeclmpsia patients are statistically not significantly different from control at (p.value > 0.05).

There is a significant increase in the means of LPL in patient group (p.value <0.001) when compared to the control group (200.10ng/dl)vs.(134.80ng/dl) respectively. While there is no statistical difference in means of LPL concentrations between subgroups.

There is a significant increase in the means of ApoC2 in patient group (p.value <0.001) when compared to the control group (186.08ng/ml) vs. (121.7ng/ml)

respectively. In addition there is no statistical difference in means of ApoC2 concentrations between subgroups.

There is a significant increase in the means of ApoE in patient group (p.value <0.001) when compared to the control group (793.2ng/ml) vs.(512.4ng/ml) respectively. In addition there is highly statistical difference in means of ApoE concentrations between subgroups.

The results of bivariate correlation analysis presented in preeclampetic patients revealed that there is significant positive correlation($P<0.001$, $r=0.5$) between LPL and Apoc2.Also there is a significant positive correlation between LPL and ApoE with (P.value <0.001, $r=0.359$) .

In conclusion increase the levels of LPL , ApoE and Apo C2 in those patients may serve as a marker for preeclampsia complication that which leads to worse consequences.

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List of Abbreviations

Abbreviation	Details
ANGPTL4	Angiopoietin Like 4
apo	Apo lipoproteins
ABCA1	ATP-binding cassette transporter A1
ABCG1	ATP-binding cassette transporter G1
HRP	Avidin-Horseradish Peroxidase
BBB	blood-brain barrier
BNB	blood-nerve barrier
BMI	Body mass index
CAA	cerebral amyloid angiopathy
CMs	Chylomicron
DPB	diastolic blood pressure
eNOS	endothelial nitric oxide synthase
ELISA	Enzyme linked immune sorbent assay
GPIHBP1	Glycosylphosphatidylinositol Anchord High density lipoprotein Binding Protein 1
HELLP	Hemolysis, Elevated Liver enzyme , Low Platelet
HSPGs	heparans sulfate proteoglycans
HL	Hepatic lipase
HDL	High density lipoprotein
HIF- 1α	Hypoxia Inducible Factor - 1α
IDL	Intermediate density lipoprotein
LRP	LDL-receptor–related protein
LCAT	lecithin cholesterol acyl transferase
Sel1L	lin-12-like protein 1
LMF1	lipase maturation factor
LPL	Lipoprotein lipase

LDL	Low density lipoprotein
LPA	Lysophosphatidic acid
NKT	natural killer T
NOS	nitric oxide synthase
OD	optical density
PIGF	placental growth factor
PLAT	Polycystin-1, Lipoxygenase, Alpha-Toxin domain
PUFA	polyunsaturated fatty acid
PE	Preeclampsia
sEng	soluble endoglin
sFlt-1	soluble fms-like tyrosine kinase 1
S1P	sphingosine-1-phosphate
SCD1	syndecan-1
SPB	Systolic blood pressure
NICE	The National Institute for Health and Clinical Excellence
TGF- β3	Transforming growth factor
TNF-α	tumor necrosis factor
VEGF	Vascular endothelial growth factor
VLDL	Very low density lipoprotein
β-FGF	β- fibroblast growth factor

Supervisor Certification

We certify that this thesis was prepared under our supervision at the College of Medicine, University of Babylon, as partial fulfillment of the requirement for the master degree of science (M.Sc.) in Clinical Biochemistry.

Prof. Dr.
Maha F. AL-Smaism

Prof. Dr.
Nadia M.AL-Hilli

In review of the available recommendation, I forward this thesis for debate by the examining committee.

Prof. Dr.

Abdulsamie Hassan Alta'ee

Head of Chemistry and Biochemistry Department

Appendix I:

Clinical signs and symptoms of preeclamptic women

Variables		Preeclampsia	
		No.	**%**
Headache		Yes: 30	**66.7**
		No: 15	**33.3**
Edema		Yes: 25	**55.6**
		No: 20	**44.4**
Nausea & Vomiting		Yes : 16	**35.6**
		No: 29	**64.4**
Blurring of vision		Yes: 4	**8.8**
		No: 41	**91.2**
Blood pressure	Mild	10	**22.2**
	Moderate	26	**57.8**
	Sever	9	**20**

Decision of Discussion Committee

We, the examination committee, certify that we have read thesis entitled " **Comparative Study of Lipoprotein Lipase Enzyme , ApoE and ApoC2 , in Pregnant women with and without preeclampsia "** and as examining committee examined the student "**Ali Mohammed kadhim "** in its content and in our opinion it is adequate with "**Excellent"** rating as a thesis for the degree of Master in Science of Clinical Biochemistry.

Prof. Dr.
Maysaa Jalal Majeed
College of Medicine/University of Baghdad
(Chairman)

Assist. Prof. Dr.
Thanaa Mohammed Juda
College of Medicine
University of Babylon
(Member)

Prof. Dr.
Bushra Jaber Al-Rubayae
College of Medicine
University of Babylon
(Member)

Prof. Dr.
Dr. Maha F. AL-Smaism
College of Medicine
University of Babylon
(Member and Supervisor)

Prof. Dr.
Nadia M. Al-Hilli
College of Medicine
University of Babylon
(Member and Supervisor)

Approved by the council of the College of Medicine

Prof. Dr.
Mohend Abbass Al-Shalah
Dean of College of Medicine/ University of Babylon

1.1.Preeclampsia

1.1.1. Definition

Preeclampsia, a human-pregnancy-specific disease defined as the occurrence of hypertension and significant proteinuria in a previously healthy woman on or after the 20 week of gestation[1]. Preeclampsia can diagnose when blood pressure elevated with proteinuria (≥ 300 mg /24 h or a urinary protein: creatinine ratio of ≥ 0.3 mg/dl in a spot urine random sample dipstick reading of 2+)[2]. Preeclampsia is a frequent complication of pregnancy that involves one of the largest organs in the human body: the vascular system. Likewise, the hallmarks of this syndrome are hypertension and other complications such as renal insufficiency, liver dysfunction, neurological complications (e.g. eclampsia and stroke), thrombocytopenia, hemolysis and fetal growth impairment can develop and may require intervention. Preeclampsia is characterized by placental hypoxia and/or ischemia, excessive oxidative stress, in association with endothelial dysfunction. Release of soluble factors from the ischemic placenta into maternal plasma plays a central role in the ensuing endothelial dysfunction that is the most prominent feature of this disease.[3] Women who have hypertensive disorders during pregnancy are at risk for intrauterine growth restriction, placental abruption, preterm birth, and cesarean birth[4]. However, the course of the disease can worsen abruptly and hypertensive disorders accounted for 15.5% of all maternal mortality. Preeclampsia is responsible for over 70 000 maternal deaths and 500 000 fetal deaths worldwide every year [5]. It has been estimated that preeclampsia complicates 2–8% of pregnancies [6]. The prevalence of preeclampsia in Iraqi ladies was 4.79% which was higher than neighboring countries such as Iran (4%) and Jordan (1.3%) [7].

1.1.2.Diagnostic Criteria for Preeclampsia

1-Blood pressure : Systolic blood pressure of ≥140 mm Hg or diastolic blood pressure of ≥ 90 mm Hg on two occasions at least 4 hours apart after 20 weeks of gestation in a woman with a previously normal blood pressure [8].

2-Proteinuria**:** Diagnostic criteria for proteinuria include at least ≥300 mg per 24 hour urine collection or Protein/creatinine ratio of ≥0.3 mg/dL or dipstick reading of 2+ (used only if other quantitative methods not available) [9].

Proteinuria is not essential for diagnosis if a severe feature (Thrombocytopenia, Renal insufficiency, Impaired liver function, Pulmonary edema) is present hypertension is sufficient for the diagnosis[10.]

1.1.3. Classification of Preeclampsia

1.1.3.1.Preeclampsia without severe features

In the past, preeclampsia without severe features was characterized as mild. However, it is recognized that preeclampsia is a spectrum and can progress in severity quite rapidly[11].

SBP ≥ 140mmHg and/or DBP ≥90 mm Hg in a previously normotensive woman with Proteinuria . It's called preeclampsia without sever feature in the absence any of the following features: cerebral symptoms (like visual disturbance, headache), right upper quadrant or epigastric pain, serum transaminase concentration ≥ twice normal, systolic blood pressure ≥160 mm Hg, and or diastolic blood pressure ≥110 mm Hg on two occasions at least four hours apart, severe thrombocytopenia (< 100,000 platelet/micro) and pulmonary edma[12] .

1.1.3.2. Severe preeclampsia

There was an agreement to define severe preeclampsia by blood pressure values ≥160 mmHg systolic or ≥110 mmHg diastolic. The amount of proteinuria was considered not useful to define the severity. The HELLP syndrome was considered a feature to include in the severe classification, and that could be identified by

1-Thrombocytopenia (platelet count less than $100{,}000 \times 10^9$/L)

2-Impaired liver function that is not accounted for by alternative diagnoses and as indicated by abnormally elevated blood concentrations of liver enzymes (to more than twice the upper limit normal concentrations), or by severe persistent right upper quadrant or epigastric pain unresponsive to medications

3- Renal insufficiency (serum creatinine concentration more than 1.1 mg/dL or a doubling of the serum creatinine concentration in the absence of other renal disease)

4- Pulmonary edema

5- New-onset headache unresponsive to medication

6- Visual disturbances[13]

Headache is included in the definition of the visual and cerebral disturbances associated with severe preeclampsia. The description of headache associated with preeclampsia include severe, persistent, worst headache ever experienced," or "not relieved with analgesics[14].

1.2.Normal Placentation

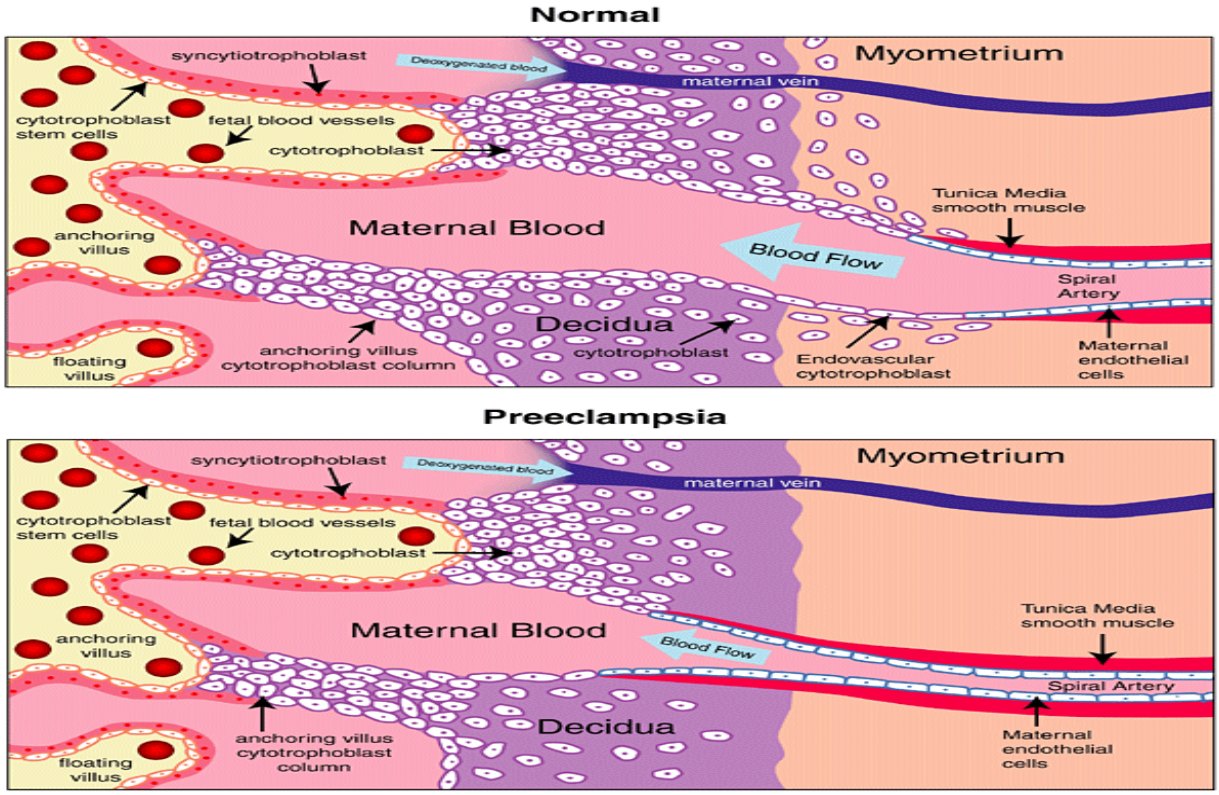
The placenta is an organ exclusively formed for pregnancy. It forms the sole surface where contact between fetus and mother is established, and where exchange of oxygen and nutrients can take place. The placenta consists of a maternal and a fetal part. The maternal part is composed of the decidua: this is former endometrium, the lining of the uterus that has grown and has become vascularized to facilitate implantation of the blastocyst. The fetal part consists of trophoblast cells derived from the outer layer of the blastocyst: they invade the decidua and build the branching structure of the villous tree. Villi are thin, protruding portions of the fetal part of the placenta. They float in the maternal decidual blood, where their specialized outer cell lining, the syncytiotrophoblast, composed of a syncytium of trophoblast cells, facilitates the exchange of oxygen and nutrients[15]. To ensure sufficient blood supply to this fast-growing organ, the arteries in the decidua and myometrium, the "spiral arteries" undergo substantial changes during early pregnancy. Fetal trophoblast cells invade the spiral arteries and replace the internal elastic lamina and underlying smooth muscle layer by loose, fibrinoid matrix[16].This results in wider spiral arteries with a lower resistance, leading to increased placental blood flow with a low arterial pressure[17].

1.3. Pathophysiology

1.3.1.Abnormal placentation

The defective placenta is attributed to the development of preeclampsia. Examination of the preeclamptic placenta reveals numerous placental infarcts and arterial sclerosis. This is accompanied by placental hypoperfusion due to altered trophoblast invasion and, thus, placental ischemia. During a healthy pregnancy, the extravillous cytotrophoblast of fetal origin invades the spiral arteries in the decidua

and myometrium. This spiral remodeling is disrupted in preeclamptic condition(Figure1-1)[18].



(Figure1-1) Normal and abnormal placentation[18].

Due to impaired spiral artery remodeling, the placenta is deprived of oxygen, which leads to a condition called placental ischemia. This favors the production of antiangiogenic factors into maternal circulation that contributes to endothelial damage. Previous study have shown that increased levels of antiangiogenic factors like tyrosine kinase-1 (sFlt-1) [19] and soluble endoglin (sEng) released by the placenta into the maternal circulation during preeclampsia contributes to endothelial dysfunction and thus cause proteinuria[20]. However, the mechanisms underlying the ischemia-induced increase in antiangiogenic factors remain unknown. A candidate molecule that may provide the missing link is hypoxia-

inducible factor-1 (HIF-1). HIF-1 is a key regulator of the response to low oxygen levels, initiating transcription of numerous genes during hypoxia. It is a heterodimeric transcription factor consisting of subunit α, which is oxygen-sensitive and rapidly degraded and inactivated during normoxia, and subunit ß, which is constitutively active[21]. HIF-1 has been shown to be overexpressed in placentas from preeclamptic women, along with sFlt-1 and sEng. Moreover, sFlt-1 and endoglin, both have been shown to be up-regulated by HIF-1.Of interest, it was observed that women living at hypoxic high altitude are more likely to develop preeclampsia and/or IUGR than women living at sea level[22].

1.3.2.Oxidative stress

Placental ischemia disrupts the balance between reactive oxygen species (ROS) and antioxidants, resulting in oxidative stress-related damage to proteins, lipids, and DNA[23]. Preserving the muscle layer of the spiral arteries resulted in intermittent placental perfusion and repeated hypoxia/reoxygenation, which significantly affects the placenta in pregnancy.Furthermore, hypoxia/reoxygenation represents a powerful stimulus for the conversion of xanthine dehydrogenase to xanthine oxidase, an important source of generation of $O_2^{\bullet-}$, which is abundantly expressed in cytotrophoblast and syncytiotrophoblast cells[24]. Placenta-derived lipid peroxides affect the circulating blood with lipoproteins, particularly low-density lipoprotein (LDL), and promote its peroxidation contributing to propagate the systemic lipid peroxidation and maternal vascular dysfunction[25]. When intracellular O2 •− production increases, •NO may also react with it, forming ONOO−. This is a powerful oxidant that can modify proteins and lipids by nitration. Additionally, peroxynitrite oxidizes BH4, resulting in the inactive molecule BH2 . In the absence of BH4, eNOS shifts from a homodimeric to a monomeric form, thus becoming uncoupled. In this free conformation, it does not synthesize •NO, but instead

generates O2 •−. In extracellulars, this uncoupling of eNOS has two important consequences: a loss of vasodilation due to the loss of •NO, and an increase in oxidative stress by the formation of O_2 •− [26].

1.3.3. Angiogenic- Antiangiogenic balance

The placenta is an organ that is highly vascularized by maternal and fetal blood vessels, and its development, maturation and functions depend on efficient vasculogenesis , angiogenesis and on cytotrophoblast invasion for spiral artery remodeling, which is called pseudo-vasculogenesis[27]. Vascular endothelial growth factor (VEGF), placental growth factor (PIGF), β- fibroblast growth factor (β-FGF), transforming growth factor β (TGF-β) and angiopoietins are some of the angiogenic proteins known to influence the vasculogenesis and angiogenesis[28]. VEGF is a cytokine synthesized by macrophages, T cells, tumor cells, and cytotrophoblasts and, thus, it is involved in various physiological and pathological conditions . VEGF isoforms include VEGF-A, VEGF-B, VEGF-C, VEGF-D and PlGF. Moreover, cells can express three main types of cellular receptors known as Flt-1 (fms-like tyrosine kinase receptor or VEGFR1), Flk-1 (kinase insert domain receptor or VEGFR2) and Flt-4 (VEGFR3), and they all have an extracellular domain, a transmembrane domain and an intracellular tyrosine kinase domain; as well as the soluble form of Flt-1 (sFlt-1), in which transmembrane and cytoplasmic domains are absent. In syncytiotrophoblast, sFlt-1 is synthesized through a splicing of mRNA of VEGRF-1 (or Flt-1) gene, which encodes proteins without the ability to bind to the VEGF or PlGF within cells, but capable of interacting with free growth factors in maternal circulation[29]. Studies indicate that the activation of Flk-1 and Flt-1 receptors would trigger signaling pathways associated with endothelial migration and proliferation, as well as vascular permeability ,formation, tubular ramification and maintenance of blood vessels; whereas Flt-4 would be related to

the development of lymphatic vessels[30]. Free bioactive VEGF levels are reported to be decreased significantly in preeclampsia, whereas sFlt-1 is found to be significantly elevated in preeclamptic pregnant women [31]. As shown in (figure 1-2)[32].

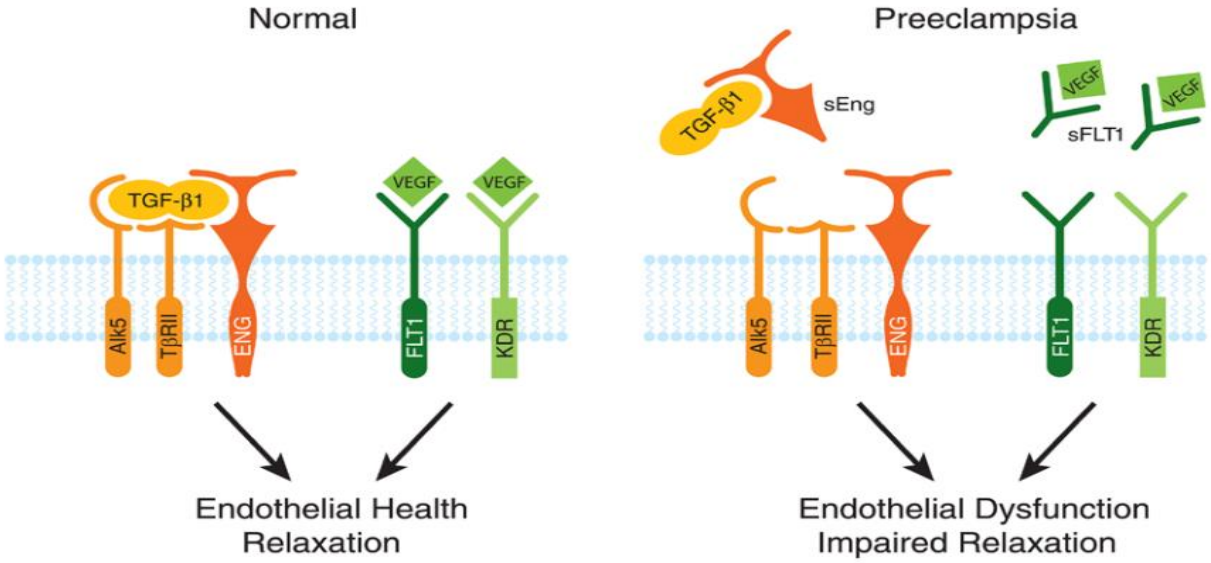


Figure (1-2) Soluble fms-like tyrosine kinase 1 (sFlt1) and soluble endoglin (sEng) causes endothelial dysfunction by antagonizing vascular endothelial growth factor (VEGF) and transforming growth factor (TGF)-β1 signaling[32].

Placental growth factor has structural homology to VEGF and is a potent angiogenic protein. PlGF is mainly secreted by syncytiotrophoblast of the placenta, which is also required for vasculogenesis throughout embryonic development during normal pregnancy. However, preeclampsia is found to be associated with decreased PlGF levels. In fact, a reduction in PlGF is noticed quite early in women who are destined to develop preeclampsia, whereas sFlt-1levels are elevated in the preeclamptic state[33] . sFlt-1 binds to both VEGF and PlGF in preeclampsia and prevents them from binding to endogenous receptors [34]. sFlt-1, when injected into mice, developed significant hypertension and proteinuria like preeclamptic

condition[35]. Several investigators have opined that sFlt-1 elevation in maternal circulation precedes the onset of clinical preeclampsia[36].

Endoglin, also known as CD105, is a membrane-bound protein that acts as a coreceptor for TGF-β1 and TGF-β3. It consists of an extracellular domain, a single transmembrane domain, and a short cytoplasmic domain and, similarly to Flt-1, it also has a soluble isoform (sEng) that has antiangiogenic properties[37] . Endoglin is a membrane-bound protein which binds with TGF-β1 and TGF- β3. This protein is found significantly in syncytiotrophoblasts, proliferating endothelial cells, hematopoietic stem cells, and stromal cells. However, sEng is a decoy receptor for TGF- β, which binds and blocks endoglin action[38]. In circulation, both sFlt-1 and sEng bind to their receptors and blocks the action of pro-angiogenic factors such as VEGF, PlGF, and TGF – β. Thus, it is clear from existing evidence that the expression of angiogenic factors during placental development is essential for the uteroplacental circulation and embryonic development[39].

1.3.5.Immunologic imbalance in preeclampsia

During preparation for embryo implantation, numbers of uterine natural killer cells (NK) rapidly increase. They produce cytokines involved in trophoblast invasion and angiogenesis instead of being cytotoxic. HLA-C on the trophoblast cells plays a major role in the recognition of trophoblast cells by uterine NK cells during trophoblast invasion. Failure of this recognition process is associated with impaired placentation and certain combinations of variants of uterine NK cell receptors and HLA-C are associated with preeclampsia[40].

In preeclampsia, there is a shift from maternal Th2 to Th1 cell activity. The cytokines produced by Th1 cells contribute to B-cell autoantibody production[41]. Preeclampsia is associated with abundance of a subset of B-cells prone to

synthesizing autoantibodies throughout pregnancy. The syndrome has been linked to angiotensin II receptor antibody production, which results in excessive stimulation of this receptor in the placenta and subsequent production of anti-angiogenic factors[40].

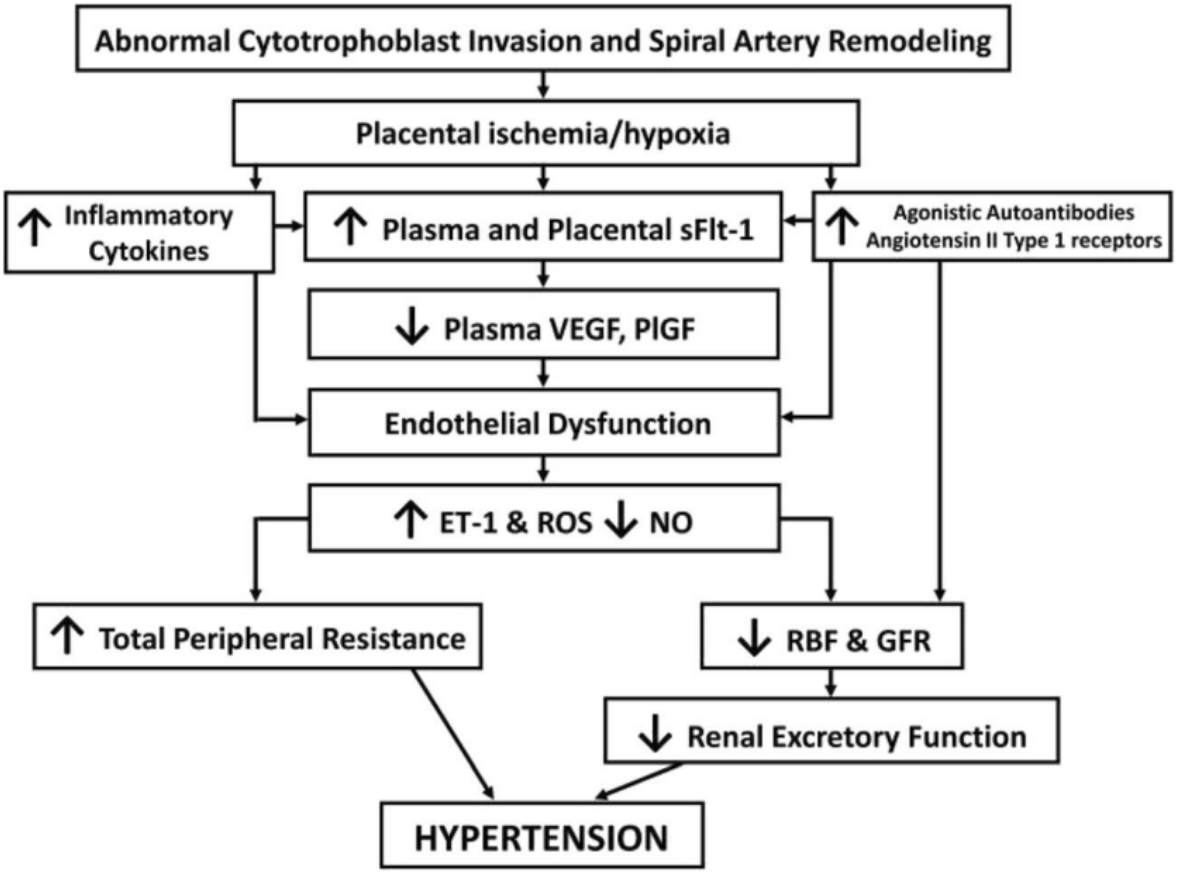


Figure (1-3) . Abnormal trophoblast invasion and spiral artery remodeling result in placental ischemia, endothelial dysfunction, and hypertension in preeclampsia[42].

1.4. Risk factor for preeclampsia

Several risk factors for preeclampsia have been identified including , a history of preeclampsia, maternal obesity, and other maternal chronic health conditions including hypertension, diabetes, and chronic heart diseases[43].

Preexisting pathological conditions such as chronic hypertension, diabetes mellitus, nephropathy, and anti-phospholipid antibody syndrome are also included ,as well as BMI ≥ 35[44] . The latter is associated with a higher incidence of multiple pregnancies and with an increase in the average age of women in their first pregnancy, which together act to increase the occurrence of preeclampsia [45]. The National Institute for Health and Clinical Excellence (NICE) proposed, a classification of the risk factors for preeclampsia as "moderate risk" and "high risk," so that it would make them tools capable of defining the group for which the immediate application of prophylactic measures would be indicated as shown in table (1-1) [46].

Table (1-1) Classification of preeclampsia risk factors as moderate and high [46].

	Moderate	High
Risk Factor	Primiparity	History of hypertensive
	Women aged 40 years or older	Chronic kidney disease
	Interdelivery interval greater than 10 years	Autoimmune diseases
	Body mass index (BMI) ≥ 35 kg/m2	Diabetes type 1 or 2
	A family history of preeclampsia	Chronic arterial hypertension
	Multiple gestations	

1.5.Lipoproteins

Triglycerides and cholesterol esters are transported in the form of lipoproteins. Triglyceride and cholesterol esters, fat soluble vitamins comprise the core of the lipoproteins and are enveloped by a layer of phospholipids, free cholesterol and proteins. The proteins [Apo proteins (apo) or Apo lipoproteins] are critical regulators of lipid transport and play a role in lipoprotein assembly, lipid transport and lipid metabolism by mediating interactions with receptors, enzymes and lipid transport proteins as shown in table (1-2) [47].

Table (1-2) Apolipoprotein ,Function and site of synthesis [47].

Apo lipoprotein	Lipoproteins	Metabolic functions	Synthesis
ApoAI	HDL, chylomicrons	Structural component of HDL, LCAT activator	Liver, intestine
ApoB48	chylomicrons	Necessary for assembly and secretion of chylomicrons from the small intestine	Intestine
ApoB100.	VLDL, IDL, LDL	Structural protein of VLDL, IDL and LDL. Ligand for LDL receptor	Liver
ApoCI	Chylomicrons, VLDL, IDL, HDL	inhibits lipoprotein binding to its receptors. Potent inhibitor of cholesteryl ester transfer protein.	Liver
ApoCII	Chylomicrons, VLDL, IDL, HDL	Activator of lipoprotein lipase	
ApoCIII	Chylomicrons, VLDL, IDL, HDL	Inhibits lipoprotein lipase; increases VLDL secretion.. Interferes with remnant lipoprotein clearance.	liver ,intestine
ApoE	Chylomicrons, VLDL, IDL, HDL	LDL receptor ligand for LDL and chylomicron remnants.	liver

1.5.1. Chylomicron

Triacylglycerol is the predominant fat in the diet, contributing 90%–95% of the total energy derived from dietary fat. Dietary fats also include phospholipids (predominantly phophatidylcholine), cholesterol and fat soluble vitamins[48]. After ingestion of a meal, dietary fat are absorbed into the cells of the small intestine and incorporated into the core of nascent CMs[49]. In the lymph and blood, CMs acquire apoCI, apoCII, apoCIII and apoE. After gaining apoCII the activator of LPL, chylomicron interacts with the enzyme and triglyceride hydrolysis is initiated. LPL mediated triglyceride hydrolysis is accompanied by a reduction in the core volume of the CMs and transfer of phospholipid, free cholesterol, apoCII and apoCIII back to HDL[50]. As shown in figure (1-4)[51].

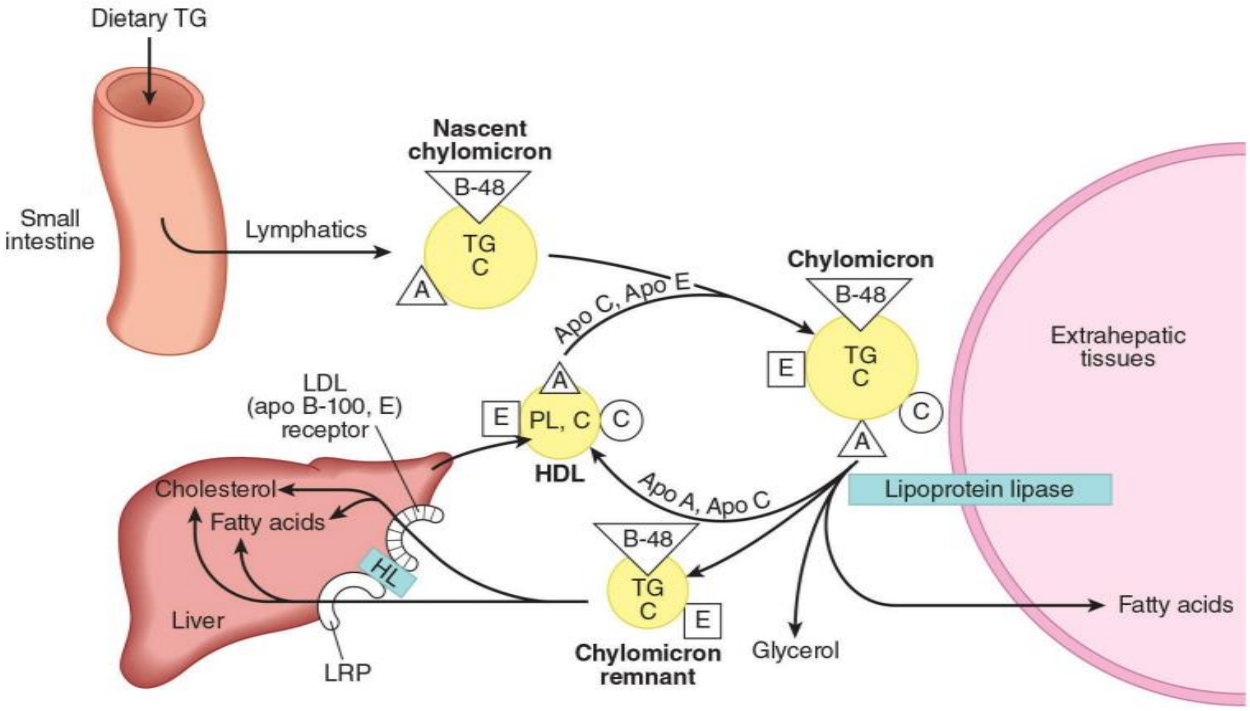


Figure (1-4) Metabolic fate of chylomicrons (A, apolipoprotein A; B-48, apolipoprotein B-48; C, apolipoprotein C; C, cholesterol and cholesteryl ester; E, apolipoprotein E; HDL, high-density lipoprotein; HL, hepatic lipase; LRP, LDL-receptor–related protein; PL, phospholipid; TG, triacylglycerol)[51].

As the chylomicron circulates, the core triglyceride undergoes hydrolysis by endothelial bound LPL with entry of fatty acids into muscle for energy production and adipocytes for storage. In the two-step model, the remaining relatively triglyceride-depleted chylomicron 'remnant' particles which are enriched in cholesteryl ester (from both dietary- and HDL derived cholesteryl ester) and apoE enriched can interact with receptors on hepatocytes and be removed from the circulation. All CMs usually disappear from the circulation within 12–14 h after a meal[50].

1.5.2.Very low density lipoprotein (VLDL)

Intestinal CMs and liver-derived VLDL represent the two classes of triglyceride rich lipoproteins responsible for the transfer of lipids to other cells of the body. CMs mediate the transport of dietary lipids whereas VLDL delivers endogeneous lipids to peripheral tissues[52]. ApoB100 is a large hydrophobic protein which is the main structural component of VLDL and LDL[53]. Once in the plasma VLDL is hydrolyzed by LPL to generate smaller denser particles and subsequently intermediate density lipoproteins (IDL). During lipolysis these remnants become enriched with HDL-derived apoE, a high affinity ligand for LDL receptor. IDL particles can undergo further catabolism by LPL to become LDL with loss of apoE. The sole remaining protein apoB100 binds to LDL receptors[54]. After VLDL has been converted to IDL, the remnant particles may be taken up by the liver directly via the LDL (apo B-100, E) receptor, or they may be further metabolized to LDL in the circulation. Only one molecule of apo B-100 is present in each of these lipoprotein particles, and this is conserved during the transformations[55].As shown in Figure (1-5)[56].

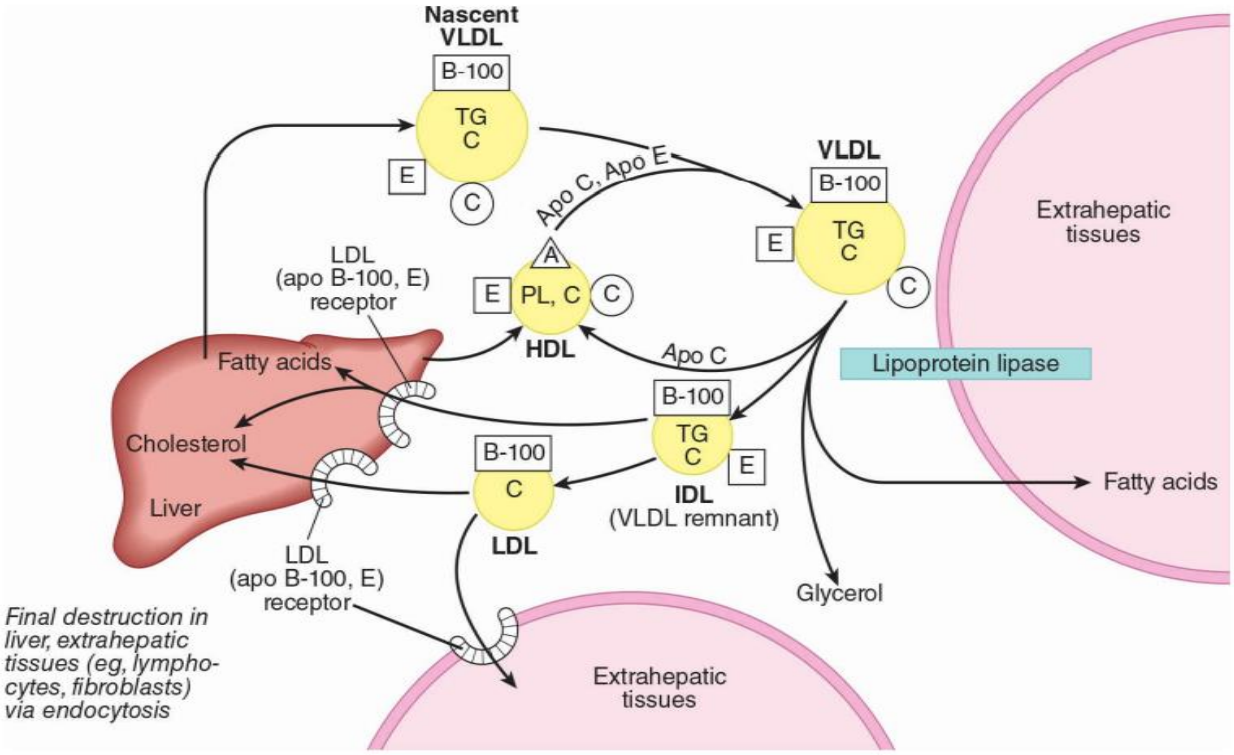


Figure (1-5).Metabolic fate of very-low-density lipoproteins (VLDL) and production of low-density lipoproteins (LDL). (A, apolipoprotein A; B-100, apolipoprotein B-100; C, apolipoprotein C; C, cholesterol and cholesteryl ester; E, apolipoprotein E; HDL, high-density lipoprotein; IDL, intermediate-density lipoprotein; PL, phospholipid; TG, triacylglycerol.) Only the predominant lipids are shown. It is possible that some IDL is also metabolized via the low-density lipoprotein receptor– related protein-1 (LRP-1.)[56].

1.5.3.Low density lipoprotein (LDL)

Low-density lipoprotein is the principal transporter of endogenous cholesterols and biologically active phospholipids (lysophosphatidic acid [LPA], sphingosine-1-phosphate [S1P], and sphingophosphorylcholine) from the liver where they are produced to the rest of the body[57]. Circulating LDL provides a constant supply of cholesterol necessary for the structural maintenance of cell membranes, cell growth, and steroid hormone synthesis.

Low-density lipoproteins are catabolized by either of 2 distinct pathways—a receptor-dependent pathway in the liver or a receptor-independent pathway in nonhepatic tissues. In the classical LDL receptor (LDLR)-dependent pathway, LDL particles interact with hepatocytes via high-affinity binding of the apolipoprotein B-100 surface protein [58]. as shown in (Figure 1-5). Approximately 30% of LDL is degraded in extrahepatic tissues and 70% in the liver[59]. Binding LDLs results in receptor-mediated endocytosis and lysosomal degradation of cholesterol esters and apolipoproteins. The LDL uptake via the receptor-independent pathway is determined by serum LDL concentration. Importantly, even at low-plasma LDL levels, LDLRs are saturated. Thus, when plasma LDL is elevated, receptor-independent uptake is greater than the amount of LDL catabolized by the liver and lipid deposition occurs. This imbalance is clinically significant because accumulation of deposited LDLs is a key event implicated in fatty streak development and atherosclerosis progression[60].

1.5.4. High density lipoprotein (HDL)

High density lipoproteins (HDL) transport cholesterol from peripheral tissues to the liver. The major apoproteins in HDL are Apo A1, with some Apo A2, Apo C and Apo E. HDL serves as a plasma reservoir of Apo C and Apo E which can be transferred to VLDL and chylomicrons and back[61]. HDL is synthesised in Liver cells and also in intestinal mucosal cells. Hepatic HDL Apo-A and apo-C are synthesised by polysomes on the rough endoplasmic reticulum (ER) and they are assembled with lipids to form the nascent HDL which is released in the circulation. Intestinal HDL in a similar manner, apo-A is synthesised by polysomes on the rough endoplasmic reticulum (ER) it is assembled with lipids to form the "nascent"- HDL which is released in circulation from intestinal mucosal cells[62].

Reverse cholesterol transfer pathway (RCT) begins when lipid-poor apolipoprotein A-I (apo A-I) is secreted from the liver or the small intestine. Apo A-I rapidly acquires phospholipid and cholesterol from cells by the ATP binding cassette transporter 1 (ABCA1) . ABCA1 is believed to pump excess cholesterol and other lipids to the outer surface of the plasma membrane, where apo A-I, in a detergent-like extraction process, removes phospholipid and cholesterol and forms nascent HDL. The form of HDL produced in this process is discoidal in shape and is named preβ-HDL based on its electrophoretic migration[63].

The free cholesterol derived from peripheral tissue cells are taken up by the HDL. The apo A-l of HDL activates LCAT (lecithin cholesterol acyl transferase) present in the plasma. Lecithin is a component of phospholipid bilayer of the HDL disc. The second carbon of lecithin contains one molecule of polyunsaturated fatty acid (PUFA). It is transferred to the third hydroxyl group of cholesterol to form cholesterol ester. The esterified cholesterol which is more hydrophobic, moves into the interior of the HDL disc.This reaction continues; till HDL becomes spherical with lot of cholesterol esters are formed. This HDL particle designated as HDL3. Mature HDL spheres are taken up by liver cells by apo A-l mediated receptor mechanism. HDL is taken up by hepatic scavenger receptor B1.Hepatic lipase hydrolyzes HDL phospholipid and TAG, and cholesterol esters are released into liver cells. The cholesterol that reaches the liver is used for synthesis of bile acids or excreted as such in bile[64]. When HDL3 remains in circulation, the cholesterol ester from HDL is transferred to VLDL, IDL and LDL by a Cholesterol Ester Transfer Protein (CETP). This will help to relieve product inhibition of LCAT so that more cholesterol can be taken up.Triacyl glycerol from VLDL, IDL and LDL is transferred to HDL in exchange for the cholesterol ester.The HDL particles that are rich in triacylglycerol and spherical are called HDL2[64].As show in figure(1-6)[65]

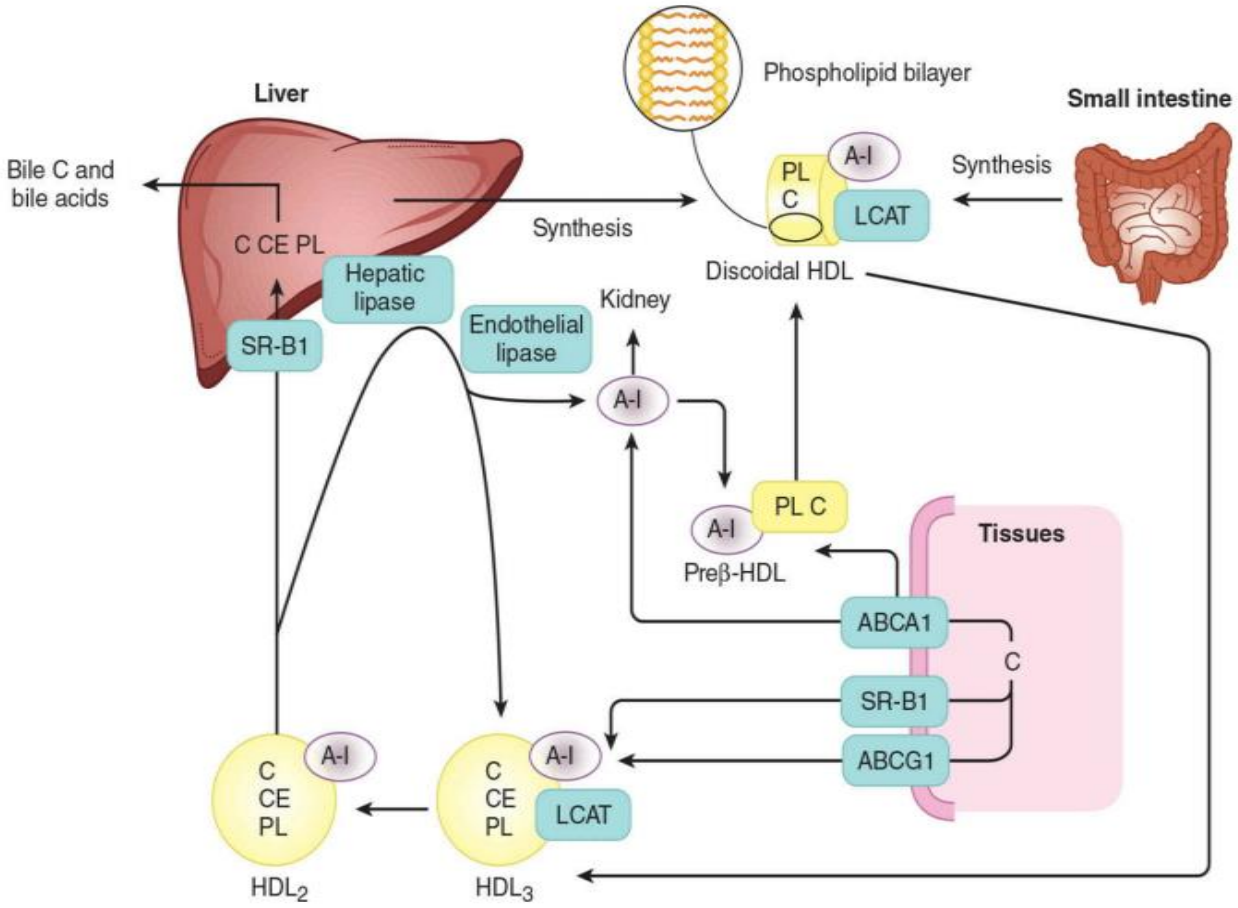
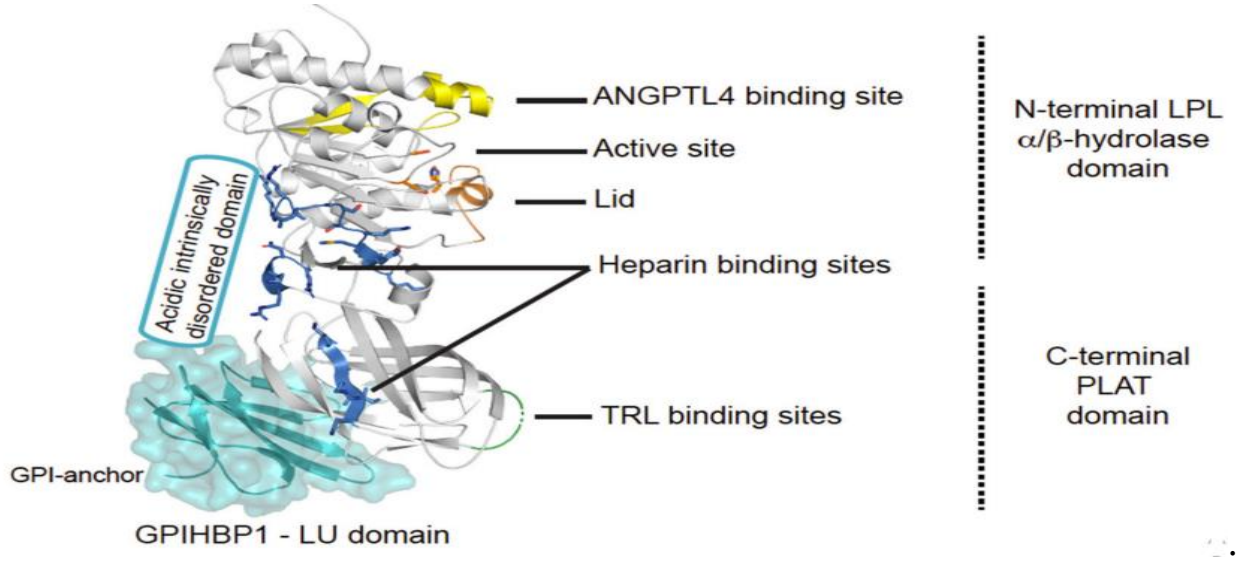


Figure (1-6) Metabolism of high-density lipoprotein (HDL) in reverse cholesterol transport[65]. (A-I, apolipoprotein A-I; ABCA1, ATPbinding cassette transporter A1; ABCG1, ATP-binding cassette transporter G1; C, cholesterol; CE, cholesteryl ester; LCAT, lecithin:cholesterol acyltransferase; PL, phospholipid; SR-B1, scavenger receptor B1.) PreβHDL, HDL2 , HDL3.

1.6. Lipoprotein Lipase

Lipoprotein lipase expression level is robust in cells and organs with a high oxidative metabolism, but it is also expressed in other tissue types, not related to intravascular lipolysis such as the spleen, testis, lung, kidneys, and brain as well as in macrophages[66] .LPL is a 55 kDa glycoprotein consisting of an N-terminal α/β-hydrolase domain and a C-terminal Polycystin-1, Lipoxygenase, Alpha-Toxin

(PLAT) domain (Figure1-7). The α/β-hydrolase domain harbors the active site that primarily hydrolyses the sn-1/sn-3 ester bonds of triglycerides, thereby releasing two unesterified fatty acids and a sn-2 monoacylglycerol[67]. The hydrolysis takes place at the luminal face of the capillary endothelium where LPL hydrolyzes the triglycerides within the neutral cores of chylomicrons and VLDLs producing chylomicron remnants and IDLs, respectively (Figure 1-4 and Figure 1-5) . The released free fatty acids (FFA) can either be used as an energy source or be re-esterified and stored in adipose tissues as triglycerides[68]



(Figure 1-7) Structural elements in LPL[69]. Cartoon representation of the human LPL•GPIHBP1(Glycosylphosphatidylinositol Anchord High density lipoprotein Binding Protein 1) complex and with the molecular surface of the LU domain of GPIHBP1 shown as a transparent light blue envelope . LPL elements implicated in ANGPTL4 (Angiopoietin Like 4) binding are highlighted in yellow. The active site containing the catalytic triad is represented with orange residues and the orange helix is the lid covering the active site responsible for substrate specificity . The triglyceride-rich lipoprotein (TRL) binding site is indicated in green and residues important for heparin binding is shown in blue .

1.6.1.Maturation and Transportation of LPL

Adipocytes and myocytes are the prime sources of LPL production and because these cells are located distantly from the capillary lumen, where LPL acts on marginated lipoproteins, it needs to be trafficked across the subendothelial space and trancytosed across the endothelial cells[70] . Accordingly, LPL secretion requires several chaperones and transporter proteins to ensure correct shuttling and folding of LPL[71] .One such protein is the lipase maturation factor (LMF1), which is essential for LPL processing since loss-of-function variants in LMF1 cause hypertriglyceridemia due to inefficient LPL secretion[72]. Besides LMF1, the suppressor of lin-12-like protein 1 (Sel1L) and syndecan-1 (SCD1) have been shown to be vital for LPL secretion[73] .As shown in figure (1-8)[69].

Upon successful secretion, LPL becomes tethered to cell surface heparans sulfate proteoglycans (HSPGs) via its heparin binding sites[74]. HSPG-bound LPL provides a storage pool that can be rapidly translocated to the capillary lumen via the endothelial cell surface bound protein GPIHBP1 . The movement of LPL towards GPIHBP1 is currently controversial and has been suggested to include random diffusion , directed diffusion on HSPG sulfation-gradients , and heparanase mediated release[75]. Interestingly, collagen XVIII with its heparin sulfate modifications may act as the proximal reservoir of LPL on the abluminal face of the capillary endothelium[76]. The expression of GPIHBP1 is restricted to capillary endothelial cells—it is absent in larger vessels—rendering the capillaries the sole relevant site for intravascular lipolysis[77].

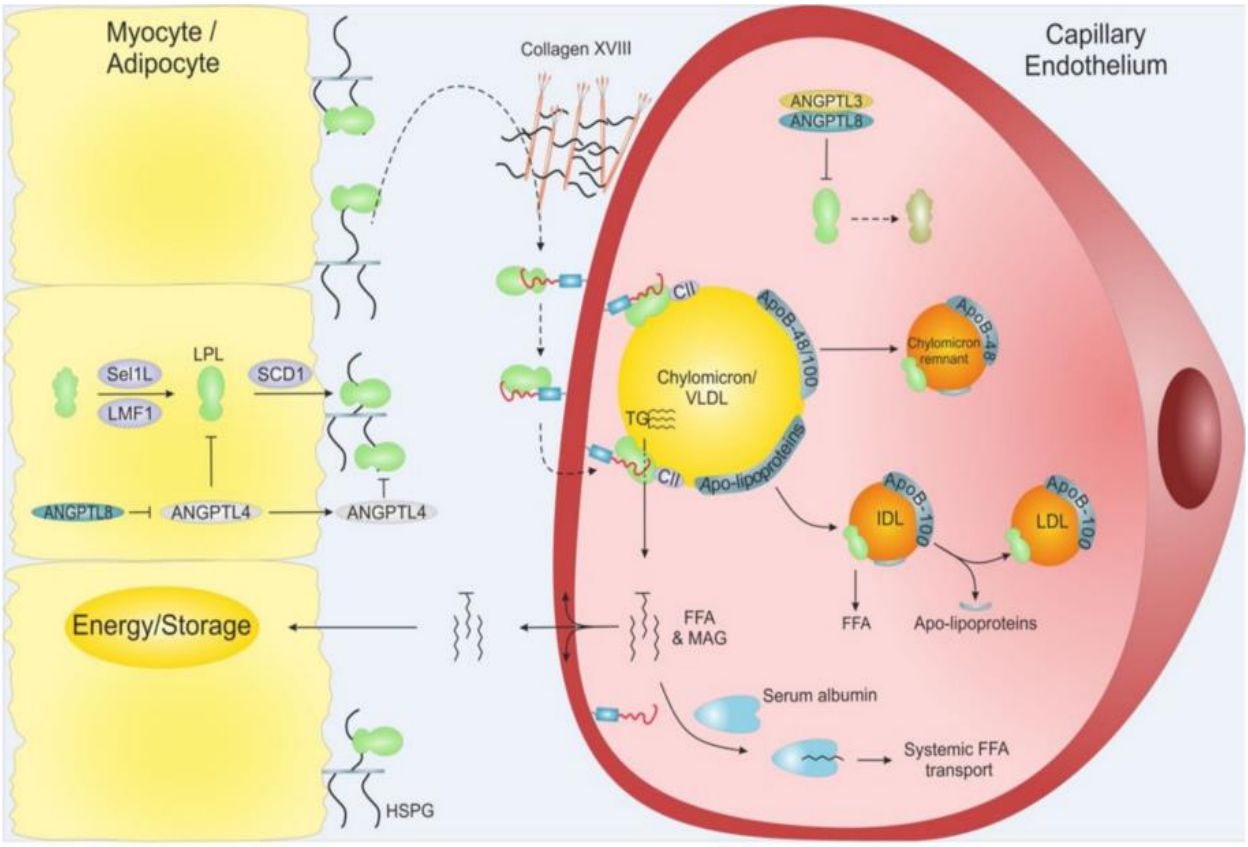


Figure (1-8) Schematic overview of LPL translocation[69]. lipase maturation factor (LMF1), lin-12-like protein 1 (Sel1L) , syndecan-1 (SCD1), ANGPTL4 (Angiopoietin Like 4),Heparan sulfate proteoglycan (HSPG).

1.6.2. Regulation of LPL by Apolipoprotein

Apolipoproteins are integrated in the maintenance of lipid homeostasis. They constitute a class of amphipathic proteins having a hydrophobic region that interacts with lipids in the lipoprotein particles, while the hydrophilic region allows interaction with soluble proteins. They provide structure-defining elements for lipoprotein particles and serve as ligands for specific receptors. Both ApoC-II and ApoA-V act as positive regulators of LPL in intravascular lipolysis, while ApoC-I and ApoC-III display an inhibitory role towards LPL[78].

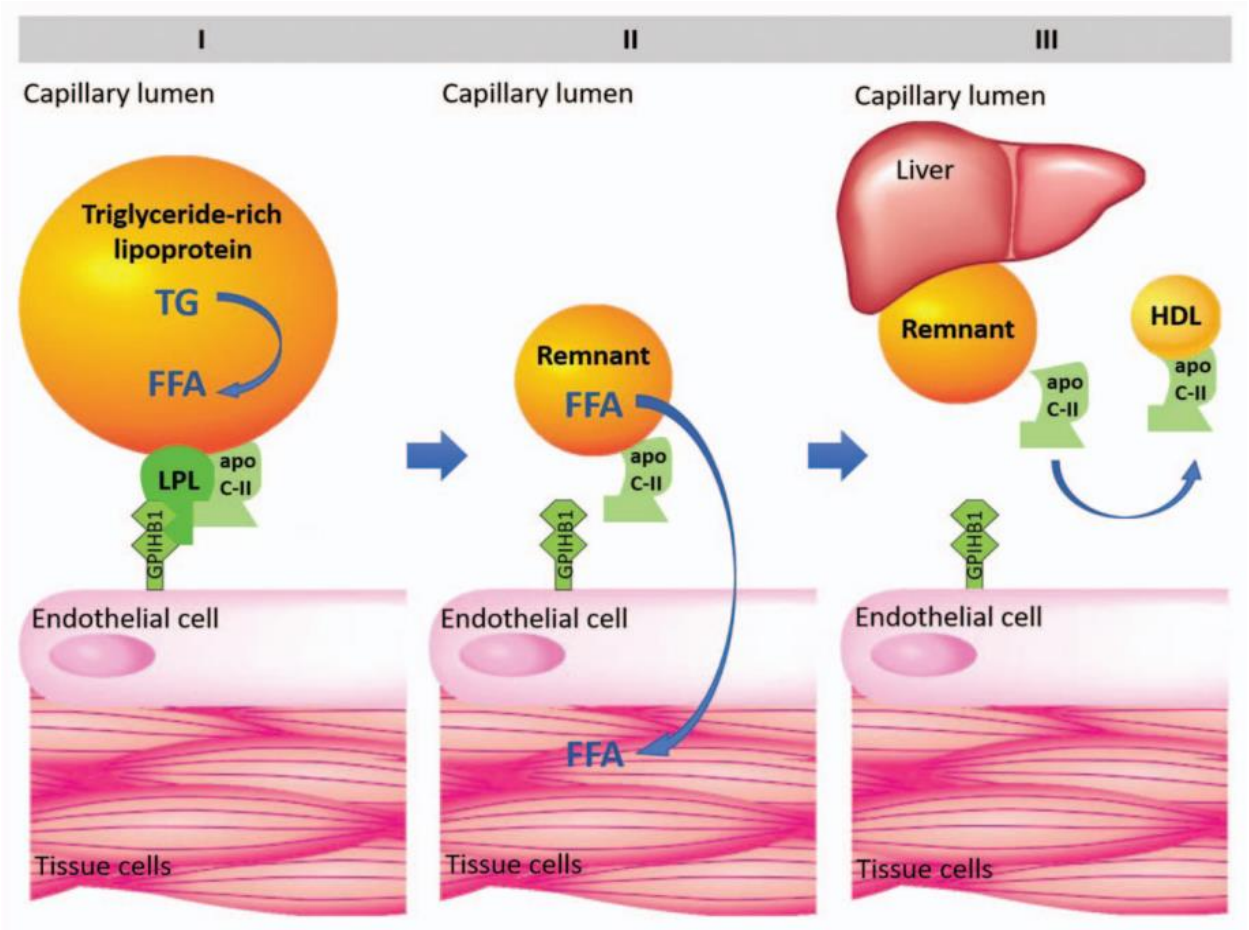
1.7. Apolipoprotein C-II

Apolipoprotein C-II is primarily expressed in the liver and secreted into plasma, but it is also produced by other tissues, including the intestine, macrophages, adipose tissue, brain, skin, lungs, retina, and retinal pigment epithelium[79]. After removal of its signal peptide and secretion into plasma, fully mature human apoC-II contains 79 amino acid residues and has a molecular weight of 8916 Da. Nuclear magnetic resonance structural studies of human apoC-II in a complex with sodium dodecyl sulfate micelles found that apoC-II contains three amphipathic a-helices[80] . The N-terminal helix, spanning residues 16–36, and a short central helix at residues 50–56 contain amphipathic helical sequences and appear to be needed for lipoprotein binding. The third helix in the C-terminus, spanning residues 63–77, is responsible for LPL activation. Although only the C-terminal fragment of apoC-II is required to activate LPL when a synthetic emulsion of long-chain TG is used as a substrate [81,82], the first two helices that bind lipoproteins are needed for maximum LPL activation when natural lipoproteins like chylomicrons are used as a source of TG substrate [83].

Study was proposed that ApoC-II regulates LPL activity in a pressure dependent model [84]. In this model, ApoC-II remains attached to the VLDL and chylomicron surfaces during LPL mediated triacylglycerol hydrolysis. As LPL consumes the neutral core TGs, the surface pressure on triglyceride rich lipoproteins (TRLs) increases. Once this surface pressure is greater than a given ApoC-II retention pressure, it leads to the extrusion of ApoC-II from the TRL. An unresolved paradox in Apo-CII biology is that over-expression of ApoC-II leads to high triacylglycerol , due to decreased lipolysis .Using the pressure dependent model, we can therefore hypothesize that high levels of ApoC-II on the TRL

surface would increase the surface pressure preventing LPL binding, thereby attenuating lipolysis, when ApoC-II is overexpressed[85].

There are several factors such as age, sex, diet that can affect plasma apoC-II concentration. ApoC-II levels increase until the age of 60 years in women, and until age 40 years in men[86] .Moreover, overweight-obese women have higher plasma apoC-II concentrations than normal weight women[87].



(Figure 1-9) Apolipoprotein-C-II-LPL-mediated lipolysis[88].

1.8. Apolipoprotein E

Apolipoprotein E, a 34 kDa glycoprotein, is a constituent of all lipoproteins except low-density lipoprotein (LDL), mainly synthesized by the liver with central role as cholesterol transporter[89] . ApoE regulates the clearance of lipoproteins from plasma and facilitates transporting lipid and cholesterols into cells through the binding to LDL receptor[90]. It is a polymorphic protein with three isoforms including: ApoE2, ApoE3, and ApoE4. ApoE3 considered to be the normal isoform and is the most common isoform, two other isoforms differ from ApoE3 by only a single amino acid; however, this single amino acid makes a significant change in their function as lipid transporter, while, ApoE3 and ApoE4 have similar affinities to bind with LDLR, ApoE2 only has almost 2% of the ApoE3 affinity for binding to LDLR[91] . Consequently lead to higher accumulation of very LDL, chylomicron, and lipids. ApoE4 increases production of cholesterol in liver and increases the level of LDL while reduces the level of high-density lipoprotein[92].

1.8.1. Functions of ApoE

Apolipoproein E is most important for lipid and lipoprotein metabolism, promoting the clearance of remnants of triglyceride-rich (apoB-containing) lipoproteins from the circulation into the liver because it is the ligand for the LDL receptor family of proteins and heparan sulphate proteoglycans (HSPG). In the brain, apoE itself assimilates and transfers lipids and affects on the amount of cerebral amyloid angiopathy (CAA). CAA is amyloid β deposition on the blood vessels in the brain and it is often associated with hemorrhagic lesions, ischemic lesions, encephalopathy, and dementia[93]. ApoE influences adipogenesis from triglyceride-rich lipoproteins [94]. Vascular function is affected in various ways by apoE: from maintaining blood-brain barrier integrity[95] to inflammatory responses [96]

ApoE also influences platelet aggregation [97] . Inflammatory effects include transformation of macrophages to M2 phenotypes and proliferation of lymphocytes and T helper cells[98]. Host responses to infections involve changes in lipid levels and lipid metabolism in the plasma. These plasma lipid changes might be mediated by three cytokines interleukin (IL)-1, IL-6, and tumor necrosis factor (TNF)-α, which induce elevated levels of triglycerides and VLDL, decreased levels of cholesterol, HDL and LDL[99].

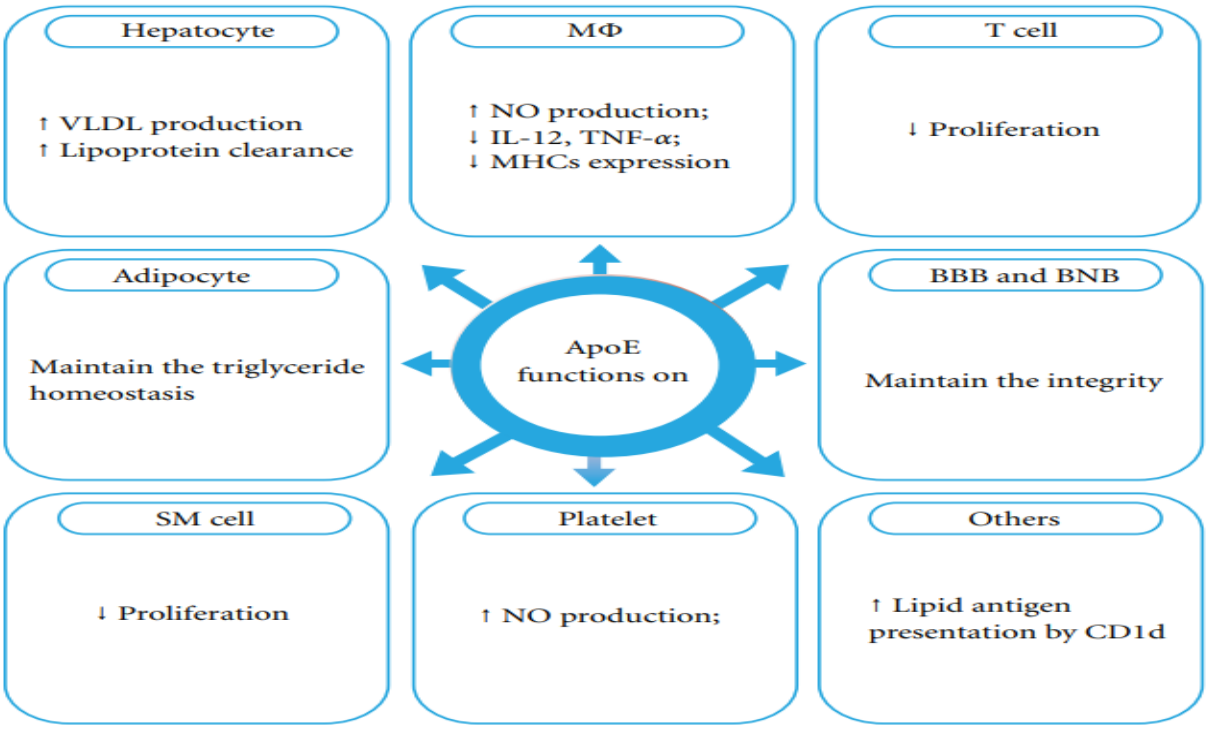


Figure (1-10) Schematic illustration of the biological properties of apoE[100]. ApoE acts on adipocytes to maintain the triglyceride homeostasis; acts on hepatocytes to promote very low density lipoprotein (VLDL) production and lipoprotein clearance; modulates the functions of macrophages (MΦ); suppresses the proliferation of T cells; maintains the integrity of blood-brain barrier (BBB) and blood-nerve barrier (BNB); inhibits the proliferation of smooth muscle (SM) cells; upregulates the production of nitric oxide (NO) of platelets; facilitates the presentation of lipid antigen by CD1 molecules to natural killer T (NKT) cells.

1.8.2. Distribution of apolipoprotein E on lipoproteins

Lipoprotein pathways and the roles of apoE in lipoprotein metabolism are illustrated in (Fig.1-11)[80]. In the fasting state apoE is found on VLDL and HDL. LDL, like IDL, carries practically no apoE. Post-prandially, chylomicrons undergo lipolysis and their remnants, like those of VLDL, are cleared rapidly. ApoE and apoCiii often coexist on apoB-containing lipoprotein, on almost half VLDL[96]. ApoCiii hinders clearance mediated by apoE[97]. Post-prandially, apoB-containing lipoproteins containing apoE can double in concentration by 4.7 hours, decrease in size to that of LDL by 6 hours and disappear by 10 hours[98].

ApoE initiates the biogenesis of HDL by accepting cholesterol and phospholipid from the ABCA1 transporter, but apoE4 is less effective than apoE3[99]. Newly formed discoidal particles, containing two molecules of apoE, gain cholesterol ester under the action of lecithin:cholesterol acyltransferase (LCAT), to almost the size of LDL. Recombinant apoE can create particles with varying composition of unesterified cholesterol, cholesterol ester and phospholipid. Large spherical apoE-containing particles are more prone to remodelling by phospholipid transfer protein than apoAi-containing particles. Even after modification by phospholipid transfer protein, prominent in cerebrospinal fluid (CSF), apoE tends to occupy larger HDL particles[100].

As shown in figure(1-11) the lipoprotein metabolism in the circulation concerns distribution of lipid from the gut and liver and recycling to the liver with little transfer of apoB-containing lipoproteins to the nervous system, though small lipoproteins containing apoE can bind receptors at the blood-brain barrier to exchange by transcytosis, supplying especially essential n-fatty acids to the brain and distributing these as well as other lipids within the nervous system[101]. ApoE is

synthesised by most cells in the nervous system and interacts with amyloid β and tau protein, apoE4 being linked with Alzheimer's disease and cerebral amyloid angiopathy[102].

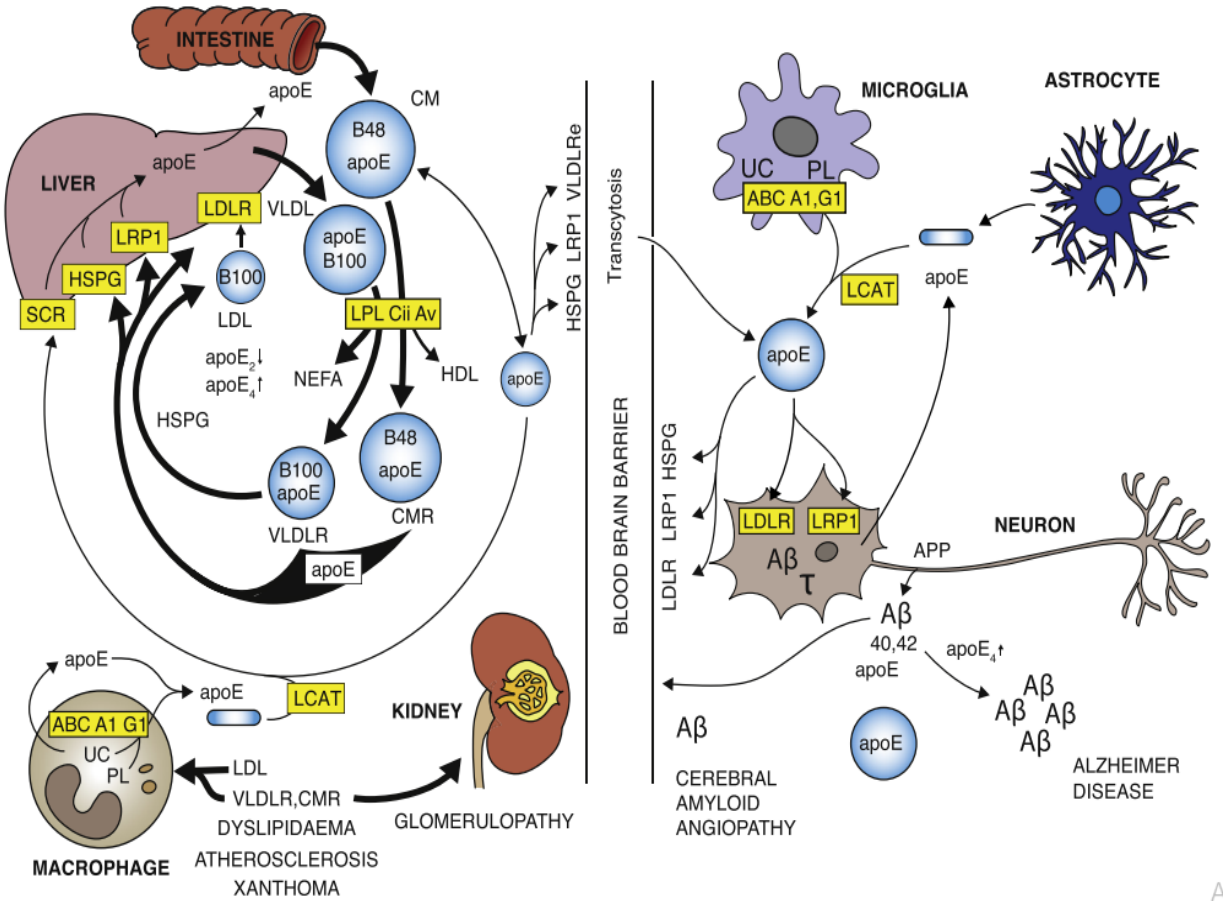
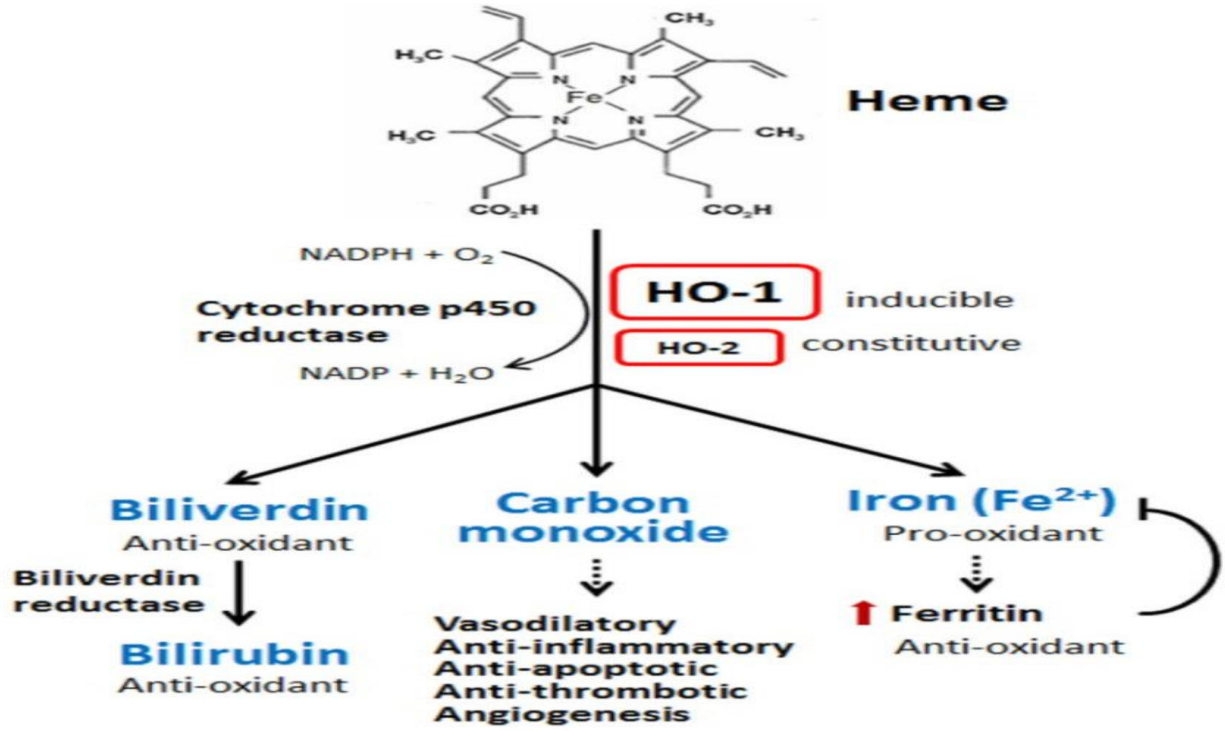


Figure (1-11) Apolipoprotein E and lipoprotein metabolism [101]. (Aβ) amyloid b protein; (ABCA1, G1), adenosine binding cassette transporters A1 and G1, (ApoE) Apolipoprotein E , (Av) Apolipoprotein Av ,(B48) Apolipoprotein B48 ,(B100) Apolipoprotein B100 ,(Cii) Apolipoprotein Cii ,(CM)chylomicron, (CMR) chylomicron remnant,(FC) free cholesterol(HDL) high density lipoprotein,(HSPG) heparan sulphate proteoglycan(LCAT) lecithin:cholesterol acyltransferase,(LDLR) low density lipoprotein receptor,(LPL) lipoprotein lipase,(LRP1)LDL receptor related protein 1(NEFA) non-esterified fatty acids(PL) phospholipid(SCR) scavenger receptor,(S) serine,(T)threonine, (t)tau protein, (UC)unesterifed cholesterol.

1.9.Liver Function Tests

1.9.1. Bilirubin

Bilirubin is derived from the breakdown of hemoglobin released from senescent erythrocytes and other hemoproteins, particularly cytochromes and several enzymes. Microsomal heme oxygenase (HO) cleaves heme at the α- methene bridge by a reaction requiring oxygen and a reducing agent, such as NADPH[103].As shown in figure (1-12)[104].



(Figure 1-12). Heme metabolism. heme oxygenase (HO)[104].

Bilirubin circulates in plasma bound to albumin. Albumin binding keeps unconjugated bilirubin in solution and prevents its diffusion into tissues and all its toxic effects. in the liver, bilirubin dissociates from albumin and is internalized by hepatocytes via facilitated diffusion. Within the hepatocyte, binding of bilirubin to glutathione- S-transferases (GSTs) inhibits its efflux, thereby increasing the net

uptake. Microsomal uridinediphosphoglucuronate glucuronosyltransferase type 1 (UGT1A1) catalyzes the transfer of glucuronic acid from UDP-glucuronate to bilirubin, forming mono- and diglucuronides. Glucuronidation makes bilirubin water soluble, reduces its toxicity, and promotes its secretion into bile. Finally, the bilirubin glucuronides are transported into the bile canaliculi by an energy-consuming process mediated by ABCC2[104].

1.9.2.Aspartate aminotransferase (AST)

In humans, AST exists as two genetically and immunologically distinct isoenzymes: cytoplasmic AST (cAST or GOT1) and mitochondrial AST (mAST or GOT2). AST has multiple metabolic functions. In the liver, 80% of AST activity is found in mitochondria and 20% in cytoplasm. Mitochondrial AST accounts for about 65% of the total AST activity in human cardiac tissue . AST has multiple metabolic functions. First, the most important metabolic function of AST is maintenance of the nicotinamide adenine dinucleotide/ reduced nicotinamide adenine dinucleotide (NAD+ / NADH) ratio in cells. Second, the AST reaction product—aspartate— is a highly active amino acid participating in numerous metabolic functions including synthesis of purine and pyrimidine bases, urea synthesis, protein synthesis and gluconeogenesis. Third, α-keto acids produced by AST reaction—alpha-ketoglutarate and oxaloacetate increase the amount of Krebs cycle intermediates contributing to maintenance of oxidative capacity of the cells and participate in gluconeogenesis depending on the metabolic needs of the cells[105].

1.9.3.Alanin aminotransferase (ALT)

Alanine aminotransferase is an enzyme that is mainly aggregated in the cytosol of the hepatocyte . ALT consists of 496 amino acids, has a half-life of 47±10

hours. Physically, the ALT enzyme catalyzes the transfer of amino groups from L-alanine to α-ketoglutarate, and the converted products are L-glutamate and pyruvate in the liver, which is a critical process of the tricarboxylic acid (TCA) cycle. In this process, the coenzyme, pyridoxal phosphate, is required as shown in figure (1-13). ALT is mainly aggregated in the cytosol of the hepatocyte. ALT activity in hepatic cells is approximately 3000 times higher than serum ALT activity . When liver injury occurs, ALT is released from injured liver cells and causes a significant elevation in serum ALT activity. ALT also exists in muscles, adipose tissues, intestines, colon, prostate, and brain ; however, the concentration of ALT in these organs is much lower than the liver[106] .

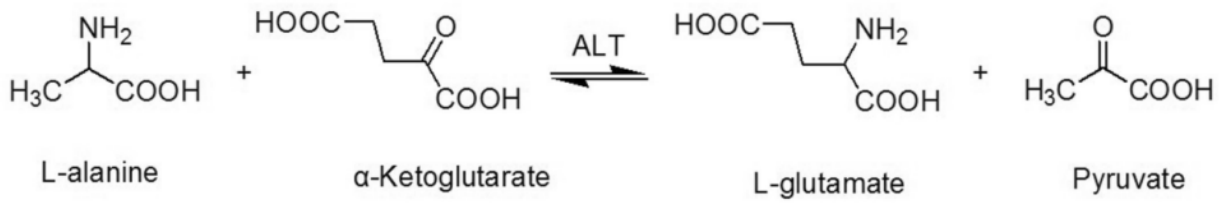


Figure (1-13) The transamination reaction catalyzed by alanine aminotransferase.

1.9.4. Alkaline phosphatase (ALP)

Alkaline phosphatases are plasma membrane-bound glycoproteins that forms a large family of dimeric enzymes, usually confined to the cell surface and hydrolyzes various monophosphate esters at a high pH optimum with release of inorganic phosphate. Mammalian alkaline phosphatases (ALPs) are zinccontaining metalloenzymes encoded by a multigene family and function as dimeric molecules. Three metal ions including two Zn^{+2} and one Mg^{+2} in the active site are essential for enzymatic activity. However, these metal ions also contribute substantially to the conformation of the ALP monomer and indirectly regulate subunit–subunit interactions . Human ALPs can be classified into at least four tissue specific forms

or isozyme mainly according to the specificity of the tissue to be expressed, termed as placental alkaline phosphatase (PLALP or Regan isozyme), Intestinal alkaline phosphatase (IALP), liver/bone/kidney alkaline phosphatase (L/B/K ALP), germ cell ALP (GCALP or NAGAO isozyme)[107].

1.10. Blood Urea

The human body normally maintains protein and amino acid homeostasis by means of processes such as protein synthesis, protein degradation, amino acid oxidation and urea production. Amino acid turnover in the body results in excessive ammonia production. In addition, ammonia is produced by intestinal urease-positive bacteria as well as constantly during amino acid metabolism. Hence, ammonia needs to be detoxified appropriately and the urea cycle performs the critical function of converting ammonia into urea. Hyperammonemia leads to cerebral edema, lethargy, anorexia, vomiting, hyperventilation (or hypoventilation), hypothermia, neurologic posturing, and coma. In Krebs cycle ornithine activates urea synthesis, which converts ammonia into urea prior to excretion via the kidney. The biochemical reaction that produces urea from ammonia is performed mainly in the liver and to a much lesser extent in the kidney. The overall energy flux of the urea cycle is shown in the following equation[108] .

$$2NH_3 + CO_2 + 3ATP + 2H2O \rightarrow NH_2CONH_2 + 2ADP + 2Pi + AMP + PPi$$

1.11. Aim of the Study:

This study aimed to:

1. To estimate serum level of lipoprotein lipase, apolipoprotein C2 and apolipoprotein E in pre-eclamptic pregnant women in comparison with normal pregnant women which may be used as marker for preeclampsia.
2. Measurement some biochemical marker (TSB, ALT, AST, ALP, Urea, Glucose) in preeclampsia to assess the severity among those patients .

2.1 Subjects

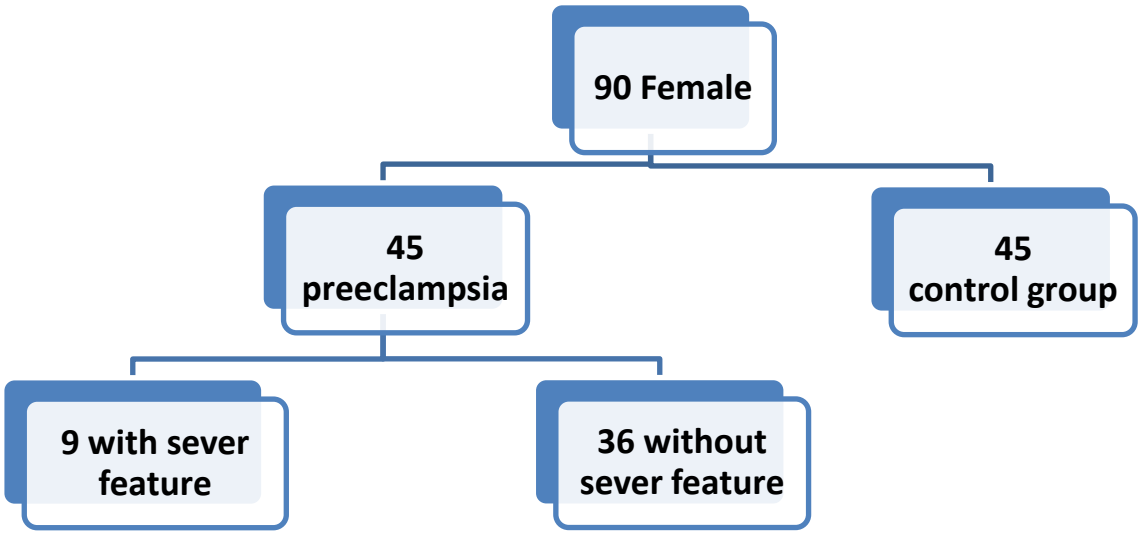
The study was carried out in Babylon Teaching Hospital for Maternity and Pediatrics , Babylon Province . The studied patients attended the outpatient clinic and Labour room. Preeclamptic women selected with age does not exceed fourty year with mean (29.1 ± 8.3) and gestational age not less than twenty weeks with mean (31.2 ± 4.2). BMI is calculated to excluded the obese women (≥30 Kg/m^2). Some patient was with previous history of preeclampsia and (75%) are primigravida . All samples were collected from October 2021 till February 2022. They were diagnosed by specialist Gynecology physicians depending on the clinical features and laboratory finding . The biochemical tests under study were performed at the laboratory of the Department of Clinical Biochemistry, College of Medicine, University of Babylon.

2.1.2 Study Design:

Case- control study.

2.1.3 Study Groups:

The study groups of the present study include (90) pregnant women , (45) of them were with preeclampsia and (45) healthy pregnant women taken as control group. In patient group (9) women with sever preeclampsia was with sever hypertension (SBP ≥160 mmHg or DBP ≥110 mmHg) and proteinuria .the remaining (36) woman was without sever feature which theirs BP was (SBP ≥ 140mmHg and/or DBP ≥90 mm Hg) with proteinuria .All participant was with no preexisting hypertension ,renal disease , diabetes mellitus and liver disease.



2.1.4 Ethical Issues:

A- Approval by scientific committee of the Clinical Biochemistry Department, College of Medicine/University of Babylon, Iraq.

B- Approval by Information Center for Research &Development of Babylon Province and Babylon Health Directorate \ Ministry of Health.

C- The objectives and methodology were explained to all subjects and verbal consent had been taken.

2.1.5 Exclusion Criteria:

A- Age over 40 years.

B- BMI ≥30 Kg/m^2

C- Diabetes type 1 or 2

D- Pre-existing hypertension or renal disease.

E- Smoking

2.1.6. Data collection

A- Questionnaire

Included sociodemographic characteristics (Age, parity ,occupation and gestational age), information about current pregnancy (symptomatology like Headache , right hypochondrial pain, edema, Blurring of vision , Nausea and Vomiting.

B-Investigation included assessment of proteinuria , haematological & biochemical profile, blood pressure . All these were done to diagnose preeclampsia and predict its severity and complication .

C-Anthropometric measurement : Included weight (Kg) , Height (m) and body mass Index(BMI) was calculated by weight (in kilograms) divided by the square of height (in meters). BMI = Weight (kg)\ Square Height (m^2)

2.2. Sample Collection:

About four millilitres of blood were obtained from each subject by vein puncture, and then pushed slowly into disposable gel separating tubes. Blood in the gel containing tubes was allowed to clot at room temperature for 15-20 minutes and then centrifuged at 5000 RPM for approximately 15 minutes then the supernatant were obtained and separate into two Eppendorf and stored at -20˚C .Urine samples were collected for measuring urine protein .

2.3. Chemicals

Chemicals and kits used in this study are listed in Table (2-1)

Table (2-1): Chemicals used in the study

No.	Chemical substance	Origin
1.	Alanine aminotransferase	Cobas C111(Germany)
2.	Alkaline phosphatase	Cobas C111(Germany)
3.	Apo Lipoprotein C2	Elabscience (USA)
4.	Apo Lipoprotein E	Elabscience (USA)
5.	Aspartate aminotransferase	Cobas C111(Germany)
6.	Glucose	Cobas C111(Germany)
7.	Lipoprotein lipase	Bioassay (China)
8.	Total serum bilirubin	Cobas C111(Germany)
9.	Urea	Cobas C111(Germany)

2.4. Instruments

The instruments and tools used in this study were shown in table (2-2)

Table (2-2) instruments and tools

No.	Instruments	Origin
1.	Centrifuge EBA 20	Hettich (Germany)
2.	Cobas C111	Germany
3.	Cylinder (50,1000 mL)	China
4.	Deep Freeze	German
5.	Disposable syringes (5 mL)	China
6.	Distillator	China
7.	ELISA washer, reader and printer	USA
8.	Eppindorf tube (1.5 mL)	China
9.	Filter Papers	China
10.	Gel Separating tube AFCO	Jordan
11.	Glass Cahn Tube (5 ml)	China
12.	Incubator Fisher scientific	USA
13.	Micropipette (100 -1000 µl)	German
14.	Micropipettes (20 -200 µl)	German
15.	Pipette tips (1ml, 0.1ml)	China
16.	Refrigerator Agur	Turkish
17.	Volumetric Flask (1000)	China

2.5. Determination OF Human Lipoprotein lipase (LPL)

2.5.1.Test Principle

This kit was based on sandwich enzyme-linked immune-sorbent assay technology. The plate was pre-coated with Bovine LPL antibody. LPL present in the sample is added and binds to antibodies coated on the wells. And then biotinylated Bovine LPL Antibody is added and binds to LPL in the sample. Then Streptavidin-HRP is added and binds to the Biotinylated LPL antibody. After incubation unbound Streptavidin-HRP is washed away during a washing step. Substrate solution is added and color develops in proportion to the amount of Bovine LPL. The reaction is terminated by addition of acidic stop solution and absorbance is measured at 450 nm.

2.5.2. Reagent Provided

Table (2-3) Reagent Provided by LPL Kit

Components	Quantity (96T)
Standard solution (80ng/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 Bovine LPL antibody	1ml x1
User instruction	1
Plate sealer	2 pics

2.5.3.Reagent Preparation

A- All reagents was brought to room temperature before use.

B- Standard : Volume of 120ul of the standard solution(480 ng\dl) was added to 120ul of standard diluent to generate (240 ng\dl)standard stock solution this labelled as one Standard tube, and mixed thoroughly at the room temperature for 15 min. Four Eppendrof tubes were labelled with 120 ng\dl, 60 ng\dl, 30 ng\dl and 15 ng\dl solutions respectively .Dilution of standard solutions was as follows:

Table (2-4): Serial dilution of stock standard solution

240 ng\dl	Standard No.5	120ul Original standard + 120ul Standard diluent
120 ng\dl	Standard No.4	120ul Standard No.5 + 120ul Standard diluent
60 ng\dl	Standard No.3	120ul Standard No.4 + 120ul Standard diluent
30 ng\dl	Standard No.2	120ul Standard No.3 + 120ul Standard diluent
15 ng\dl	Standard No.1	120ul Standard No.2 + 120ul Standard diluent

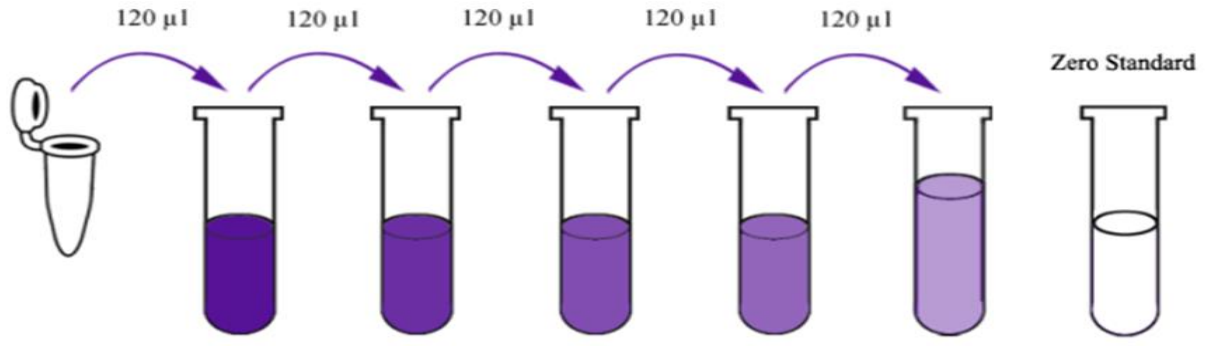


Figure (2-1) Serial dilutions of standard concentration of lipoprotein lipase.

C-Wash Buffer : A Volume of 20ml of Wash Buffer Concentrate (25x) was added to deionized or distilled water to yield 500 ml of 1x Wash Buffer.

2.5.4. Procedure

1- A volume of 50 ul of standard solutions 240ng/dl, 120ng/dl, 60ng/dl, 30ng/dl, 15ng/dl , respectively were added into the standard wells.

2- A volume of 40 ul of Sample was added to sample wells and then added 10ul Bovine LPL antibody to sample wells, then added 50 ul streptavidin-HRP to sample wells and standard wells. The plate was sealed with a cover, and incubated at 37 °C for 60 min.

3- The sealer was removed and the plate washed 5 times with wash buffer by automated washer.

4- A volume of 50ul substrate solution A was added and then added substrate solution B to each well .The plate was sealed with a cover and incubated at 37°C for 10 min in the dark .

5- A volume of 50 µl of Stop solution was added into each well and mixed thoroughly, the blue color was changed into yellow immediately then the optical density (O.D. value) absorbance was read at 450 nm in a microplate reader immediately after adding the stop solution.

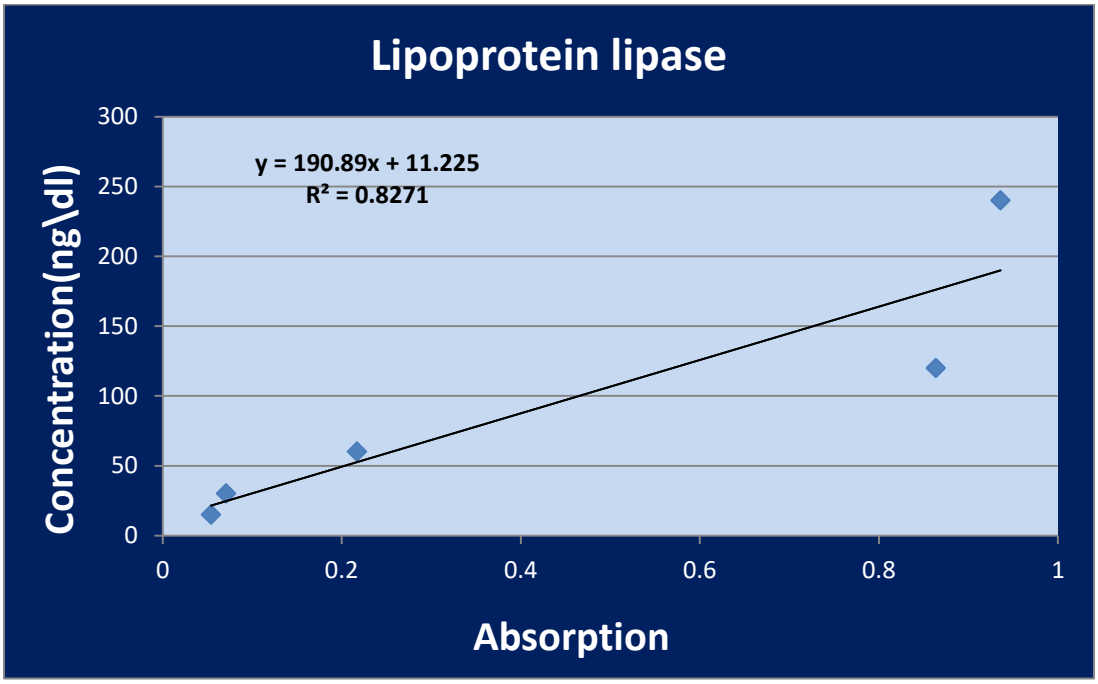


Figure (2-2) Standard Curve for Lipoprotein lipase ng/dl

2.6. Determination of Human Apolipoprotein C2

2.6.1. Test principle

This kit was based on sandwich enzyme-linked immune-sorbent assay technology. The micro ELISA plate in this kit was pre-coated with an antibody specific to Human ApoC2. Samples (or Standards) were added to the micro ELISA plate wells and combined with the specific antibody. Then a biotinylated detection antibody specific for Human ApoC2 and Avidin-Horseradish Peroxidase (HRP) conjugate are added successively to each micro plate well and incubated. Free components are washed away. The substrate solution is added to each well. Only those wells that contain Human ApoC2, biotinylated detection antibody and Avidin-HRP conjugate will appear blue in color. The enzyme-substrate reaction is terminated by the addition of stop solution and the color turns yellow. The optical density (OD) is measured spectrophotometrically at a wavelength of 450 nm ± 2 nm. The OD value is proportional to the concentration of Human ApoC2.

2.6.2 Reagent provided

Table (2-5) Reagent provided by Apolipoprotein C2 Kit

Components	Quantity (96T)	Storage
Micro ELISA Plate (Dismountable)	96T	-20℃, 6 months
Reference Standard	2 vials	
Concentrated Biotinylated Detection Ab (100×)	1 vial, 120 μL	
Concentrated HRP Conjugate (100×)	1 vial, 120 μL	-20°C(shading light), 6 months
Reference Standard & Sample Diluent	1 vial, 20 mL	2-8°C, 6 months
Biotinylated Detection Ab Diluent	1 vial, 14 mL	
HRP Conjugate Diluent	1 vial, 14 mL	
Concentrated Wash Buffer (25×)	1 vial, 30 mL	
Substrate Reagent	1 vial, 10 mL	2-8°C(shading light)
Stop Solution	1 vial, 10 mL	2-8°C
Plate Sealer	5 pieces	
Product Description	1 copy	
Certificate of Analysis	1 copy	

2.6.3. Reagent preparation

A- All reagents was brought to room temperature before use.

B- Wash Buffer : A Volume of 30 mL of Concentrated Wash Buffer was added to 720 ml deionized or distilled water to yield 750ml of Wash Buffer

C- Standard : Firstly the concentrated standard was centrifuged at 2500 RPM for 1 min. A volume of 1.0 ml of Sample / Standard dilution buffer was added into Standard tube and leaved for 10 min to dissolve fully .This was produced a working solution of 200ng/mL. Six labeled Eppendorf tubes with100ng/ml, 50ng/ml, 25ng/ml, 12.5ng/ml, 3.12ng/ml,0 ng/mL was prepared respectively. A volume of 500 uL of the Sample / Standard dilution buffer was added into each tube. A volume of 500 of the above 200ng/ml standard solution was added into 1st tube and mixed thoroughly and produced 100 ng/mL working solution. A volume of 500 uL was transferred from the former tube into the latter one according to this step.

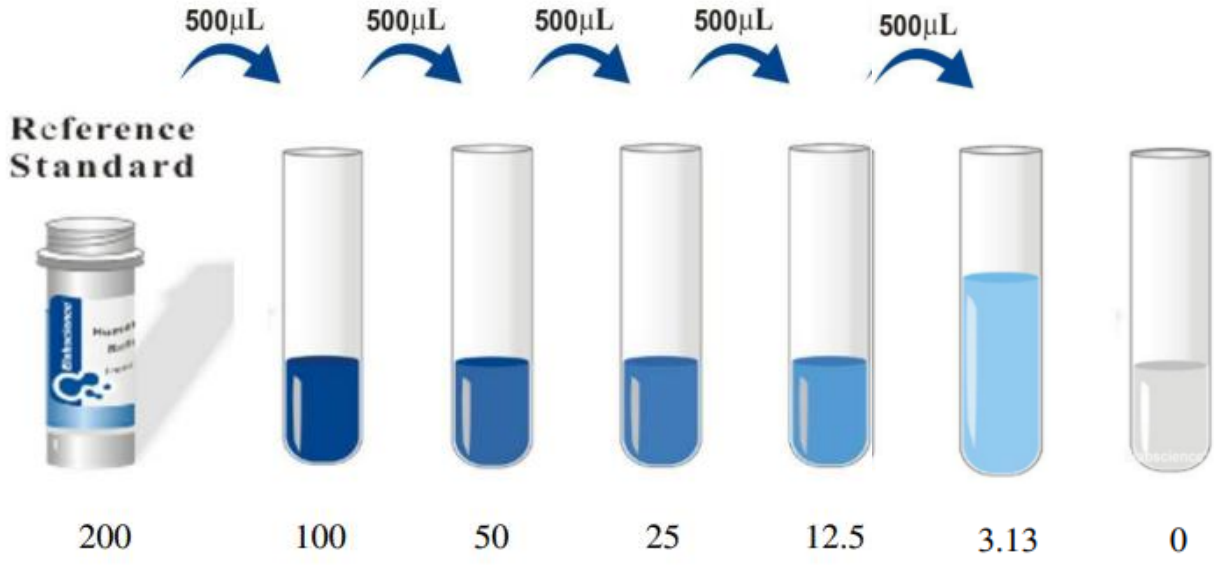


Figure (2-3) Serial dilutions of standard concentration of Apolipoprotein C2.

D- Preparation of Biotin-detection Antibody working solution :The solution was prepared within 1 hour before the experiment. The total volume of the working solution was calculated by 0.1 ml / well × quantity of wells. A volume 0.1 ml of Biotin-detection antibody was added in to 9.9 ml of the Antibody dilution buffer, this was diluted as 1:100 and mix thoroughly.

E-Preparation of Concentrated HRP Conjugate working solution: The solution was prepared within 30min before the experiment. The total volume of the working solution was calculated by 0.1 ml / well × quantity of wells. A volume of 0.1 ml of HRP was added in to 9.9 ml of the HRP dilution buffer, and was added as 1:100 and mixed thoroughly.

2.6.4.Assay procedure

1-The wells was determined for diluted standard and sample. Volume of 100 µL from each dilution of standard and sample was added into the appropriate wells and the plate was covered with the sealer and incubated for 90 min at 37℃.

2.And Without wash directly volume of 100 µL from Biotinylated Detection Ab working solution was added to each well and the plate was covered with the sealer and incubated for 60 min at 37℃.

3. the solution from each well was decanted and volume of 350 µL from diluted wash buffer was added to each well. Microplate washer were used in this step and the wash was repeated 3 times .

4. HRP Conjugate working solution was added to each well and the plate was covered with the sealer and incubated for 30 min at 37℃.

5. The liquid was decanted from each well and wash process in step 3 was repeated 5 times.

6. 90 µL of substrate to each well and the plate was covered with a new sealer and incubated for about 15 min at 37°C. the plate was Protected from light.

7. A volume of 50 µL of Stop Solution was added to each well .

8- The optical density (OD value) was determined for each well at once with a micro-plate reader set to 450 nm.

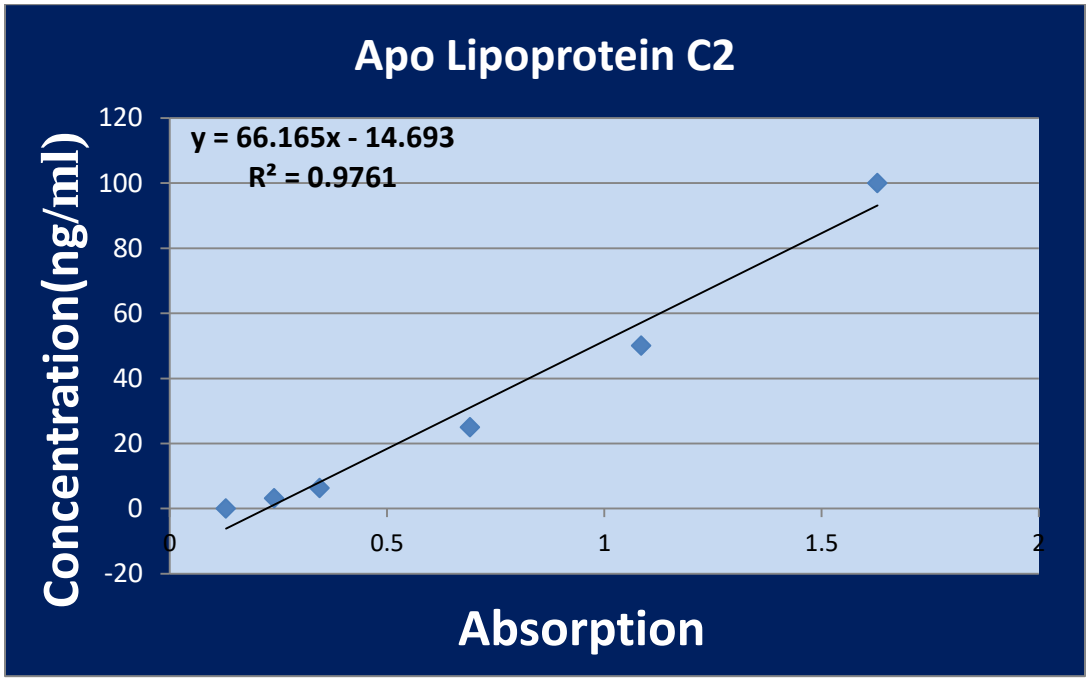


Figure (2-4) Standard Curve for apolipoprotein C2 ng/ml

2.7. Determination of Human apolipoprotein E (Apo E)

2.7.1 Reagent Provided

Table (2-6) Reagent provided by Apolipoprotein E Kit

Components	Quantity (96T)	Storage
Micro ELISA Plate (Dismountable)	96T	-20℃, 6 months
Reference Standard	2 vials	
Concentrated Biotinylated Detection Ab (100×)	1 vial, 120 Ml	
Concentrated HRP Conjugate (100×)	1 vial, 120 Ml	-20°C(shading light), 6 months
Reference Standard & Sample Diluent	1 vial, 20 Ml	2-8°C, 6 months
Biotinylated Detection Ab Diluent	1 vial, 14 mL	
HRP Conjugate Diluent	1 vial, 14 mL	
Concentrated Wash Buffer (25×)	1 vial, 30 mL	
Substrate Reagent	1 vial, 10 mL	2-8°C(shading light)
Stop Solution	1 vial, 10 mL	2-8°C
Plate Sealer	5 pieces	
Product Description	1 copy	
Certificate of Analysis	1 copy	

2.7.2.Test principle

This kit was based on sandwich enzyme-linked immune-sorbent assay technology. The micro ELISA plate in this kit was pre-coated with an antibody specific to Human ApoE. Samples (or Standards) were added to the micro ELISA plate wells and combined with the specific antibody. Then a biotinylated detection antibody specific for Human ApoE and Avidin-Horseradish Peroxidase (HRP) conjugate are added successively to each micro plate well and incubated. Free components are washed away. The substrate solution is added to each well. Only those wells that contain Human ApoE, biotinylated detection antibody and Avidin-HRP conjugate will appear blue in color. The enzyme-substrate reaction is terminated by the addition of stop solution and the color turns yellow. The optical density (OD) is measured spectrophotometrically at a wavelength of 450 nm ± 2 nm. The OD value is proportional to the concentration of Human ApoE.

2.7.3. Reagent preparation

A- All reagents was brought to room temperature before use.

B- Wash Buffer : A Volume of 30 mL of Concentrated Wash Buffer was added to 720 ml deionized or distilled water to yield 750ml of wash Buffer.

C- Standard : Firstly the concentrated standard was centrifuged at 2500 RPM for 1 min. A volume of 1ml of Sample / Standard dilution buffer was added into standard tube and leaved for 10 min to dissolve fully .This was produced a working solution of 1500 ng/mL. Six labeled Eppendorf tubes with750ng/ml, 375ng/ml, 187.5ng/ml, 93.75ng/ml, 23.44ng/ml, 0 ng/mL was prepared respectively. A volume of 500 uL of the Sample / Standard dilution buffer was added into each tube. A volume of 500 of the above 1500 ng/ml standard solution was added into 1st tube and mixed thoroughly and produced 750 ng/mL working solution. A volume of 500 uL was transferred from the former tube into the latter one.

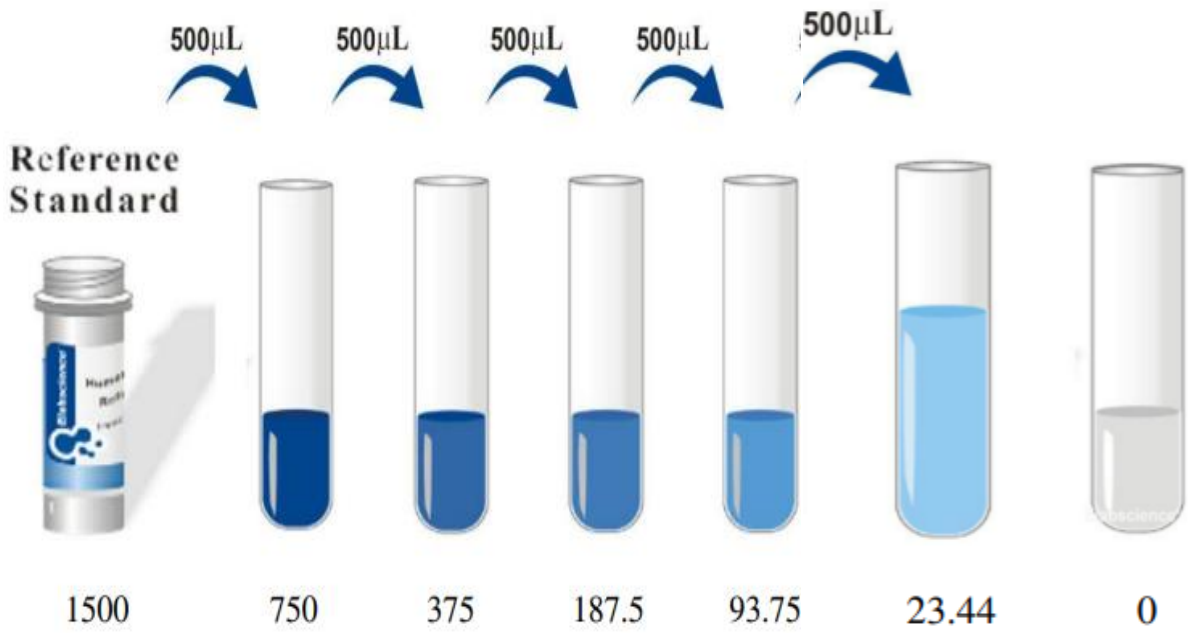


Figure (2.5) Serial dilutions of stock standard solution

D- Preparation of Biotin-detection Antibody working solution :The solution was prepared within 1 hour before the experiment. The total volume of the working solution was calculated by 0.1 ml / well × quantity of wells. A volume 0.1 ml of Biotin-detection antibody was added in to 9.9 ml of the Antibody dilution buffer, this was diluted as 1:100 and mix thoroughly.

E-Preparation of Concentrated HRP Conjugate working solution: The solution was prepared within 30min before the experiment. The total volume of the working solution was calculated by 0.1 ml / well × quantity of wells. A volume of 0.1 ml of HRP was added in to 9.9 ml of the HRP dilution buffer, and was added as 1:100 and mixed thoroughly.

2.7.4.Assay procedure

1-The wells was determined for diluted standard and sample. Volume of 100 µL from each dilution of standard and sample was added into the appropriate wells and the plate was covered with the sealer and incubated for 90 min at 37℃.

2.And Without wash directly volume of 100 µL from Biotinylated Detection Ab working solution was added to each well and the plate was covered with the sealer and incubated for 60 min at 37℃.

3. the solution from each well was decanted and volume of 350 µL from diluted wash buffer was added to each well. Microplate washer were used in this step and the wash was repeated 3 times .

4. HRP Conjugate working solution was added to each well and the plate was covered with the sealer and incubated for 30 min at 37℃.

5. The liquid was decanted from each well and wash process in step 3 was repeated 5 times.

6. 90 µL of substrate to each well and the plate was covered with a new sealer and incubated for about 15 min at 37°C. the plate was Protected from light.

7. A volume of 50 µL of Stop Solution was added to each well .

8- The optical density (OD value) was determined for each well at once with a micro-plate reader set to 450 nm.

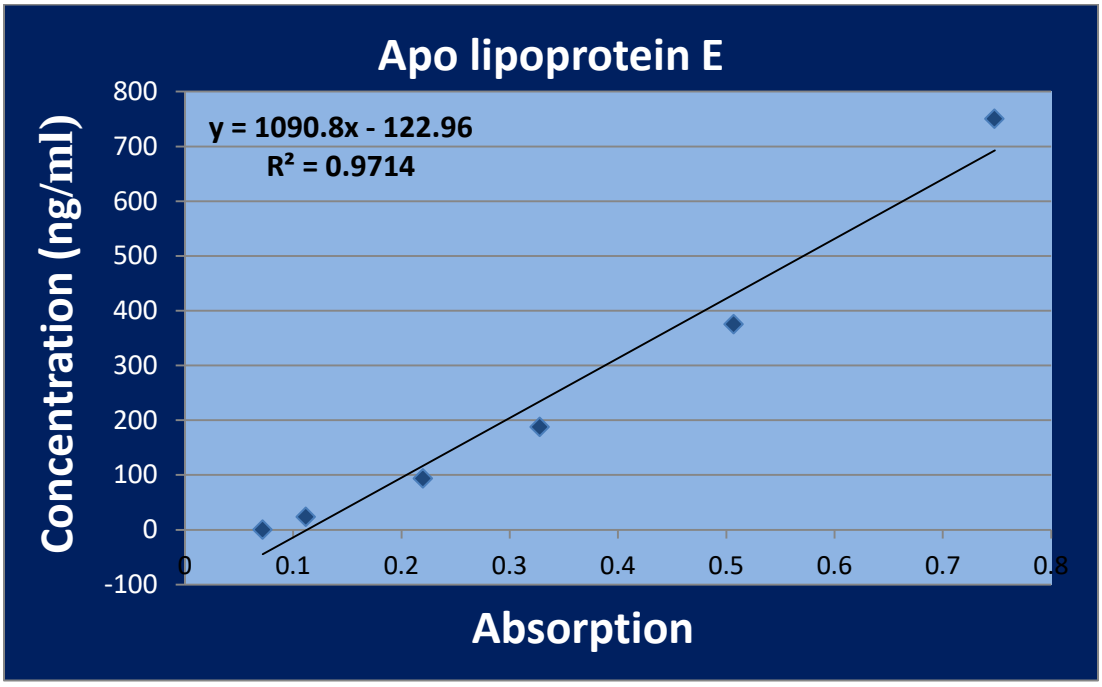


Figure (2-6) Standard Curve for Apolipoprotein E ng/ml

2.8. Biochemical Assessments

Total serum bilirubin , AST, ALT, ALP, Urea and glucose was measured quantitatively by automated technique on the Cobas C111 instruments . The instrument use absorption photometry for determining the amount of absorbance in a fluid and the absorbance is used to calculate the concentration in the solution . The same automated procedure steps for Cobas C111 instrument was applied for all tests and as the following steps

1- The new QC-card was readed and reagents was applied in cobas C111 instrument
2- Sample cuvette was applied in the specified sample rack according to sequence number and sample ID that applied previously.
3- As soon as pressed the START key the analyzer initiate testing.
4- The instrument automatically calculates the results.

2.8.1. Determination of Total serum bilirubin

2.8.1.1.Principle (Colorimetric diazo method)

Total bilirubin, in the presence of a suitable solubilizing agent, is coupled with a 3.5 dichlorophenyl diazonium ion in a strongly acidic medium. The color intensity of the red azo dye formed is proportional to the total bilirubin and can be determined photometrically.

2.8.1.2.Reagents

- Reagent (1): Phosphate ; detergent; stabilizers; pH 1.0
- Reagent (2): 3, 5 - dichlorophenyl diazonium salt.

2.8.2. Determination of Aspartate Aminotransferase (AST)

2.8.2.1.Principle

The aspartate aminotransferase (AST) assay uses enzymatic activation. AST in a sample catalyzed the transfer of an amino group between L-aspartate and 2-oxoglutarate to form oxaloaacetate and L-glutamate. The oxaloacetate then reacts with NADH, in the presence of malate dehydrogenase (MDH), to form NAD+. Pyridoxal phosphate serves as a coenzyme in the amino transfer reaction.The rate of the NADH oxidation is directly proportional to the catalytic AST activity. It is determined by measuring the decrease in absorbance.

$$\text{L-Aspartate} + \text{2-oxoglutarate} \xrightarrow{\text{AST}} \text{oxaloacetate} + \text{L-glutamate}$$

$$\text{Oxaloacetate} + \text{NADH} + H^{+} \xrightarrow{\text{MDH}} \text{L-malate} + NAD^{+}$$

2.8.2.2.Reagents

- Reagent (1): TRIS buffer; L-aspartate; MDH
- Reagent (2): NADH; 2-oxoglutarate

2.8.3. Determination of Alanine Aminotransferase (ALT)

2.8.3.1.Principle

Alanine aminotransferase (ALT) catalyzes the reaction between L-alanine and 2-oxoglutarate. The pyruvate formed is reduced by NADH in a reaction catalyzed by lactate dehydrogenase (LDH) to form L-lactate and NAD+ . The rate of the NADH oxidation is directly proportional to the catalytic ALT activity. It is determined by measuring the decrease in absorbance.

$$\text{L-Aalanine + 2-oxoglutarate} \xrightarrow{\text{ALT}} \text{Pyruvate + L-glutamate}$$

$$\text{Pyruvate + NADH + } H^{+} \xrightarrow{\text{LDH}} \text{L-Lactate + } NAD^{+}$$

2.8.3.2.Reagents

- Reagent (1): TRIS buffer; L-alanine ; LDH
- Reagent (2): NADH; 2-oxoglutarate

2.8.4. Determination of alkaline phosphatase (ALP)

2.8.4.1.Principle

In the presence of magnesium and zinc ions, p-nitrophenyl phosphate is cleaved by phosphatases into phosphate and p-nitrophenol. The p-nitrophenol released is directly proportional to the catalytic ALP activity. It is determined by measuring the increase in absorbance.

$$\text{p-nitrophenyl phosphate +} H_2O \xrightarrow{\text{ALP}} \text{phosphate + p-nitrophenol}$$

2.8.4.2.Reagents

- Reagent (1): 2-Amino-2-methyl-1-propanol; magnesium acetate; zinc sulfate; N-(2-hydroxyethyl)- ethylenediamine triacetic acid
- Reagent (2): p-nitrophenyl phosphate

2.8.5. Determination of blood urea

2.8.5.1.Principle

Urea is hydrolyzed by urease to form ammonium and carbonate. In the second reaction, 2-oxoglutarate reacts with ammonium in the presence of glutamate dehydrogenase (GLDH) and the coenzyme NADH to produce L-glutamate. In this reaction, two moles of NADH are oxidized to NAD for each mole of urea hydrolyzed. The rate of decrease in the NADH concentrations is directly proportional to the urea concentration in the specimen. It is determined by measuring the absorbance at 340nm.

$$\text{Urea} + 2H_2O \xrightarrow{\text{Urease}} 2NH_4 + CO2$$

$$NH_4 + \text{2-Oxoglutarate} + \text{NADH} \xrightarrow{\text{GLDH}} \text{L-glutamate} + NAD^+ + H_2O$$

2.8.5.2.Reagents

- Reagent (1): NaCl 9% TRIS buffer; L-alanine ; LDH ; GLDH
- Reagent (2): TRIS buffer, pH 8.6; 2-oxyoglutarate; NADH; urease

2.8.6. Determination of blood glucose

2.8.6.1. Principle

Hexokinase (HK) catalyzes the phosphorylation of glucose by ATP to form glucose-6-phosphate and ADP. To follow the reaction, a second enzyme, glucose-6-phosphate dehydrogenase (G6PD) is used to catalyze oxidation of glucose-6-phosphate by NAD + to form NADH. The concentration of the NADH formed is directly proportional to the glucose concentration.

$$\text{Glucose} + \text{ATP} \xrightarrow{\text{HK}} \text{G-6-P} + \text{ADP}$$

$$\text{G-6-P} + \text{NAD}^{+} \xrightarrow{\text{G6PD}} \text{gluconate-6-P} + \text{NADH} + \text{H}^{+}$$

2.8.6.2.Reagents

- Reagent (1): TRIS buffer, Mg2+ , ATP, NADP
- Reagent (2): HEPES buffer , Mg2+ , HK , G-6-PDH

2.9. Statistical Analysis

Statistical analysis was carried out using SPSS version 21. Continuous variables were given as (Mean ± SD) while categorical variables were provided as frequencies and percentages. Student t-test was used to compare means between two groups and the correlation test (Pearson test) was performed to find the association between variables. P-value less than 0.05 was considered as significant. Receiver operating characteristic (ROC) curve was used to evaluate the diagnostic value of NAG, B2M and ACR. The sensitivity and specificity of biochemical parameter and calculate the optimal cutoff according to “Youden Index” by select the point that is closest to the top-left corner of the ROC curve. The area under the curve (AUC) provides a useful tool to compare different biomarkers as Table 2-7.

Table (2-7) List of AUC ranges and their classification levels AUC Range

	AUC Range Classification Level	
1-	0.90 - 1.00	**Excellent**
2-	0.80 - 0.90	**Good**
3-	0.70 - 0.80	**Fair**
4-	0.60 - 0.70	**Poor**
5-	0.50 - 0.60	**Failure**

3.1. General characteristics of studied groups

3.1.1. Age of participants:

The mean age ± SD of participants of preeclampsia group and control group were calculated and showed there is no significant differences (P. value > 0.05) between the matched groups, as shown in table (3-1) and figure (3-1).

Table (3-1): General characteristics of studied groups.

Variable	Preeclampsia Mean ± SD*		Normal pregnant Mean ± SD	p. value
Age (years)	29.1 ± 8.3[a]		26.8 ± 4.02	**> 0.05**
Gestational age (Weeks)	31.2 ± 4.2 [a]		28.9 ± 4.4	**> 0.05**
Parity N (%)	**Primigravida**	34 (75.5%)	21 (46.6%)	***X2*: 270 p.value: <0.001**
	Multigravida	11 (24.5%)	24 (53.4%)	
Previous history of Preeclampsia	**Yes: N (%)**	8 (17.8%)	5 (11.2%)	**X^2: 289 p. value: <0.001**
	No: N (%)	37 (82.2%)	40 (88.8%)	

X^2: chi-square**; ***: standard deviation **;** [a]: two independent t. test.

The maternal age of ≥35 years are associated with a significantly increased risk of preeclampsia this may be related to the progressive vascular endothelial damage that occurs with maternal aging and obstruction of maternal spiral arteriolar lumina by atherosis[109].

3.1.2. Gestational age of participants:

Table (3-1) and figure (3-1) shows there were no statistically significance difference (P. value > 0.05) between the means of gestational age in preeclampsia and control groups (31.2 ± 4.2week) (28.9 ± 4.4week) respectively .

The gestational age specific incidence of preeclampsia increased sharply with gestational age. The rate of early-onset disease (<34 weeks' gestation) was substantially lower than the rate of late-onset disease (gestation of ≥ 34weeks). Several factors, including chronic hypertension, African-American race, and congenital abnormities conferred a relatively higher risk for early-onset , whereas other factors such as diabetes mellitus, nulliparity, and young maternal age were associated with a higher risk of late-onset preeclampsia. Early-onset preeclampsia conferred a substantially higher risk for adverse birth outcomes than late-onset preeclampsia[110].

3.1.3. Parity of participants:

The percentage of parity for participants of preeclampsia group divided as primigrivida (75.5%), multigravida (24.5%) and control group divided as primigravida (46.6%), multigrivida (53.4%). The results were calculated and showed highly significant variances at (p. value <0.001) between the matched groups, as shown in table (3-1).

The incidence of preeclampsia in multiparous was also varied but lower than nulliparaous. The study conducted by Lisonkova and Joseph found that nulliparous women had 1.8 times the risk of preeclampsia compared to multiparous [111]. In contrast, a study in India revealed that parity was not found to be associated with preeclampsia[112]. The etiology of preeclampsia in the first pregnancy is associated with the role of immunological factors. In the first pregnancy the formation of blocking antibodies against placental antigenic site may be impaired, thus increases the risk of preeclampsia. Beside the existence of a foreign protein, the fetus or placental agent could evoke an immunological response. An immune response disorders can lead the syndrome of preeclampsia[113].

3.1.4. Previous history of preeclampsia of participants:

The study reveals that (17.8%) of patient group with previous history of preeclampsia while (11.2%) of control have preeclampsia in previous as shown in table (3-1) . Study mentioned by Sharami and his colleagues, which reveal that the previous history of preeclampsia increased the risk of developing mild and severe types of preeclampsia[114].

3.2.Biochemical Assessments:

3.2.1. Liver function tests in preeclamptic patients and healthy control group

Preeclampsia patients had a significantly higher total bilirubin (TSB), AST (aspartate aminotransferase), ALT (alanine aminotransferase) and alkaline phosphatase in contrast to control group with highly statistical significance difference (p.value <0.001) . The average serum level of urea and glucose in preeclmpsia patients are statistically not significantly with control at (p.value > 0.05) as shown in Table (3-3).

The study that conducted by Deniz and his colleague, was revealed high Serum bilirubin level in preeclamptic patient in contrast with control group [115]. Maryam and her colleague, found a considerable increase in liver enzymes in patients with preeclampsia and showed that high levels of AST and ALT can be considered to categorize the severity of preeclampsia[116]. All these studies are with agreement with the results of our study.

Table (3-2): Serum biochemical parameters in preeclampsia and normal pregnant women.

Variables	**Preeclampsia** Mean ± SD*	**Controls** Mean ± SD*	**Statistical test P. value**
S.TSB (mg/dl)	0.95 ± 0.05	0.54 ± 0.2	**<0.001**
S.AST (U/L)	33.68± 18.2	18.24 ± 3.3	**<0.001**
S.ALT (U/L)	45.8 ± 22.3	18.4 ± 5.1	**<0.001**
S.ALP (U/L)	150.6 ± 57.8	117.3 ± 17.05	**<0.001**
S. Urea (mg/dl)	24.8 ±7.5	21.9 ± 5.3	**>0.05**
S.Glucose (mg/dl)	108.3 ± 28.6	98.1 ± 9.5	**>0.05**

***SD:** standard deviation

3.3. Biochemical Parameters:

3.3.1. Serum Lipoprotein Lipase (LPL) concentration (ng/dl) among preeclampsia patients and control groups:

Preeclampsia patients had a significantly higher LPL than the controls, 200.10 vs. 134.80 ng/dl respectively. Therefore, the mean of LPL was highly significant, as ($P< 0.001$) and there is no significance difference in means of LPL concentrations between subgroups with ($P> 0.05$). as shown in Table (3-4) and figure (3-2).

Table (3-3): Serum LPL (ng/dl) concentration in preeclampsia patients and healthy control groups.

Parameter	Subject	Mean ± SD	p. value
LPL (ng/dl)	**Preeclampsia (45)**	200.10 ± 27.15	**<0.001**
	Normal pregnant (45)	134.80 ± 11.88	

The study on LPL that conducted by Procopciuc and his colleague disagreement with our study , was revealed reduce LPL serum activity, increasing hypertriglyceridemia and decreasing HDL levels, with a probable accumulation of chylomicrons and VLDL at the arterial level leading to a greater predisposition to preeclampsia [117].

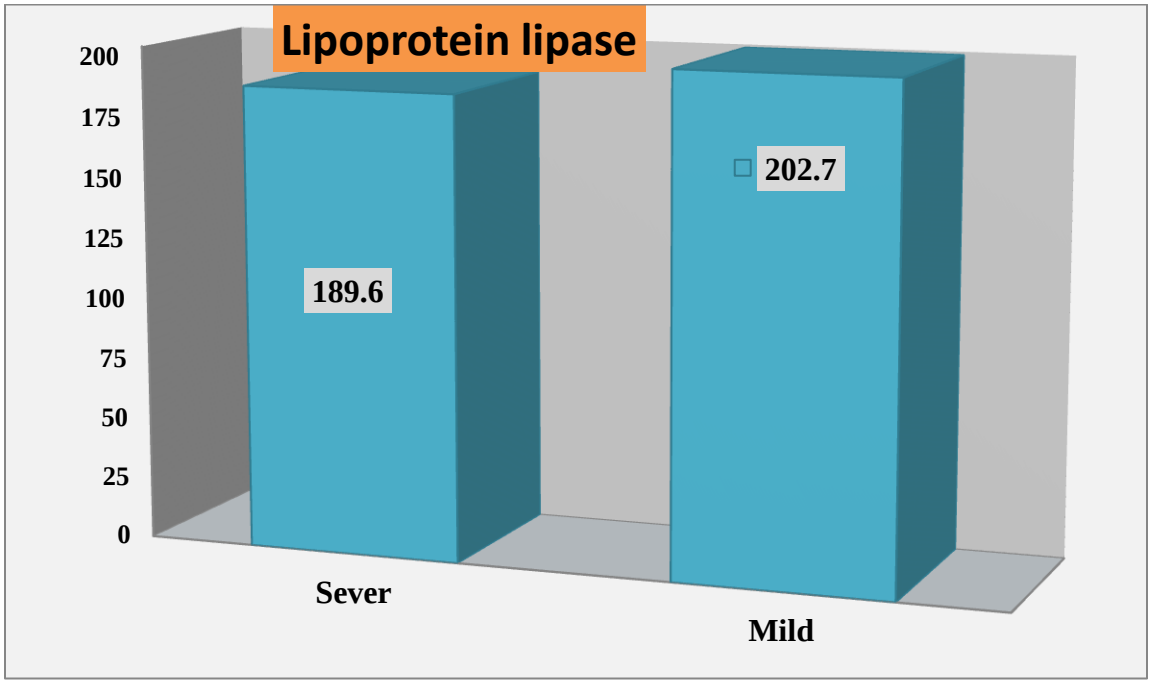


Figure (3-1): serum lipoprotein lipase concentration among preeclampsia subgroups.

Lipoprotein lipase anchored in the luminal surface of the vascular endothelium normally hydrolyzes triglycerides to glycerol and fatty acids available for extrahepatic tissues. However, the amount of LPL and whether it increases or decreases may differ in various tissues at late gestation: LPL activity decreases in adipose tissue but increases in placenta and mammary gland. [118] . Two hypotheses have been proposed to explain the influence of dyslipidemia on the pathogenesis of preeclampsia: 1. Triglycerides accumulate in endothelial cells, which triggers a decreased release of prostaglandins and nitric oxide and consequently causes endothelial dysfunction. 2. Preeclampsia may be accompanied by a dysregulation of lipoprotein lipase activity[119].

3.3.2. Serum Apo-lipoprotein C2 concentration (ng/ml) among preeclampsia patients and control groups:

The mean of serum levels of apolipoprotein C2 in preeclampsia patients (186.08 ng/ml) and controls (121.7 ng/ml) are presented in Table (3-5).The mean of apolipoprotein C2 was highly significance as (P<0.001). There is no statistical difference in means of apolipoiprotein C2 concentrations between subgroups as with (P >0.05) shown in figure (3-3)

Table (3-4): serum ApoC2 (ng/ml) concentration in preeclampsia and normal pregnant women.

Parameter	Subject	Mean ± SD	p. value
ApoC2 (ng/ml)	**Preeclampsia (45)**	186.08 ± 18.8	**<0.001**
	Normal pregnant (45)	121.7 ± 39.3	

ApoC2 (apolipoprotein C2)

Increase level of ApoC-II leads to HTG, due to decreased lipolysis. Using the pressure dependent model, we can therefore hypothesize that high levels of ApoC-II on the TRL surface would increase the surface pressure preventing LPL binding, thereby attenuating lipolysis, when ApoC-II is overexpressed[120].

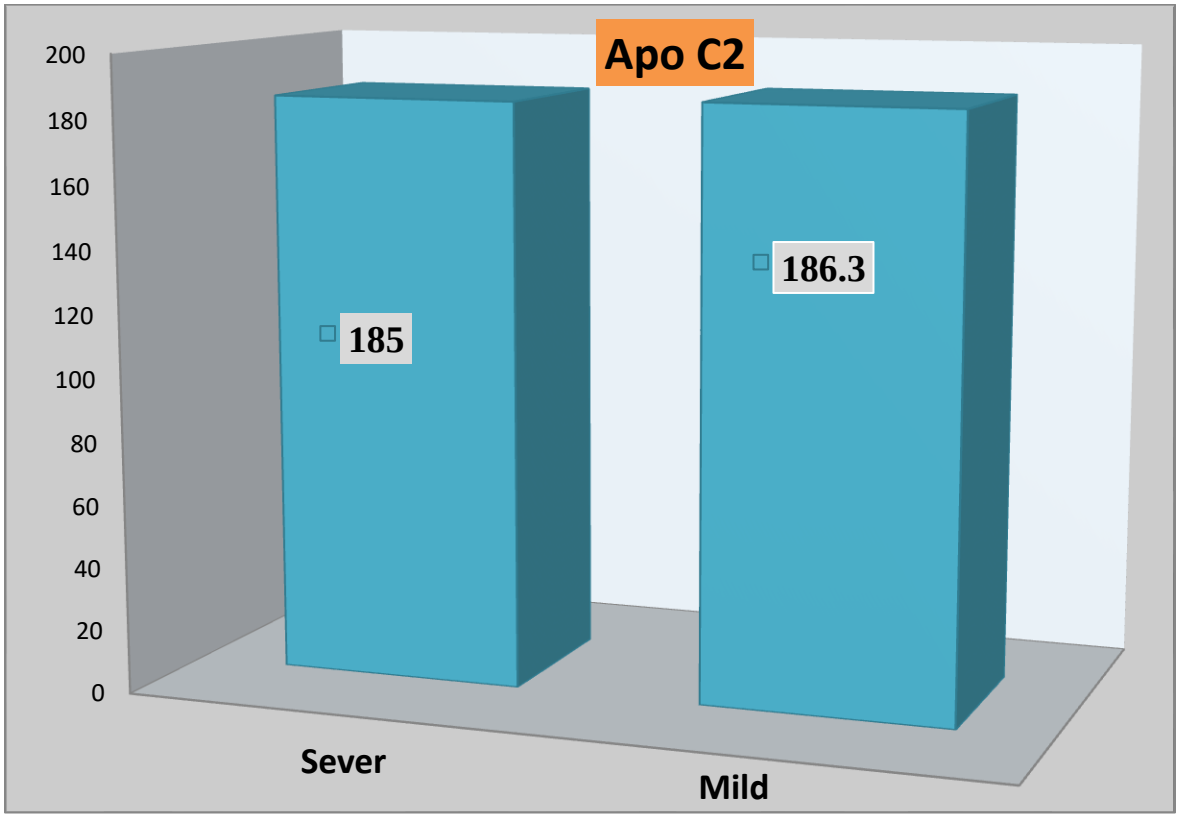


Figure (3-2): serum Apolipoprotein C2 concentration among preeclampsia subgroups.

3.3.3. Serum Apolipoprotein E concentration (ng/ml) among preeclampsia patients and control groups:

Preeclampsia patients had a significantly higher apolipoprotein E than the controls, 793.2ng/ml vs. 512.4ng/ml, respectively. Therefore, the mean of apolipoprotein E was highly significant, as ($P< 0.001$), as shown in Table (3-6) . There is statistical significance difference in means of Apo E concentrations between subgroups with ($P <0.05$) as shown in figure (3-4).

Table (3-5): serum ApoE (ng/ml) concentration in preeclampsia and normal pregnant women.

Parameter	Subject	Mean ± SD	p. value
ApoE (ng/ml)	**Preeclampsia (45)**	793.2 ± 152.8	**<0.001**
	Normal pregnant (45)	512.4 ± 133.3	

Apolipoprotein E isoforms and gene variants have been postulated and highlighted as potential predictors of preeclampsia development[121].Study by Timalsina and her colleague was revealed significantly higher concentrations of serum total cholesterol, triacylglycerol were found in the preeclamptic cases compared to pregnant controls [122].The high levels of serum TG in preeclamptic women are believed to be due to high circulating levels of Apo E that interfere with TG clearance by interfering with lipoprotein lipase activator, Apo C-II[123].

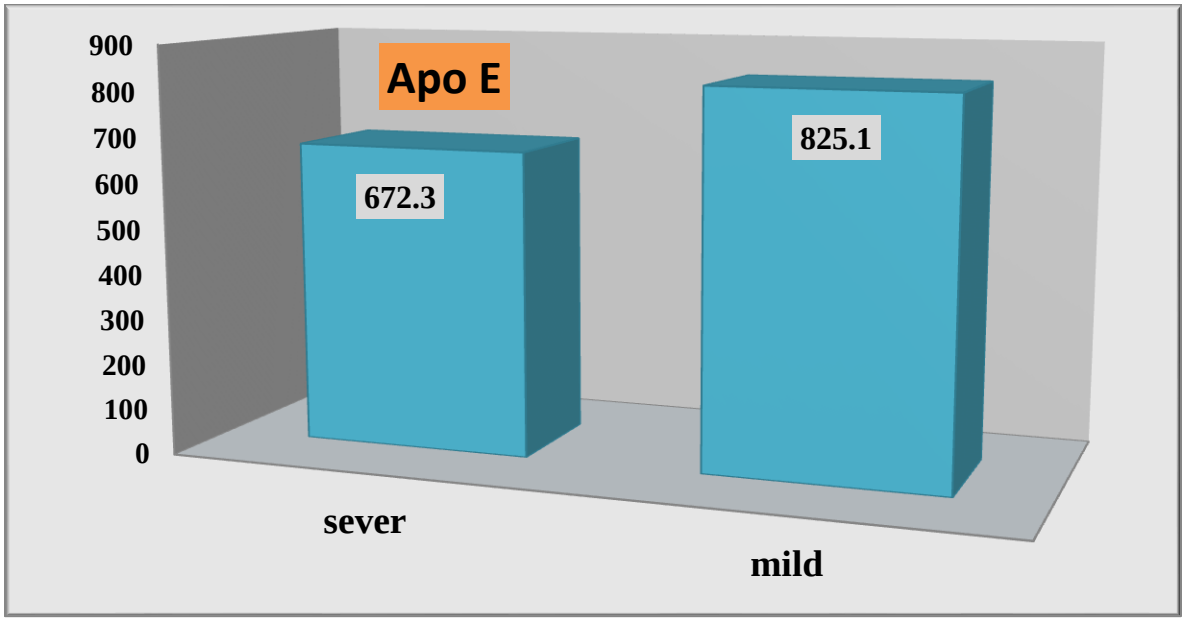


Figure (3-3): serum Apolipoprotein E concentration among preeclampsia subgroups.

Serrano and his colleagues showed the strongest associations between apolipoprotein E (ApoE) with pre-eclampsia, particularly for those cases with an early onset[121]. Mao concluded that, elevated lipid metabolism and inflammatory/apoptosis parameters suggest a potentially significant role of apoE in preeclampsia pathology, as well as a relationship between iNOS and preeclampsia progression[124]. The study that conducted by Reza Ahmadi and his colleague was indicates a protective role for Apo E2 allele against severe preeclampsia that might be through high antioxidant capacity of this allele. ApoE possessed an antioxidant activity likely due to its capacity to chelate metal ions. Furthermore, this activity was shown to be allele-specific (e2 > e3 > e4)[125].

3.4. Correlation Analysis among the study parameters:

The results of bivariate correlation analysis is shown in the Table (3-7).

Table (3-6): Correlation between Biochemical Parameters

Parameters	Correlation coefficient R	P-value
LPL Vs. Apo C2	0.5	**<0.001**
LPL Vs. Apo E	0.359	**<0.001**

3.4.1.Correlation between lipoprotein lipase and apolipoprotein C2

The study result regarded lipoprotein lipase were found to be positive correlated with apolipoprotein C2 concentration among preeclampsia patients as shown in figure (3-6).

Lipoprotein lipase acts as a molecular bridge associating with lipoproteins and promoting their binding to heparan sulfate proteoglycan. LPL is activated by apolipoprotein C2 (Apo C2) which binds to the protein and is required for efficient lipolysis[126].Kersten concluded that by human genetic and in vitro studies have provided overwhelming support for a plasma TG-lowering effect of ApoC2 via stimulation of LPL activity[127].

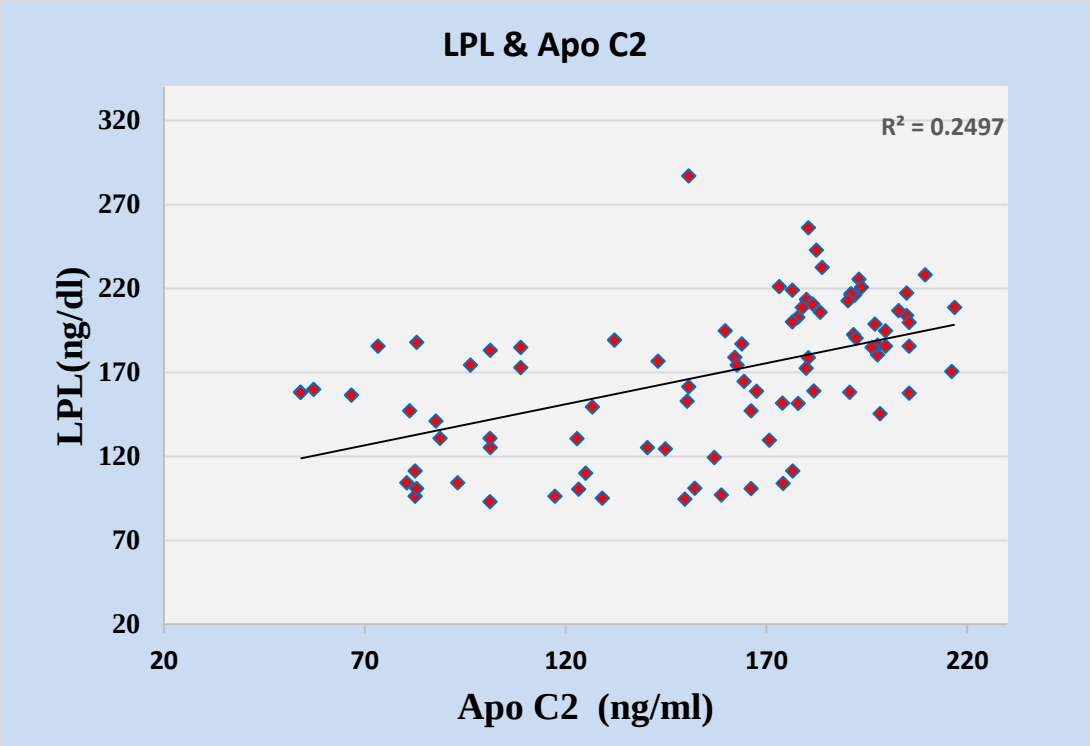


Figure (3-4): correlation of lipoprotein lipase and apolipoprotein C2.

3.4.2. Correlation between lipoprotein lipase and apolipoprotein E:

The study result regarded lipoprotein lipase were found to be positively correlated with apolipoprotein E concentration as shown in figure (3-7). Study by Whitacre and his colleagues concluded that the level of ApoE was negatively correlated with lipid hydrolysis by modulating LPL activities .However, some studies have agreement with our results which showed that human ApoE added to human plasma significantly activated human LPL, whereas anti-ApoE antibodies decreased activity [128].

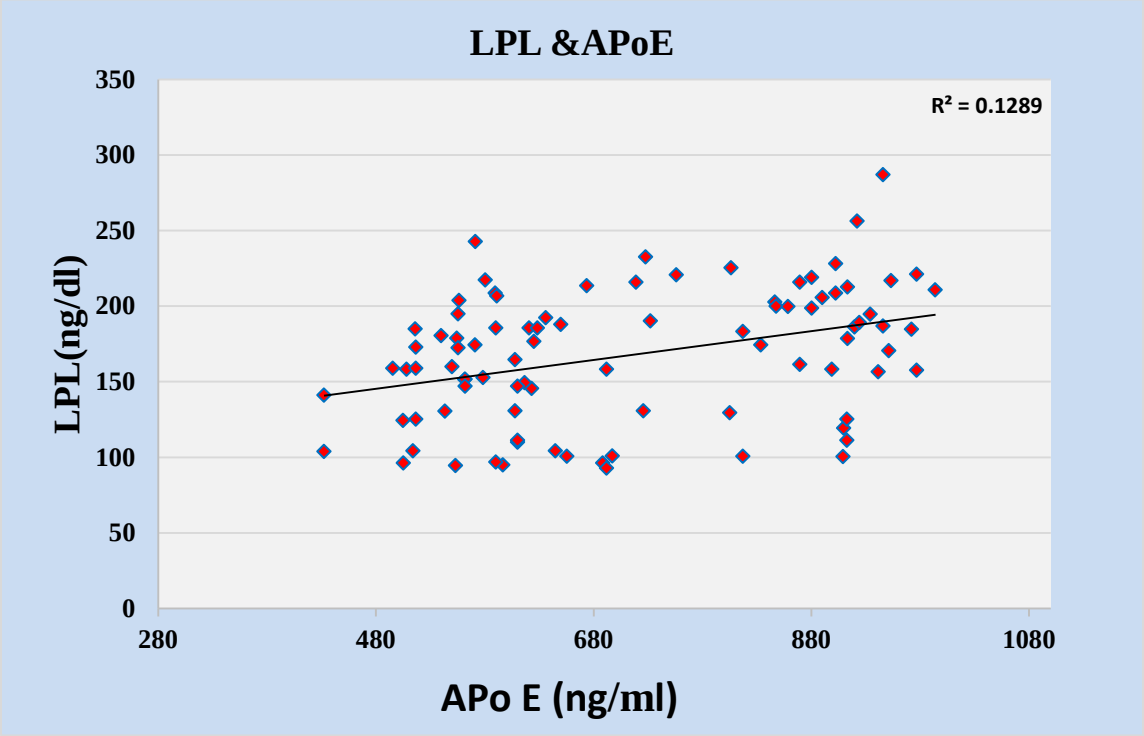


Figure (3-5): correlation of lipoprotein lipase and apolipoprotein E .

3.5. 1.ROC Curve of lipoprotein lipase (LPL).

ROC curve for the sensitivity and specificity of LPL (ng/dl) for diagnosis of preeclampsia in pregnant women, (Cut-off point was ≥ 173.5 ng/dl), AUC=0.89, P 0.001, CI (0.90-0.98), the sensitivity and the specificity was 86.7 %, 85.7 % respectively, as shown in figure (3-7).

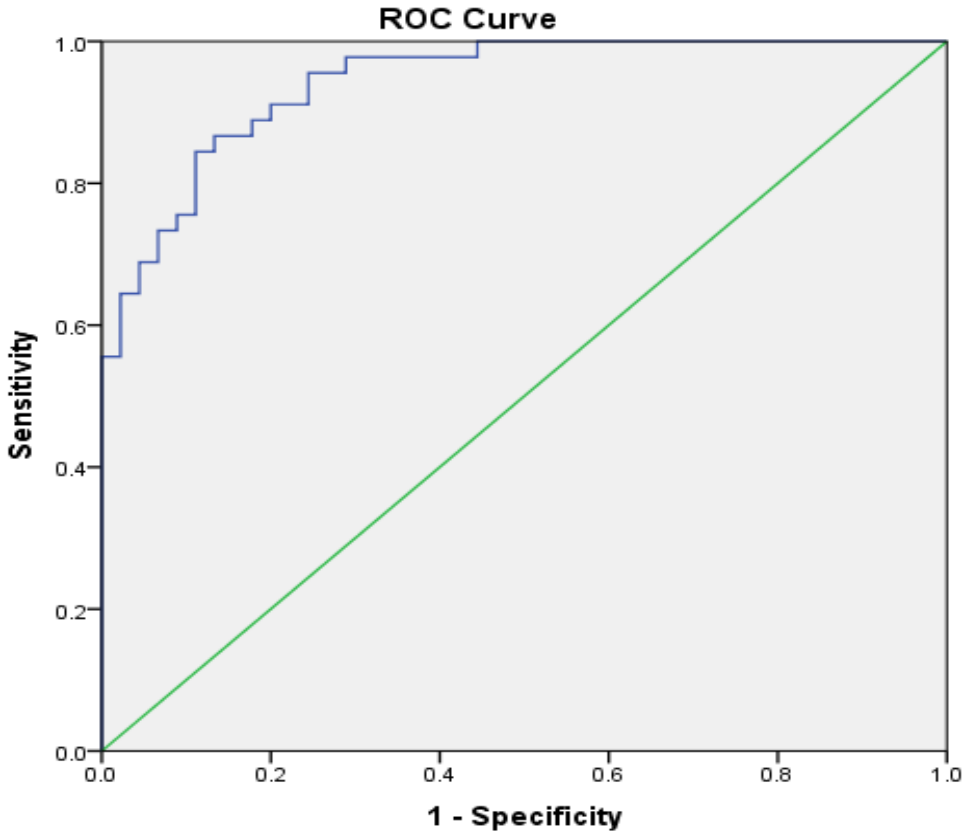


Figure (3-6) ROC Curve of lipoprotein lipase (LPL).

For LPL, our results stated that good diagnostic value in the diagnosis of preeclampsia in pregnant women

3.5.2. ROC Curve of Apo C2.

ROC curve for the sensitivity and specificity of Apo C2 (ng/ml) for diagnosis of preeclampsia in pregnant women, (Cut-off point was ≥ 175.3 (ng/ml), AUC=0.82, P 0.001, CI (0.89-0.98), the sensitivity and the specificity was 80 %, 89.9 % respectively, as shown in figure (3-8).

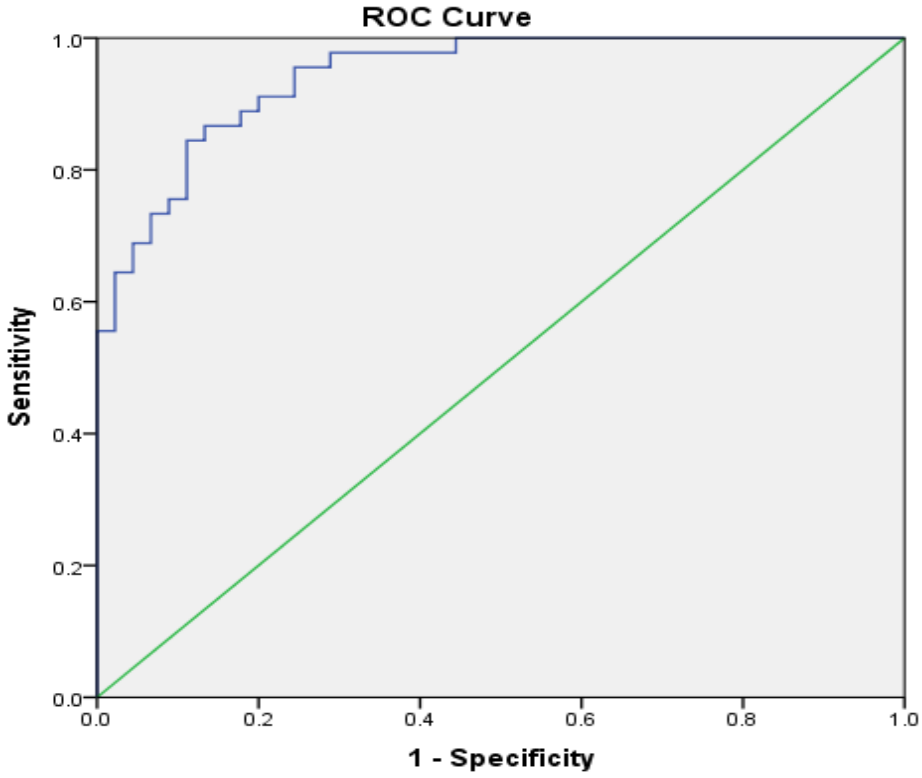


Figure (3-7) ROC Curve of Apo C2.

For Apo C2, our results stated that good diagnostic value in the diagnosis of preeclampsia in pregnant women.

3.5.3. ROC Curve of Apo E.

ROC curve for the sensitivity and specificity of Apo C2 (ng/ml) for diagnosis of preeclampsia in pregnant women, (Cut-off point was ≥ 708.2 (ng/ml), AUC=0.78, P 0.001, CI (0.68-0.87) the sensitivity and the specificity was 68.9 %, 80.0 % respectively, as shown in figure (3-9).

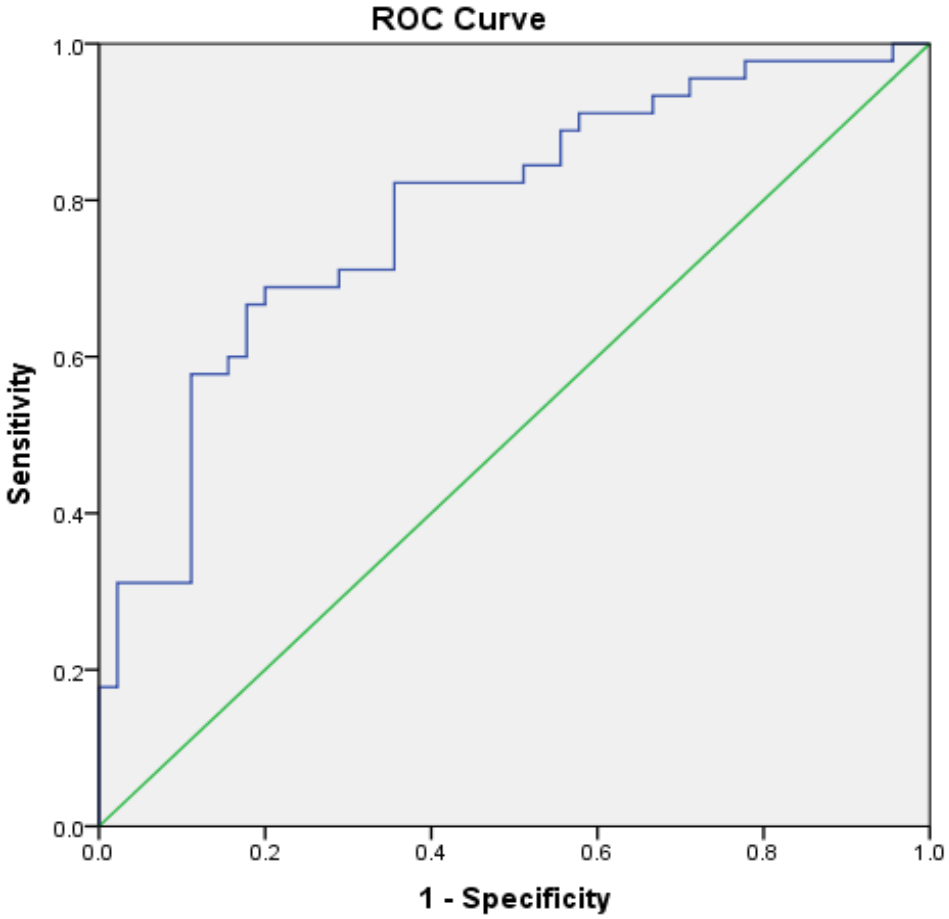


Figure (3-8) . ROC Curve of Apo E.

For Apo E, our results stated that fair diagnostic value in the diagnosis of preeclampsia in pregnant women

4.1.Conclusions

The study concluded that:

- Increase the levels of LPL , ApoE and Apo C2 in those patients may serve as a marker for preeclampsia complication that which leads to worse consequences.
- Apolipopotien E and Apo C2 modulates LPL activity and could be a relevant factor in the pathophysiology of preeclampsia .

4.2. Recommendations:

- Large sample size for study pregnant preeclampsia are recommended.
- Study the genetic variation of LPL ,Apo E and Apo C2 among preeclampsia patients to confirm elevation of levels with its gene expression.

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