ASSESSMENT OF MATERNAL SERUM DOCOSAHEXAENOIC ACID LEVELS IN PRE-ECLAMPSIA AND UNCOMPLICATED PREGNANCIES

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DECLARATION

I, **Dr.V.Hariraj,** Post Graduate, Department of Biochemistry, Government Mohan Kumaramangalam Medical College, Salem, solemnly declare that the dissertation titled **"ASSESSMENT OF MATERNAL SERUM DOCOSAHEXAENOIC ACID LEVELS IN PRE-ECLAMPSIA AND UNCOMPLICATED PREGNANCIES"** was done by me at Government Mohan Kumaramangalam Medical College and Hospital under the expert guidance and supervision of my Professor and Head of the Department **Dr. P. JOSEPHINE LATHA, M.D.** The dissertation is submitted to The Tamil Nadu Dr. M.G.R Medical University, towards partial fulfillment of requirement for **M.D. BIOCHEMISTRY Degree (Branch XIII).**

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ASSESSMENT OF MATERNAL SERUM DOCOSAHEXAENOIC ACID LEVELS IN PRE-ECLAMPSIA AND UNCOMPLICATED PREGNANCIES

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ABBREVATIONS

ADAM	A Disintegrin& A Metalloprotease-12
ASA	Acetyl Salicylic Acid
ARCD	Age Related Cognitive Decline
ALA	Alpha Linolenic Acid
APP	Amyloid Precursor Protein
AA	Arachidonic Acid
ADMA	Asymmetric Dimethylarginine
CHOD-POD	Cholestrol Oxidase-Peroxidase
CHD	Coronary Heart Disease
DBP	Diastolic Blood Pressure
DDAH	Dimethylarginine Dimethylaminohydrolase
DHA	Docosahexaenoic Acid
EPA	Eicosapentaenoic Acid
ELISA	Enzyme Linked Immune Sorbent Assay
GOD-POD	Glucose Oxidase- Peroxidase
GLDH	Glutamate Dehydrogenase
HO	Heme Oxygenase
HB	Hemoglobin
HELLP	Hemolysis, Elevated Liver Enzymes, Low Platelets
HDL	High Density Lipoprotein

H_2O_2	Hydrogen Peroxide
HHE	Hydroxyhexenal
HNE	Hydroxynonenal
IL	Interleukin
IUGR	Intra Uterine Growth Restriction
LCPUFA	Long Chain Polyunsaturated Fatty Acid
LDL	Low Density Lipoprotein
MMC	Mild Memory Complaints
NPD	Neuroprotectin D
PlGF	Placental Growth Factor
PP-13	Placental Protein-13
PE	Preeclampsia
PAPP-A	Pregnancy Associated Plasma Protein A
PI	Pulsatility Index
RCT	Randomized Control Trial
RBC	Red Blood Cell
RPF	Renal Plasma Flow
S-Flt-1	Soluble Feline McDonough Sarcoma-(Fms-) like Tyrosine Kinase-1
SBP	Systolic Blood Pressure
TNF	Tumor Necrosis Factor
VEGF	Vascular Endothelial Growth Factor

INTRODUCTION

Preeclampsia is a pregnancy complicating multi-organ disease which is characterized by a classical triad of hypertension, proteinuria and edema.

Preeclampsia usually begins after 20 weeks of pregnancy in a woman whose blood pressure had been normal previously. It can lead to serious, even fatal, complications to both mother and baby.

Preeclampsia is associated with an increased risk of placental abruption, preterm birth, fetal intrauterine growth restriction (IUGR), acute renal failure, cerebrovascular and cardiovascular complications, disseminated intravascular coagulation, and maternal death. Therefore, the need to provide an early diagnosis of Preeclampsia is vital[(1)].

EPIDEMIOLOGY

GLOBAL BURDEN OF PRE-ECLAMPSIA

The incidence of preeclampsia is estimated to be between 3 and 10% of all pregnancies.

As a leading cause of maternal mortality, preeclampsia and related hypertensive disorders of pregnancy claims nearly 76,000 mothers and 5,00,000 babies life worldwide every year. To raise awareness, maternal health organizations host World Preeclampsia Day on 22 May yearly[(2)].

PREECLAMPSIA INCIDENCE IN INDIA

The incidence is low in Haryana (33%) and high in Tripura (87.5%).

Various risk factors of preeclampsia and its prevalence odds ratios are[3]

RISK FACTOR	ODDS RATIO	CONFIDENCE INTERVAL
Twin pregnancy	1.53	1.12-2.09
Tobacco smoking	1.91	1.19-1.91
Terminated pregnancy	1.38	1.30-1.48
Diabetes	1.89	1.44-2.49
Asthma	2.05	1.59-2.65
Residing in Eastern part of India	2.10	1.89-2.33
Residing in Central part of India	1.37	1.26-1.50
Residing in Northeastern part of India	1.49	1.27-1.75

Additional data on global impact of preeclampsia:

- Preeclampsia is a common factor for preterm delivery and data shows almost 20% of all neonatal intensive care admissions are due to PE.
- Preeclampsia results in 16% of maternal deaths in low- and -middle income countries, where 99% of pregnancy-related deaths occur

World Preeclampsia Day's theme - **"Be prepared before lightning strikes"** - highlights the importance of early recognition of symptoms because preeclampsia can occur quickly, without warning.

AIMS AND OBJECTIVES

The aim of this study is to assess the serum levels of Docosahexaenoic acid of preeclamptic mothers in comparison with normotensive mothers.

REVIEW OF LITERATURE

1. Dominik.et al conducted a meta-analysis of randomized controlled trials (RCTs) to find the effect of Eicosapentaenoic acid & Docosahexaenoic acid (EPA+DHA) on coronary heart disease (CHD). They also conducted another meta-analysis of prospective cohort studies to estimate the association between intake of EPA+DHA and the risk of developing CHD. In their study they found out that EPA+DHA is associated with reducing the risk of CHD, particularly among higher-risk population in RCT[(4)].

2. Karin.et.al conducted a systematic review and meta-analysis on DHA and adult memory. The study was conducted to find the effect of DHA intake, alone or along with EPA on episodic memory domains, in 18 years and above healthy individuals. Subjects free of any neurologic disease, with or without Mild Memory Complaints (MMC), were included in the study. Episodic memory outcomes of adults with MMC increased considerably with DHA/EPA supplementation and found to be statistically significant[(5)].

3. Rakesh.et al conducted a systematic review and meta-analysis on DHA supplementation and its role in preventing Age-Related Cognitive Decline (ARCD) in individual cognitive domains. They found out supplementing DHA does not have a role in preventing/retarding ARCD of memory, attention, executive function and working memory[(6)].

4. Marcus.et al conducted a study to estimate the effect of DHA on amyloid β production by multiple pleiotropic mechanisms. β- amyloid peptide(Aβ) accumulation is the characteristic of Alzheimer disease, which is generated by β- and γ-secretase processing of amyloid precursor protein(APP). They analyzed the effect of DHA on amyloidogenic and nonamyloidogenic processing, found out DHA reduces amyloidogenic processing by reducing β- and γ-secretase activity. They also found out DHA increases protein stability of α-secretase activity leads to increased nonamyloidogenic processing. Thus they concluded, DHA has a pleiotropic effect, DHA mediated Aβ reduction is due to combined multiple effects, not the consequence of single major mechanism[7].

5. Susan.et al conducted a study on DHA supplementation and pregnancy outcomes. In their study they have assessed maternal and newborn DHA level, gestational duration, birth weight and length after administrating 600mg/day of omega-3 long-chain polyunsaturated fatty acids (LCPUFAs) especially DHA. In their study they compared DHA supplementation with placebo intake. Mean DHA intake was 469mg/day. When compared with placebo, DHA supplementation results in higher maternal and cord RBC-phospholipid –DHA,longer gestation, greater birth weight, length. They also found out DHA supplementation results in less number of infants born at <34 week of gestation and reduced hospital stay for preterm infants compared with placebo group. They have concluded, 600mg/day of DHA supplementation especially in the last half of pregnancy results in

increased gestation duration, infant size. Reduction in early preterm and very-low birth weight has also been noted[8].

6. Fatemeh.et al conducted a systemic review and meta-analysis on the efficacy of n-3 fatty acids supplementation on the prevention of pregnancy induced-hypertension or preeclampsia. Main aim of the study was to examine the effect of supplementation with EPA, and/or DHA, and/or ALA during pregnancy on the pregnancy-induced hypertension or preeclampsia. In their study they have concluded that the n-3 fatty acid supplements are an effective strategy to prevent the incidence of preeclampsia in women with low-risk pregnancies[9].

7. Alice.et al conducted a pilot study to find the role Played by Salicylic Acid and Omega 3 in the Placental Vascular Resistance Mechanism. The aim of their study was to evaluate uterine artery resistance and pulsatility indices, bilateral notch in pregnant women presenting identifiable risk factor for developing PE, who use omega 3 in association, or not, with ASA. They found out comparison between ASA use in association, or not, with omega did not show any difference in preeclampsia, prematurity, oligohydramnios, IUGR or hospitalization in neonatal ICU frequency. There were no cases of fetal death or HELLP Syndrome in both groups. In their study they concluded omega 3 use in association with ASA has increased the uterine artery resistance and pulsatility indices, though, it did not make any difference in primary and secondary outcomes[10].

8. Paige.et al conducted a meta-analysis of randomized controlled trials on Long-Chain Omega-3 Fatty Acids Eicosapentaenoic Acid and Docosahexaenoic Acid and Blood Pressure.The objective of their meta-analysis study was to examine the effect of EPA+DHA on blood pressure in RCT, without upper dose limits and including food sources. EPA+DHA provision when compared with placebo, shows reduced systolic blood pressure of 1.52mm Hg with 95% confidence interval (CI) and diastolic blood pressure of 0.99mm Hg with 95% CI. The strongest effects of EPA+DHA were observed among untreated hypertensive subjects with fall in systolic blood pressure of 4.51mm Hg, and diastolic blood pressure of 3.05mm Hg[(11)].

9. Maria.et al conducted a RCT on the Effect of DHA Supplementation during Pregnancy on Maternal Depression and Neurodevelopment of Young Children. Objective of the study was to determine the neurodevelopmental outcome of children in women with high levels of depressive symptoms with increasing DHA during the last half of pregnancy. They observed there is no difference between the two group women with high levels of depressive symptoms during the first 6 months postpartum. They concluded the use of DHA-rich fish oil capsules during pregnancy compared with vegetable oil capsules did not result in lower levels of postpartum depression in mothers, improved cognitive and language development in the offspring during early childhood[(12)].

10. Yuhua.et al conducted a meta-analysis study to estimate the efficacy of LCPUFA especially EPA and DHA in the improvement of depression. Their study showed that when compared with placebo, EPA-pure and EPA- major formulations have better clinical benefits in the improvement of depression than DHA-pure and DHA-major formulations[13].

HYPERTENSIVE DISORDERS OF PREGNANCY

According to National High Blood Pressure Education Program Working Group (NHBPEP), Hypertension is defined as

1) Systolic BP of ≥ 140 mmHg and/or

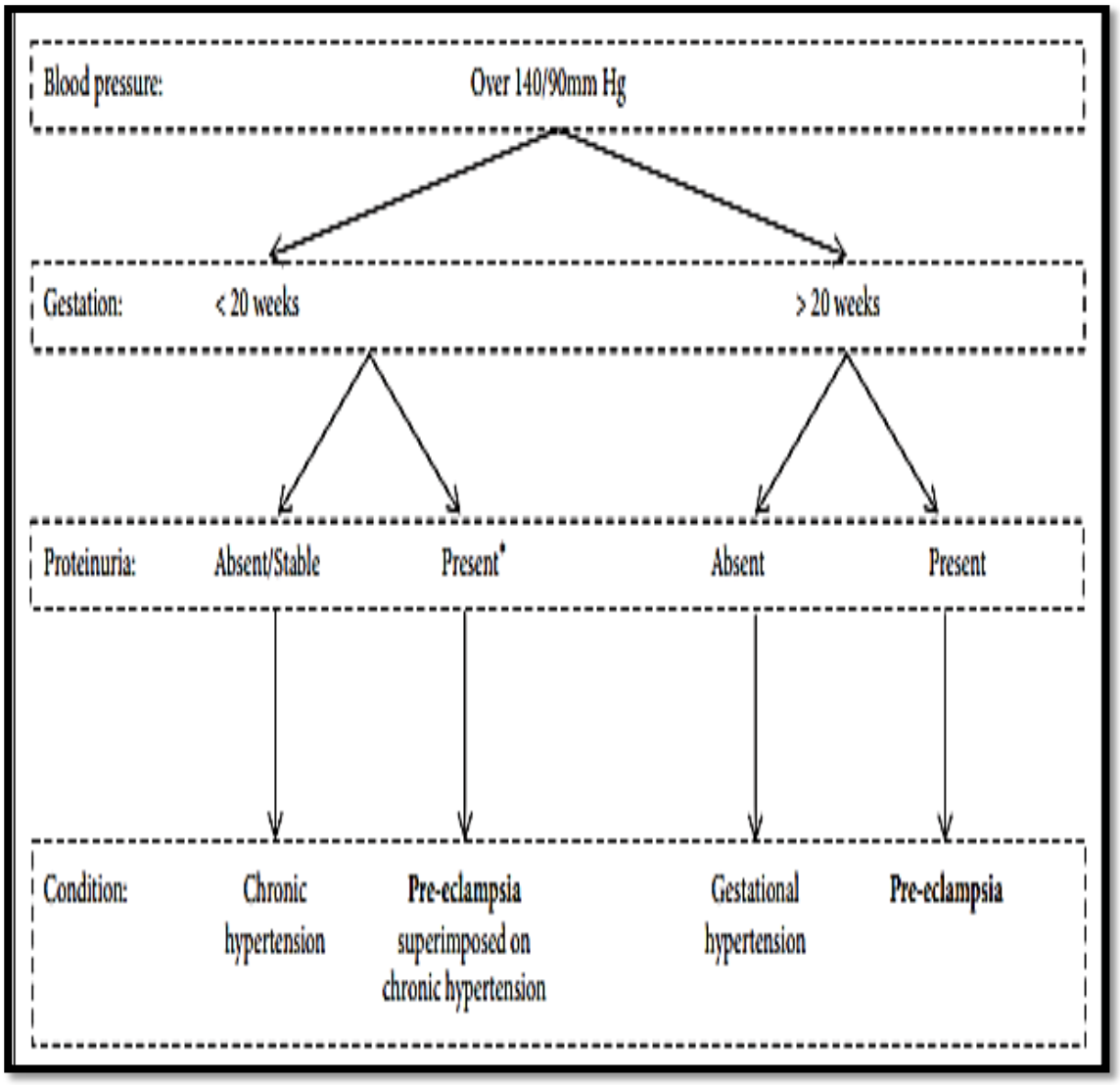
2) Diastolic BP of ≥ 90 mmHg (Korotkoff V) measured in 2 occasions 4 – 6 hours apart within a week. Increase in Systolic BP of 30mmHg or Diastolic BP of 15 mmHg above the patient's baseline is the diagnostic criteria for Hypertension.

Hypertension is classified according to severity as follows:

- MILD
- MODERATE
- SEVERE

1) MILD HYPERTENSION: Systolic Blood pressure 140-149 mmHg, Diastolic Blood Pressure 90 – 99 mmHg.
2) MODERATE HYPERTENSION: Systolic Blood pressure 150-159 mmHg, Diastolic Blood Pressure 100-109 mmHg.
3) SEVERE HYPERTENSION: Systolic Blood PRESSURE 160 mmHg or greater, Diastolic Blood Pressure 110mmHg or greater[14].

Figure 1: CLASSIFICATION OF HYPERTENSIVE DISORDERS IN PREGNANCY



Hypertensive disorders during pregnancy are classified into 4 categories as:

1. Gestational hypertension
2. Preeclampsia and eclampsia
3. Chronic hypertension
    a) Essential
    b) Secondary
4. Preeclampsia superimposed on chronic hypertension

GESTATIONAL HYPERTENSION:-

Gestational hypertension, formerly known as pregnancy-induced hypertension or PIH. It is the new onset of hypertension after 20 weeks of gestation.

The diagnosis requires that the patients have:

a) Elevated blood pressure (systolic ≥140 or diastolic ≥90mm Hg, the latter measured using the fifth Korotkoff sound) in previously normal blood pressures.
b) No protein in the urine
c) No manifestations of preeclampsia/eclampsia.

PREECLAMPSIA:-

Preeclampsia is defined as the presence of a systolic blood pressure (SBP) ≥140 mm Hg or a diastolic blood pressure (DBP) ≥ 90 mm Hg, on two occasions at least 4 hours apart in a previously normotensive patient associated with proteinuria> 300mg/L in 24 hour urine collection (or) 1+ by qualitative urine examination.

ECLAMPSIA:-

Convulsions occurring in a patient with preeclampsia.

CHRONIC HYPERTENSION:-

Chronic hypertension is high blood pressure that either occurs before pregnancy, is diagnosed within the first 20 weeks of pregnancy, or does not resolve by the 12-week postpartum checkup. Further classified into-

a) ESSENTIAL HYPERTENSION - diagnosed when there is no apparent underlying cause for chronic hypertension.

b) SECONDARY HYPERTENSION - caused by renal parenchymal disease, endocrine disorders, renovascular disease or coarctation of aorta.

PREECLAMPSIA SUPERIMPOSED ON CHRONIC HYPERTENSION:-

Superimposed preeclampsia (on chronic hypertension) is characterized by

(1) new onset proteinuria (≥300 mg/24 h) in a woman with hypertension but no proteinuria before 20 weeks gestation.

(2) sudden increase in proteinuria or BP, or a platelet count of less than 100,000/mm^3, in a woman with hypertension and proteinuria before 20 weeks of gestation[(1)].

PREECLAMPSIA

Preeclampsia (PE) is a multisystem, pregnancy specific disorder characterized by the development of hypertension and proteinuria (elevated levels of protein in the urine) after 20 weeks of gestation. PE is a leading cause of maternal, perinatal (from the 20th week of gestation to the 4th week after birth), and fetal/neonatal mortality and morbidity worldwide.

PE can have an early onset (starting before 34 weeks of gestation) or late onset (after 34 weeks of gestation). Further PE is classified as mild or severe, depending on the severity of the symptoms present[15].

High blood pressure and protein in the urine are key features. Blood pressure that exceeds 140/90 millimeters of mercury (mm Hg) or greater measured on two occasions, at least four hours apart is considered abnormal.

Symptoms include pedal edema, seizures, upper abdominal pain, usually under ribs on the right side, nausea or vomiting, reduced urine output.

The disease is more common among nulliparous women, in women who conceive through assisted reproduction techniques and also in women affected by autoimmune disorders[16].

Figure 2: CLASSIFICATION OF PREECLAMPSIA

Symptom	Mild PE	Severe PE
Blood Pressure	Systolic ≥140 mm Hg or diastolic ≥90 mm Hg, over 20 weeks of gestation (in a woman with previously normal blood pressure)	Systolic ≥160 mm Hg or diastolic ≥110 mm Hg (on two occasions at least six hours apart; in a woman on bed rest)
Proteinuria	24-hour urine collection protein ≥0.3 g (urine dipstick test ≥1+)	24-hour urine collection protein ≥5 g (urine dipstick test ≥3+; in two random urine samples collected at least four hours apart)
Others	N.A.	(i) Oliguria (ii) Cerebral or visual disturbances (iii) Pulmonary oedema or cyanosis (iv) Epigastric or right upper quadrant pain (v) Impaired liver function (vi) Thrombocytopenia (vii) Intrauterine growth restriction

PATHOPHYSIOLOGY:

Pathophysiology of PE has been described as two stages.

Stage 1→ Placentation abnormalities

Stage 2→The Maternal Syndrome

PLACENTATION ABNORMALITIES: STAGE I

Placenta plays a crucial role in the pathogenesis of the disease. Pathologic examination of placentas of preeclamptic pregnancies reveals placental infarcts and sclerotic narrowing of arteries and arterioles. They are characteristed by diminished endovascular invasion by cytotrophoblasts and inefficient remodeling of the uterine spiral arterioles.

There is no obvious gross pathologic changes seen in the placenta of preeclamptic women.

Doppler study of Uterine artery which assess the pulsatility index (PI) shows increased uterine vascular resistance well before the clinical signs and symptoms develops[17].

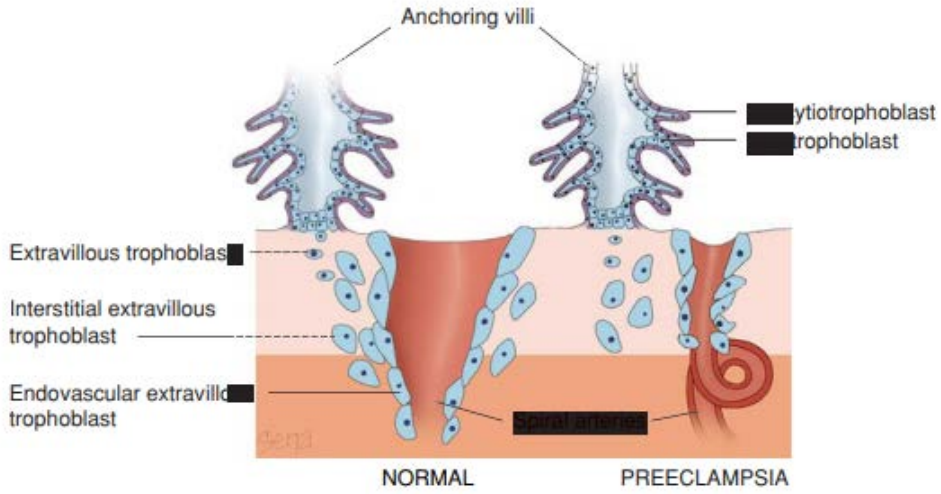
Mechanical constriction of the uterine arteries produces hypertension, proteinuria. Glomerular endotheliosis plays a causative role for placental ischemia in the pathogenesis of preeclampsia[18].

Good angiogenesis is required for normal placentation for the supply of oxygen and nutrients in the fetus. Various pro- and antiangiogenic factors are released by developing placenta. Placental angiogenesis is defective in preeclampsia, leading to failure of the cytotrophoblasts to convert from a epithelial to endothelial phenotype[18,19].

Normally, invasive cytotrophoblasts downregulate the expression of adhesion molecules of epithelial origin, adopt a cellsurface adhesion phenotype specific to endothelial cells, a process referred to as pseudovasculogenesis[19,20]. In preeclampsia, cytotrophoblast cells fail to undergo this switching of cell-surface integrins and adhesion molecules. This abnormal cytotrophoblast differentiation an early defect, leads to placental ischemia.

Hypoxia-inducible factor-1 is upregulated in preeclampsia and plays a central role in the abnormal differentiation phenotype of preeclampsia[21,22].

Figure 3: PLACENTAL IMPLANTATION IN NORMAL VS PREECLAMPSIA



In NORMAL- proliferation of extravillous trophoblasts from an anchoring villus invade the deciduas and extend into the walls of the spiral arteriole to reduce the endothelium and muscular wall to create a dilated low resistance vessel.

In PREECLAMPSIA- defective implantation is characterized by incomplete invasion of the spiral arteriolar wall by extra villous trophoblasts. This results in a small-caliber vessel with high resistance to flow.

The Maternal Syndrome: Stage II

Abnormal placentation as a result of failure of trophoblast remodeling of uterine spiral arterioles leads to the release of secreted factors enter the mother's circulation, culminating in the clinical signs and symptoms of preeclampsia.

Clinical manifestations of preeclampsia are due to glomerular endotheliosis, increased vascular permeability, a systemic inflammatory response that results in end-organ damage and hypoperfusion. These clinical manifestations occur after the 20th week of pregnancy[17].

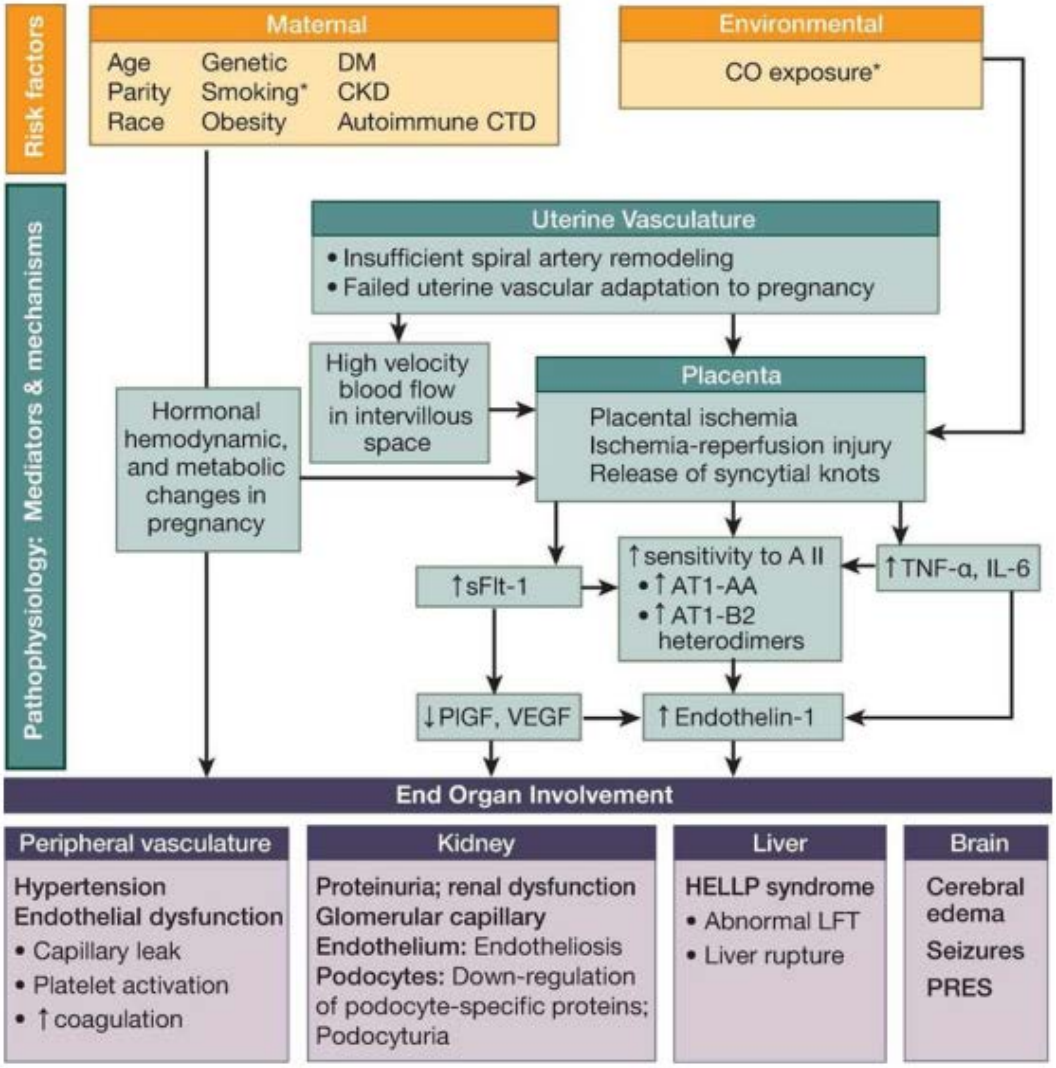
Decrease in systemic vascular resistance primarily secondary to vasodilation is the prime cause for decrease in both systolic and diastolic BP during normal pregnancy. Relaxin, released from ovaries under the influence of human chorionic gonadotrophin (HCG), regulates nitric oxide synthase (NOS), enzyme generates nitric oxide (NO) from arginine, via the endothelial endothelin β receptor[23].

In preeclampsia, derangement of endothelial-derived vasoactive factors result in the predominance of vasoconstrictors (endothelin, thromboxane A2) over vasodilators (NO, prostacyclin).

Elevated circulating levels of asymmetric dimethyl arginine, an endogenous inhibitor of NOS, seen in pregnancies complicated by preeclampsia[24,25,26]

In normal pregnancy, all components of RAS are upregulated, but resistance to the pressor effects of angiotensin II (AngII) allows for normal to low BP[24,25].

Figure 4: ETIOLOGY OF PREECLAMPSIA

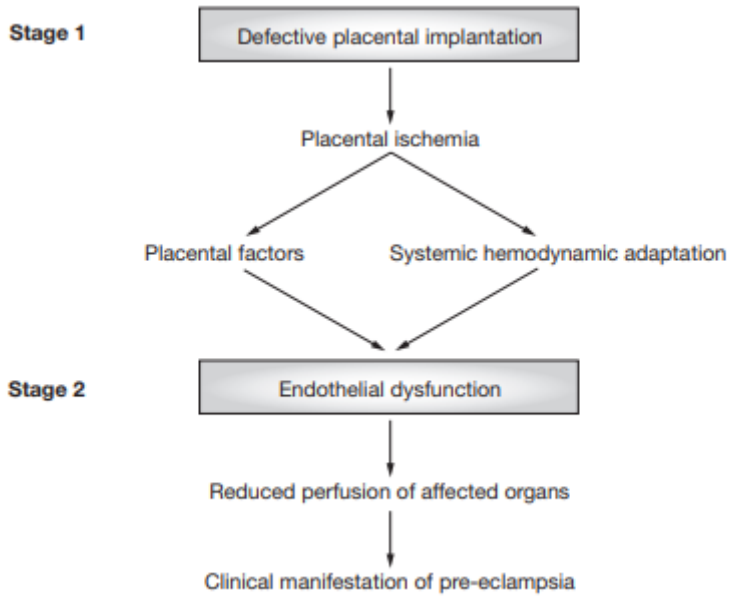


Angiotensin II, binds to both angiotensin type 1 receptor (AT1R) and angiotensin type 2 receptor (AT2R). AT1R mediates vasoconstriction, sodium and water, retention, fibrosis and inflammation whereas AT2R has opposing actions mediating vasodilation, anti-inflammation, natriuresis and antifibrosis. Angiotensin-(1–7) [Ang-(1–7)] is one another active hormone within the RAS. Ang-(1–7) is cleaved from angiotensin II by ACE2 (angiotensin-converting enzyme 2). Ang-(1-7) is significantly decreased in women with preeclampsia compared to normal pregnant control subjects[27,28].

Preeclamptic women produce IgG autoantibody which will stimulate the AT1 receptor[29].

The IgG autoantibody stimulate heterodimerization between AT1 receptor and B2 receptor for bradykinin[30]. This plays an important role in the increased vascular sensitivity to angiotensin. It induces production of reactive oxygen species (ROS), which block cytotrophoblast invasion and this leads to shallow trophoblastic implantation[31]. This autoimmune activity wanes after delivery[29].

Figure 5: PATHOPHYSIOLOGY OF PREECLAMPSIA



STAGE 1- Abnormal placentation due to lack of dilatation of the uterine arterioles.

STAGE 2- Maternal syndrome is a function of the circulatory disturbance caused by systemic maternal endothelial cell dysfunction resulting in vascular reactivity, activation of coagulation cascade and loss of vascular integrity.

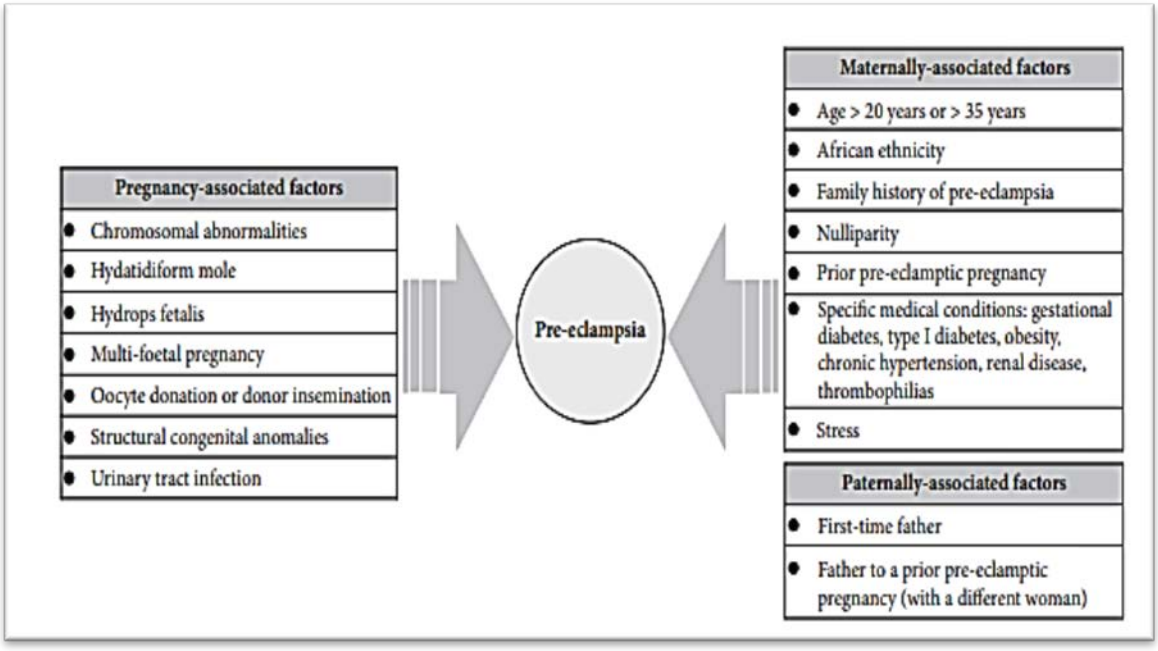
RISK FACTORS FOR PE:

Various risk factors for pre-eclampsia has been documented. Some of them are[32,33,34]

HIGH RISK FACTORS:-

i. Previous preeclampsia(PE)
ii. Previous early onset PE and preterm delivery at < 34 weeks of gestation
iii. PE in more than one prior pregnancy
iv. Chronic kidney disease
v. Autoimmune disease such as systemic lupus erythematosis or antiphospholipid syndrome
vi. Type 1 or 2 diabetes
vii. Chronic hypertension

Figure 6: RISK FACTORS ASSOCIATED WITH PREECLAMPSIA



MODERATE RISK FACTORS:-

i. First pregnancy
ii. Pregnancy interval for more than 10 years
iii. Reproductive technologies
iv. Family history of PE (mother or sister)
v. Excessive weight gain in pregnancy
vi. Gestational trophoblastic disease
vii. Multiple pregnancies
viii. Age 40 years or older
ix. Body mass index of 35kg/m^2 or more at first visit
x. Increased pregnancy triglycerides
xi. Family history of early onset cardiovascular disease
xii. Lower socioeconomic status
xiii. Cocaine and methamphetamine use
xiv. Nonsmoking.

According to NICE guidelines, the presence of two moderate-risk factors or a single high-risk factor has to be considered for prophylactic measures of pregnant women.

CLINICAL FEATURES:

Healthy pregnant women exhibit marked glomerular hyperfiltration, above normal, non-gravid levels by 40 to 60%[35,36]. This hyperfiltration is due to depression of plasma oncotic pressure (Π_{GC}) in the glomerular capillaries.

The reduction of oncotic pressure in pregnancy is due to two phenomena. The first is hypervolemia-induced hemodilution which lowers the protein concentration of plasma entering glomerular microcirculation. The second is an enhanced rate of RPF. Hyperperfusion of glomeruli blunts the extent to which the oncotic pressure can increase along the glomerular capillaries during filtrate formation. In preeclampsia, variable degrees of renal insufficiency are associated with characteristic glomerular lesion, "glomerular endotheliosis."[37]

Preeclampsia is differentiated from gestational hypertension by the presence of proteinuria and is the most common cause of nephrotic syndrome in pregnancy. The quantity of protein that is excreted in the urine varies widely. Significant protein excretion is defined as 300 mg in a 24-h urine collection or ≥1+ on urine dipstick testing of two random urine samples that are collected at least 4 h apart[38].

Presence of glomerular proteins of intermediate size, such as albumin, alone or in combination with tubular proteins, such as β2-microglobulin, reflecting the tubular damage occuring in severe preeclampsia [39,40].

Maternal complications:

Cardiovascular and cerebrovascular diseases, liver and kidney failure, placental abruption, disseminated intravascular coagulation, and hemolysis, elevated liver enzyme levels, and low platelet levels (HELLP) syndrome[41].

Fetal complications:

Fetal complications includes fetal growth restriction with oligohydramnios, non-reassuring fetal status, preterm delivery, low birth weight, severe birth asphyxia, stillbirth, and intrapartum death.

BIOMARKERS OF PREECLAMPSIA

For the diagnosis of Preeclampsia, its ability to find the severity, few markers have been used[14,42]. They are:

a) Placental growth factor (PlGF)
b) Soluble Feline McDonough Sarcoma- (fms-) like tyrosine kinase-1 (sFlt-1)
c) Asymmetric dimethylarginine (ADMA)
d) PAPP-A (Pregnancy Associated Plasma Protein A)
e) β human chorionic gonadotrophin (β HCG)
f) PP-13 (Placental Protein-13)
g) Inhibin A and Activin A
h) Soluble endoglin
i) ADAM-12 (A Disintegrin & A Metalloprotease-12)
j) Cystatin C
k) Pentraxin 3
l) P-selectin

PLACENTAL GROWTH FACTOR (PlGF):

i. PlGF belongs to the vascular endothelial growth factor (VEGF) family of proteins and it is secreted as a glycosylated protein. PlGF has angiogenic and mitogenic properties, induce proliferation, migration, and activation of endothelial cells[43,44].

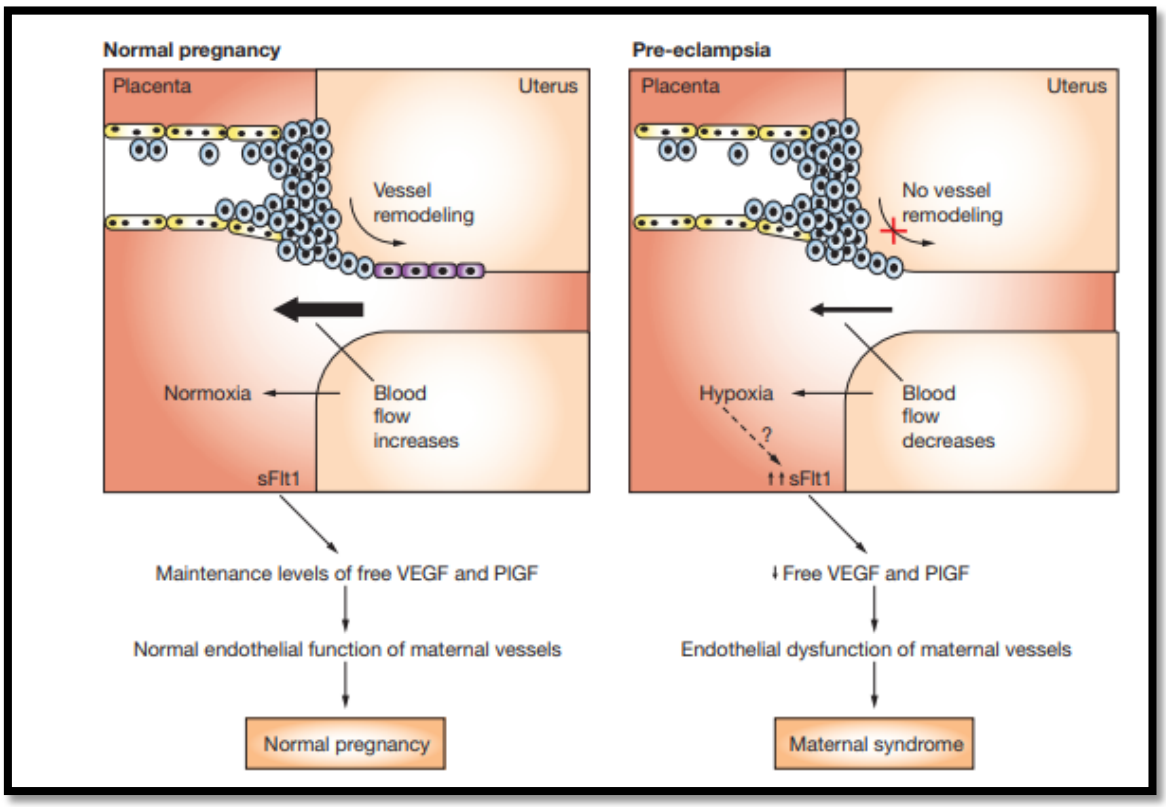
ii. The proangiogenic activity of VEGF family of proteins, including PlGF, is by binding and activation of tyrosine kinase receptors[45]. The receptors bind the proteins with high affinity, are the fms like tyrosine kinase receptor [(Flt-1) also known as VEGF receptor 1, VEGFR1] and kinase domain region (KDR or VEGFR2).

SOLUBLE FMS-LIKE TYROSINE KINASE-1 (sFlT-1):

i. sFlt-1, the soluble splice variant of Flt-1, is secreted into the circulation which acts as an antiangiogenic factor by antagonising and neutralizing PlGF and VEGF. This is done by binding of sFlt-1 to PlGF & VEGF and inhibiting their interaction with endothelial receptors on the cell surface[46,47,48].

ii. sFlt-1 levels were higher in fetus born to mothers with Preeclampsia, the sFlt-1 concentrations measured in umbilical samples were low compared to the maternal sFlt-1 concentrations. This shows that the fetus does not contribute to the elevated maternal sFlt-1 concentration in Preeclampsia. Thus increase in circulating sFlt-1 concentration in mothers with PE indicates sFlt-1 originates primarily from the placenta[49].

Figure 7: ROLE OF sFlT-1 IN PREECLAMPSIA



In NORMAL- Most of sFlt-1 produced by placenta is released into blood. It binds to both VEGF and PlGF thereby reducing its free levels in the blood by working as a soluble antagonist of both factors, maintaining normal endothelial function of maternal vasculature.

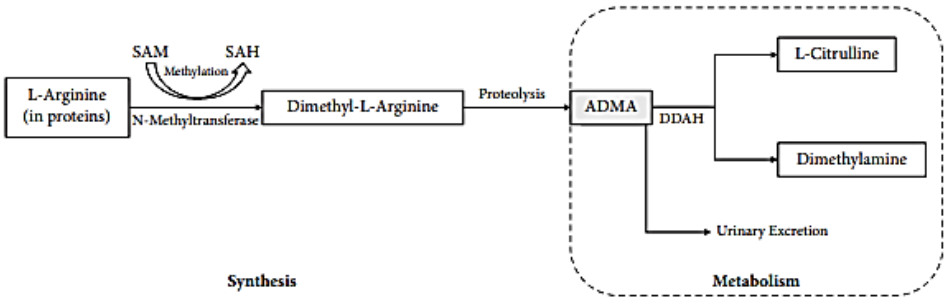
In PREECLAMPSIA- placenta releases large amount of sFlt-1 than normal placenta, depriving the vasculature of kidney, liver, brain and other organs of essential maintenance signals, thereby triggering the maternal vascular dysfunction of preeclampsia.

ASYMMETRIC DIMETHYL ARGININE (ADMA):

i. ADMA is an endogenous competitive inhibitor of NOS. NOS is responsible for the synthesis of nitric oxide in endothelial cells as it catalyses the conversion of L-arginine to L-citrulline and NO. ADMA is an analogue of L-arginine which is also synthesized and released by endothelial cells[(50)].

ii. Dimethylargininase also known as dimethylarginine dimethylaminohydrolase (DDAH), catalyses the hydrolysis of ADMA. Decreased levels or inhibition of DDAH results in higher levels of ADMA in the circulation and which causes gradual vasoconstriction. This is because the increased level of ADMA in the circulation results in reversible inhibition of endogenous NO synthesis which leads to endothelial dysfunction. The low levels of NO result in increased systemic vascular resistance and blood pressure[51].

Figure 8: SYNTHESIS OF ADMA



Methylation of arginine residues with the help N-methyltransferase produces ADMA which converts the methyl donor S-adenosylmethionine (SAM) to S-adenosylhomocysteine (SAH). ADMA is partly eliminated via urine excretion. However, ADMA is mainly eliminated through its metabolism to citrulline and dimethylamine by enzyme DDAH.

PAPP-A (PREGNANCY ASSOCIATED PLASMA PROTEIN A):

i. PAPP-A is an insulin like growth factor binding protein protease derived from syncytiotrophoblast, used in risk calculation for chromosomal abnormalities like Down's syndrome.

ii. PAPP-A regulates the bioavailability of free IGF at the placental decidual interface during implantation. Low concentrations of PAPP-A in the first trimester of pregnancy are highly associated with chromosomal aneuplodies. Apart from chromosomally normal pregnancies, low maternal serum PAPP-A is associated with increased risk for development of Preeclampsia[52].

β HUMANCHORIONIC GONADOTROPIN (β HCG):

i. β-hCG is a promoter of cell growth and differentiation in the embryo. They are secreted by syncytiotrophoblastic cells of the placenta and its primary function is maintaining the vascular supply of placenta during pregnancy[53].

ii. In normal pregnancies, until 9 to 10 weeks its level increases, decreasing afterwards. However, studies show that in those who develop Preeclampsia, β-hCG levels continue to be elevated well beyond to the second trimester.

iii. It has a low predictive value for Preeclampsia[54].

PLACENTAL PROTEIN 13 (PP-13):

i. PP-13 is a dimer protein belongs to the galectin super-family, which is a member of carbohydrate binding proteins called β-galactoside-specific lectins. They are highly expressed in the placenta, specifically by the syncytiotrophoblast[55].

ii. PP-13 is involved in the remodelling of common fetomaternal blood-spaces by binding to proteins between the placenta and the endometrium.

iii. Serum PP-13 increases to double or triple of their values before deliveryin normal pregnancy, whereas low concentrations of PP-13 during 5-7 weeks predicts the onset of Preeclampsia[56].

INHIBIN-A AND ACTIVIN-A:

i. Inhibin-A and Activin-A are hormones basically glycoproteins. They are produced by the fetoplacental unit involved in the feedback loop regulating hCG levels during pregnancy.

ii. Both Inhibin-A and Activin-A are increased in the maternal blood during first trimester of patients who develop Preeclampsia later, thus serves as a marker for early diagnosis of preeclampsia compared to pregnant women with normal pregnancies [34].

SOLUBLE ENDOGLIN:

i. Soluble endoglin is an auxiliary co-receptor of transforming growth factor β2 (TGFβ2).

ii. It interferes with binding of TGF-β1 to its receptor, playing a role in the alterations in vasculogenesis and angiogenesis leading to Preeclampsia

iii. Effects include production of nitric oxide, capillary formation by endothelial cells, vasodilation, hypoxia as well as oxidative stress[57].

A DISINTEGRIN AND METALLOPROTEASE 12 (ADAM-12):

i. ADAM12 is a placenta-derived member of ADAM protein family, takes part in placental growth and development.

ii. It is the most upregulated transcription factor in placental tissues in women with Preeclampsia[58].

iii. Reduced ADAM12 levels were noted between 8 to 14 weeks in pregnancies complicated by Preeclampsia, hence a potiential early biomarker for Preeclampsia[59].

CYSTATIN C:

i. Cystatin C is an established marker for renal function, increasing as the glomerular filtration rate falls.

ii. In Preeclampsia, placental expression of Cystatin C is increased at the mRNA and protein levels, indicating increased synthesis and secretion of Cystatin C protein. This leads to elevated maternal plasma Cystatin C levels in Preeclampsia[60].

PENTRAXIN 3 (PX3):

i. Pentraxin 3 (tumor necrosis factor-stimulated gene-14) belongs to the family as C-reactive protein and serum amyloid P component. Pentraxin 3 consists of 381 amino acids.

ii. The maternal inflammatory response in Preeclampsia results in increased levels of pentraxin 3, an inflammatory marker having the same molecular class as that of C-reactive protein[61].

P-SELECTIN:

i. P-selectin belongs to the selectin family of cell surface adhesion molecules. P-selectin is expressed by platelets and endothelial cells upon activation.

ii. They play a crucial role in inflammatory reactions by recruitment and activation of circulating leucocytes, and also in coagulation by generation of leukocyte-derived “bloodborne” tissue factor[62].

iii. P-selectin is rapidly shed from the cellular membrane of activated platelets, and thus contribute to most of the soluble isoform of the molecule found in plasma.

iv. Preeclampsia is associated with extensive platelet activation. Activated platelets release P-selectin-exposing micro-particles with procoagulant activity, detected in the peripheral blood of women with Preeclampsia[63].

v. Soluble P-selectin is observed in higher amounts in serum of patients with preeclampsia[64].

DOCOSAHEXAENOIC ACID

Essential fatty acids are fatty acids that cannot be synthesized within the human body, therefore must be obtained from the diet.

There are nearly 20 edible fatty acids, of which, omega-3 and omega-6 fatty acids are not synthesized by the body, hence supplemented through diet.

LC-PUFAs or long chain polyunsaturated fatty acids are those which contain 8-20 carbon atoms.

They are classified based on the position at which double bond is present from the methyl end as:

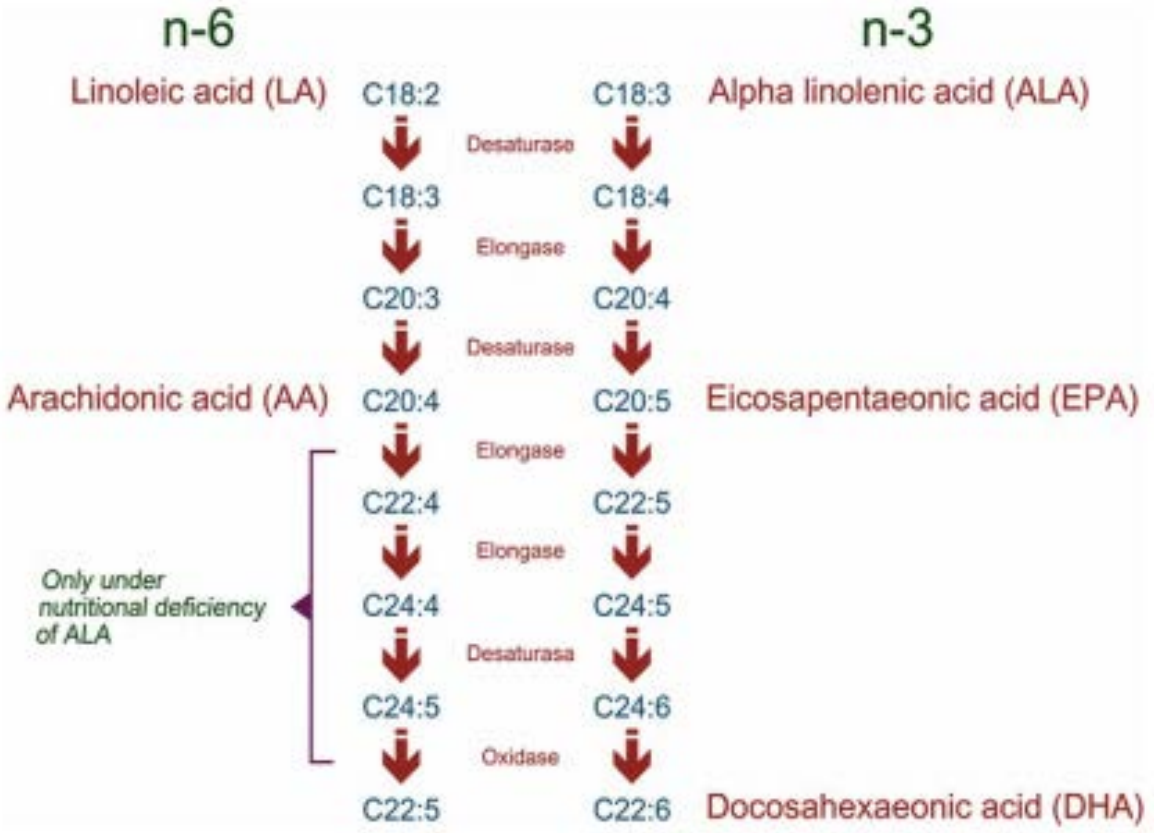
a) omega-3 (ω−3) fatty acids
b) omega-6 (ω−6) fatty acids

Fatty acids that are not saturated with hydrogen (H) atoms (and contain more than one double bond between the atoms) are called ‘polyunsaturated fatty acids’ (PUFAs)[65].

There are three major essential fatty acids:

1. alpha- linolenic acid(ω-3)
2. arachidonic acid(ω-6)
3. linoleic acid(ω-6)

Figure 9: NATURAL ORIGIN OF DHA IN THE FOOD CHAIN



SOURCES OF EFA

Omega-6 fatty acids

Food sources of linoleic acid (LA) include vegetable oils, such as soybean, safflower, corn oil as well as nuts, seeds, and some vegetables. Animals, but not plants, can convert LA to arachidonic acid (AA). Therefore, AA is present in small amounts in meat, poultry, and eggs.

Omega-3 fatty acids

Flaxseeds, walnuts, and their oils are the richest dietary sources of alpha-linolenic acid (ALA). Canola oil is also an excellent source of ALA.

Oily fish, such as herring and salmon, are the major dietary source of long-chain omega-3 polyunsaturated fatty acids, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA)[66].

Trans fatty acids (trans fats) are made through hydrogenation to solidify liquid oils. Heating omega-6 oils to high temperatures creates trans fats.

Trans fats increase the shelf life of oils and are found in vegetable shortenings and in some margarines, commercial pastries, fried foods, crackers, cookies, and snack foods. The intake of trans fatty acids increases blood LDL-cholesterol ("bad cholesterol"), decreases HDL cholesterol ("good cholesterol"), and raises the risk of coronary heart disease[67].

FUNCTIONS OF EFA

The three main **omega-3 fatty acids** are

1) alpha-linolenic acid (ALA)
2) eicosapentaenoic acid (EPA)
3) docosahexaenoic acid (DHA)

Omega-3s are important components of the membranes that surround each cell in your body. Docosahexaenoic acid levels are high in retina (eye), brain, and sperm cells. The energy required for various body functions are also derived from Omega-3 fatty acids[68].

DOCOSAHEXAENOIC ACID (DHA)

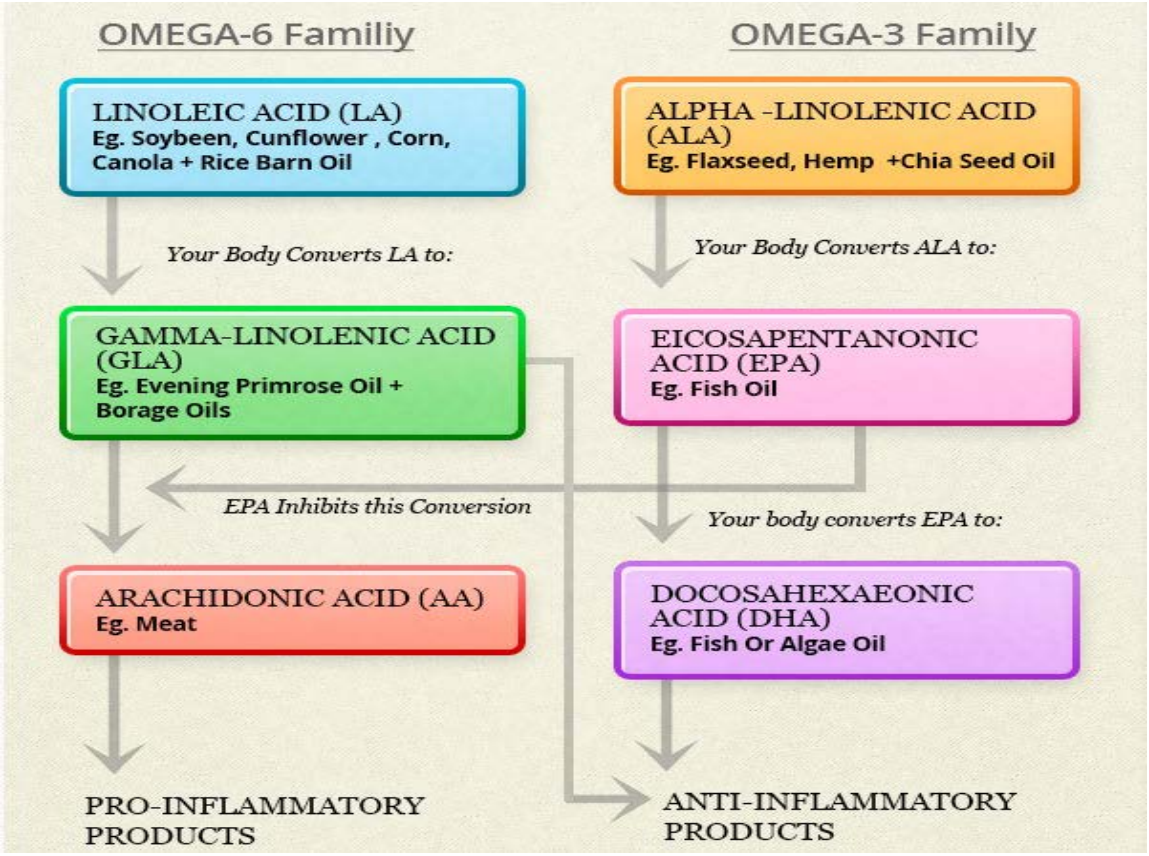
Docosahexaenoic acid is a long chain polyunsaturated essential fatty acid. During pregnancy, Docosahexaenoic acid is essential for the placentation. Apart from implantation, Docosahexaenoic acid is necessary for normal brain development of the fetus.

Fetus accumulates all of the omega-3 and omega-6 fatty acids from the mother by placental transfer only.

DHA is of high interest due to its highly unsaturated structure (six double bonds, the fatty acid most unsaturated in our body) and cell location, mostly concentrated at sn-2 position of phospholipids forming cell membranes, providing a great fluidity to these structures[(69)].

DHA is proposed to have a significant role in human evolution, process characterized by increased size and complexity of the brain tissue and by development of mental, behavioral and motor skills with cognitive components.

Figure 10: ESSENTIAL FATTY ACID PATHWAY



During pregnancy and the early stage of childhood DHA plays a crucial role in brain and retinal development, directly affecting the cognitive function and the visual acuity of child. The capacity of human brain to synthesize DHA from its precursor, ALA, is very low. Less than 1% of ALA consumed is converted into DHA in the liver by enzymatic process[(70)].

DHA is the most abundant n-3 LCPUFA in the central and peripheral nervous system. It is present in large amounts in phospholipids of brain gray matter. DHA plays a crucial role in neurogenesis and synaptogenesis, specifically in fetal development and during the first two years of life. Fetal DHA accretion occurs actively as pregnancy proceeds but is most active during the third trimester. Hence,

the nutritional status of DHA for pre-gestational mother, during pregnancy and lactation represents a critical step in the brain and visual development of the child.

Neonates having higher concentrations of DHA in umbilical plasma phospholipids have longer gestational length in comparison with neonates having low concentration. During pregnancy, DHA supplementation increases the expression of fatty acid transport proteins and thus increases the transport of n-3 LCPUFA through the placenta to fetus via blood. Pregnant women who consumed DHA (2.2 g/day), from the 20th week of pregnancy until partum, with no adverse effects, the children delivered showed better visual and coordination capacity. When DHA is supplemented with 5-methyltetrahydrofolate (400 μg/day), cognitive benefits were prolonged until 6.5 year-old[71].

High levels of DHA in the mother, particularly in breast milk, directly correlate with better growth and development of the brain and visual system of the children. Perinatal supplementation of DHA in children reduces the risk of lower scores on IQ in children from families with very low income.

DHA is essential for the neuronal structure and also for the neuronal signaling. It also has a neuroprotective property against cerebral aging, neurodegenerative diseases and cerebrovascular diseases, particularly in the injury produced by ischemia-reperfusion episodes.

Neuroprotectin D-1 (NPD-1) derivative of DHA is primarily responsible for the neurological benefits associated to DHA protection. Protective mechanism of DHA are:

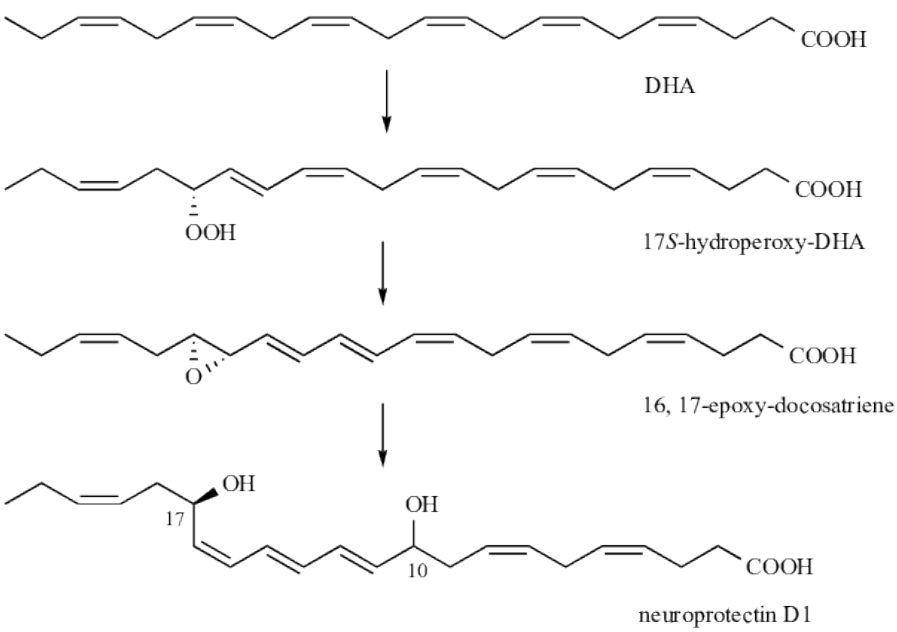
a) to maintain the integrity and function of the neuronal membranes

b) to preserve neuronal signaling pathways

c) to significantly reduce neuronal death

DHA present in the phospholipids of neuronal membranes, will be released into the neuronal cytoplasm during adverse conditions by the action of the enzyme phospholipase A2. Released DHA is converted into NPD-1 by the enzyme 15-lipoxygenase[(69)].

Figure 11: FORMATION OF NEUROPROTECTIN



Formation of NPD-1 is stimulated by various factors

a) increase in oxidative stress induced by H_2O_2

b) presence of tumor necrosis factor-alpha (TNF-α) and interleukin 1-beta (IL-1β)

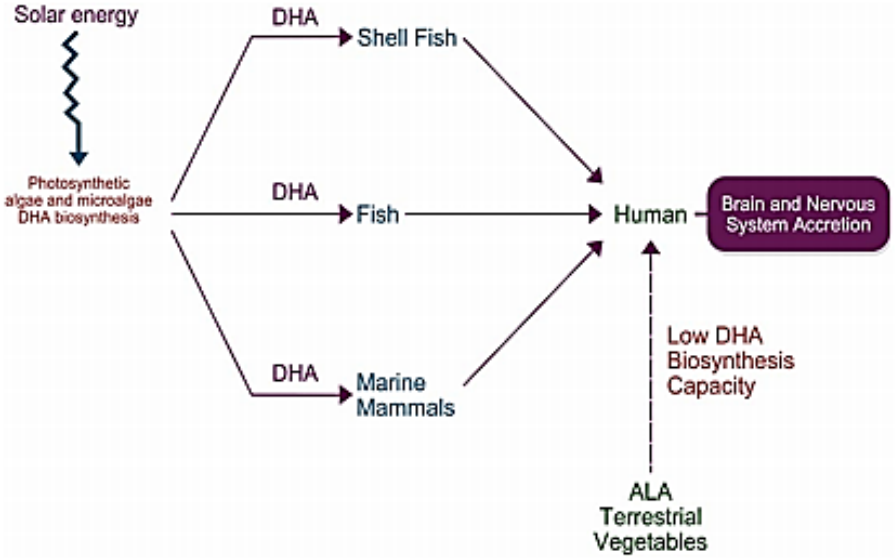
c) during brain ischemia-reperfusion episodes

NPD-1 has the properties of

a) reducing the generation of proinflammatory cytokines
b) reducing the formation of β-amyloid peptide, a cytotoxic structure which is neurotoxic and oxidative stress promoter, disrupts synaptogenesis and induces neuronal apoptosis
c) stimulating the expression of anti-apoptotic genes
d) reducing the expression of pro-apoptotic genes

β- amyloid peptide production is inhibited by alpha secretase whose secretion is favored by NPD-1[(69)].

Figure 12: n-6 and n-3 FATTY ACID SYNTHESIS



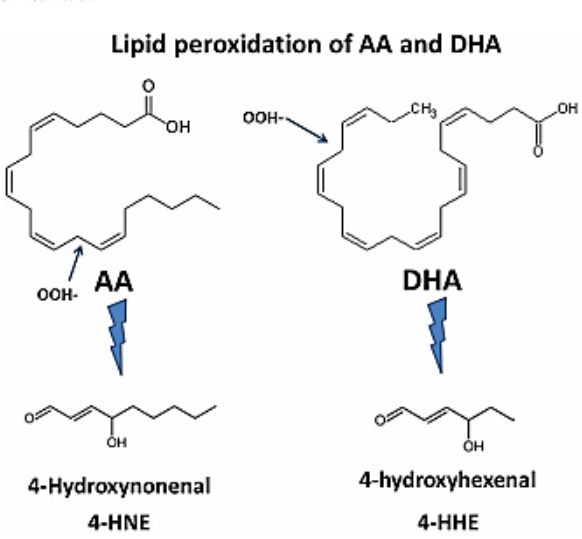
Tissue DHA levels of women are higher than men, due to increased capacity to synthesize DHA from ALA due to oestrogenic stimuli of enzymes desaturase and elongase. Trans fatty acid interrupts the availability of n-3 LCPUFA.

Figure 13: BENEFICIAL EFFECTS OF DHA

Lipid peroxidation of polyunsaturated fatty acids in membrane phospholipids by free radicals results in the release of hydroxyl-alkenals such as 4-hydroxynonenal (4-HNE) from AA and 4-hydroxyhexenal (4-HHE) from DHA respectively.

HNE and HHE exhibit adaptive response by protecting neurons against oxidative stress induced by H_2O_2 and 6-hydroxydopamine (a neural toxin) through activation of Nrf2 pathway. These compounds induce the production of heme oxygenase-1 (HO1), a potent antioxidant enzyme downstream of Nrf2/ARE activation. DHA-induced HO-1 occurs in multiple organs, including brain, kidney, liver, heart and skeletal muscle[(69)].

Figure 14: LIPID PEROXIDATION OF AA AND DHA



MATERIALS AND METHODS

During the period of December 2018 – November 2019 a case control study was conducted at Government Mohan Kumaramangalam Medical College Hospital, Salem.

All patients were informed about the study and consent was obtained from each individual. Institutional Ethical Committee permission was obtained regarding the procedures involving the patients.

STUDY POPULATION

The study consisted of 40 Preeclamptic patients taken as cases, 40 Normotensive ante-natal mothers as controls.

INCLUSION CRITERIA

1. All Primi pregnant mothers between 18 to 28 years of age.
2. BP $\geq$ 140/90 mm Hg with proteinuria.
3. Gestational age between 32 to 36 weeks.

EXCLUSION CRITERIA

1. Known Hypertensive patients
2. Known Epileptic patients
3. Known Diabetic patients
4. Patients having any other systemic illness
    a) Liver disorder
    b) Renal disorder

c) Thyroid disorder

d) Heart Disease

e) Leukemia

f) Haemolysis

g) Pancreatitis

5. Chronically ill patients.

All study participants were explained about the study protocol.

SAMPLE COLLECTION

5ml of blood was collected with minimal stasis from antecubital vein. Venous blood for estimation of DHA and other routine investigations was collected using a red top clot activator tube from all the participants and serum was separated after centrifugation at 3000 rpm for 15 minutes and aliquoted into an eppendorf tube and stored at -20°C and not thawed until itwas analyzed for DHA.

Venous sample for the estimation of hemoglobin and platelet count was collected in dipotassium EDTA tube.

Routine blood investigations like sugar, urea, creatinine, uric acid, total protein, albumin were performed in semi auto analyser.

Estimation of the analytes were done as follows:

ANALYTE	METHOD
BLOOD SUGAR	GOD-POD Method
BLOOD UREA	GLDH Method
SERUM CREATININE	MODIFIED JAFFE'S Method
SERUM TOTAL CHOLESTROL	CHOD-PAP Method
SERUM TRIGLYCERIDES	GLYCEROL 3 PHOSPHATE OXIDASE Method
SERUM HDL	PHOSPHOTUNGSTIC ACID Method
PLATELET COUNT	AUTOMATED BLOOD CELL ANALYSER
TOTAL PROTEIN	BIURET Method
SERUM ALBUMIN	BROMOCRESOL GREEN Method
SERUM URIC ACID	URICASE Method
HEMOGLOBIN	AUTOMATED BLOOD CELL ANALYSER
DHA	ELISA method

DHA ESTIMATION

Test Principle

This assay employs the competitive inhibition enzyme immunoassay technique. The microtiter plate provided in this kit has been pre-coated with Docosahexaenoic Acid (DHA) protein. Standards or samples are then added to the appropriate microtiter plate wells with a biotin-conjugated antibody specific to

Docosahexaenoic Acid (DHA). Next, Avidin conjugated to Horseradish Peroxidase (HRP) is added to each microplate well and incubated. After TMB substrate solution is added. The enzyme-substrate reaction is terminated by the addition of sulphuric acid solution and the color change is measured spectrophotometrically at a wavelength of 450nm ± 10nm. The concentration of Docosahexaenoic Acid (DHA) in the samples is then determined by comparing the OD of the samples to the standard curve.

Sample collection and storage

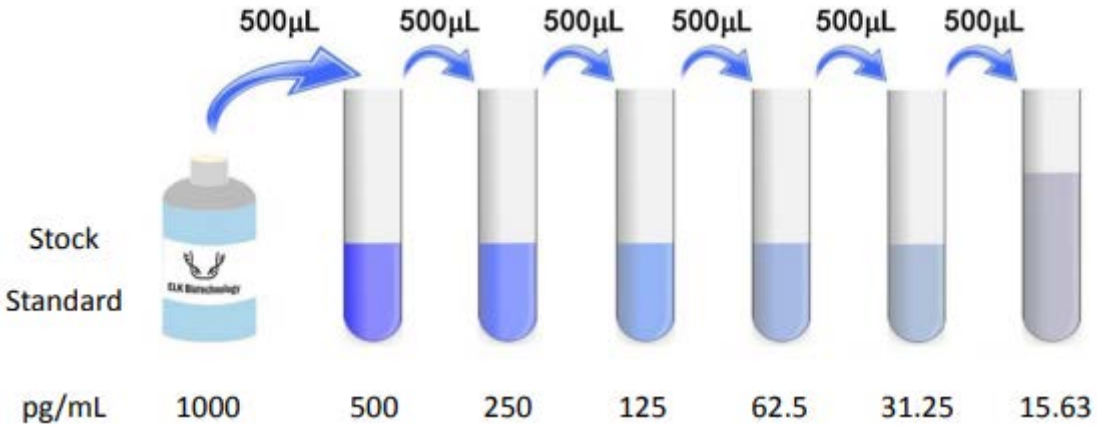
Serum - Using a serum separator tube and samples were allowed to clot for two hours at room temperature or overnight at 4°C before centrifugation for 20 minutes at approximately 1000×g. Freshly prepared serum was assayed immediately or store samples in aliquot at -20°C or -80°C for later use. Repeated freeze/thaw cycle was avoided.

Reagent preparation

1. All kit components and samples were brought down to room temperature (18-25°C) before use.
2. 25x wash buffer was diluted into 1x working concentration with double steaming water.
3. Biotinylated-Conjugate (1x) was Centrifuged before opening the vial. Biotinylated-Conjugate requires a 100-fold dilution. It was done by diluting 10µl of Biotinylated-Conjugate with 990µl of Biotinylated-Conjugate diluent.

Standard -Standard was reconstituted with 1.0mL of Standard Diluent and kept for 10 minutes at room temperature, shaken gently (not to foam). The concentration of the standard in the stock solution is 1000pg/mL.7 tubes containing 0.5mL Standard Diluent was taken and the diluted standard was used to produce a double dilution series according to the picture shown below. Each tube was mixed thoroughly before the next transfer. 7 points of diluted standard such as 1000 pg/mL, 500 pg/mL, 250 pg/mL, 125 pg/mL, 62.5 pg/mL, 31.25 pg/mL, 15.63 pg/mL was set up, and the last tube with Standard Diluent has the blank as 0 pg/mL.

Figure 15: DILUTION OF DHA STANDARD



4. Streptavidin-HRP (1x) – The vial was centrifuged before opening. Streptavidin-HRP requires a 100-fold dilution. It was done by diluting 10µL of Streptavidin-HRP with 990µL of HRP Diluent.
5. **TMB substrate** – Needed dosage of the solution was aspirated with sterilized tips.

Assay Procedure:

1. All reagents and samples were brought down to room temperature before use.
2. 50 µL of Standard or Sample was added to each well. Then 50 µL of Biotinylated-Conjugate (1x) was added to each well. Wells were mixed and covered with the adhesive films provided, incubated for 60 minutes at 37°C.
3. Each well was aspirated and washed, repeating the process for a total of three washes. Wash was done by filling each well with Wash Buffer (250 µL) using an autowasher. After the last wash, remaining Wash Buffer, if any, was removed by aspirating or decanting. The plate was inverted and blotted against clean paper towels.
4. 100µL of Streptavidin- HRP (1x) was added to each well. Wells were covered with the adhesive films provided and incubated for 30 minutes at 37°C.
5. Each well was aspirated and washed, repeating the process for a total of five washes. Wash was done by filling each well with Wash Buffer (250 µL) using an autowasher. After the last wash, remaining Wash Buffer, if any, was removed by aspirating or decanting. The plate was inverted blotted against clean paper towels.
6. 100µL of Substrate Solution was added to each well. Wells were incubated for 15-20 minutes at 37°C in the dark.
7. 50 µL of Stop Solution was added to each well. The first four wells

containing the highest concentration of standards develop obvious blue color. Plate was gently tapped for thorough mixing.

8. The optical density of each well was determined within 5 minutes, using a microplate reader set to 450 nm.

Sensitivity : 4.93 pg/mL

Detection range : 15.63-1000 pg/mL

Specificity : This assay has high sensitivity and excellent specificity for detection of DHA. No significant cross-reactivity or interference between DHA and analogues was observed.

GLUCOSE ESTIMATION

Method : Glucose oxidase peroxidase (GOD/POD), End point

Principle:

$$\text{Glucose} + O_2 + H_2O \xrightarrow{\text{Glucose oxidase}} \text{Gluconic acid} + H_2O$$

$$H_2O_2 + \text{Phenol} + 4\text{AAP} \xrightarrow{\text{Peroxidase}} \text{Quinonemine dye} + H_2O$$

Assay procedure:

	Blank	Standard	Test
Working Reagent	1000 µl	1000 µl	1000 µl
Standard	-	10 µl	-
Sample	-	-	10 µl

Mixed well and incubated at 37^{o}C for 15min. At wavelength of 540nm, absorbance of the test and standard were read against reagent blank. Pink colored Quinonemine dye was obtained which was proportionate to glucose concentration.

Reference range :

Fasting glucose : 70 –100 mg/dl

Post prandial glucose : 80 – 140mg/dl

BLOOD UREA ESTIMATION

Method : GLDH - Urease method, Kinetic assay

Principle :

Urea is hydrolysed by urease to produce ammonia and carbon dioxide. The ammonia produced combines with alpha-oxoglutarate and NADH in the presence of glutamate dehydrogenase to produce glutamate and NAD.

$$\text{Urea} + H_2O \xrightarrow{\text{urease}} 2NH_4 + CO_2$$

$$NH_4 + \text{NADH} + H^+ + \text{2-oxoglutarate} \xrightarrow{\text{GLDH}} \text{Glutamate} + \text{NAD}$$

Assay procedure:

	Blank	Standard	Test
Working reagent	1000 µl	1000 µl	1000 µl
Standard	-	10 µl	-
Sample	-	-	10 µl

Mixed well and the absorbance was measured at 340 nm. The initial rate of decrease in absorbance is directly proportional to the concentration of urea in the sample.

Reference Range:

Serum Urea : 15- 40 mg/dl

SERUM CREATININE ESTIMATION

Method : Modified Jaffe's Method

Principle :

Creatinine reacts with alkaline picrate to form a orange yellow compound. The absorbance of the orange -yellow color formed is directly proportional to the concentration of creatinine in the sample. It is measured at 505nm.

Creatinine concentration: 2 mg/dl

Assay Procedure :

	Blank	Standard	Test
Working reagent	1000 µl	1000 µl	1000 µl
Standard	-	100 µl	-
Test	-	-	100 µl

Mixed well and the absorbance of the test and standard were read against reagent blank at wavelength of 505 nm.

Reference range:

Male : 0.7 – 1.4 mg/dl

Female : 0.6 – 1.2 mg/dl

ESTIMATION OF TOTAL CHOLESTEROL

Method: Cholesterol oxidase - PAP, end point

Principle:

$$\text{Cholesterol ester + water} \xrightarrow{\text{Cholesterol esterase}} \text{Cholesterol + Fatty acid}$$

$$\text{Cholesterol + oxygen} \xrightarrow{\text{Cholesterol oxidase}} \text{Cholest-4-en-3one} + H_2O_2$$

$$2H_2O_2 + \text{Phenol} + 4\text{Amino antipyrine} \xrightarrow{\text{Peroxidase}} \text{Quinoneimine Dye} + 4H_2O$$

Assay procedure:

Working reagent	1000 µl	1000 µl	1000 µl
Distilled water	10 µl	-	-
Standard	-	10 µl	-
Sample	-	-	10 µl

Mixed well and incubated for 10 min at room temperature. The absorbance of the test and standard were read against reagent blank at wavelength of 505 nm. Absorbance of formed quinoneimine is directly proportional to cholesterol concentration.

Calculation:

$$\text{Cholesterol (mg/dl)} = \frac{\text{Absorbance of test x Concentration of standard}}{\text{Absorbance of standard}}$$

Reference Range:

Serum / Plasma

Age	mg/dl
2-12 months	60-190
≥ 1 year	110-230
Adults	< 200

Interference:

HB upto 200mg/dl, ascorbate upto 12mg/dl, bilirubin upto 10mg/dl and Triglycerides upto 700 mg/dl do not interfere with the test.

ESTIMATION OF SERUM TRIGLYCERIDES

Method : GPO-PAP method, endpoint

Principle:

The following reactions occur in the assay system

$$\text{Triglycerides} + H_2O \xrightarrow{\text{Lipoprotein Lipase}} \text{Glycerol} + \text{free fatty Acids}$$

$$\text{Glycerol} + \text{ATP} \xrightarrow{\text{Glycerol Kinase}} \text{Glycerol-3-phosphate} + \text{ADP}$$

$$\text{Glycerol-3-phosphate} + O_2 \xrightarrow{\text{Glycerol-3-Phosphate Oxidase}} \text{DAP} + H_2O_2$$

$$H_2O_2 + 4AAP + 3,5\text{-}DHBS \xrightarrow{\text{Peroxidase}} \text{Quinonemine dye} + 2H_2O$$

ATP - Adenosine Tri Phosphate

4AAP - 4 Amino Anti Pyrine

DHBS - 3, 5 Dichloro -2 Hydroxy Benzene Sulfonate

Triglyceride standard concentration - 200 mg/dl

Assay procedure:

Reagents	Blank	Standard	Test
Working reagent	1000 µl	1000 µl	1000 µl
Distilled water	10 µl	-	-
Standard	-	10 µl	-
Sample	-	-	10 µl

Mixed and incubated for 10 min. Absorbance were read at 505nm (500- 540 nm) for standard and against reagent blank. The intensity of Quinoemine dye formed is proportional to the Triglyceride concentration.

Calculation:

$$\text{Triglycerides (mg/dl)} = \frac{\text{Absorbance of test x concentration of std (mg/dl)}}{\text{Absorbance of standard}}$$

Reference range:

Serum/Plasma	mg/dl
Normal fasting level	25-160

Linearity - upto 1000 mg/dl

Sensitivity - 2 mg/dl

Interference:

HB upto 300 mg/dl, ascorbate upto 3 mg/dl, bilirubin upto 20 mg/dl do not interfere with the test.

HDL CHOLESTEROL ESTIMATION

Method: Phosphotungstic acid method, endpoint

Principle:

Chylomicrons, LDL and VLDL are precipatated from serum by phosphotungstate in the presence of divalent cations such as magnesium. The HDL cholesterol remnants are unaffected in the supernatant and is estimated using cholesterol reagent.

$$\text{Serum} \xrightarrow[Mg^{2+}]{\text{Phosphotungstate}} \text{HDL} + (\text{LDL} + \text{VLDL} + \text{Chylomicrons})$$

HDL cholesterol standard - 25 mg/dl

Precipitation:

Precipitation of LDL,VLDL and chylomicrons done as follows

Test	250 µl
Precipitating reagent	500 µl

After mixed well, the reaction mixture was allowed to stand for 10min at room temperature, then centrifuged at 4000 rpm for 10 min. A clear supernatant

was obtained. The concentration of HDL cholesterol in the sample was determined using the obtained supernatant.

Assay procedure:

Reagents	Blank	Standard	Test
Cholesterol reagent	1000 µl	1000 µl	1000 µl
Distilled water	50 µl	-	-
HDL Standard	-	50 µl	-
Supernatant	-	-	50 µl

Mixed well and incubated for 10 min at room temperature. At 505nm, the absorbance of the standard and the test samples were read against reagent blank.

Calculation:

$$\text{HDL cholesterol (mg/dl)} = \frac{\text{Absorbance of test}}{\text{Absorbance of standard}} \times \text{conc of std} \times \text{dilution factor}$$

$$= \frac{\text{Absorbance of test}}{\text{Absorbance of standard}} \times 25 \times 3$$

Linearity - upto 125 mg/dl

Reference Range:

Male - 30-65 mg/dl

Female - 35-80 mg/dl

Interference:

High triglyceride concentration above 300mg /dl cause interference with the assay. Bilirubin and ascorbate at high concentrations interfere with precipitation.

ESTIMATION OF PLATELET COUNT

Method : Automated blood cell analysis

Principle : Electronic Impedance

Cell counting principle is based on the detection and measurement of changes in electrical resistance produced by cells as they transverse a small aperture. Cells suspended in an electrically conductive diluents such as saline are pulled through an aperture (orifice) in a glass tube. In the counting chamber, or transducer assembly, low-frequency electrical current is applied between an external electrode (suspended in the cell dilution) and an internal electrode (housed inside aperture tube). Electrical resistance between the two electrodes, or impedance in the current, occurs as the cells pass through the sensing aperture, causing voltage pulses that are measurable.

ESTIMATION OF TOTAL PROTEIN

Method: Biuret Method, End point

Principle:

Protein in serum reacts with copper ions in biuret reagent to form colored complex in an alkaline medium. The colour produced is measured at 545nm which is directly proportional to the concentration of protein in the sample.

Protein Standard: 10 g/dl

Assay Procedure:

20µl of serum was added with 1ml of reagent, mixed, incubated for 10 minutes at room temperature and absorbance of standard and sample read against reagent blank at 545 nm.

Calculation:

$$\text{Serum Total protein in g/dl} = \frac{(\text{Abs.T})-(\text{Abs.B})}{(\text{Abs.S})-(\text{Abs.B})} \times 10$$

Reference Range:

Serum Total protein : 6.4-8.3 g/dl

Linearity : Upto 15g/dl

ESTIMATION OF ALBUMIN

Method : Bromocresol green (BCG) Method, End point

Principle:

In acidic medium, albumin in serum reacts with bromocresol green in reagent to form colored complex. The color produced is directly proportional to the concentration of albumin in the sample which ismeasured at 630nm.

Albumin standard: 2 g/dl

Assay Procedure:

1ml of reagent was added with 10µl of serum, mixed, incubated for 1minute at room temperature. At 630nm, the absorbance of standard and sample read against reagent blank.

Calculation:

$$\text{Serum Albumin in g/dl} = \frac{(\text{Abs.T})-(\text{Abs.B})}{(\text{Abs.S})-(\text{Abs.B})} \times 2$$

Reference Range:

Serum albumin : 3.5-5.0 g/dl

Linearity : upto 7 g/dl

ESTIMATION OF SERUM URIC ACID

Method: Uricase method

Principle:

$$\text{Uric acid} + 2H_2O_2 + O_2 \xrightarrow{\text{Uricase}} \text{Allantoine} + CO_2 + H_2O_2$$

$$2H_2O_2 + \text{4-Aminophenzone} + \text{TOOS} \xrightarrow{\text{Peroxidase}} \text{Red quinone} + 2H_2O$$

Uric acid standard: 6mg/dl

Assay procedure:

	Blank	Standard	Sample
Distilled water	20µl	-	-
Standard	-	20µl	-
Sample	-	-	20µl
Working reagent	1000µl	1000µl	1000µl

Mixed well and incubated for 5 min at room temperature. At 550nm, the absorbance of the standard and the test samples were read against reagent blank. The final color is stable for 30 minutes.

Calculations:

$$\text{Serum uric acid (mg/dl)} = \frac{(\text{Abs.T})-(\text{Abs.B})}{(\text{Abs.S})-(\text{Abs.B})} \times 6$$

Linearity: 20mg/dl

Reference range:

Male: 4.4-7.6mg/dl

Female: 2.3-6.6mg/dl

ESTIMATION OF HEMOGLOBIN

Method: Automated blood cell analysis

Hemoglobin is measured directly by a modification of cyanmethemoglobin method (as hemoglobins are converted to cyanmethemoglobin by potassium ferricyanide). A nonionic detergent is added for rapid red cell lysis and to minimize turbidity caused by cell membranes and plasma lipids. Poorly mixed sample, high WBC count, hyperlipidemia, hypergammaglobulinemia and cryoglobulinemia can cause falsely elevated hemoglobin levels.

STATISTICS

Statistical analysis was done using Graph Pad Prism software 8.2.1and p value was fixed at <0.05 as significant. Continuous data has been expressed as Mean and Standard Deviation.

Independent t test was used to compare the mean value of biochemical parameters between cases and controls.

RESULTS

Serum Docosahexaenoic acid (DHA), blood sugar, blood urea, serum creatinine, serum total protein, serum albumin, serum lipid profile, serum uric acid, platelet count, hemoglobin levels were estimated in cases and controls.

TABLE NO: 1

MEAN SUGAR LEVEL BETWEEN CASES AND CONTROLS

S.No		N	Mean	SD	P-Value
1	Cases	40	104.68	8.684	0.396
2	Controls	40	103.03	8.604	

The mean value of Sugar in cases was 104.68±8.684 and that of controls was 103.03±8.604 which was found to be statistically not significant by independent t test. The p value was >0.05.

CHART NO: 1

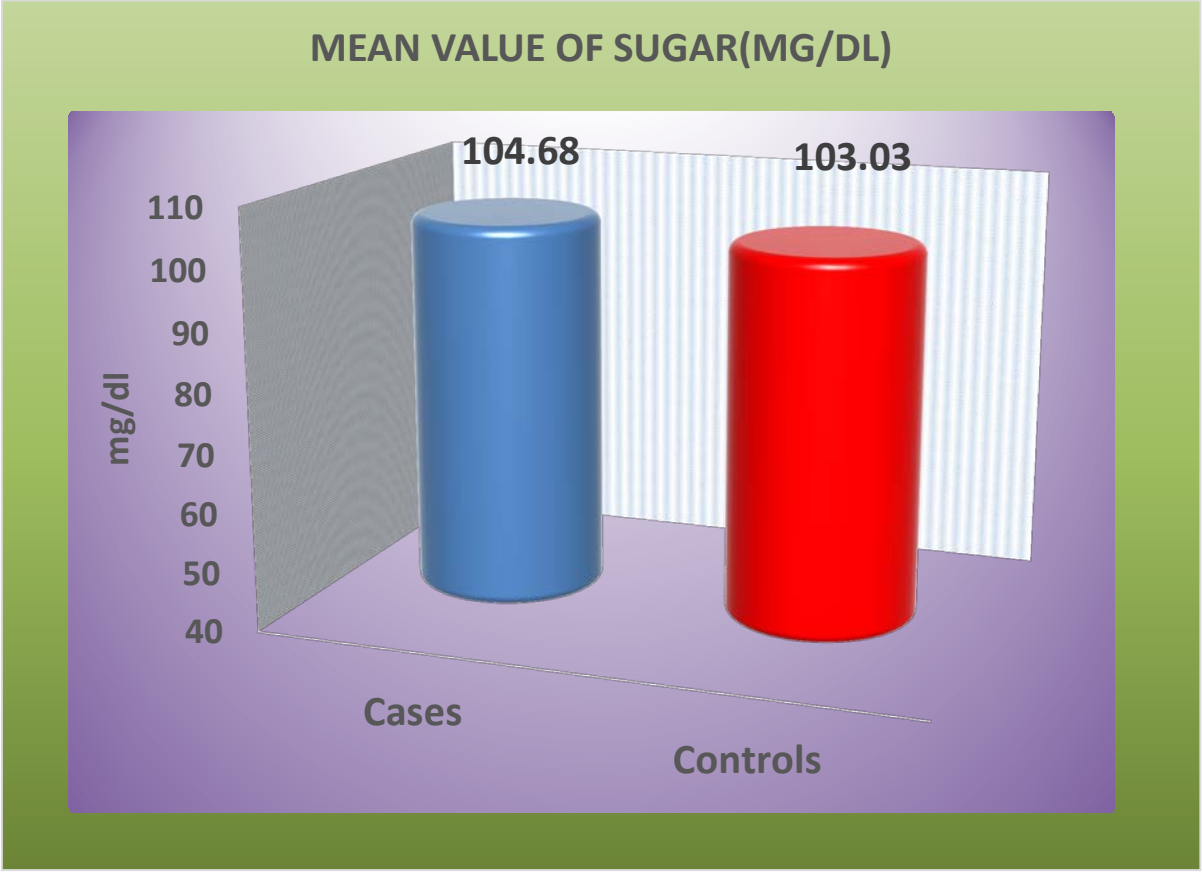

MEAN VALUE OF SUGAR(MG/DL)
104.68
103.03
110
100
90
80
70
60
50
40
mg/dl
Cases
Controls

TABLE NO:2

MEAN UREA LEVEL BETWEEN CASES AND CONTROLS

S.No		N	Mean	SD	P-Value
1	Cases	40	30.45	5.359	0.854
2	Controls	40	30.68	5.567	

The mean value of Urea in cases was 30.45±5.359 and that of controls was 30.68±5.567 which was found to be statistically not significant by independent t test. The p value was >0.05.

CHART NO: 2

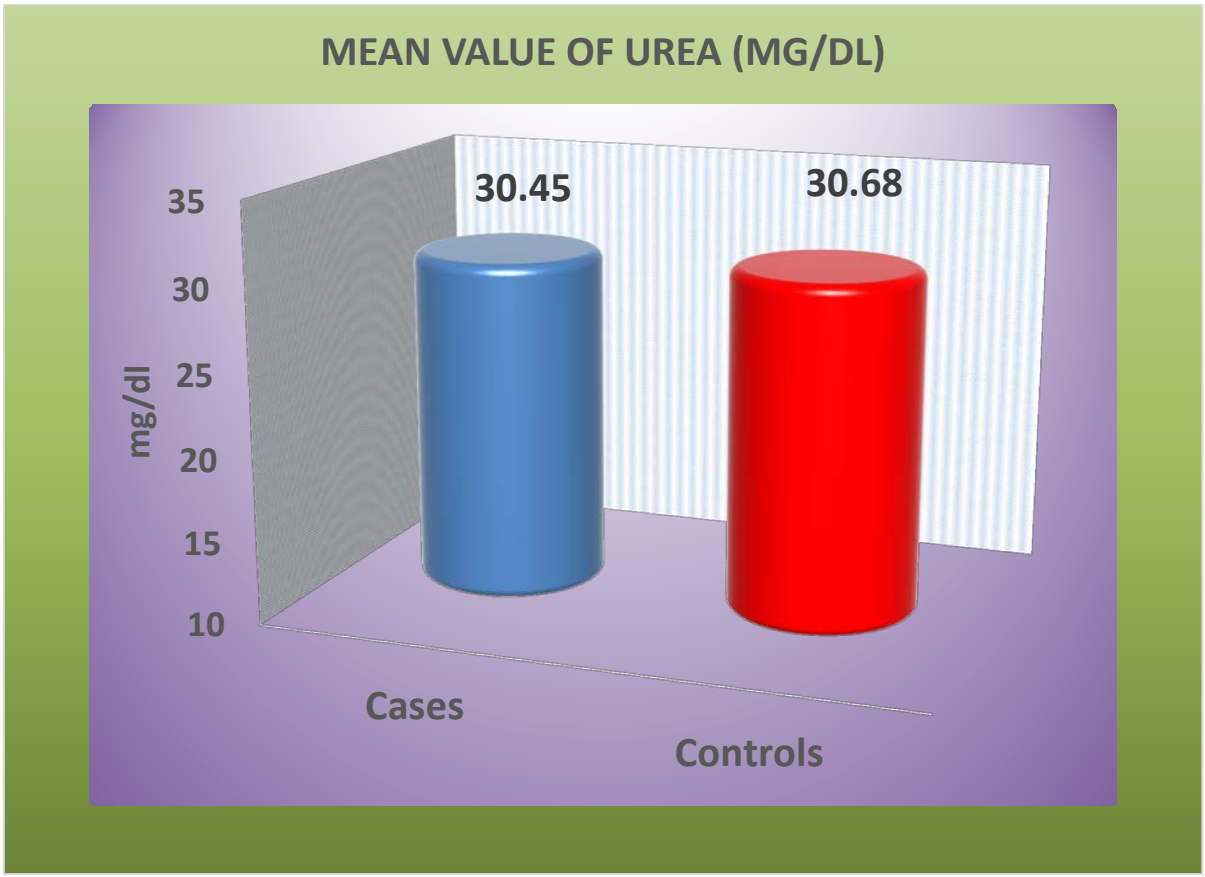

MEAN VALUE OF UREA (MG/DL)
35
30
25
20
15
10
mg/dl
30.45
30.68
Cases
Controls

TABLE NO:3

MEAN CREATININE LEVEL BETWEEN CASES AND CONTROLS

S.No		N	Mean	SD	P-Value
1	Cases	40	1.75	0.55	< 0.000
2	Controls	40	0.84	0.15	

The mean value of Creatinine in cases was 1.75±0.55 and that of controls was 0.84±0.15 which was found to be statistically significant by independent t test. The p value was <0.05.

CHART NO: 3

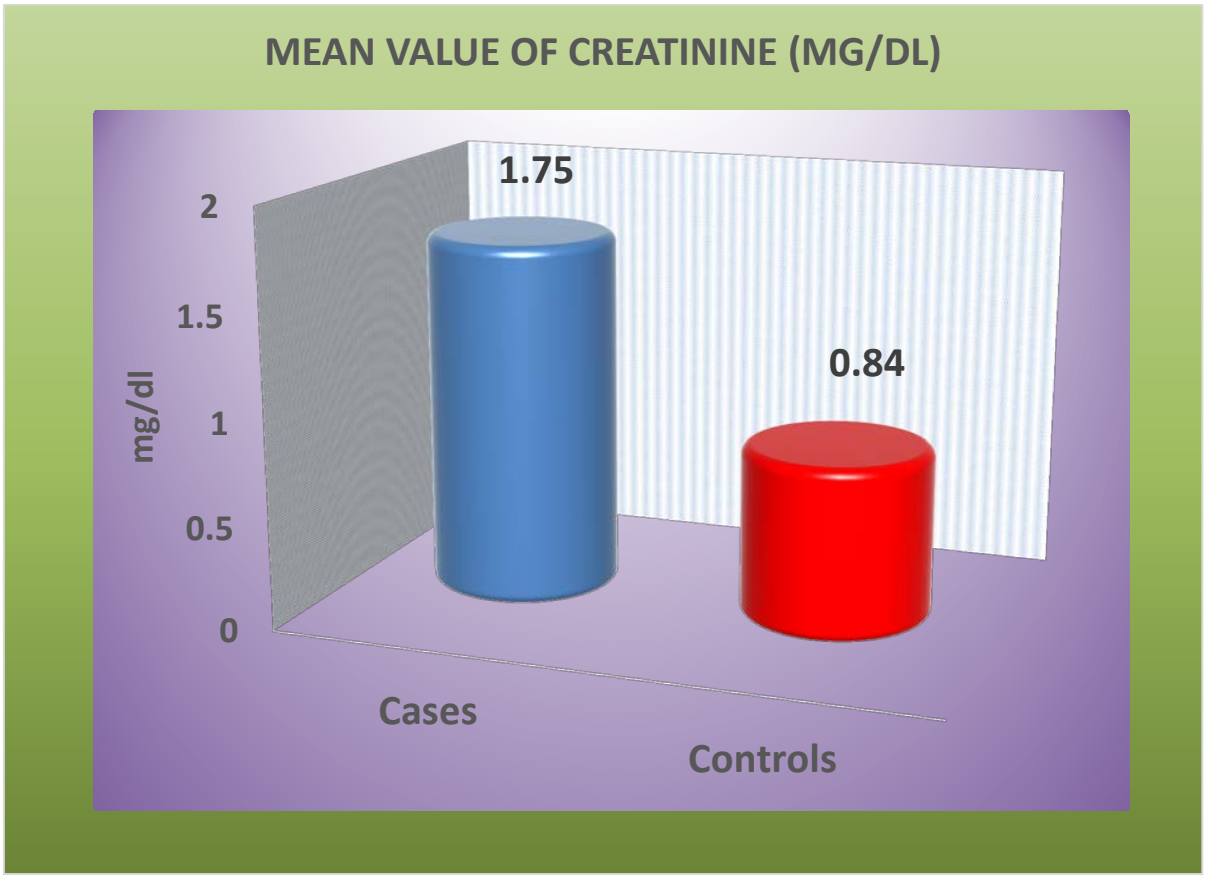

MEAN VALUE OF CREATININE (MG/DL)
1.75
0.84
2
1.5
1
0.5
0
mg/dl
Cases
Controls

TABLE NO:4

MEAN TOTAL CHOLESTEROL LEVEL BETWEEN CASES AND CONTROLS

S.No		N	Mean	SD	P-Value
1	Cases	40	124.43	38.604	>0.05 (0.694)
2	Controls	40	121.15	35.524	

The mean value of Total cholesterol in cases was 124.43±38.604 and that of controls was 121.15±35.524 which was found to be statistically not significant by independent t test. The p value was >0.05.

CHART NO: 4

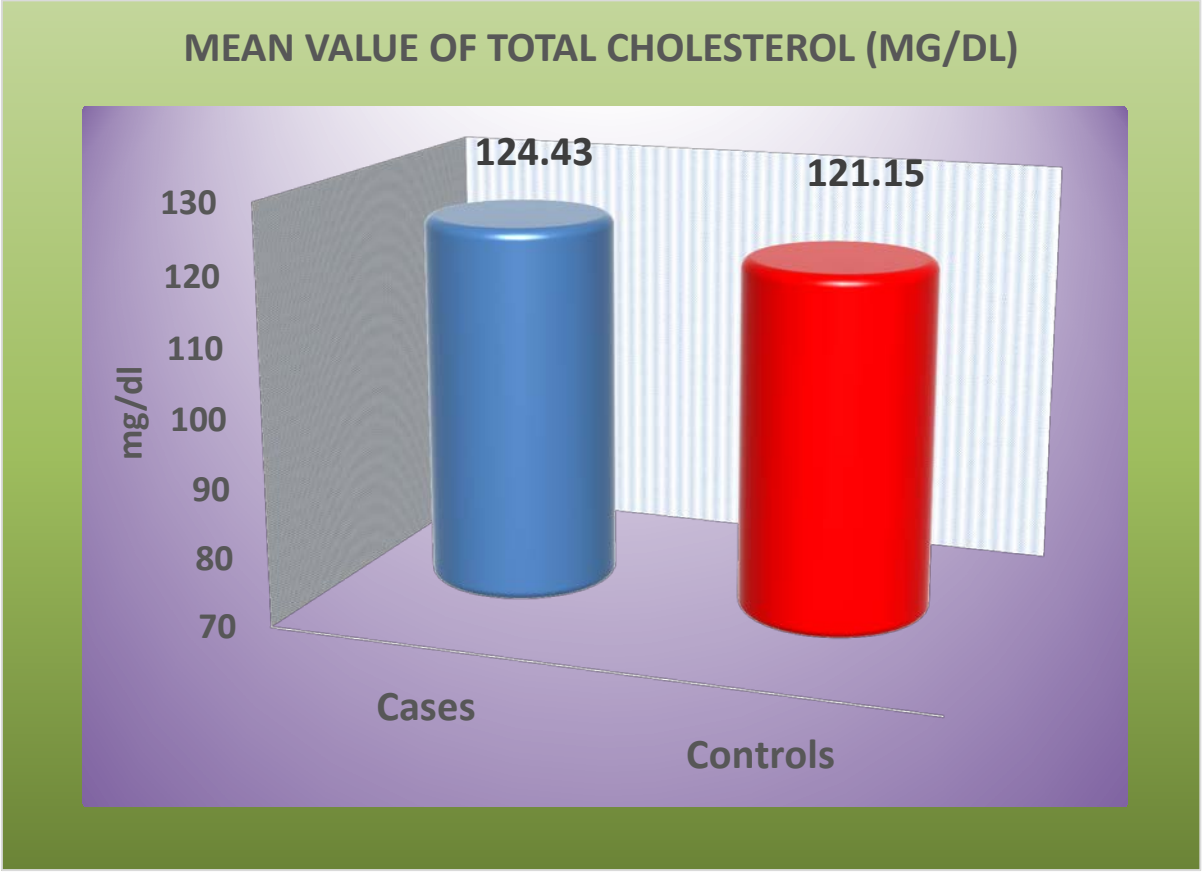

MEAN VALUE OF TOTAL CHOLESTEROL (MG/DL)
124.43
121.15
130
120
110
100
90
80
70
mg/dl
Cases
Controls

TABLE NO:5

MEAN TGL LEVEL BETWEEN CASES AND CONTROLS

S.No		N	Mean	SD	P-Value
1	Cases	40	108.83	22.316	>0.05 (0.584)
2	Controls	40	106.08	22.463	

The mean value of TGL in cases was 108.83±22.316 and that of controls was 106.08±22.463 which was found to be statistically not significant by independent t test. The p value was >0.05.

CHART NO: 5

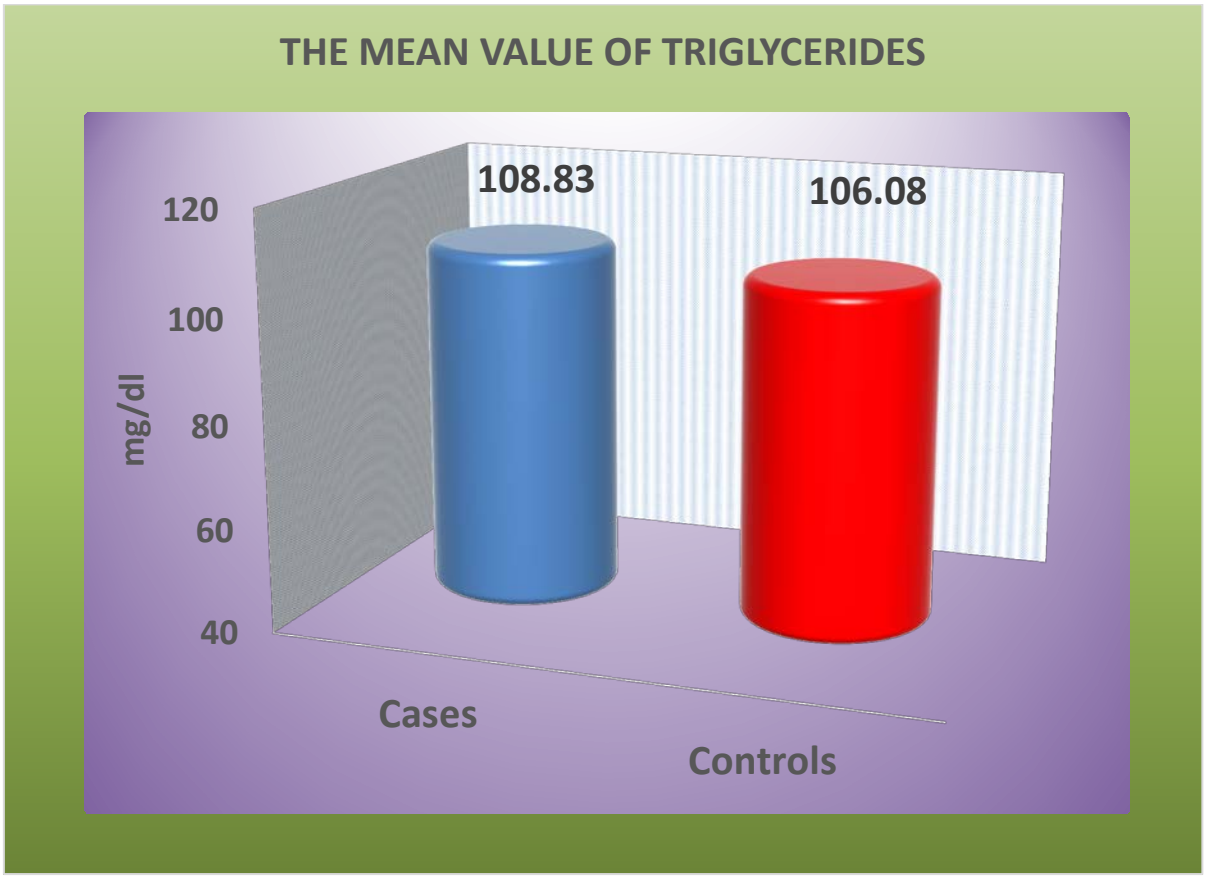

THE MEAN VALUE OF TRIGLYCERIDES
108.83
106.08
120
100
80
60
40
mg/dl
Cases
Controls

TABLE NO:6

MEAN HDL LEVEL BETWEEN CASES AND CONTROLS

S.No		N	Mean	SD	P-Value
1	Cases	40	56.53	13.529	>0.05 (0.895)
2	Controls	40	56.93	13.603	

The mean value of HDL in cases was 56.63±13.529 and that of controls was 56.93±13.603 which was found to be statistically not significant by independent t test. The p value was >0.05.

CHART NO: 6

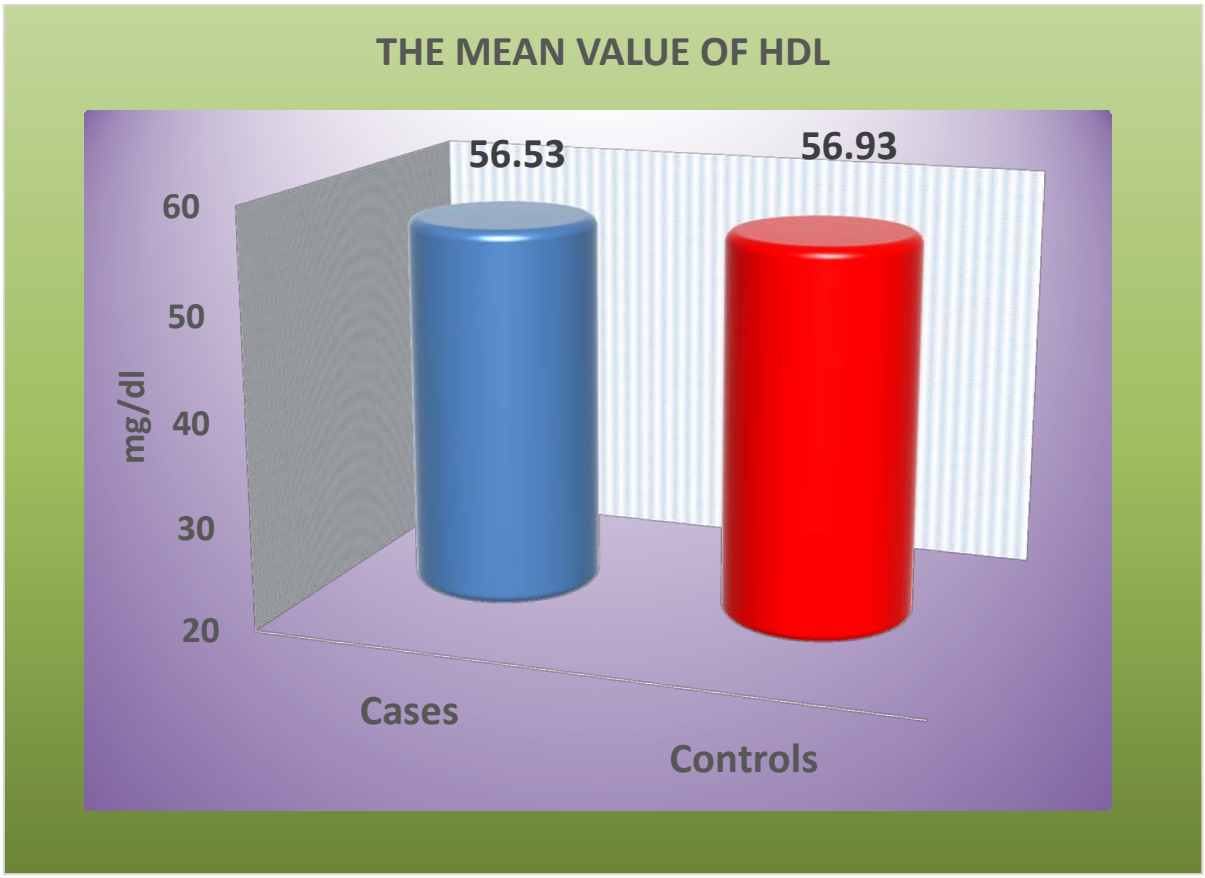

THE MEAN VALUE OF HDL
56.53
56.93
60
50
40
30
20
mg/dl
Cases
Controls

TABLE NO:7

MEAN PLATELET COUNT BETWEEN CASES AND CONTROLS

S.No		N	Mean	SD	P-Value
1	Cases	40	2.725	0.605	>0.05(0.64)
2	Controls	40	2.790	0.6328	

The mean value of Platelet count in cases was 2.725±0.605 and that of controls was 2.79±0.6328 which was found to be statistically not significant by independent t test. The p value was >0.05.

CHART NO: 7

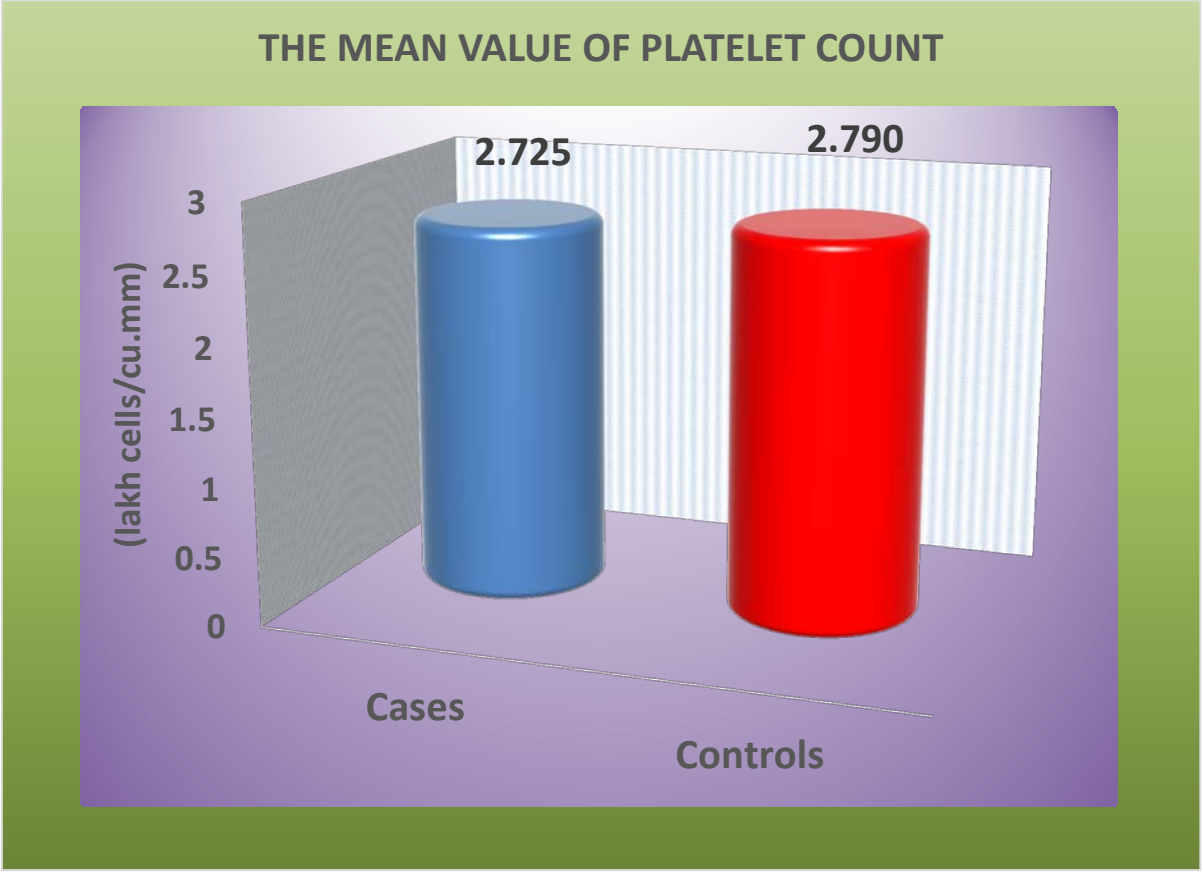

THE MEAN VALUE OF PLATELET COUNT
2.725
2.790
3
2.5
2
1.5
1
0.5
0
(lakh cells/cu.mm)
Cases
Controls

TABLE NO:8

MEAN TOTAL PROTEIN LEVEL BETWEEN CASES AND CONTROLS

S.No		N	Mean	SD	P-Value
1	Cases	40	5.208	0.5146	< 0.05
2	Controls	40	7.405	0.4432	

The mean value of Protein in cases was 5.208±0.5146 and that of controls was 7.405±0.4432 which was found to be statistically significant by independent t test. The p value was <0.05.

CHART NO: 8

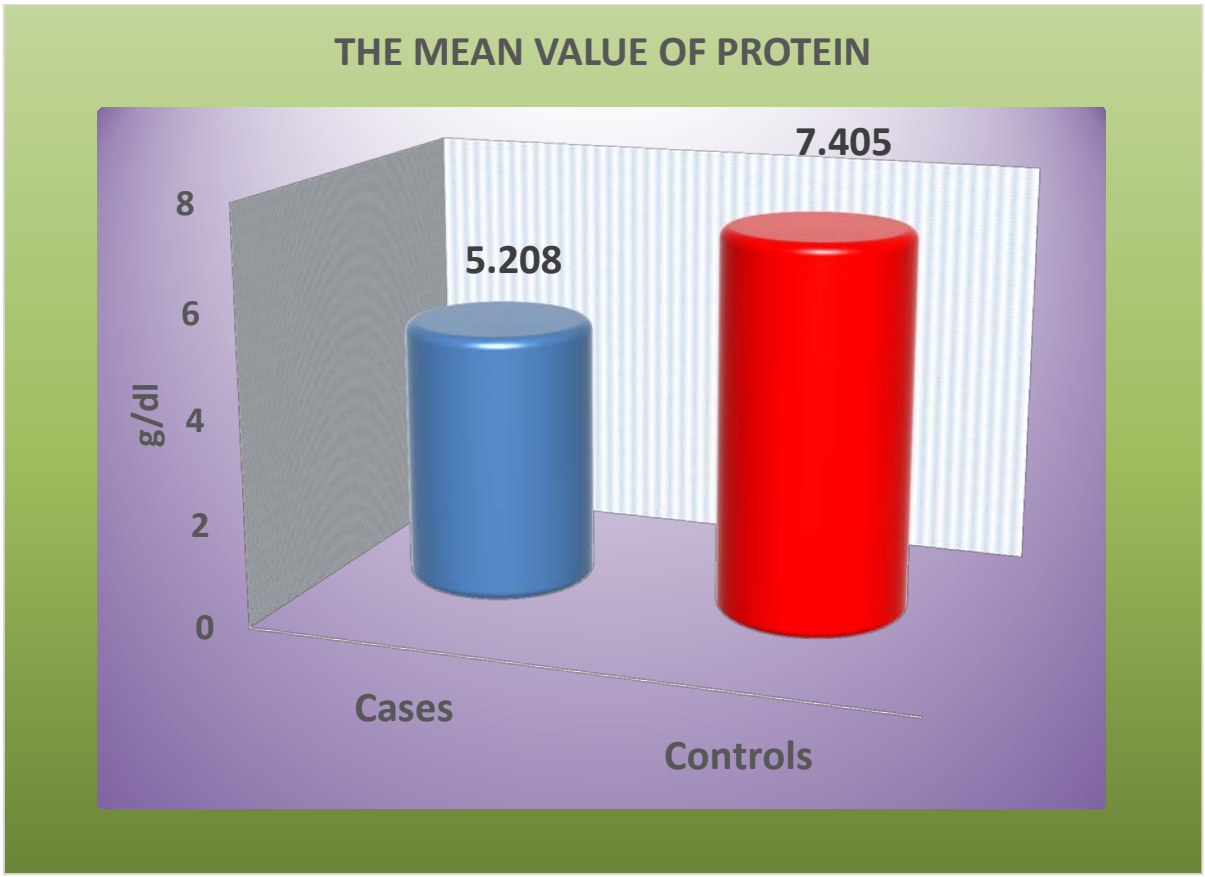

THE MEAN VALUE OF PROTEIN
7.405
5.208
8
6
4
2
0
g/dl
Cases
Controls

TABLE NO:9

MEAN ALBUMIN BETWEEN CASES AND CONTROLS

S.No		N	Mean	SD	P-Value
1	Cases	40	3.085	0.7698	< 0.05
2	Controls	40	4.27	0.4542	

The mean value of Albumin in cases was 3.085±0.7698 and that of controls was 4.27±0.4542 which was found to be statistically significant by independent t test. The p value was <0.05.

CHART NO: 9

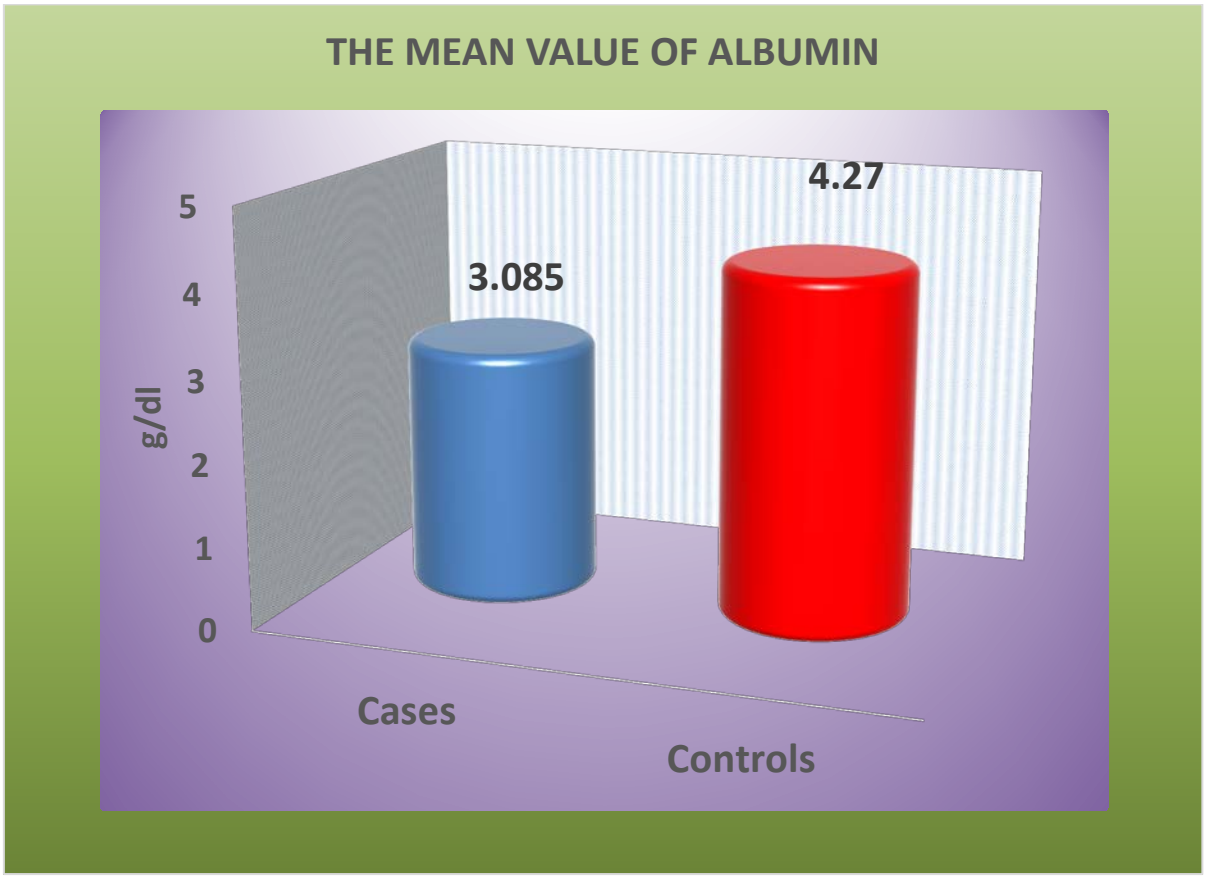

THE MEAN VALUE OF ALBUMIN
5
4
3
2
1
0
g/dl
3.085
4.27
Cases
Controls

TABLE NO: 10

MEAN URIC ACID LEVEL BETWEEN CASES AND CONTROLS

S.No		N	Mean	SD	P-Value
1	Cases	40	6.338	1.5302	< 0.05
2	Controls	40	3.83	1.0537	

The mean value of Uric Acid in cases was 6.338±1.5302 and that of controls was 3.83±1.0573 which was found to be statistically significant by independent t test. The p value was <0.05.

CHART NO: 10

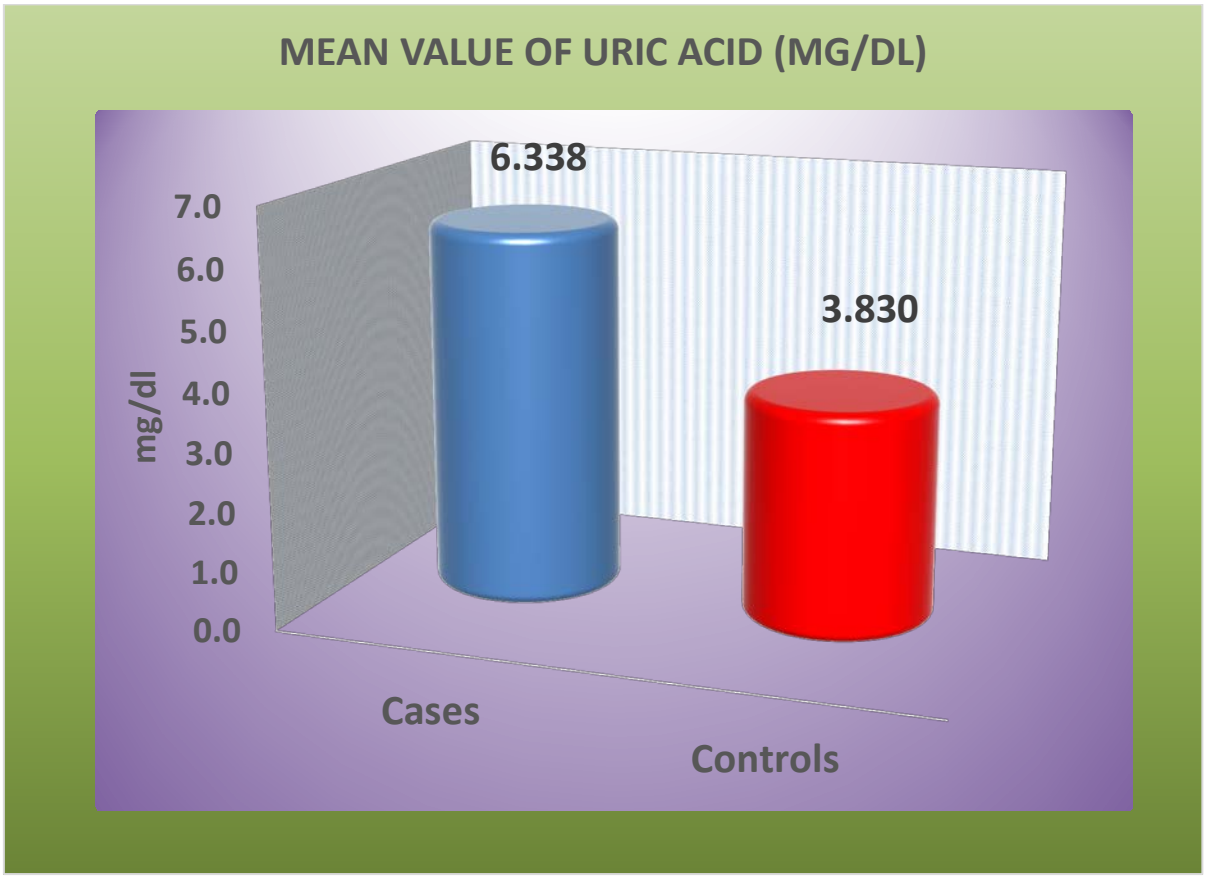

MEAN VALUE OF URIC ACID (MG/DL)
6.338
3.830
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
mg/dl
Cases
Controls

TABLE NO: 11

MEAN HB LEVEL BETWEEN CASES AND CONTROLS

S.No		N	Mean	SD	P-Value
1	Cases	40	12.525	1.0796	0.505
2	Controls	40	12.348	1.2802	

The mean value of Hemoglobin in cases was 12.525±1.0796 and that of controls was 12.348±1.2802 which was found to be statistically not significant by independent t test. The p value was >0.05.

CHART NO: 11

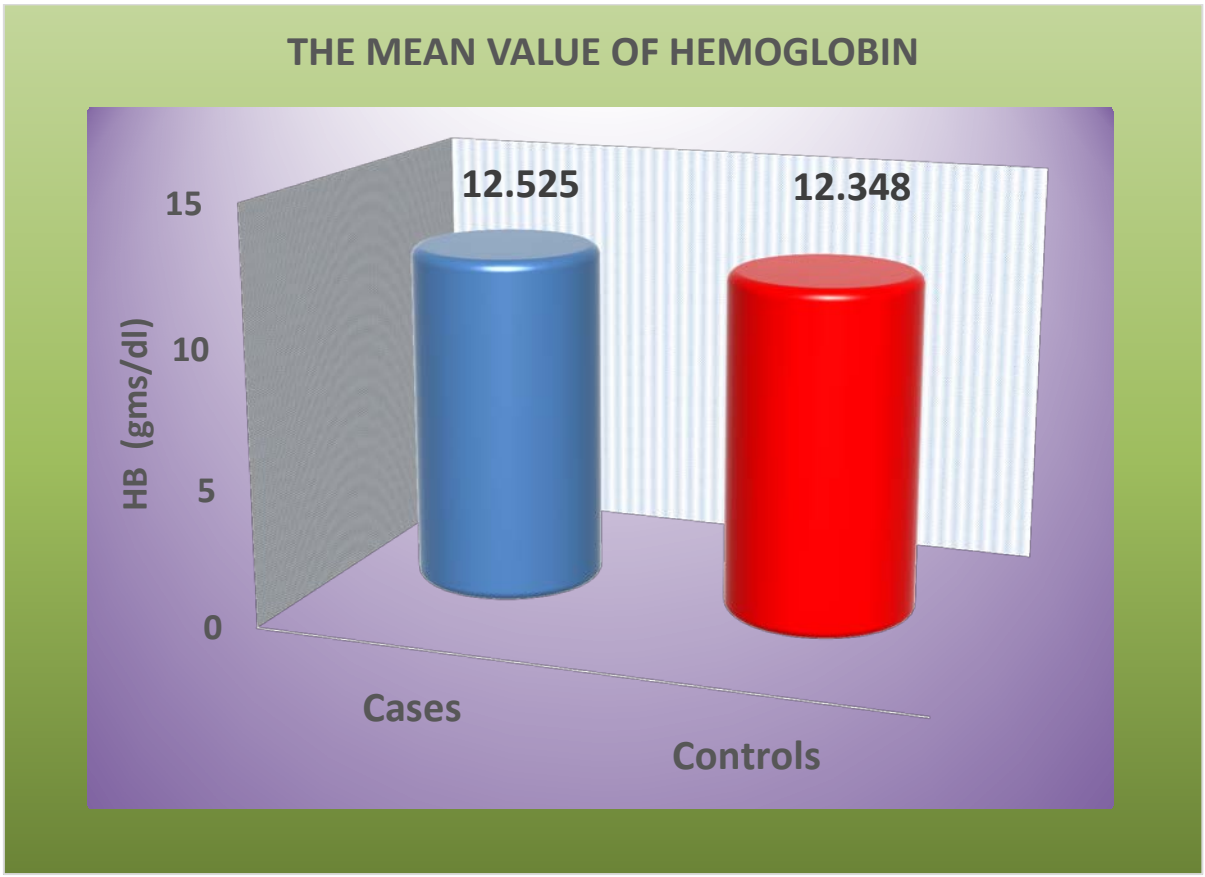

THE MEAN VALUE OF HEMOGLOBIN
12.525
12.348
15
10
5
0
HB (gms/dl)
Cases
Controls

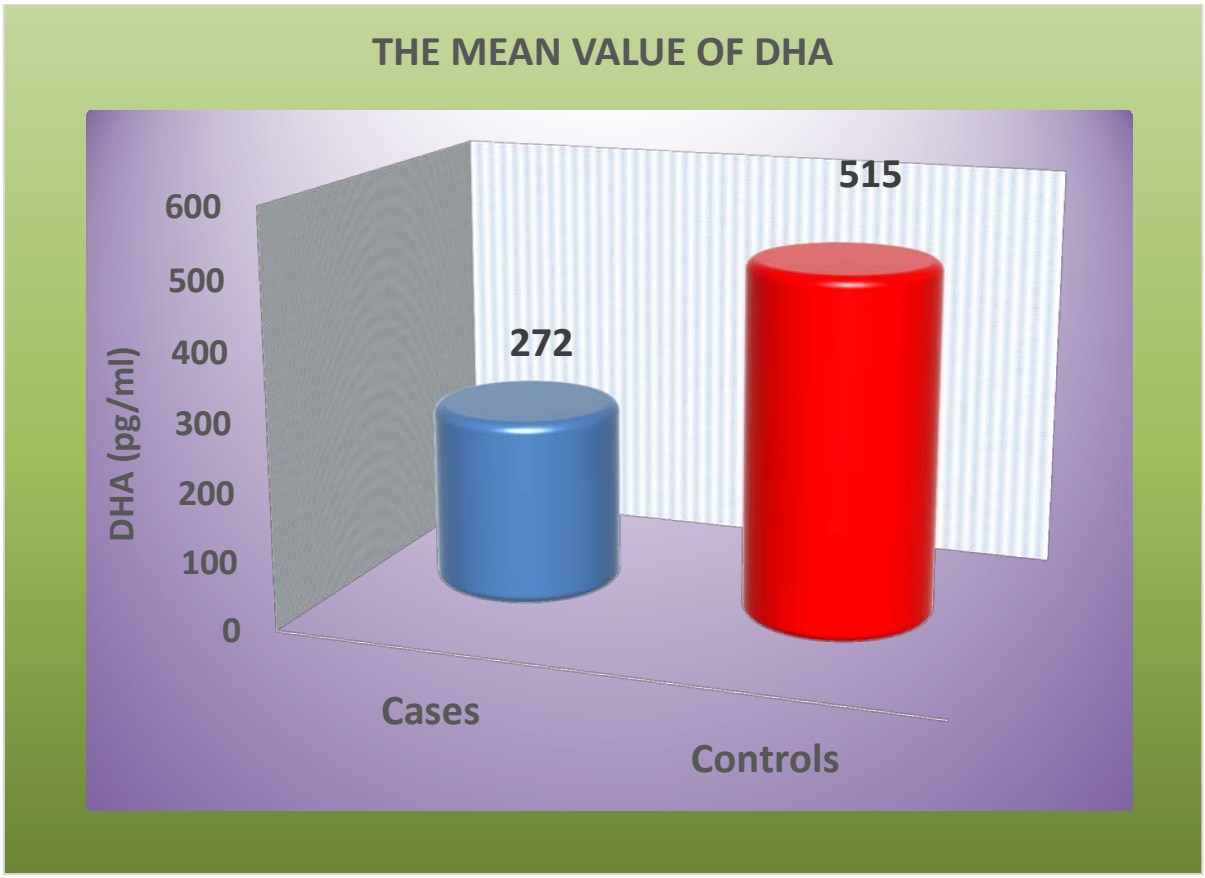
TABLE NO:12

MEAN DHA LEVEL BETWEEN CASES AND CONTROLS

S.No		N	Mean	SD	P-Value
1	Cases	40	271.935	147.9252	< 0.05
2	Controls	40	515.365	291.5948	

The mean value of DHA in cases was 271.935±147.9252 and that of controls was 515.365±291.5948 which was found to be statistically significant by independent t test. The p value was <0.05.

CHART NO: 12


THE MEAN VALUE OF DHA
DHA (pg/ml)
600
500
400
300
200
100
0
272
515
Cases
Controls

DISCUSSION

Preeclampsia is a pregnancy related hypertensive disorder. It is characterized by increased Blood Pressure (≥140/90 mmHg on two different occasions when taken four hours apart), proteinuria with or without edema after 20 weeks of gestation in a previously normotensive mother. This disease is of great importance because it causes increased morbidity and mortality to both mother and child. Therefore it is necessary to assess and develop new biomarker which can help in the early diagnosis and prevention of further progression of the disease[1].

Docosahexaenoic acid is a long chain polyunsaturated fatty acid having 22 carbon atoms. It is an essential fatty acid that comes under omega-3 (ω-3) family having 6 double bonds. Docosahexaenoic acid is essential for the placentation. Apart from implantation, Docosahexaenoic acid is necessary for normal brain development of the fetus.

There is a higher ratio of DHA in cord to maternal blood compared with other fatty acids, and this reflects a higher placental DHA transfer. The maternal-fetal transfer of fatty acids is a slow process which requires ≥ 12 hrs. Hence a high intake of dietary DHA appears to be essential for placental uptake and transfer. Placental messenger RNA expression of fatty acid transport protein (FATP-4) correlates with levels of DHA in cord blood, compatible with role of FATP-4 in DHA transfer. Impaired maternal-fetal LCPUFA transport during pregnancy leads to abnormal placental function.

In our study we compared various biochemical markers between preeclamptic mothers with control group.

The mean DHA level of preeclamptic group was 271.93pg/ml and that of normal pregnant mothers were 515.36pg/ml. The mean difference between two groups were 243.43pg/ml. The p value of mean difference between two groups was found to be <0.05. This means that the serum DHA levels were significantly reduced in preeclamptic mothers compared to normotensive pregnant mothers.

The mean creatinine level of preeclamptic group was 1.75mg/dl and that of normal pregnant mothers were 0.84mg/dl. The mean difference between two groups were 0.91mg/dl. The p value of mean difference between two groups was found to be <0.05. This means that the serum creatinine levels were significantly elevated in preeclamptic mothers compared to normotensive pregnant mothers.

The mean uric acid level of preeclamptic group was 6.33mg/dl and that of normal pregnant mothers was 3.83mg/dl. The mean difference between two groups was 2.50mg/dl. The p value of mean difference between two groups was found to be <0.05. This means that the serum Uric acid levels were significantly elevated in preeclamptic mothers compared to normotensive pregnant mothers.

The mean serum protein levels of preeclamptic group was 5.20g/dl and that of normal pregnant mothers were 7.40g/dl. The mean difference between two groups were 2.2g/dl. The p value of mean difference between two groups was found to be <0.05. This means that the serum protein levels were significantly reduced in preeclamptic mothers compared to normotensive pregnant mothers.

The mean serum albumin of preeclamptic group was 3.08g/dl and that of normal pregnant mothers were 4.27g/dl. The mean difference between two groups were 1.19g/dl. The p value of mean difference between two groups was found to be <0.05. This means that the serum albumin levels were significantly reduced in preeclamptic mothers compared to normotensive pregnant mothers.

Other parameters including blood sugar, blood urea, serum total cholesterol, serum triglycerides, serum HDL, platelet count, hemoglobin were compared between the two groups and found to be not statistically significant.

In our study we found that serum DHA levels were significantly low in preeclamptic mothers compared to normal pregnant mothers. Vanessa et al conducted a study to find out the association of Compromised Maternal Synthesis of Long-Chain Polyunsaturated Fatty Acids and preeclampsia. They observed Concentrations of n−6 and n−3 long-chain polyunsaturated fatty acids were 23% to 60% lower in erythrocytes in PE. Additionally they found out, mRNA expression of Δ-5 and Δ-6 desaturase and very long-chain fatty acid elongase in subcutaneous adipose tissue was lower in PE than controls[(72)].

Keith et al conducted a study to find the role of serum uric acid as a prognostic indicator of the severity of maternal and fetal complications in hypertensive pregnancies. In their study, they observed there is a significant elevation in serum uric acid levelsin both the gestational hypertensive group and the preeclamptic group of women over normotensive pregnant women group. They concluded, though Uric acid levels were significantly elevated in women with

gestational hypertension and preeclampsia compared to normotensive pregnant women, was not a good prognostic indicators of the severity of the maternal or fetal complications[73].

During pregnancy, DHA requirements are increased mainly during the last trimester. Maternal DHA concentration is physiologically reduced by 50%, possibly due to a decreased intake but mainly because of the increased maternal blood volume and enhanced placental and fetal requirements. As fetal LC-PUFA synthesis is limited, its concentration depends mainly on placental transfer. Mechanism of placental LC-PUFA uptake from maternal circulation and transport is not clear but involves binding proteins and placental enzymes. The placental transfer has a high variability as shown in cord DHA levels compared to maternal DHA status. This can be explained by the intake regimens, the duration of DHA supplementation, life style, maternal BMI and placental disorders for a small proportion. The maternal to fetal DHA transfer is facilitated by placental lipases and fatty acid binding proteins. As the need for DHA is increased during the third trimester, a daily supplementation could be recommended. Many studies have reported the benefits of an adequate DHA status during pregnancy and lactation[74].

CONCLUSION

Our study shows that serum DHA levels were reduced among preeclamptic mothers compared to normotensive mothers. So the estimation of serum DHA level may aid in early diagnosis and prevention of further progression of preeclampsia.

Supplementation of long chain polyunsaturated fatty acids especially DHA during pregnancy and its outcome is another special topic and it needs further detailed research work.

LIMITATIONS

- Smaller sample size.
- Only patients of gestational age between 32 to 36 weeks were included.
- Only primi mothers were included.

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ANNEXURE

GOVERNMENT MOHAN KUMARAMANGALAM
MEDICAL COLLEGE & HOSPITAL
SALEM, TAMILNADU
College: Phone No.0427-2383313 Fax No:0427-2383193
E-Mail ID: deangmkmcslm@gmail.com
Hospital: Phone No: 0427 - 2210674, 2210757 Fax : 0427 - 2210876
E-Mail ID: msgmkmchsalem@gmail.com

Communication of Decision of the Institutional Ethics Committee(IEC)

Ref. No. GMKMC&H/4341/IEC/02/2018-72 **Date: .11.2018**

Protocol title	"ASSESSMENT OF MATERNAL SERUM DOCOSAHEXAENOIC ACID LEVELS IN PRE-ECLAMPSIA AND UNCOMPLICATED PREGNANCIES"
Guide/Principal Investigator	Dr.R.RANGARAJAN, MD (BIOCHEMISTRY) Head of the, Dept. of Biochemistry, GMKMCH, Salem-01
Primary Investigator	Dr.V.HARIRAJ, First year Post Graduate MD (BIOCHEMISTRY), GMKMC, Salem-30.
Name & Address of Institution	Govt. Mohan Kumaramangalam Medical College & Hospital, Salem, Tamil Nadu.
Type of Review	☑ New review ☐ Revised review ☐ Expedited review
Date of review (D/M/Y)	09.10.2018
Date of previous review, if revised application:	Nil
Decision of the IEC	☑ Recommended ☐ Recommended with suggestions ☐ Revision ☐ Rejected
Suggestions/ Reasons/ Remarks:	Nil
Recommended for a period of :	From December 2018 to November 2019

Please note *

- Inform IEC immediately in case of any Adverse events and Serious adverse events.
- Inform IEC in case of any change of study procedure, site and investigator
- This permission is only for period mentioned above. Annual report to be submitted to IEC.
- Members of IEC have right to monitor the trial with prior intimation.

G.M.K.M.C. SALEM-30

Signature of Member Secretary

By E-Mail / RPAD

From

Dr.R. BALAJINATHAN, MD.,
Dean,
Govt. Mohan Kumaramangalam
Medical College, Salem – 30.

To

The Controller of Examination,
The Tamil Nadu Dr.M.G.R. Medical University,
No.69, Anna Salai,
Guindy, Chennai – 600 023.

Ref.No.1697/MEI(PG)/2018, Dated :16.12.2020

Sir,

Sub : Medical Education – Govt. Mohan Kumaramangalam Medical College, Salem – 3rd year Post Graduate Students in Biochemistry of this College – Requesting in change of Dissertation Guide – Requisition submitted – Forwarded - Regarding.

Ref : Application submitted by the individuals dated 01.12.2020.

****

I am forwarding herewith the following Post Graduate Students studied in Md (Biochemistry) of this College, requesting to change the name of the guide in their dissertation, for your kind perusal and necessary action.

1) Dr.V. Hariraj
2) Dr.M. Ganesan
3) Dr.C. Janani

This is for your kind information and necessary further action.

DEAN 16/12/20

Encl: Original Requesting letters – 3

Copy to the above individuals

..... through the HOD of Biochemistry of this College.

Copy to the HOD of Biochemistry of this College.

PROFORMA

ASSESSMENT OF MATERNAL SERUM DOCOSAHEXAENOIC ACID LEVELS IN PRE-ECLAMPSIA AND UNCOMPLICATED PREGNANCIES

Name: Age/sex: Op/Ip no:

Presenting complaints:

Past History: DM HT Thyroid Epilepsy TB

Previous History of Surgery: Yes / No

Personal History:

Menstrual History:

General Examination:

Pallor / Icterus / Pedal Edema / Lymphadenopathy

BP: Wt: Ht:

System Examination:

P/A: CVS: RS: CNS:

Lab Investigations:

Blood Sugar(mg/dl)

Blood Urea (mg/dl)

Serum Creatinine (mg/dl)

Serum Uric acid (mg/dl)

Total Cholesterol (mg/dl)

HDL (mg/dl)

TGL (mg/dl)

Total Protein (g/dl)

Albumin (g/dl)

Hemoglobin (mg/dl)

Platelet Count (lakh cells/cubic mm)

DHA (pg/ml)

PATIENT CONSENT FORM

STUDY TITLE :

ASSESSMENT OF MATERNAL SERUM DOCOSAHEXAENOIC ACID LEVELS IN PRE-ECLAMPSIA AND UNCOMPLICATED PREGNANCIES

DEPARTMENT OF BIOCHEMISTRY, GMKMCH, SALEM

PARTICIPANT NAME: AGE: SEX: IP.NO

I confirm that I have understood the purpose of the above study. I have the opportunity to ask the question and all my questions and doubts have been answered to my satisfaction.

I have been explained about the possible complications that may occur during and after medical procedure. I understand that my participation in the study is voluntary and that I am free to withdraw at any time without giving any reason.

I understand that investigator, regulatory authorities and the ethical committee will not need my permission to look at my health records both in respect to the current study and any further research that may be conducted in relation to it, even if I withdraw from the study. I understand that my identity will not be revealed in any information released to third parties or published, unless as required under the law. I agree not to restrict the use of any data or results that arise from the study. I hereby consent to participate in this study.

Time :

Date :

Place :

Patient name;

Signature / Thumb Impression

Name and signature of the Investigator

ஆராய்ச்சி ஒப்புதல் படிவம்

பெயர்	:	தேதி	:
வயது	:	உள்நோயாளி எண்	:
பாலினம்	:	ஆய்வு சேர்க்கை எண்	:

இந்த ஆய்வின் நோக்கம் மற்றும் விவரங்கள் முழுமையாக எனக்கு தெளிவாக விளக்கப்பட்டது. இவ்ஆயிவில் இருந்து நான் எந்த நேரமும் பின்வாங்கலாம் என்பதையும் அதனால் எனக்கு எந்த பாதிப்பும் இல்லை என்பதையும் தெளிவாக புரிந்து கொண்டேன்.

முடிவுகளை அல்லது கருத்துகளை வெளியிடும் போதோ அல்லது ஆய்வின் போதோ என்னுடைய பெயரையோ அல்லது அடையாளங்களையோ வெளியிட மாட்டார்கள் என்பதையும் அறிந்து கொண்டேன்.

இந்த ஆய்வில் எவ்வித நிர்பந்தமும் இன்றி எனது சொந்த விருப்பத்தின் பேரில் நான் பங்கு பெறுகின்றேன்.

நான் சுயநினைவுடனும் முழு சுதந்திரத்துடனும் இந்த மருத்துவ ஆராய்ச்சியில் சேர்த்துக் கொள்ள சம்மதிக்கின்றேன்.

ஆராய்ச்சியாளர் ஒப்பம் **பங்கேற்பாளர் ஒப்பம் (அ)**

இடது பெருவிரல் ரேகை

MASTER CHART - CONTROL

S.NO	AGE	SUGAR (mg/dl)	UREA (mg/dl)	CREATININE (mg/dl)	URIC ACID (mg/dl)	TOTAL CHOLESTROL (mg/dl)	HDL (mg/dl)	TRIGLYCERIDES (mg/dl)	TOTAL PROTEIN (g/dl)	ALBUMIN (g/dl)	HB (gms/dl)	PLATLET COUNT (lakh cells/cu.mm)	PROTEINURIA	DHA (pg/ml)
1	25	92	21	0.7	2.9	69	41	78	7.4	5	13.1	3.2	NIL	751.5
2	18	102	23	0.8	2.4	152	54	92	6.4	3.6	10.1	1.9	NIL	175.8
3	19	105	28	1	2.8	163	75	111	7.9	3.9	12.6	2.6	NIL	250.5
4	21	107	38	1.1	4	115	68	97	6.9	4.2	13.1	1.9	NIL	350.6
5	25	98	40	0.9	5	109	42	123	7.1	4.1	13.8	2.8	NIL	800.3
6	18	92	26	0.7	4	98	39	138	6.9	3.8	12.8	3.1	NIL	653.4
7	18	118	34	0.6	2.6	132	77	108	7.3	4.6	12.9	2.7	NIL	8.9
8	20	109	29	0.9	2.9	198	39	127	7.9	3.6	12	2.6	NIL	95.9
9	22	108	38	0.6	3.1	114	45	135	7.8	4.8	9.5	3.4	NIL	698.1
10	24	97	37	0.8	3.8	95	55	78	7.7	3.9	12	2.6	NIL	950.3
11	25	93	26	0.8	4.5	78	57	112	6.9	4.7	13.1	3.8	NIL	287.5
12	18	100	29	1.1	5.1	139	62	125	6.8	3.9	13.5	2.4	NIL	875.6
13	19	98	34	1	5.2	142	69	98	7.9	4.2	12.5	1.9	NIL	615.7
14	21	108	26	0.6	5.8	157	78	45	7.5	4.3	12	3.4	NIL	702.8
15	25	119	35	0.9	4.9	78	46	67	7.9	4.8	13.5	3.7	NIL	325
16	18	105	29	0.8	4.6	63	68	112	8.1	3.8	9.9	2.8	NIL	901.2
17	19	116	31	0.7	3.8	96	49	134	7.6	3.9	12.9	2.5	NIL	402.7
18	21	104	34	0.7	2.6	145	67	78	7.2	3.5	13.7	3.4	NIL	702.5
19	25	95	26	0.8	2.9	115	39	89	7.3	4.8	10.9	1.9	NIL	652.2
20	18	93	25	0.9	2.8	109	41	122	8.2	4.6	13.5	2	NIL	130.1
21	18	104	27	0.6	3.7	98	44	114	6.9	4.9	13.6	3.8	NIL	156.9
22	20	100	34	0.6	4.2	132	54	102	7.3	4.7	13.9	3.2	NIL	265.7
23	22	109	39	0.8	3.2	198	68	132	7.9	4.6	12.9	1.9	NIL	963.8
24	24	108	24	0.9	4.9	113	77	78	7.8	3.9	12.8	2.6	NIL	706.5
25	25	119	29	0.7	5.4	99	49	92	7.7	3.7	12	2.3	NIL	213.8
26	18	105	38	0.8	6	69	68	111	7.1	3.9	10.2	2.2	NIL	402
27	19	102	27	0.9	4.3	152	54	97	6.9	4.5	13.1	2	NIL	312.6
28	21	104	28	0.9	2.8	163	75	123	7.3	4.6	12.1	3.6	NIL	201.3
29	25	95	25	0.8	2.7	115	68	138	7.9	4.9	13	3.8	NIL	785.4
30	18	93	36	1.1	5.3	109	42	108	7.8	3.9	12.5	2.9	NIL	358.6
31	21	104	24	1	4.6	98	39	127	7.1	4.6	13.5	2.4	NIL	598.9
32	25	115	39	1.1	4.8	132	77	135	6.9	4.2	12.4	3.5	NIL	159.1
33	18	103	27	1	2.6	198	39	78	7.3	4.1	12.6	2.3	NIL	869.3
34	18	88	23	0.9	3.8	114	45	112	7.1	3.9	13.5	3.6	NIL	148.5
35	20	88	26	0.8	2.7	95	55	125	6.9	3.7	10.2	3.7	NIL	987.6
36	22	98	32	0.9	2.6	78	57	98	7.3	4.6	13.4	2.7	NIL	324.7
37	24	92	39	0.7	3.6	139	62	102	7.9	3.8	12.3	2.6	NIL	714.8
38	25	118	35	0.8	4.6	142	69	132	7.8	4.6	11	2.9	NIL	452.6
39	18	109	38	1.1	2.9	157	78	78	7.7	4.7	9	1.9	NIL	825.2
40	21	108	28	0.9	2.8	78	46	92	6.9	5	12.5	3.1	NIL	836.7

MASTER CHART – CASES

S.NO	AGE	SUGAR (mg/dl)	UREA (mg/dl)	CREATININE (mg/dl)	URIC ACID (mg/dl)	TOTAL CHOLESTROL (mg/dl)	HDL (mg/dl)	TRIGLYCERIDES (mg/dl)	TOTAL PROTEIN (g/dl)	ALBUMIN (g/dl)	HB (gms/dl)	PLATLET COUNT (lakh cells/cu.mm)	PROTEINURIA	DHA (pg/ml)
1	21	98	39	0.9	5.2	132	39	112	4.7	2.5	12.9	3.6	1+	200.2
2	25	92	24	1.6	4.9	198	77	125	4.3	3.6	13.7	3.7	2+	175.7
3	18	118	29	1.1	5.6	114	39	98	5.7	2.3	10.9	2.7	1+	250.5
4	18	109	38	2.1	7.3	95	45	45	5.6	2.9	13.5	2.6	1+	350.2
5	20	108	27	1.9	6.4	78	55	67	5.9	2.8	13.6	2.9	1+	352.1
6	22	97	28	2.3	8.2	139	57	112	5.3	3.1	13.9	1.9	1+	470.9
7	24	93	25	1.7	4.9	142	62	134	4.9	2.5	12.9	3.1	1+	560.7
8	25	119	36	1.1	5.8	157	69	78	5.2	3.5	12.8	1.9	2+	95.8
9	18	105	24	0.9	7.8	78	78	89	4.6	3.4	12	2.6	2+	102.5
10	21	116	40	1.8	7.1	63	46	122	4.7	3.6	10.2	1.9	1+	375.8
11	25	104	26	2	4.2	96	68	114	5.2	2.5	13.1	2.8	2+	287
12	18	95	34	1.5	5.6	145	49	102	5	2.6	12.1	3.1	1+	98.6
13	18	93	29	1.2	9	115	67	132	5.1	5.8	13	2.7	2+	102.3
14	20	104	38	1.8	8.5	109	39	78	4.4	3.4	12.5	2.6	2+	425.4
15	22	100	37	1.2	4.6	98	41	92	5.5	3.5	13.5	3.4	1+	325.6
16	24	109	26	2.1	4.7	132	44	111	5.8	2.6	12.9	2.6	1+	78.9
17	18	108	29	2.1	5.2	198	54	97	4.6	2.4	12.8	2.6	2+	402.5
18	19	119	34	2.3	5	109	68	123	5.7	2.9	12	1.9	1+	39.8
19	21	107	26	2.4	8.3	98	77	138	5.7	2.8	10.2	2.8	1+	652.5
20	25	98	35	1.7	6	132	49	108	4.9	3.5	13.1	3.1	1+	130.6
21	18	92	29	2.3	6.1	198	68	127	5.6	2.4	12.1	2.7	1+	156.3
22	18	118	31	2.2	5.8	114	54	135	4.9	2.6	12.2	2.6	1+	265.4
23	20	109	34	2.4	8.1	95	75	78	5.8	3.4	12.5	3.4	2+	95.6
24	22	108	26	0.8	7.6	78	68	112	5.3	2.5	13.5	2.6	2+	45.9
25	24	97	25	2.1	5.6	139	42	125	4.6	2.7	12.4	3.8	1+	213.1
26	25	93	27	2.3	8.9	142	39	111	4.2	3.4	12.6	2.4	2+	402.3
27	18	100	34	2.4	4.7	157	77	97	5.6	2.6	13.5	1.9	1+	312.5
28	19	98	39	2.4	4.3	78	39	123	5.3	3.7	13.1	3.4	2+	201.6
29	21	108	24	2.3	5.7	63	45	138	4.9	2.6	13.8	3.7	2+	401.7
30	22	119	29	1.5	5.6	132	55	108	5.2	5.8	12.8	2.8	1+	358.8
31	24	105	38	1.7	8.2	198	57	127	4.9	3.4	12.9	2.5	1+	456
32	18	116	27	1.6	7.6	113	62	135	5.6	3.5	12	3.4	2+	159.2
33	19	104	28	1.1	8.2	99	69	78	6	2.6	9.5	1.9	1+	357.7
34	21	95	25	2.4	9	69	78	112	5.9	2.4	12	2	1+	148.5
35	25	93	36	2.3	4.6	152	46	125	4.7	2.9	13.1	3.8	1+	369.2
36	18	104	24	0.8	4.7	163	68	98	4.9	2.8	13.5	3.2	1+	324.1
37	18	100	39	1.4	5.2	115	49	102	5.8	3.5	12.5	1.9	1+	159.9
38	20	109	27	0.7	5	132	67	132	6	2.4	12	2.6	2+	452.7
39	22	108	23	1.2	8.3	198	39	135	5.7	2.6	13.5	1.9	2+	265.8
40	24	119	29	2.4	6	114	41	78	4.6	3.4	9.9	2	1+	253.5