"STUDY OF SERUM HOMOCYSTEINE LEVELS IN PREECLAMPSIA AND RELATION TO ITS SEVERITY AND OBSTETRIC OUTCOME"

By

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Dr. NAGA JYOTHI. S

LIST OF ABBREVIATIONS

ACE	Angiotensin converting enzyme
ACOG	American College of Obstetrics and Gynaecology
ALT	Alanine transaminase
AST	Aspartate transaminase
AT	Angiotensin
BP	Blood pressure
CNS	Central nervous system
DBP	Diastolic blood pressure
DNA	Deoxyribonucleic acid
EDRF	Endothelial derived relaxing factor
FDA	Food and drug administration
FFA	Free fatty acids
GFR	Glomerular filtration rate
GHTN	Gestational hypertension
Hcy	Homocysteine
HLA	Human leukocyte antigen
LDH	Lactate dehydrogenase
MAP	Mean arterial pressure
Methyl THF	Methyl tetrahydrofolate
MS	Methionine synthase

mTHFR	Methyl tetrahydrofolate reductase
NF-KB	Nuclear factor kB
NO	Nitric oxide
NTD	Neural tube defects
PAI	Plasminogen activator inhibitor
PE	Pre-eclampsia
PGE2	Prostaglandin E2
PGI2	Prostacyclin
PIH	Pregnancy induced hypertension
PET	Pre-eclamptic Toxaemia
PLGF	Placental growth factor
RNA	Ribonucleic acid
SAM	S-adenosyl methionine
SBP	Systolic blood pressure
sFLT	Soluble fms – like tyrosine kinase
TG	Triglycerides
Th	T.helper lymphocytes
tHCY	Total homocysteine

TNF	Tumour necrosis factor
TXA2	Thromboxane A2
USA	United States of America
VEGF	Vascular endothelial growth factor

ABSTRACT

"STUDY OF SERUM HOMOCYSTEINE LEVELS IN PREECLAMSIA AND RELATION TO ITS SEVERITY AND OBSTETRIC OUTCOME"

Background and Objectives :

Hypertensive disorder in pregnancy is a common disease. The incidence of pregnancy induced hypertension (PIH) in India range from 5-15%. Though the exact cause of PE is still undecided, endothelial dysfunction with associated intense vasospasm has been implicated in its causation.

Recently homocysteine, a metabolite of essential aminoacid methionine, has been postulated to produce oxidative stress and endothelial cell dysfunction. The present study is aimed at the estimation of homocysteine concentration in both pre-eclamptic and normotensive pregnant women, thereby deducing its relation in causation of pre-eclampsia.

Elevated homocysteine levels comprise an independent and incremental risk for vascular disease, direct endothelial toxicity, failure of nitric acid oxide release and platelet abnormalities. Homocysteine may prove to be the missing link in the etiology of pre-eclampsia.

Objectives of the study :

1) To show a relationship between serum homocysteine levels in preeclampsia.

2) To find if any correlation between homocysteine levels and severity of preeclampsia

3)To associate levels of homocysteine in preeclampsia and obstetric outcome.

Study design :

A prospective case-control study conducted in the Department of Obstetrics and Gynecology at R.L. Jalappa Hospital and Research centre, Tamaka, Kolar, from March 2015 to July 2016.

Materials and Methods :

The present study was carried out in 90 pregnant women of whom 45 were Preeclampsia(cases) and 45 were normotensive pregnant women(controls) admitted to R.L.Jalappa Medical College, Kolar were selected consecutively as and when they presented with the application of inclusion and exclusion criteria. Informed consent was obtained from all the subjects and the study was approved by the ethical committee of the institute. A standard proforma was used to collect the data. Five ml of blood samples are collected in plain vacutainer from control and preeclamptic subjects. Samples are centrifuged at 3000 x g to separate serum and stored at -20 $^{\circ}$C in ultra freezer until analysis. Maternal serum homocysteine levels were measured by ELISA method.

Results :

In the present study of 45 preeclamptic women and 45normotensive pregnant women in whom the serum homocysteine levels were compared. Majority of the subjects were in the age group of 21-25years in both the groups. All the ninety subjects were primigravida. We have found that there is a statistically significant correlation between serum homocysteine levels and severity of PE i.e. the mean serum Hcy levels in controls(7.9 ±1.3µmol/l),mild preeclampsia (14.5±3.2µmol/l) and severe preeclampsia(19.6± 3.9µmol/l).

Serum homocysteine levels increased with increasing severity of PE with p-value being highly significant ($p<0.001$). Significant positive correlation was found between serum homocysteine levels and SBP and DBP i.e. with increase in SBP and DBP there was significant increase in serum homocysteine in preeclampsia. In our study, hyperhomocysteinemia was associated with poor pregnancy outcome in preeclampsia; IUGR(48.9%), Preterm(28.9%) and SGA(15.6%).

Interpretation and Conclusion :

Homocysteine concentration decreases during normal pregnancy, but this does not occur in preeclampsia. In preeclampsia there is increase in levels of homocysteine compared to normotensive women. In PET, homocysteine levels further increase with increasing severity of preeclampsia. Hyperhomocysteinemia is associated with poor pregnancy outcome.

Key words : Homocysteine, Normotensive, Preeclampsia

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INTRODUCTION

Preeclampsia is an obstetric condition characterized by hypertension and proteinuria. This obstetric complication causes preterm delivery, intrauterine growth restriction, maternal and fetal morbidity and mortality.[1,2] . In 1998, National Center for Health Statistics showed hypertension was the most common medical risk factor in pregnancy. The worldwide incidence of pre-eclampsia is 5-7% of all pregnancies.[3]The incidence is still higher in India of around 8-10%.[4] As per the World Health Report the maternal mortality during pregnancy and puerperium is around 12%. In developing countries, hypertension accounts for 17% of direct obstetric deaths.[5] Mortality rate of preeclampsia in the developing and developed countries varies, it has been recorded that approximately eight hundred women die from pregnancy and child birth related complications around the world every day.[6]

Preeclampsia is multifactorial. Till date its etiology is indefinite. Studies conducted on animal models to know the pathophysiology of preeclampsia reported that abnormal trophoblast invasion, oxidative stress, inappropriate maternal vascular damage and anomalous maternal-fetal immune interactions play an important role.[7]

Though the exact cause of pre-eclampsia is still undecided, endothelial dysfunction with associated intense vasospasm has been implicated in its causation. Recently homocysteine, a sulfur containing essential aminoacid has been implicated as a missing link in causation of preeclampsia. Current hypothesis states that increased levels of homocysteine promote oxidative stress which might damage the vascular endothelium of the developing placenta, thereby increasing contractile response and production of pro-coagulants and vasoconstriction.[8] Further, homocysteine levels is known to increase with increasing severity of preeclampsia.

Present study is aimed at to shed light on Homocysteine, which can be a missing link in the pathogenesis of preeclampsia and also to deduce its effect in relation to severity of pre-eclampsia.

AIMS AND OBJECTIVES

1) To show a relationship between serum homocysteine levels in preeclampsia.

2) Find if any correlation between homocysteine levels and severity of preeclampsia

3) To associate levels of homocysteine in preeclampsia and obstetric outcome.

REVIEW OF LITERATURE

In the past, hypertensive disorders were regarded as one of the toxemias of pregnancy.. In the last 20-30 yrs, a considerable number of names are introduced including preeclamptic toxemia(PET), preeclampsia(PE), pregnancy induced hypertension(PIH) and gestational hypertension(GHTN) (Gant and Worley [9])

DEFINITION AND CLASSIFICATION[10,11]

Definition :

Pre-eclampsia is a pregnancy specific syndrome seen after 20 weeks of gestation, characterized by elevation of blood pressure of ≥ 140mm Hg systolic or ≥ 90 mm of Hg diastolic with proteinuria.

According to ACOG[10] :

Hypertension in pregnancy is defined as systolic blood pressure ≥ 140 mmHg or a diastolic blood pressure ≥ 90 mmHg or both measured on atleast two different occasions 6 hours apart with the patient in left lateral recumbent position.

Proteinuria is diagnosed when its urinary excretion equals or exceeds 300mg in 24 hours or the ratio of protein to creatinine measured in a single voided urine is ≥ 3.0(each measures in mg/dl).

Classification :

Classification of pregnancy hypertension promulgated by NHBPEP – National High Blood Pressure Education Program – 2000 is currently accepted worldwide[3].

1. Gestational hypertension
2. Pre-eclampsia and Eclampsia syndrome
3. Pre-eclampsia superimposed on chronic hypertension
4. Chronic hypertension of any etiology

Diagnostic criteria for Pregnancy associated hypertension[3]

CONDITION	CRITERIA REQUIRED
Gestational Hypertension	BP ≥140/90 mmHg after 20 weeks in previously normotensive women
Preeclampsia- Hypertension and Proteinuria	- ≥300mg/24hr, or -Protein: creatinine ratio ≥ 0.3 or -Dipstick 1+ persistent[a]
	OR
Thrombocytopenia	Platelets < 100,000/µL
Renal insufficiency	Creatinine > 1.1mg/dl or doubling of base[b]
Liver involvement	SerumTransaminase(AST&ALT) levels twice normal
Cerebral symptoms	Headache, visual disturbances, convulsions
Pulmonary edema	-----

[a]Recommended only if sole available test.

[b]No prior renal disease.

Modified from the American College of Obstetricians and Gynaecologists, 2013b.

Diagnosis of Hypertensive disorders complicating pregnancy :

Gestational hypertension :

New onset of hypertension during pregnancy after 20 weeks of gestation wherein BP ≥ 140/ 90 mm Hg without proteinuria or other features of preeclampsia. Usually in this condition blood pressure returns to normal by 12 weeks postpartum.

Pre-eclampsia :

Minimum criteria :

Blood pressure ≥ 140/90 mm Hg after 20 weeks gestation with associated Proteinuria ≥ 300mg/24 hours or ≥ 1 + dipstick.

Severe features of Preeclampsia

Systolic blood pressure of ≥160mmHg or diastolic blood pressure of ≥110mmHg on two occasions at least 6 hours apart while the patient is at bed rest. Thrombocytopenia confirmed with platelet count <100,000/µl. Impaired liver function proved by abnormally elevated liver enzymes to twice normal concentration in blood. Severe persistent right upper quadrant or epigastric pain, not relieved by medication and not accounted for by alternative diagnosis, or both. Progressive renal insufficiency(serum creatinine concentration greater than 1.1mg/dl or a doubling of serum creatinine concentration in absence of renal disease. Presence of Pulmonary edema or new onset cerebral or visual disturbances

Eclampsia :

Eclampsia is defined as new onset of grand mal seizures in preeclamptic condition. The cause for convulsion in woman with preeclampsia, cannot be attributable to another disorder. Approximately 10% of preeclamptic women do not develop seizures until 48hours postpartum(Sibai2005).

Superimposed pre-eclampsia on chronic hypertension :

In this condition, woman with preexisting chronic hypertension develops one or more features of preeclampsia for first time during pregnancy after 20 weeks.

Chronic hypertension :

Hypertension diagnosed either pre -conceptionally or before 20th week of pregnancy (or) defined retrospectively as hypertension persisting for more than 12 weeks postpartum.

Pre-eclampsia is further classified into Non severe and severe forms as follows[3]:

Abnormality	Non severe[a]	Severe
Diastolic blood pressure	<110 mm Hg	≥ 110 mm Hg
Systolic blood pressure	<160 mm Hg	≥ 160 mm Hg
Proteinuria[b]	None to positive	None to positive
Headache	Absent	Present
Visual disturbances	Absent	Present
Upper abdominal pain	Absent	Present
Oliguria	Absent	Present
Convulsions (eclampsia)	Absent	Present
Serum creatinine	Normal	Elevated
Thrombocytopenia	Absent	Present
Serum transaminase elevation	Minimal	Marked
Fetal growth restriction	Absent	Obvious
Pulmonary edema	Absent	Present

[a] Includes "mild" and "moderate" hypertension not specifically defined.

[b] Most disregard degrees of proteinuria being non severe or severe.

Risk factors for preeclampsia:

The following are the possible risk factors for preeclampsia:

Extremes of reproductive age(< 20 or 35- 40years) , nulliparity , Previous history of preeclampsia and family history of preeclampsia

Placental abnormalities: molar pregnancy, multiple gestation, placental ischemia.

Obesity, pre existing chronic hypertension, chronic renal disease, diabetes milletus Thrombophilias: Anti-phospholipid Antibody syndrome, protein C,S deficiency, factor V Leiden mutation. Genetic and Immunological factors also play consistent role in preeclampsia.

Etiology and Pathogenesis of pre-eclampsia[3]

Several studies have been conducted to know the exact etiology, till date it is still unclear but many proposed the following causes for preeclampsia. They are abnormal trophoblastic invasion of uterine blood vessels, endothelial dysfunction, immunological maladaptive tolerance between maternal and paternal and fetal tissues, Maternal maladaptation to cardiovascular or inflammatory changes of normal pregnancy, Genetic factors including inherited predisposing genes as well as epigenetic influences etc

Pre-eclampsia- a Two-stage disorder Hypothesis :

Observations that abnormal interfaces between maternal, paternal, and fetal tissues may cause pre-eclampsia have led to hypothesis that the syndrome is a two stage disorder.

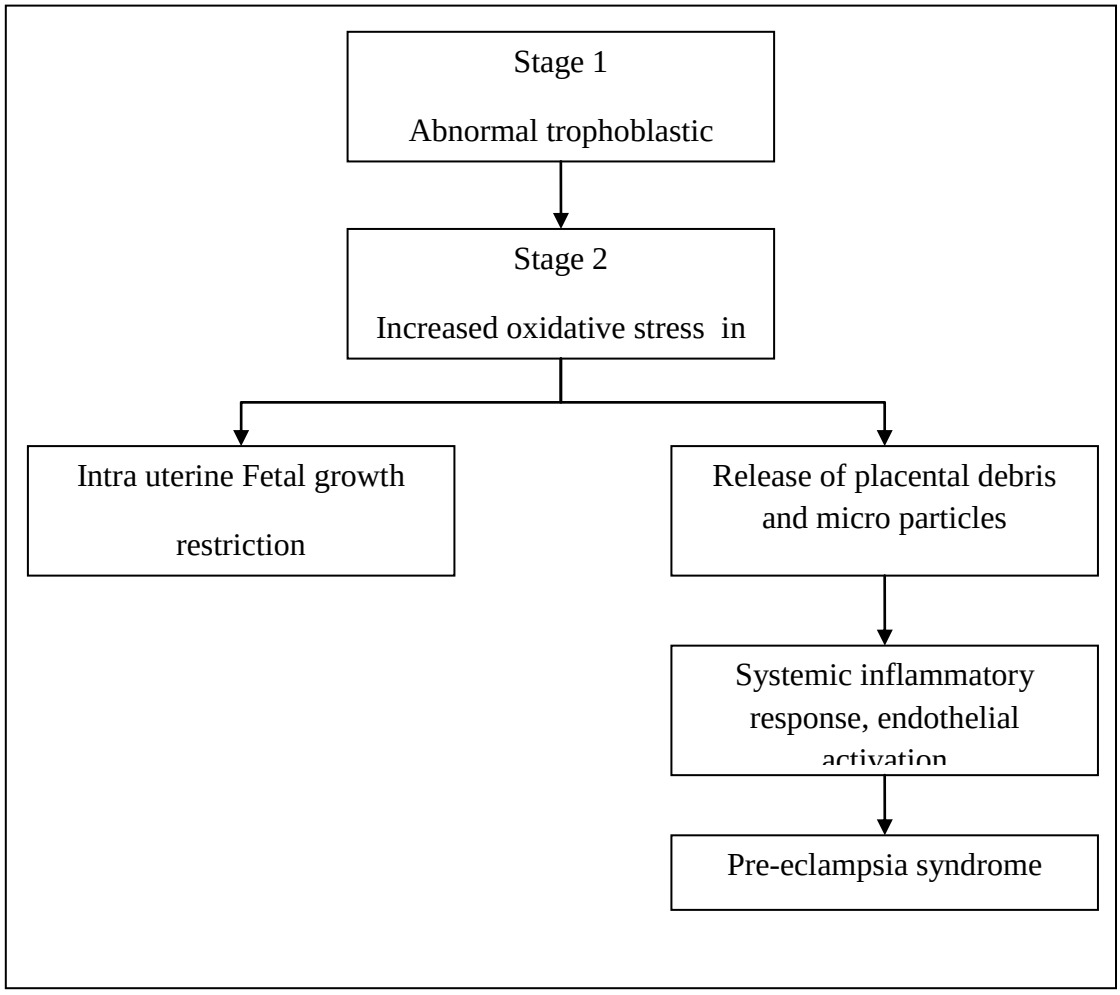


Figure 1: TWO STAGE MODEL OF PREECLAMPSIA

Stage 1 is preclinical and characterized by incomplete trophoblastic vascular invasion leading to remodeling of uterine spiral arteries that causes placental hypoperfusion and hypoxia.

Stage 2 is caused by release of placental factors into the maternal circulation that incite systemic inflammatory response and endothelial activation.

Etiology [3] :

Theories in the causation of pre-eclampsia

1) Abnormal trophoblastic invasion :

In normal implantation, the uterine spiral arterioles are invaded by endovascular trophoblasts thereby they undergo extensive remodeling. These endovascular trophoblasts increase the vessel diameter by replacing the vascular endothelial and muscular linings .

In pre-eclampsia, however, there is incomplete trophoblastic invasion of decidual vessels, but not myometrial vessels, become lined with endovascular trophoblasts. The deeper myometrial arterioles do not lose their endothelial lining and musculoelastic tissue, and their mean external diameter is only half that of vessels in normal placenta.. The magnitude of defective trophoblastic invasion of the spiral arteries correlates with the severity of the hypertensive disorder.

Endothelial damage, insudation of plasma constituents into vessels walls, proliferation of myointimal cells, and medial necrosis are the electron microscopic changes seen in the early preeclamptic stage. This electron microscopic changes in the arteries of implantation site was first observed by De Wolf and coworkers(1980). Lipid is laden first in myointimal cells and then within macrophages. Such lipid-laden cells and associated findings, were referred to as atherosis. Subsequently, blood vessels affected by atherosis develop aneurysmal dilatation.

Thus, the abnormally narrow spiral arterioles impairs blood flow to the placenta. Diminished perfusion and a hypoxic environment eventually lead to release of placental debris that incites a systemic inflammatory response.

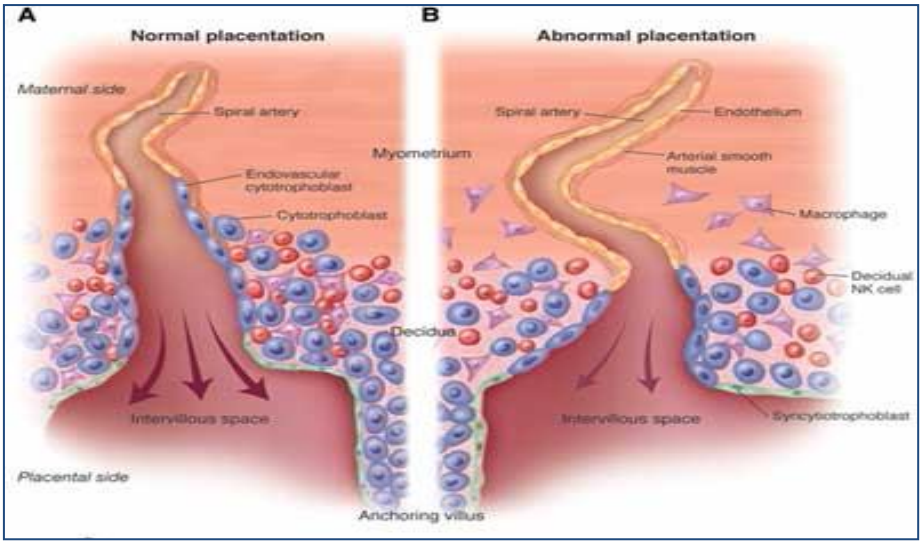


Figure-2:

A. Depicts normal placentation and spiral arterioles in normal Pregnancy

B. Depicts abnormal placentation and spiral arterioles in Preeclampsia

2) Immunological factors :

Theory projected by Erlebacher,2013 that loss of maternal immune tolerance to paternally derived placental and fetal antigens, or perhaps its dysregulation account for pre-eclampsia syndrome.

Absence of blocking antibodies :

The risk of pre-eclampsia is noticeably enhanced in circumstances in which formation of blocking antibodies to placental antigenic sites might be impaired. In this scenario, there would be higher risk in first pregnancy. Tolerance dysregulation also explains an increased risk when the paternal antigenic load is increased, as in molar pregnancy, trisomy 13. Conversely, women previously exposed to paternal antigens, such as a prior pregnancy – with the same, but not different partner are "immunized" against pre-eclampsia.

Immune maladaptation :

Redman and colleagues (2014) reviewed the possible role of immune maladaptation in the pathophysiology of preeclampsia. . In women destined to be preeclamptic, extravillous trophoblast early in pregnancy express reduced amounts of

immunosuppressive human leukocyte antigen G (HLA-G). This may contribute to defective placental vascularization in stage 1. During normal pregnancy, T-helper (Th) lymphocytes are produced so that type 2 activity is increased in relation to type 1 – termed as type 2 bias. Th 2 cells promote humoral immunity, whereas Th 1 cells stimulate inflammatory cytokine secretion[12]. Beginning in the early second trimester in women who develop pre-eclampsia, Th 1 action is increased and the Th1 / Th2 ratio changes[13].

3) Endothelial cell activation :

Endothelial cell dysfunction plays a central role in the pathophysiology of pre-eclampsia.

The normal endothelium produces :

a) Nitric oxide (earlier known as the endothelium derived relaxing factor, EDRF), a derivative of L-arginine is a potent vasodilator and maintains the normal low pressure vasodilator state which is characteristic of fetoplacental perfusion. Research on pregnant animal model have similar clinical picture of preeclampsia on withdrawal of nitric oxide (Conrad 1989)). But few studies have reported that before the onset of hypertension there was no decrease in nitric oxide production or release and thus the changes in the nitric oxide concentrations appear to be the consequence of hypertensive disorders and not the inciting event.[14,15,16]

b) PGI_2 is also a powerful vasodilator and inhibits aggregation of platelets. In normal pregnancy there is increased production of endothelial prostacyclin which results in blunted pressor response.

c) Elevated homocysteine levels increases the risk of pre-eclampsia by three fold in early pregnancy [3].

Wang and Colleagues (1991) reported progressive increase in the PGI_2 : TXA2 ratio and vitamin E : Lipid peroxide ratio in normotensive pregnancies . These ratios are inverted and an increase in TXA 2 results in increased vasospasm and platelet destruction and increased lipid peroxides cause endothelial damage and finally lead to preeclamptic syndrome.[17]

In pre-eclampsia, there is an imbalance between vasodilators and the vasoconstrictors. It is believed to be a state of relative PGI_2 deficiency and TXA2 dominance. [18]

4) ROLE OF VASOACTIVE AGENTS :

Normally, pregnant women have refractoriness to vasopressor substances like . Angiotensin II, nor epinephrine, vasopressin. In pre-eclampsia this refractoriness is lost and there is increased vascular reactivity.[3]

The following vasoactive agents cause various changes in preeclampsia:

(i)Prostaglandins : There is evidence that in preeclampsia , there is decreased production of prostacyclin (PGI_2) mediated by phospholipase A_2 and simultaneous increased secretion of TXA_2 by platelets, resulting in vasoconstriction.[19]

(ii)Endothelins : Endothelin-1(ET-1), is a potent vasoconstrictor and profoundly increased in pre-eclampsia.[20]

Vascular endothelial growth factor (VEGF) :

It is a glycoprotein found in the human placenta which causes vasculogenesis and is required for microvascular permeability. VGEF is found to be increased in pre-eclampsia and restore the uteroplacental blood flow to normal as a compensatory mechanism.[3,21]

5) Inflammatory changes and oxidative stress :

In preeclampsia, oxidative stress is evidenced in both maternal circulation and placenta. In this hypothesis, endothelial cell dysfunction leads to intense state of activated leucocytes in the maternal circulation in preeclampsia.[22] Cytokines like tumour necrosis factor-alfa, interleukins contribute to oxidative stress by formation of free radicals and lipid peroxides that injure endothelial cells, and modify their nitric oxide production.[23]

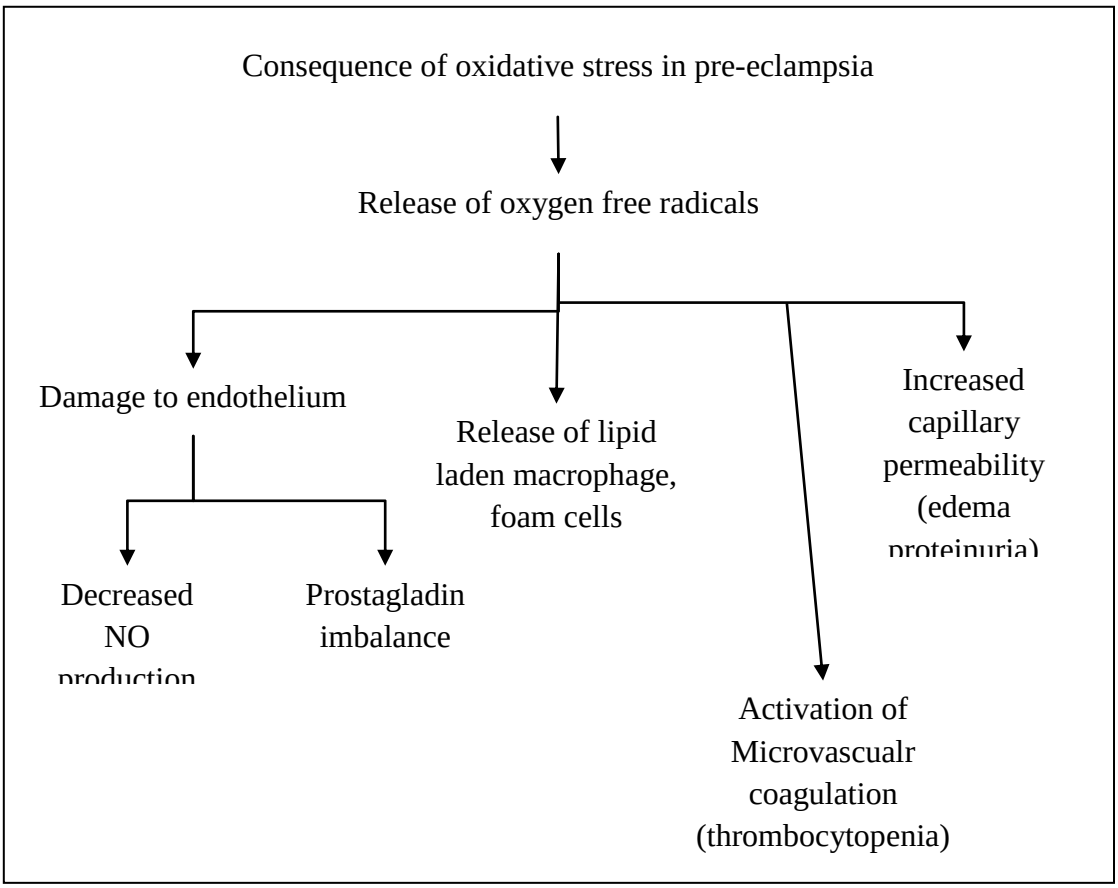


Figure 3: Effects of oxidative stress in Preeclampsia

With above observations, reduction of oxidative stress, a modulation of immune response with some therapeutic strategies may play a central role in management of preeclampsia

6) Nutritional factors[3] :

Studies have shown that in the general population a diet high in fruits and vegetables that have antioxidant activity is associated with decreased blood pressure.

Zhang and associates (2002) reported that the incidence of pre-eclampsia was doubled in women whose daily intake of ascorbic acid was less than 85 mg.

Villar and coworkers (2006) showed that calcium supplementation in populations with a low dietary calcium intake had a small effect to lower perinatal mortality rates, but no effect on the incidence of pre-eclampsia.

According to the 2013 Task force trials, the antioxidant vitamin C and E supplementation showed no beneficial effects.

7) Genetic factors[3] :

Pre-eclampsia is a polygenic disorder. Ward and Lindheimer reported an incident risk for pre-eclampsia of 20-40% for daughters of pre-eclamptic mothers; 11-37% for sisters of preeclamptic women; 22-47% in twin studies.

There is an extensive list of other variables that affect genotypic and phenotypic expression of the preeclampsia syndrome.

Some of them are as follows:

a) Multiple genotypes: maternal and paternal (fetal and placental)
b) Associated disorders such as diabetes and characteristics such as parity.
c) Genomic ethnicity: frequency of polymorphisms, genetic drift, founder effect and selection.
d) Gene-gene interaction: specific alleles or products of two or more genes affect one another and thus the phenotype.
e) Epigenetic phenomena: variations III expression of a functional stable gene for example, monozygotic twin differences.
f) Gene-environmental interactions: these are infinite.

8) HOMOCYSTEINE :

One of the hypothesis in the etiology of pre-eclampsia is endothelial dysfunction secondary to the per oxidation of membrane lipids.

Experimental evidence suggests that hyperhomocystenemia can predispose to atherogenicity and subsequently contributes to the development of preeclampsia.[24] Homocysteine may prove to be the missing link in the etiology of preeclampsia.

Pathophysiology of preeclampsia[25]

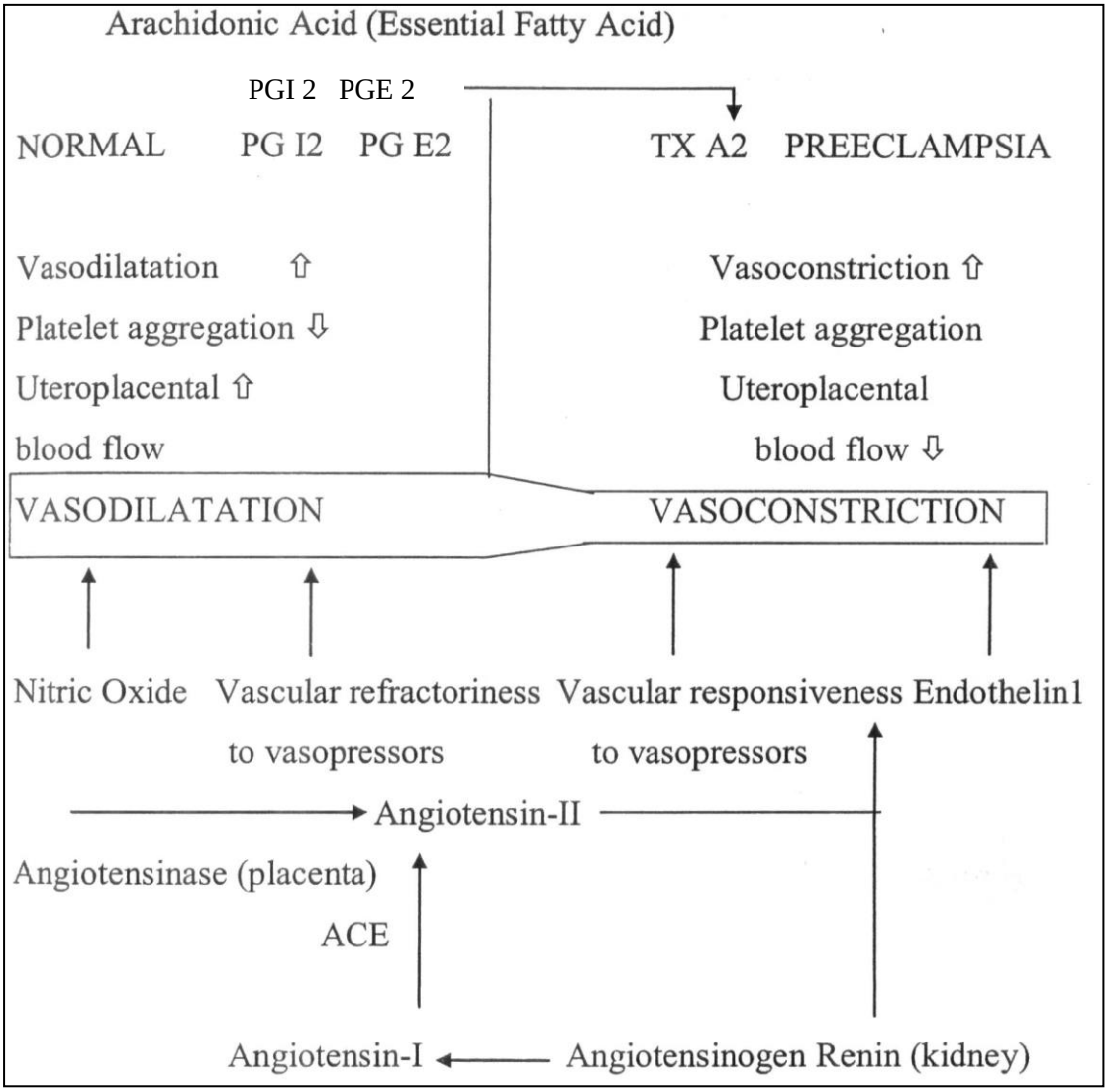


Figure 4: Schematic representation of pathophysiology of preeclampsia

The pathophysiological process of preeclampsia is very complex and occur as a result of abnormal maternal response to pregnancy. All mechanisms put forth for preeclampsia are interconnected, provide positive feedback loops leading to further vascular damage thus establishing a vicious cycle. This vicious cycle can be triggered from any point as the interrelations will ensure that the other processes are activated.[17]

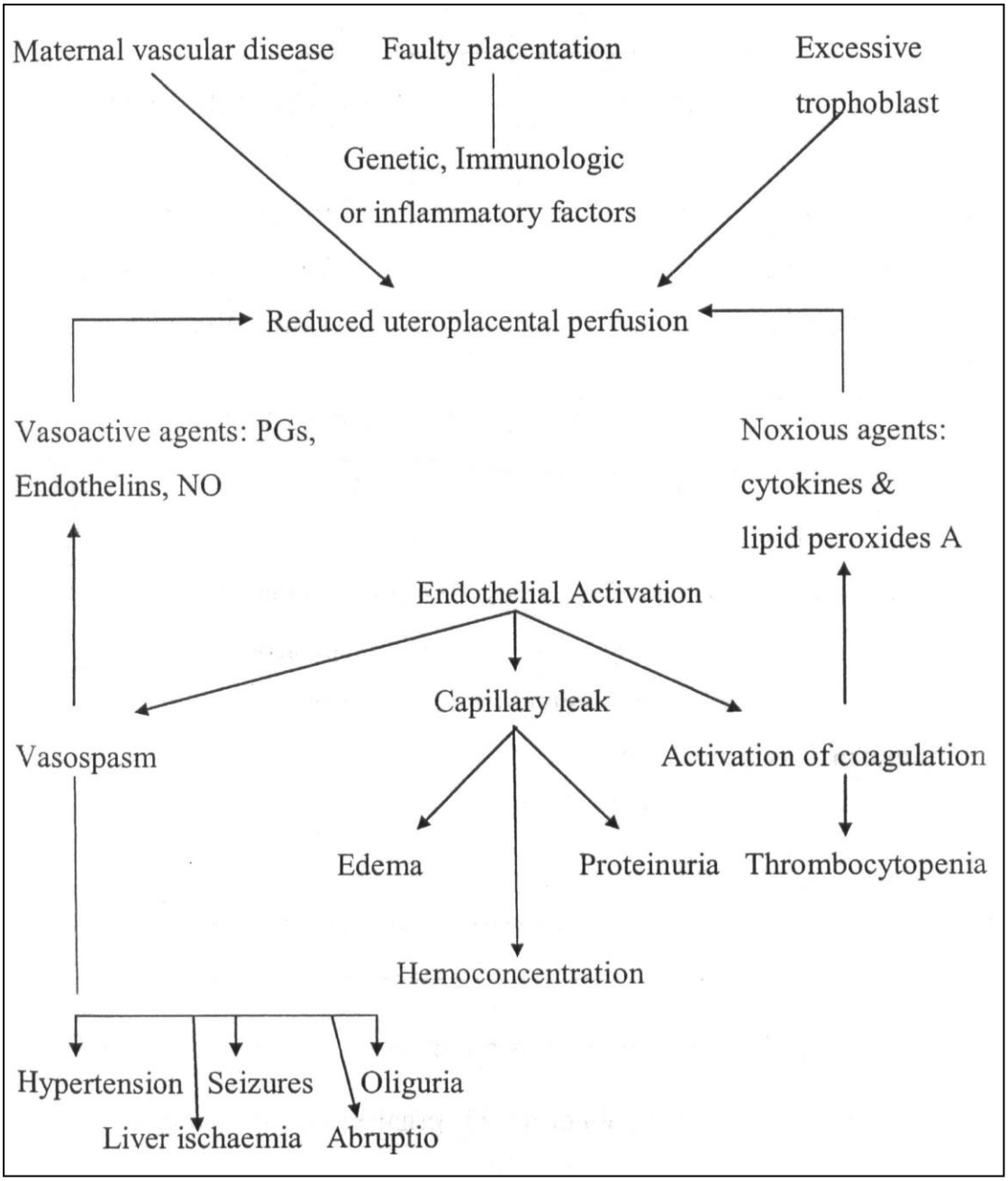


Figure 5: Schematic representation of pathophysiology of preeclampsia

Vasospasm[3]: is basic to the pathophysiology of pre eclampsia-eclampsia. This concept was first advanced by Volhard (1918) based on direct observations of small blood vessels in the nail beds, ocular fundi, bulbar conjunctivae and histological changes in various organs. Endothelial activation causes vascular constriction leads to increased resistance to blood flow and arterial hypertension. Endothelial damage leads to interstitial leakage of blood constituents like platelets and fibrinogen and are deposited sub endothelially[3].

Increased Pressor Responses:

Development of refractoriness to the pressor effects of Angiotensin-II is observed in normal pregnancy. This blunted response is due to down regulation of the angiotensin receptors present in the vascular smooth muscle. Normal vascular refractoriness is maintained by balance between the production and metabolism of the vasoactive prostaglandins. In preeclampsia, the ratio of prostanoids to TXA2 is reversed i.e. the PGI2 and PGE2 production is markedly decreased and that of TXA2 increased[3].

But in preeclampsia there is an increased sensitivity to vasoactive agents, which may be secondary to increased density of angiotensin receptors and imbalance in the prostaglandin production thus leading to vasoconstriction. This increased response antedates all other changes.[18]

In normal pregnancy, spiral arteries in the placental bed undergo a series of physiological changes. They are lined by the cytotrophoblast, which breaks down the endothelium, internal elastic lamina and the muscular coat of the vessel and is largely replaced by fibrinoid material. Virtually every spiral artery undergoes this by the end of the I trimester. Early in II trimester, a second wave of trophoblastic invasion occurs and transforms the myometrial segments of the spiral arteries. With loss of the musculoelastic coat there is a loss of sensitivity to the vasopressors. These changes convert vessels from one of high pressure, high resistance to one of low pressure, low resistance flow.[17,25]

In pre eclampsia about 30% - 50% of the spiral arterioles of the placental bed escape endovascular trophoblastic remodeling. Myometrial segments remain unchanged. Thus primary invasion is partial and secondary invasion fails to occur or is limited resulting in reduced fetoplacental perfusion.

PATHOPHYSIOLOGICAL CHANGES

Hemodynamic Changes[3]

a) Due to the generalized vasospasm in preeclampsia, decreased in cardiac preload is observed.
b) Increase in the cardiac after load which is due to increased systemic vascular resistance.

c) Decreased cardiac output because of increased after load.
d) MAP is raised due to an increase in cardiac output and vascular resistance.

Hematological Changes

a)Thrombocytopenia:

Most common hematological finding seen in about 10-25% of those with preeclampsia/eclampsia. The frequency and intensity of thrombocytopenia. It can occur without other evidences of coagulation disturbances. It probably occurs as a result of an immunologically mediated process or more likely due to increased platelet deposition at the site of endothelial damage. (Pritchard et al, 1976). There is an increased platelet activation and consumption and simultaneous increase in platelet production. It reflects the severity of the pathological process. Lower the platelet count, higher is the maternal and fetal morbidity and mortality. After delivery, the platelet count will progressively increase and return to normal[3].

Abnormalities in the coagulation system:

The possibility of a hypercoagulable state associated with pre eclampsia was first suggested by the presence of fibrin and thrombin found in the microvasculature and various organs. Initiation of the coagulation cascade with fibrin formation in the microvasculature may occur secondary to an endothelial injury that develops in response to vasospasm.

Changes which imply hypercoagulation in pre eclampsia are:

Increased:

- Activity of the intrinsic pathway factors
- Increased FVIIIa: FVIIIc activity, indicating thrombin formation
- Thrombin: Antithrombin III ratio
- Increased platelet aggregation
- Increased PAI-1 and decrease in PAI-2 (In normal pregnancy, both PAI-1&2 increased)

Decreased:

- Anti thrombin III
- Fibrinogen

Studies have shown an increased incidence of pre eclampsia with deficiencies of certain antithrombotic factors like protein S and activated protein C resistance (2-8 fold rise).[26]

Fibrinolytic System:

As a result of the endothelial damage, tissue plasminogen activator is released thus inducing the fibrinolytic system.

Erythrocyte destruction is evidenced by the presence of schistocytosis, spherocytosis, reticulocytosis and hemoglobinuria which are due to microangiopathic haemolysis[3].

Endocrine Changes:

In Pre Eclampsia the renin-angiotensin-aldosterone axis is said to be suppressed. In spite of a decrease in the aldosterone levels in PE, there is an increase in the sodium retention. This is explained by an increase in the deoxycorticosterone (which is formed from placental progesterone)[3].

Fluid and Electrolyte Changes:

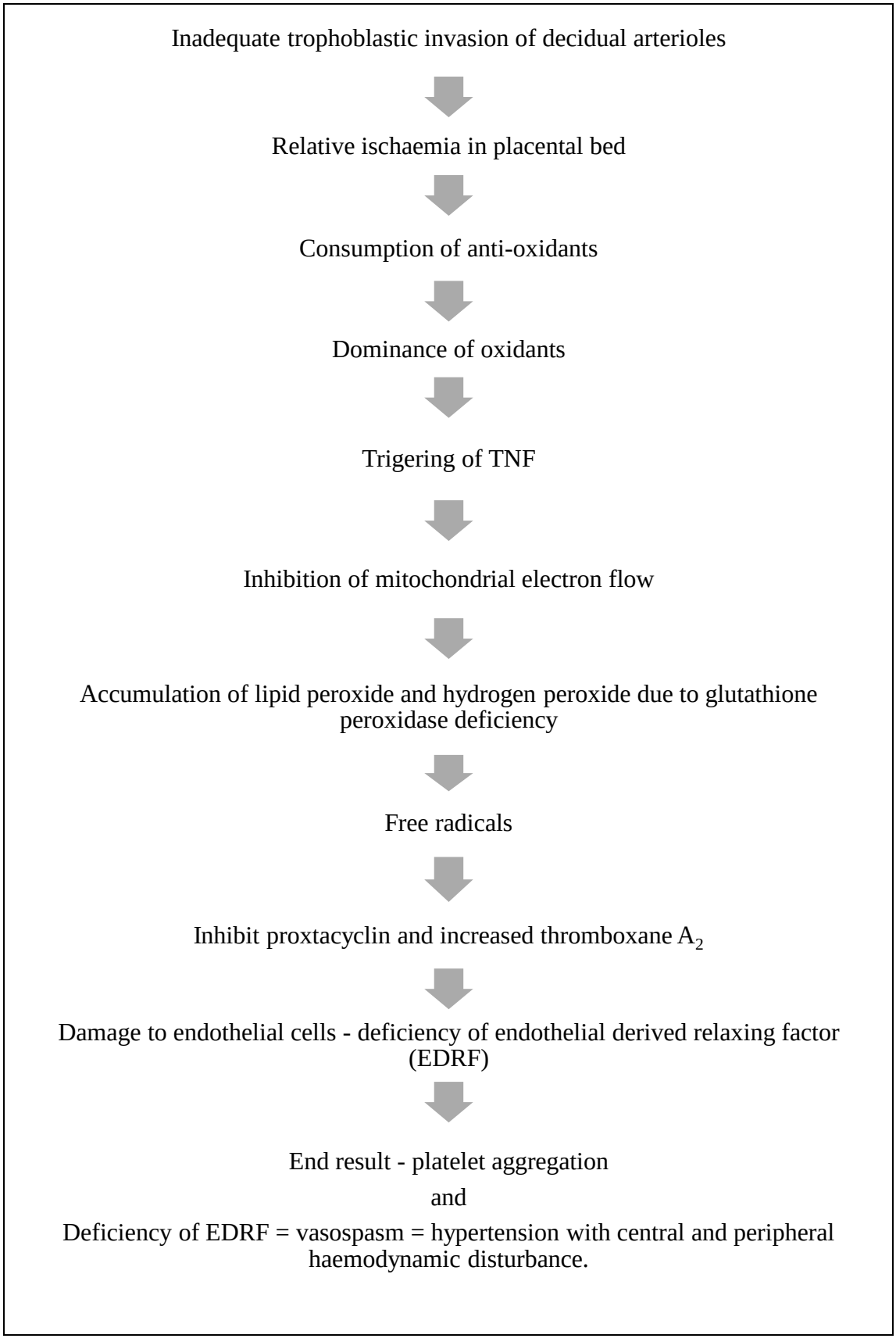
There is an increase in the extracellular fluid volume which occurs as result of endothelial injury → proteinuria → decrease in the colloid oncotic pressure → edema.

There is usually not much of electrolyte disturbances unless there is a fluid overload or excess administration of diuretics[3].

Metabolic changes:

Pre eclampsia may be the pregnancy associated expression of an underlying metabolic syndrome. High BMI is an established risk factor for preeclampsia especially if it is central obesity which is in turn related to insulin resistance.[17]↑ Insulin resistance → ↑FFAs, TGs → hyperlipidemia→Vascular damage

Figure 6: Sequence of pathophysiology in preeclampsia



PREDICTORS OF PIH

The various predictors of PIH can be broadly classified as non laboratory methods and laboratory methods.

I) NON-LABORATORY METHODS:

1. Parity and family history[3,27,28] : Historic and demographic factors can be used to assign a relative risk of pre eclampsia. The most easily ascribed risk factor is nulliparity. The incidence of pre-eclampsia ranges from 3% to 7% for nulliparas and 1% to 3% for multiparas.[29] Late pregnancy hypertension in 24.2% of first viable pregnancies was noted[30].

Angiotensinogen gene variant 7235 is associated with higher incidence of PIH. Other studies did not confirm this.[28,31]

2. Serial blood pressure measurements from early pregnancy : Recording of blood pressure in early pregnancy is of paramount importance and a normal blood pressure in early pregnancy, changing over to higher level from middle of pregnancy onwards raises the possibility of oncoming PIH. Even if the blood pressure remains below the internationally accepted level of 140 / 90 mm Hg, the serial rise of systolic pressure by 20 mm Hg and rise of the diastolic pressure by 10 mm Hg would warrant closer observation.[32]

3. Mean arterial pressure and 24 hour blood pressure monitoring in early pregnancy:

It was found that diastolic and mean blood pressure elevated between 9 and 12 weeks (P-0.01) with sensitivity ranging from 16% to 57% and specificity from 75% to 89%.[33] An increase in the DBP as early as 9 to 12 weeks was found.[34] A study arrived at a sensitivity of 8% and a positive predictive value of 23%, while the negative predictive value ranged from 81 % to 85%. The correlation between second-trimester blood pressure and subsequent development of pre-eclampsia has been confirmed by several others.[3,35]

Awake and sleeping blood pressure are higher in mid pregnancy in women who later experience the development of pre-eclampsia or gestational hypertension.[36,37]

4.Provocative Pressor test: The use of various pressor tests to predict development of preeclampsia dates to the landmark publications of Gant et al.

a) ***Angiotensin sensitivity test :*** The largest reported evaluation of the angiotensin sensitivity test was that of Kaul Lauson. A 20 mmHg rise in diastolic pressure was used as the definition of a response and the dosage required to achieve this response was termed the effective pressor dose. The overall results were similar to those of Gant et al in terms of sensitivity and specificity, but the predictive values were lower. However, because of the low practicability of the test, it may not be recommended as a screening method in routine prenatal care.[38,39,40]

b) **Roll over test*:*** The most studied pressor test was pioneered by Gant et al. It measures the hypertensive response in women between 28-32 weeks gestational age who are resting in left lateral position and then roll over to the supine position. Gant et al., reported that a normotensive woman at 18 to 22 weeks gestation are likely to develop hypertension later in pregnancy if her diastolic blood pressure rises by 20 mm Hg or more within 5 minutes after changing from the left lateral to the supine position. This is called the roll over test or supine pressor test. Data from studies direct that although the Roll over test is not a perfect predictor, its advantages recommend usage in population with high risk factors.[41,42]

c) **Hand grip test (isometric exercise test) *:*** Isometric exercise causes general sympathetic activation and increase systemic arterial pressure in a healthy adult.[43,44] In this test the subjects are required to compress an inflated cuff of sphygmomanometer for 3 minute period with maximal effort and 50% of maximal voluntary contraction. It employs the same principle by squeezing the ball. A rise of 25 mmHg in diastolic blood pressure is considered to be of positive predictive value. In conclusion, by use of a very simple Handgrip test early in gestation, we are able to predict PIH with the highest sensitivity.[41,45]

5. Forearm venous tone : Normotensive pregnancy was associated with progressive venodilatation. As the hypertension become manifest, the women

became relatively venoconstricted (P<.0001). It is a simple noninvasive method may be useful in the detection of women who are at increased risk.[46]

6. Uterine Artery Doppler velocimetry : Measurement of uteroplacental vascular resistance during Doppler ultrasound evaluation of uterine artery impedance in the second trimester has been used as an early screening test for preeclampsia.[47,48] A two-step screening test beginning at 18 to 22 weeks was used and its sensitivity for prediction of preeclampsia was 78 percent, but the positive predictive value was only 28 percent.[49]

Uterine artery Doppler velocimetry with serum levels of placental growth factor, activin A and fibronectin was evaluated and was found potentially useful as predictors of PE.[50]

II) LABORATORY METHODS:

1. Serum uric acid: One of the earliest laboratory findings of preeclampsia is hyperuricemia .Serum uric acid is said to be a sensitive indicator to assess the prognostic significance of fetal well being. Serum uric acid is a better indicator than blood pressure as an index of fetal prognosis. The use of serum uric acid as a prognostic index of fetal well being in preeclampsia is not well evaluated. There is contradictory information regarding their association. It was found to be predictive in a small case control study of high-risk women.[51] In contrast, it was found that serum uric acid levels did not vary significantly before the detection of hypertension in another study.[52] Another study showed hyperuricemia in patients with hypertensive disorders of pregnancy is a strong risk factor for several maternal and perinatal complications.[53]

2. Fibronectin : Endothelial cell activation is likely the cause of elevated serum cellular fibronectin levels in some women with preeclampsia.[54] A study did four week sampling beginning at 16 weeks in 378 low-risk nulliparas. The women who subsequently developed preeclampsia had significantly higher levels by 12 weeks but the positive predictive value was only 29 percent, however, the negative predictive value was 98 percent.[55]

3. Coagulation activation : A study found that high platelet volumes to be a marker of impending preeclampsia.[56] However, there was substantive overlap with normotensive women. In a case-control study, the PAI-1 : PAI-2 ratio was found to be predictive of preeclampsia in high-risk women.[51]

4. Oxidative stress : Increased level of lipid peroxides, coupled with decreased activity of antioxidants in women with preeclampsia, have raised the possibility that markers of oxidative stress might predict preeclampsia.[3] Other markers are a variety of pro-oxidants or potentiators of pro-oxidants, including iron, transferrin and ferritin; blood lipids, including triglycerides, free fatty acids, and lipoproteins and antioxidants, including ascorbic acid and vitamin E. Some of these have been studied clinically. It was reported that women with elevated serum homocysteine levels around mid pregnancy had a three to fourfold risk of preeclampsia. Although clinical studies substantiate this association, they have not shown elevated serum homocysteine levels to be a useful predictor.[57,58]

5. Cytokines : There are over 50 cytokines and a number of these are elevated in preeclampsia. A number of these have been evaluated in clinical trials but none have yet proved sufficiently predictive.[59,60]

6. Placental peptides : As a result of the inflammatory cascade, a number of peptides are produced by the placenta and some may prove to be markers for prediction of preeclamspia. Those studied include corticotropin releasing hormone, chorionic gonadotropin, activin A and inhibin A.[61,62] They are variably elevated and there is substantive overlap with normal pregnant women. It was reported that activin A and inhibin A were increased markedly in women who developed preeclampsia. Conversely, other investigators have reported significant overlap of activin A and inhibin A levels in normotensive and preeclamptic pregnancies.[63,64] The two placentally derived angiogenic factors VEGF and PLGF, regulate placental development. Excessively elevated serum levels of placental protein sFlt 1 have been reported in women with preeclampsia[65]. A study reported that PLGF levels in early pregnancy were not predictive.[66] Combined first-trimester serum levels of PLGF and sFlt 1 were highly predictive of subsequent preeclampsia.[67,68]

7. Fetal DNA : Maternal serum levels of cell-free fetal DNA were elevated at two stages. Screening for fetal DNA in earlier pregnancy may be predictive of subsequent preeclampsia, but that elevations after 28 weeks indicate impending disease.[69]

8. Urinary calcium/ creatinine ratio as a predictor of PIH: It is a well documented fact that in PIH glomerular damage does occur and it affects the renal hemodynamics. Various clearance tests are used to know the GFR. The degree of glomerular damage can be measured by studying GFR and evaluating endogenous creatinine clearance.[70]

Renal excretion of calcium is markedly increased during normal pregnancy. Urinary calcium excretion in normal pregnancy is 350-620 mg/day, compared to 100-250 mg/day in non pregnant women. Excretion usually increases during each trimester, with maximum levels reached during the third trimester.

Several recent studies have shown a marked reduction in the excretion of urinary calcium in patients with preeclampsia when compared to normotensive controls. It is found that urinary calcium excretion can be markedly decreased early in the course of preeclampsia, even before the clinical appearance of signs and symptoms.

The etiology of hypocalciuria in preeclampsia is unknown. It has been speculated that hypocalciuria may result from decreased dietary intake, decreased intestinal absorption, increased calcium uptake by the fetus and placenta, or intrinsic renal tubular dysfunction. The involvement of the renal system in PIH in the form of endotheliosis and the alteration in renal functions is the basis for using urinary calcium/creatinine ratio as a predictor of PIH.[71]

HOMOCYSTEINE

The words homocysteine and homocystines were coined by Du Vigneaud and co-workers who discovered these compounds 60 years ago, to designate respectively, the reduced (sulfhydryl) and the oxidized (disulfide) forms of these homologues of cysteine and cystine.

STRUCTURE OF HOMOCYSTEINE:

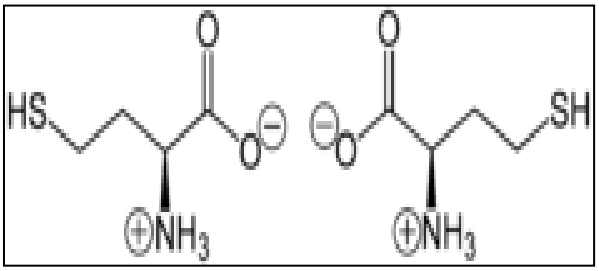


Figure 7: Structure of Betaline form of (S)-homocysteine (left) and (R)-homocysteine(right)

Introduction

The advent of simple assays has changed homocysteine measurement from a research tool to a standard and routine clinical test.

The history of homocysteine

Homocysteine was first described by Butz and du Vigneaud in 1932. Homocysteine is a sulphur containing amino acid that is closely related to the essential amino acid methionine and cysteine.

An association between elevated homocysteine levels and human disease was first suggested in 1962 by Carson and Nell.[72] They had found high homocysteine concentrations in the urine of some children with mental retardation. The elevated homocysteine levels in these patients were caused by severe enzyme defects blocking the homocysteine metabolism.

This condition, homocystinuria, was later found to be associated with premature occlusive cardiovascular disease, even in childhood, and about 25% of the patients die before the age of 30 of cardiovascular events.

In 1969, McCull[73,74] described the vascular pathology in these patients, including smooth muscle proliferation, progressive arterial stenosis, and haemostatic changes.

During the last 15 years it has been thoroughly documented that also moderately elevated homocysteine levels[75] in serum or plasma is a strong and independent risk factor for occlusive arterial disease, and of venous thrombosis. As many as 50% of patients with stroke, and other atherothrombotic disease have high homocysteine levels (over 15µmol/L).[76]

Plasma homocysteine is normally lower throughout pregnancy than in non pregnant state.[77] Homocysteine concentrations are directly correlated with albumin concentration, which decrease during pregnancy and further decrease in pregnant women taking folic acid supplementation. Vollset et al[78] reported that hyperhomocystenemia may be an important biological marker for adverse outcome in pregnancy and even possibly a cause of or a contributor to the complications of pregnancy. An increased risk of preeclampsia, preterm delivery, very low birth weight, neural tube defects and clubfoot occurs in women with hyperhomocysteinemia.[78]

Many studies[79] have also found an association between elevated homocysteine levels and impaired cognitive performance and dementia. Several prospective studies have now shown that folate and/or vitamin B12 status or elevated levels of homocysteine, even within the currently accepted reference range, predisposes for the development of dementia, or increases the rate of disease progress. An association with depression and other neuropsychiatric disorders[80] is also found. There is also much focus on the association between carcinogenesis and impaired homocysteine metabolism.

The recent identification of several, fairly common polymorphisms affecting the genes of enzymes participating in the homocysteine metabolism, and resulting in decreased enzyme activity has also lent the research some impetus. Some of these variations are shown to be associated with increased incidence of the above mentioned conditions, such as birth defects.

Plasma homocysteine is already a proven predictor of cardiovascular as well as non-cardiovascular morbidity and mortality. The next step will be to reduce the incidence of these conditions by monitoring homocysteine levels.

Biochemistry of homocysteine [81]

All homocysteine found in human organisms is formed during the metabolism of methionine in the methionine cycle. Homocysteine is metabolized through two pathways; re-methylation or trans-sulphuration.

METHIONINE CYCLE

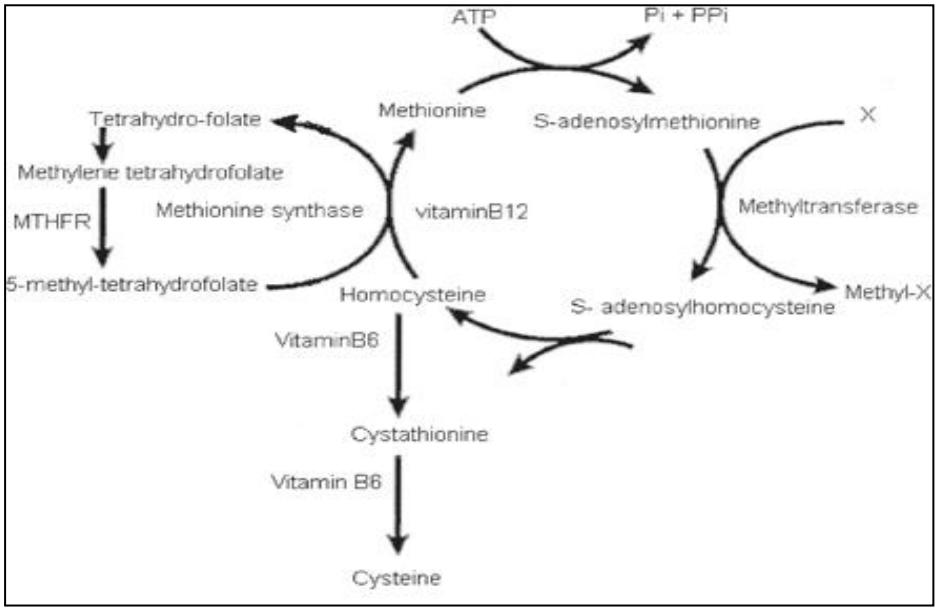


Figure 8: Metabolism of methionine and homocysteine

The remethylation of homocysteine is directly dependent on the enzyme methionine synthase to which vitamin B12 is a co-factor and methyl tetrahydrofolate (methylTHF) a substrate. This reaction is indirectly regulated by the activity of methylenetetrahydrofolate reductase (MTHFR), as this enzyme mediates the formation of methylTHF. This reaction therefore has a strong, indirect influence on the remethylation of homocysteine.

In a few tissues, predominantly the liver and kidneys, an alternative pathway for the re-methylation of homocysteine to SAM exists, but the

majority of tissues, including the CNS, are entirely dependent on the MethyleneSynthase - mediated recycling of homocysteine.

The remaining homocysteine is converted in trans-sulphuration pathway to cysteine in two reactions requiring vitamin B6 as a co-factor. Cysteine is a precursor to glutathione, the major cellular redox buffer. The trans-sulphuration pathway also directs Hcy to degradation and its ultimate removal as sulphate via urine.

Several common genetic variations of the gene for enzymes involved in the homocysteine metabolism are described in the scientific literature. A thermolabile form of MTHFR due to one such polymorphism (C677T polymorphism) affects 10-20% of most populations in its homozygote form. It results in decreased enzyme activity and causes moderately increased Hcy. The impact of tHcy is, however, dependent on folate status and tHcy can generally be normalized by increased folate intake.

The interplay between the individual genetic background and environmental factors in the pathogenesis of many disorders is currently the subject of intense research. The concept emerges that an individual's genetic make-up may substantially affect an individual's functional vitamin status.

Thus, disturbances in homocysteine metabolism, (either caused by deficiency of co-factor(s) or by some genetic enzyme defect) normally results in a cellular accumulation of homocysteine, with subsequent increase in Hcy levels in the circulation.

Homocysteine exists in several forms. The sum of all homocysteine forms is termed total homocysteine, abbreviated tHcy. During the last decade, several assays for tHcy have been developed. The recent introduction of enzyme immunoassays now allows determination of tHcy in most routine laboratories.

Why is homocysteine harmful?[81]

The methyl group of SAM is required for over 100 known reactions, including methylation of nucleic acids (DNA and RNA), proteins, phospholipids, myelin, polysaccharides, Choline and catecholamines.

It is understandable that reduced methylation capacity may have found effects on cellular growth, differentiation, and function. This may be critical in many situations, as in the ageing brain, where neurochemical processes related to methylation may be declining, in psychiatric and neurological diseases, for the rapidly growing fetus and infant, and also for carcinogenesis by reducing DNA repair. Studies on children with severe inborn errors resulting in defective methyl group synthesis, support the theory that deficient methylation is one of the leading causes of demyelination.

The synthesis of glutathione is dependent on the trans-sulphuration of homocysteine. Glutathione is an important endogenous antioxidant. It protects many cellular components against oxidative damage and other types of injury. Glutathione may also have protective vascular effects possible by interaction with nitric oxide.

Finally, certain forms of homocysteine itself are proposed to have oxidative effects and to react with proteins leading to protein damage.

Why do homocysteine levels increase?

Plasma tHcy increases throughout life in both sexes. Before puberty both sexes have low and similar levels (mean values of about 6 mmol/L). During puberty, levels increase, and more in boys than in girls. At the same time, tHcy distribution starts to show a distortion in certain populations with more high levels occurring.

Throughout life, mean Hcy increases by 3-5 mmol/L. At the age of 40-42, mean values are about 11 and 9 mmol/L in men and women, respectively.

After the menopause, the gender - related differences in tHcy diminish, but concentrations remain lower in women than in men. The gender disparity may be explained by hormonal status, more muscle mass in men and gender-related lifestyle differences.

During pregnancy, tHcy concentrations are reduced by up to 50%. Higher plasma volume, or increased metabolic rate and glomerular filtration, and fetal Hey metabolism may be the explanation.

The higher tHcy concentrations seen in the elderly[82] may be caused by many factors such a malabsorption owing to prevalent atrophic gastritis or insufficient nutritional supply of vitamins, lower nutritional intake, a slowdown of the metabolism, reduced kidney function and other physiological, age-related changes. Moreover, many drugs interact either by reducing the absorption of co-factors, or by increasing the catabolism of the vitamins. Certain diseases also influence the homocysteine metabolism. Nutritional and other lifestyle factors are important determinants of tHcy, and may explain the observed variation between different populations.

Smoking, high alcohol intake and coffee consumption also interact by increasing the catabolism of vitamins or reducing the absorption of them. Several other lifestyle factors are also of importance for the homocysteine metabolism. Lack of physical exercise, obesity and even stress, are also associated with hyperhomocysteinemia.[83] The plasma levels of tHcy are thus influenced by many factors.

Therefore, several factors may contribute to a patient's hyperhomocystenemia, even if vitamin status, primarily of folate, vitamin B12 and B6, is a major determinant. Enzyme defects[84], disturbed distribution of the vitamins, which are actively transported by means of specific transport proteins and receptors, interaction with lifestyle factors, diseases and drugs, or a combination, can thus impair the homocysteine metabolism with various disturbances as a consequence. Increased tHcy levels are a sensitive marker of such disturbances.

Many of the factors causing hyperhomocystenemia, for instance, unhealthy lifestyle factors, can be eliminated. The diagnosis of hyperhomocystenemia could therefore be used as an incentive of the patient to opt for a healthier lifestyle.

C) HOMOCYSTEINE AND ATHEROTHROMBOSIS INTRODUCTION

In 1969 McCully[73,82] made the clinical observation linking elevated plasma homocysteine concentrations with vascular disease. He reported autopsy evidence of extensive arterial thrombosis and atherosclerosis in children with elevated plasma homocysteine concentrations and

homocystinuria. On the basis of this observation, he proposed that elevated plasma homocysteine (hyperhomocystenemia) can cause atherosclerotic vascular disease. The term "homocysteine" is used to define the combined pool of homocysteine, mixed disulfides involving homocysteine, and homocysteine thiolactone found in the plasma of patients with hyperhomocystenemia.

Subsequent investigations have confirmed McCully's hypothesis and it has recently become clear that hyperhomocystenemia is an independent risk factor for atherosclerosis and atherothrombosis. Although severe hyperhomocysteinemia[85] is rare mild hyperhomocystenemia occurs in approximately 5 to 7 percent of the general population. Patients with mild hyperhomocystenemia have none of the clinical signs of severe hyperhomocystenemia or homocystinuria and are typically asymptomatic until the third or fourth decade of life when premature coronary artery disease develops, as well as recurrent arterial and venous thrombosis. Abundant epidemiologic evidence[86] has demonstrated that the presence of mild hyperhomocystenemia is an independent risk factor for atherosclerosis in the coronary, cerebral and peripheral vasculature. Although the molecular mechanism by which homocysteine or a related metabolite promotes atherothrombosis is unknown, the epidemiological evidence of the association of hyperhomocystenemia with atherothrombotic vascular disease is convincing.

Pathophysiological Mechanisms of Hyperhomocystenemia

Experimental evidence[87] suggests that the atherogenic propensity associated with hyperhomocystenemia results in endothelial dysfunction and injury followed by platelet activation and thrombus formation. Studies in humans[88] and animals demonstrate that homocysteine-induced atherosclerosis is characterized by substantial platelet accumulation and platelet-rich thrombus formation in areas of endothelial injury. Harker[89] and colleagues have proposed that homocysteine-induced endothelial injury exposes the sub endothelial matrix, which in turn leads to platelet activation. Lentz and colleagues have demonstrated that diet-induced hyperhomocystenemia in primates leads to impaired vasomotor regulation in vivo and endothelial antithrombotic function ex vivo. These findings are supported by the work of Celermajer and colleagues, who demonstrated impaired endothelium-dependent

vasodilatation, and also by van den Berg and colleagues[90], who demonstrated impaired endothelial anticoagulant function in young patients with hyperhomocystenemia and peripheral vascular disease. Although the exact mechanism of endothelial dysfunction is unknown, there is growing evidence that homocysteine exerts its effects by promoting oxidative damage.

Homocysteine is rapidly auto-oxidized when added to plasma forming homocysteine, mixed disulfides and homocysteine thiolactone. Potent reactive oxygen species, including super oxide and hydrogen peroxide, are produced during the auto-oxidation of homocysteine, and hydrogen peroxide (along with the hydroxyl radical) in particular, has been implicated in the vascular toxicity of hyperhomocyst(e)inemia. There is extensive evidence that homocysteine(e)ine-induced endothelial - cell injury in vitro is largely due to the generation of hydrogen peroxide. Harker[89] and colleagues have proposed that homocysteine induced endothelial cell injury mediated by hydrogen peroxide exposes the underlying matrix and smooth muscle cells, which in turn proliferate and promote the activation of platelets and leukocytes.

Auto-oxidation of homocysteine produces other cytotoxic reactive oxygen species, including the superoxide anion radical and hydroxyl radical.[91]Superoxide-dependent formation of the hydroxyl radical has been shown to initiate lipid peroxidation, an effect that occurs at the level of the endothelial plasma membrane and within lipoprotein particles. Homocysteine auto-oxidation has been shown to support the oxidation of low-density lipoprotein through the generation of the superoxide anion radical.

Although the precise molecular mechanism is unknown, homocysteine causes endothelial dysfunction at several levels. Homocysteine alters the normal antithrombotic phenotype of the endothelium by enhancing the activities of factor XII and factor V and depressing the activation of protein C. Homocysteine also inhibits the expression of thrombomodulin, induces the expression of tissue factor, and suppresses the expression of heparan sulfate by the endothelium. All of these effects ultimately facilitate the formation of thrombin and create a prothrombotic environment.

The production of endothelial-derived nitric oxide is also adversely affected by homocysteine. Our group has previously shown that normal endothelial cells detoxify homocysteine by releasing nitric oxide, which combines with homocysteine in the presence of oxygen to form S.nitroso-homocysteine. Nitrosation of the sulfhydryl group of homocysteine inhibits sulfhydryl-dependent generation of hydrogen peroxide. S-nitroso-homocysteine is also a potent platelet inhibitor and vasodilator. This protective effect of nitric oxide is eventually compromised as long-term exposure to hyperhomocyst(e)inemia damages the endothelium sufficiently to limit nitric oxide production. Impaired endothelial production of nitric oxide leave the endothelium vulnerable to unopposed homocysteine-mediated oxidative injury. Homocysteine may also decrease the bioavailability of nitric oxide by impairing its synthesis. Homocysteine promotes lipid peroxidation, which may subsequently decrease the expression of endothelial nitric oxide synthase and directly degrade nitric oxide. Studies have recently shown that homocysteine (but not cysteine) suppresses the expression of cellular glutathione peroxidase by endothelial cells, and this effect promotes lipid peroxidation by the reactive oxygen species elaborated during the oxidation of homocysteine.

In addition to promoting atherosclerosis through endothelial injury or dysfunction homocysteine is also a potent mitogen for vascular smooth-muscle cells. Harker and colleagues demonstrated that infusion of homocysteine into baboons results in the formation of atheromata Exposure of homocysteine leads to a marked increase in vascular smooth muscle proliferation in vitro, an effect that is due in part to an increase in the expression of messenger RNA of cyclin D1 and cyclin A. It has been recently demonstrated that homocysteine increased nitric oxide production in vascular smooth-muscle cells by activating the transcription factor NF-κB. It appeals that NF-κB is activated by a homocysteine generated reactive oxygen species. Since NF-κB activity is essential for the proliferation of vascular smooth-muscle cells, these data suggest that homocysteine-mediated activation of NF-κB contributes to the mitogenic effect of homocysteine.

Homocysteine also directly damages the vascular matrix by affecting the biochemical and biosynthetic functions of vascular cells. Homocysteine

thiolactone, a highly reactive anhydrous by product of homocysteine oxidation, combines with low-density lipoprotein to form aggregates that are taken up by intimal macrophages and incorporated into foam cells within nascent atheromatous plaques. There is however, some doubt that thiolactone can form in sufficient concentrations in vivo to evoke these effects. Recently. Jakubowski[92] showed that cells deficient in cystathionine β-synthase produce more homocysteine thiolactone in culture than normal cells and that the thiolactone is incorporated into cellular and secreted proteins through lysine acylation by the activated carboxyl group of the thiolactone. McCully[82] has suggested that in this microenvironment homocysteine thiolactone facilitates the conversion of mitochondrial thioretinaco ozonide to thiolactone, thereby impairing oxidative phosphorylation and promoting the proliferation and fibrosis of smooth muscles. This homocysteine induced disturbance in oxidative metabolism also leads to overproduction of oxidative radicals that subsequently induce intimal injury, activate elastase and increase calcium deposition. Homocysteine may also contribute the deposition of sulfated glycosaminoglycan in the matrix; it appears that the sulfur group of homocysteine thiolactone is incorporated into phosphoadenosine phosphosulfate, which ultimately leads to the formation of sulfated glycosaminoglycans.

The recently observed multiplicative increase in the risk of vascular disease in the presence of traditional risk factors and hyperhomocyst(e)inemia may in part be related to the effect of homocysteine on lipid peroxidation. The vascular cytotoxicity of oxidized low-density lipoprotein has been linked to its content of lipid peroxidation products. Homocysteine increased the formation of highly atherogenic oxycholesterols, increase lipid peroxidation, and increased the oxidation of low-density lipoprotein in vitro. These observations suggest a potential role of antioxidant therapy in ameliorating homocysteine dependent oxidative vascular injury, however, this therapeutic approach has not yet been tested in prospective clinical trials.[93]

Association of hyperhomocysteinemia and pre-eclampsia.

One of the most favoured hypothesis of the aetiology of pre-eclampsia is the endothelial dysfunction secondary to the peroxidation of membrane

lipids. Decreased antioxidant activity and increased lipid peroxides was shown clearly in pre-eclampsia.

Serum concentrations of homocysteine decrease during normotensive pregnancy parallel to the physiologic fall of albumin concentration and folic acid supplementation, but increases in pre-eclampsia like pregnancy complications.[94,95,96] Hyperhomocysteinemia here might be a cause rather than just a marker of adverse pregnancy outcome. In a study conducted on early pregnancy losses, hyperhomocystinuria was shown to decrease total vessel surface and hence to disrupt placental perfusion. [97]

In normal endothelium, nitric oxide (NO) suppresses the smooth muscle proliferation in vessel walls. Decreased NO activity by the effect of homocysteine might contribute to the pathology in those patients.

Hyperhomocysteinemia increases the risk of atherosclerosis through a mechanism involving oxidative damage. When added to the plasma, homocysteine is readily oxidized to form homocysteine, homocysteine mixed disulfides and homocysteine thiolactone leading to the formation of oxygen radicals and lipid peroxidation. [98]

Endothelial cells detoxify homocystein by NO and S-nitrosothiol compounds. In addition, hyperhomocysteinemia decreases NO production probably through increasing synthesis of asymmetric dimethylarginine which is an endogenous inhibitior of NO synthesis.[99,100] S-nitrosylation decreases the oxygen radical producing capacity of homocysteine and converts this molecule to a potent vasodilator, S-nitroso-homocysteine. This protective effect of NO is attenuated in long term leaving endothelium more susceptible to oxygen radicals. Increased concentrations of oxygen radicals also decrease the bioavailability of NO, which amplifies their effect on endothelium. Homocysteine, on the other hand decreases the activity of glutathione peroxidase enzyme, which plays an important role in the detoxification of lipid peroxides. As a result, endothelial dysfunction worsens.[98]

It is speculated that high concentration of homocysteine in preeclampsia is associated with increasing total oxidant and decreasing antioxidant activities

which might be the mechanism of endothelial injury and hence vasospasm.[101,102]

It has been speculated that, folic acid supplement could prevent the unwanted effects of homocysteine in pre-eclampsia.[103,104]

TREATMENT OF HYPERHOMOCYSTEINEMIA

Homocysteine lowering therapy

Vitamin supplementation (folate, B12 and B6) appears to be safe and efficient in reducing plasma Hcy levels. Use of vitamin is associated with lowered risk of vascular disease in general population.

Several studies[89] have demonstrated that 0.65 to 10mg folic acid per day along and together with vitamin B12 and/or vitamin B6 reduce the fasting and post, methionine load plasma Hcy levels by 25-50 percent both in healthy subjects and those with vascular disease.

In a study by Ubbink et al[92] 100 men with hyperhomocysteinemia (>16.3 umol/L) were randomly assigned to five groups and treated with a daily dose of placebo, folic acid (0.65mg), vitamin B12 (0.4 mg), vitamin B6 (10 mg) or a combination of the three vitamins for six weeks. The results showed that folic acid supplement reduced plasma Hcy by 41.7 percent, whereas daily vitamin B12 supplement lowered Hey levels by 14.8% and both were significant. The daily vitamin B6 dose did not significantly reduce plasma Hey levels. The combination of the three vitamins reduced Hcy levels by 49.8 percent, which was not significantly different from the reduction achieved by folate supplement alone. The results suggest that folate deficiency is the major cause for the elevated plasma Hcy levels in Western population.

Generally, a folate intake from food and supplements 200-250 µg/day is occasionally associated with hyperhomocysteinemia. Whereas, an intake of 300-400 µg/day ensures normal to low plasma Hcy in majority of the subjects. A higher concentration may be necessary in patients with reduced renal function and folate deficient subjects.

Food and Drug Administration[84] USA (FDA) made it mandatory that all the cereal grain products have to be folate fortified with 140 µg per 100 g product. A significant reduction in plasma Hcy concentration was observed after feeding breakfast cereal products fortified with folic acid in patients with coronary heart disease.

REVIEW

- The study was conducted on 90 women, Study group I consisted 30 pregnant normotensive women; study group II consisted 30 pregnant women with PE. Serum homocysteine was measured in all subjects using fluorescence polarization immunoassay. Results showed control group had the highest mean homocysteine levels, while the study group I had least mean homocysteine levels ($p<0.001$). Levels were significantly higher in subjects with BP > 146/100 mmHg as compared to those with BP > 140/90mmHg and <146/100mmHg ($p=0.017$). Hyperhomocysteinemia was observed in pre-eclamptic females; it was also found that homocysteine levels were directly correlated with severity of preeclampsia.[105]
- 8 studies measured total serum homocysteine concentration before the clinical onset of preeclampsia (1876 women), whereas 17 studies measured it after the onset (1773 women). Overall, there were higher serum homocysteine concentrations among pregnant women with preeclampsia than among those with uncomplicated pregnancies.[106]
- Plasma homocysteine was measured in 40 nulliparas, 20 Preeclampsia cases and 20 cases without preeclampsia at the time of their delivery. Mean plasma homocysteine levels in the 20 nulliparous women with preeclampsia were significantly higher than in the 20 nulliparous women without preeclampsia (8.66 ± 3.05 versus 4.99 ± 1.11 µmol/L, $p<0.001$).[107]
- A case control study comparing non pregnant women with an obstetric history of placental vasculopathy (study group - 101 women) with non pregnant women (control group 92 women) matched for age and occupation. Increased risk for placental vasculopathy was found in the study group with elevated homocysteine.[108]
- Antepartum blood samples were collected > or = to 6 hours after the last meal from 33 women with normal, uncomplicated pregnancies and 21 women with preeclampsia. The mean value of total plasma homocysteine in PE was significantly higher than that observed in normal pregnancy ($p<0.4$).[109]

- Plasma homocysteine concentration was determined for all available stored samples at 26 and 37 weeks gestation. Mean homocysteine levels in women with PIH and PE were similar to those of the control subjects at 26 weeks gestation but were significantly higher at 37 weeks gestation. II trimester plasma homocysteine concentration do not predict the subsequent development of PIH and PE.[110]
- In a cross sectional study including 155 normal women, homocysteine was measured in the I, II and III trimesters (study) and in non pregnant women (controls). Serum homocysteine concentration decreases during pregnancy (when compared with non pregnant controls) which occurs in association with physiological fall in albumin during pregnancy as well as with folic acid supplementation.[111]
- A study conducted on 26 normal pregnancies and 60 pregnancies with evidence of placental vascular disease i.e., 19 → preeclampsia, 17 → umbilical placental vascular disease and 24 → with both. The maternal plasma homocysteine levels were significantly higher in all 3 affected groups, compared with the normal pregnancy group.[112]
- Study on 1049 nulliparous women was done from whom serum was collected at 16 weeks gestation. At 16 weeks gestation, concentration of homocysteine in women who developed PE (6.99 µmol/L), was similar to those who remained normotensive (6.91 µmol/L).[113]
- A prospective study of 28 preeclampsia patients matched with 26 normal controls of the same gestational age found that the mean levels of homocysteine were significantly elevated in the preeclamptic than in control group (11.11 v/s 6.40 µmol/l, $p<0.001$). There were no differences between the groups regarding the levels of folic acid (11.12 V/s 9.73 ng/ml $p=0.55$) and vitamin B12 (295.76 Vs 356.15 pg/ml) $p=0.43$.[114]
- Antepartum blood samples were collected ≥ 6 hours after the last meal from 33 women with normal, uncomplicated pregnancies and 21 women with preeclampsia. The plasma sample was analyzed for concentration of total homocysteine.The mean value of total plasma homocysteine in preeclampsia was significantly higher than that observed in normal

pregnancy and it is suggested that homocysteine plays a role in promoting endothelial dysfunction in pre-eclampsia.[115]

- Venous blood samples of 20 preeclamptic women and 20 healthy pregnant controls were collected. Plasma malondialdehyde and homocysteine concentrations were measured and the correlation between them was investigated. Plasma malondialdehyde and homocysteine concentrations were higher in pre-eclamptic patients ($p<0.05$) and a positive correlation between these parameters was found (r 0.777, $p<0.01$, n:20).[116]
- Studies conducted by Metin Ingec etal., has shown an increased plasma Hcy concentration in severe preeclampsia and eclampsia, but not in mild preeclampsia.[117]
- Cross sectional study done by Khosrowbeygi A etal., showed an increase in maternal serum levels of total Hcy in both mild and severe preeclampsia compared with normal pregnancy and elevated Hcy concentrations with the severity of preeclampsia.[118]
- A cross sectional study carried out by Ferdausai et al, A total number of 50 PE patient [Severe PE (23) & Mild PE (27)] and 50 pregnant women without PE were selected. Fasting serum total homocysteine (tHcy) concentration was estimated by fluorescence polarization immunoassay (FPIA) method. Mean serum homocysteine concentration in severe PE, mild PE and pregnant women without PE were 11.5 ± 4.58 mol/L, 10.43 ± 5.12mol/L and 5.70± 1.30mol/L respectively. Serum homocysteine was significantly increased in severe PE and mild PE in comparison to without PE group . However severe PE and mild PE group cases did not differ with respect to serum homocysteine.[119]
- Karunashree etal., in their case control study conducted in antenatal ward during 2007-2008 included group A of 50 pregnant women with preeclampsia and group B of 50 normotensive pregnant women. Serum homocysteine levels were measured by ELISA method and they observed homocysteine levels to be significantly higher in pregnancy with preeclampsia which resulted in significant effect on pregnancy outcome. [120]

MATERIALS AND METHODS

Source of data :

In the present study, a total number of 90 Pregnant women were included out of which 45 were Preeclampsia(cases) and 45 were normotensive pregnant women(control) who attended Department of Obstetrics and Gynecology in R L Jalappa Hospital and Research Centre, attached to Sri Devaraj Urs Academy Of Higher Education, Tamaka, Kolar between March 2015 to July 2016.

Study design -- Case control study

Sample size : 90 - 45 control group

- 45 study group

Collection of data :

Data was collected by patient evaluation, which was done by detailed history taking and clinical examination through structured proforma specially designed for this study.

All 90 pregnant women were primi gravidae and divided into 2 groups.

45 – pregnant women with pre-eclampsia (study group).

45 – normotensive pregnant women (control group)

Inclusion criteria :

1) Preeclampsia defined as blood pressure constantly greater than 140/90 mmHg with proteinuria with no urinary tract infection and with no previous history of hypertension.

2) Primigravida with singleton pregnancy and gestational age of 28-40 weeks.

Control group :

The control group includes normotensive primigravida with gestational age beyond 28weeks.

Study group :

All primigravida beyond 28 weeks of gestation with preeclampsia diagnosed as per National High Blood Pressure Education Programme working group (NHBPEP) Classification were included in the study group.

Exclusion criteria :

Pregnant women with

- Diabetes mellitus
- Chronic hypertension
- Renal or liver disease
- H/o thromboembolism
- Neural tube defects
- Repeated miscarriage
- Abruptio placenta
- Preterm labor and delivery
- H/o smoking
- H/o previous medical illness.
- Anemia
- Multigravida
- Gestational hypertension
- Women who were on folic acid and Vit B12 supplements.

The subjects in the two groups were age and gestational age matched, were included in the study after obtaining the ethical committee approval of the institute and patient information consent. A standard proforma was used to collect the data.

Subjects in control and study group underwent detailed clinical examination and following investigations.

- Urine for albumin, sugar and microscopy.
- Complete hemogram
- Blood grouping and Rh typing
- HIV and HBsAg
- RBS, blood urea, serum creatinine, serum uric acid

- Liver function tests (if required)

Special investigation:

- Serum homocysteine levels.

Specimen collection

Five ml of fasting blood samples are collected in plain vacutainer from control and preeclamptic subjects. Samples are centrifuged at 3000 x g to separate serum and stored at -20°C in ultra-freezer until analysis. Homocysteine is measured by ELISA method.

Estimation of serum homocysteine level

The method used for assay of serum homocysteine level was Enzyme Linked ImmunoSorbent Assay (ELISA) with the aid of Micro ELISA plate reader.

Assay method :

The kit uses a double- antibody sandwich enzyme- linked immunosorbent one-step process assay(ELISA) to assay the level of homocysteine (Hcy) in samples.

Assay procedure:

All specimens and reagents were allowed to reach room temperature at 25ºC and mixed thoroughly by gentle inversion before use. Reagents were prepared by 20× dilution of washing buffer : distilled water, diluted by 1: 20,or 1 copy of the 20× washing buffer plus 19 copies of the distilled water. The micro plates were marked, 50µl of the standard was added to 6 standard wells and 40µl of special diluent added to sample wells before adding 10µl of samples of controls and cases. Then 50µl of Horseradish peroxide(HRP) is pipetted into each well. The plate was sealed and gently shaked, then incubated at 37°C for 60 minutes. Automatic washing method was used which discards excess liquid, followed by drying and filling each well with diluted washing liquid. This washing method is repeated for 5 times. Then 50µl of chromogen solution A later 50µl of chromogen B were added. The plate was incubated again for 10 minutes at 37°C. Finally stop solution of 50µl was added into each well to stop the reaction (the blue color changed to yellow immediately).The optical density (OD) was measured at 450nm wavelength within 15 minutes after adding stop solution. Calculated the standard curve linear regression equation and the

corresponding OD values of the sample on the regression equation to calculate the corresponding sample's concentration. Final result was multiplied by five times.

STATISTICAL ANALYSIS:

Data was entered into Microsoft excel data sheet and was analyzed using SPSS 22 version software. Categorical data was represented in the form of Frequencies and proportions. **Chi-square test of Fischer's exact test** (for 2x2 tables only) was used as test of significance for qualitative data.

Continuous data was represented as mean and standard deviation. **Independent t test or Mann Whitney U test** was used as test of significance to identify the mean difference between two quantitative variables and qualitative variables respectively.

Graphical representation of data: MS Excel and MS word was used to obtain various types of graphs such as bar diagram, Pie diagram and Scatter plots.

p value (Probability that the result is true) of <0.05 was considered as statistically significant after assuming all the rules of statistical tests.

Pearson correlation or Spearman's correlation was done to find the correlation between two quantitative variables and qualitative variables respectively.

Correlation coefficient (r)	**Interpretation**
0 - 0.3	Positive Weak correlation
0.3-0.6	Positive Moderate correlation
0.6-1.0	Positive Strong correlation
0 to (-0.3)	Negative Weak correlation
(-0.3) to (-0.6)	Negative Moderate Correlation
(-0.6) to – (1)	Negative Strong Correlation

Statistical software: MS Excel**,** SPSS version 22 **(**IBM SPSS Statistics, Somers NY, USA) was used to analyze data. EPI Info (CDC Atlanta), Open Epi, Med calc and Medley's desktop were used to estimate sample size, odds ratio and reference management in the study.

RESULTS

The present study is carried out from March 2015 to July 2016 to estimate serum homocysteine levels in pre-eclampsia and its relation to severity and perinatal outcome. The total of 90 cases were studied and results obtained are presented as here under:

Table 1: Characteristics of control and study groups.

Parameter	Control group (n=45)	Study group (n=45)	p value
Age (years)	24.8± 3.2	23.9 ± 3.3	0.114
Gestational age (weeks)	37.5±1.4	37.3± 1.2	0.523
Systolic Blood pressure(mmHg)	113.1±7.8	150.5± 11	< 0.001*
Diastolic Blood pressure (mmHg)	74.4 ± 4.6	103±11.3	< 0.001*

Results are presented as Mean ± SD.

Table 1 shows there is no significant differences in age , gestational age between control and study groups. The mean age in control group was 24.8 years and in study group was 23.9 years. Women in study group had higher mean systolic and diastolic blood pressure ($p < 0.001$).

Table 2: Age distribution of subjects between two groups

		Groups			
		Control group		Study group	
		No. of Subjects (n)	Percentage(%)	No. of subjects(n)	Percentage(%)
Age (years)	20	5	11.1%	11	24.4%
	21 to 25	20	44.4%	22	48.9%
	26 to 30	20	44.4%	12	26.7%

$\chi 2 = 4.345$, df = 2, p = 0.114

Majority of subjects in both the groups were in the age group 21 to 25 years. In control group,44.4% of the subjects and 48. 9% subjects of study group belong to 21- 25 years age group. There was no significant difference in age distribution between two groups.

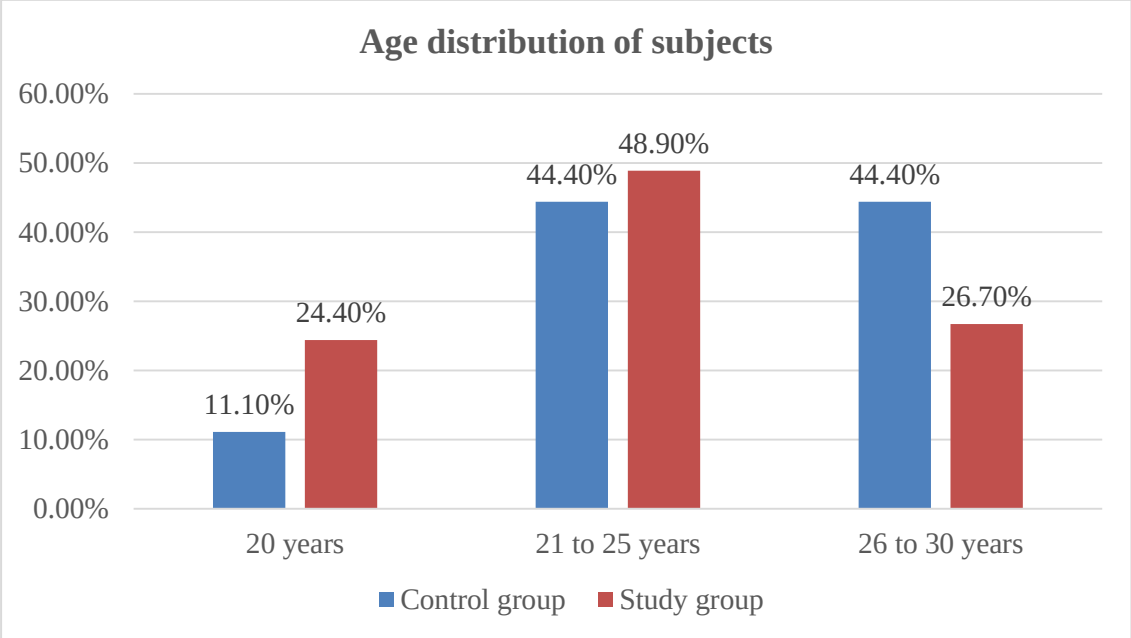


Chart 1: Age distribution of subjects between two groups

Table 3: Gestational age between two groups

		Gestational age (weeks)	P value
		Mean ±SD	
Groups	Control group	37.5 ± 1.4	0.523
	Study group	37.3 ±1.2	

Mean period of gestation among controls was 37.5 ±1.4 weeks and in cases was 37.3 ± 1.2 weeks. There was no significant difference in period of gestation between two groups.

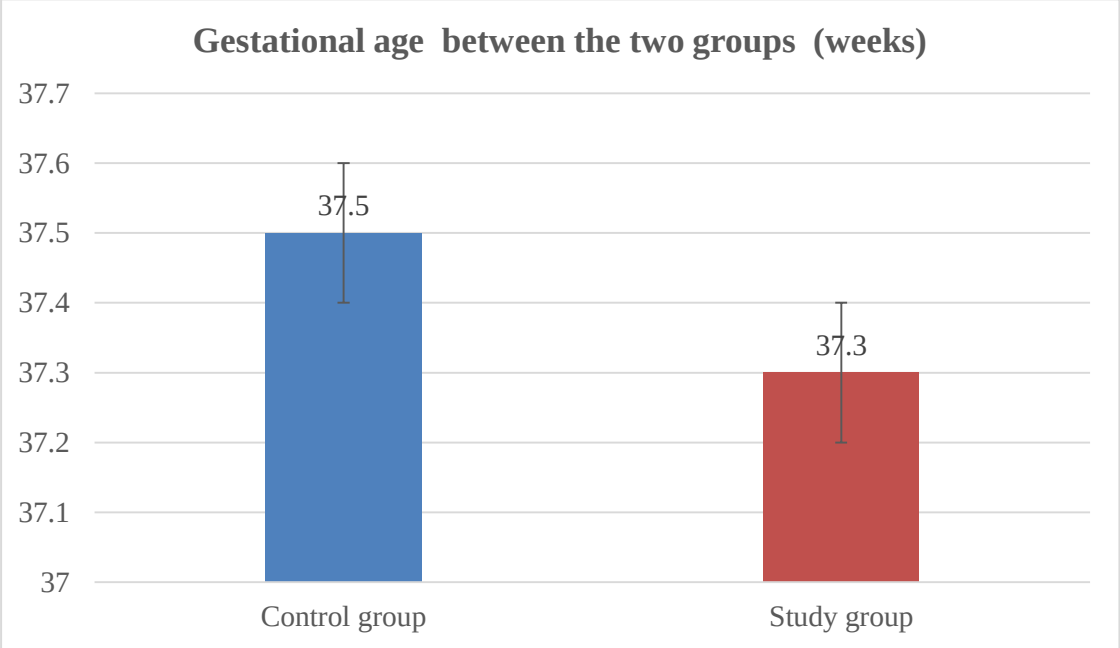


Chart 2 : Bar diagram showing Period of Gestation between two groups

Table 4: Mode of Delivery between two groups

		Group(n=90)			
		Control Group(n=45)		Study Group (n=45)	
		n	%	n	%
Mode of Delivery	Vaginal delivery	40	88.9%	40	88.9%
	LSCS	5	11.1%	5	11.1%

$\chi 2 = 0.00$, df = 1, p = 1.000

In both cases and controls 88.9% delivered at term vaginally and 11.1% were delivered by LSCS. This was no difference in mode of delivery between two groups.

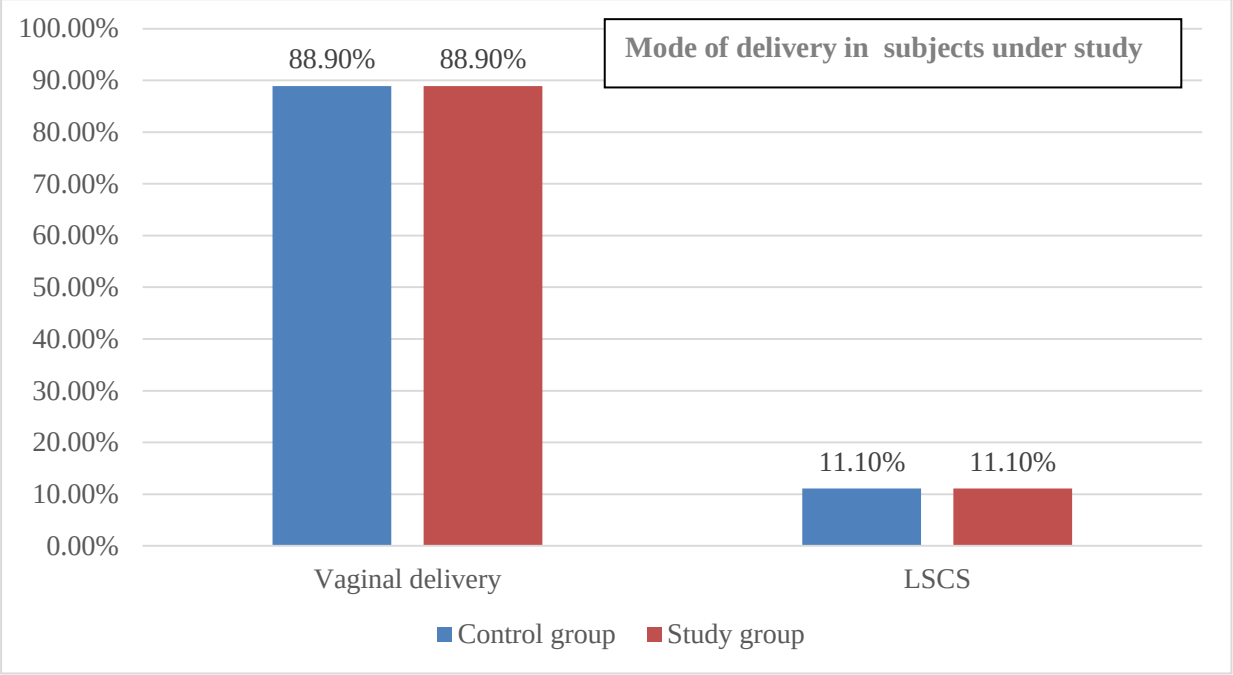


Chart 3: Bar diagram showing Mode of Delivery between two groups

Table 5 : Distribution of study group based on Severity of preeclampsia

		Study group (n=45)	
		No. of subjects(n)	Percentage (%)
Preeclampsia severity	Mild	20	44.4%
	Severe	25	55.6%

Total number of subjects in study group were 45.Among them, 44.4% had mild preeclampsia and 55.6% had severe preeclampsia.

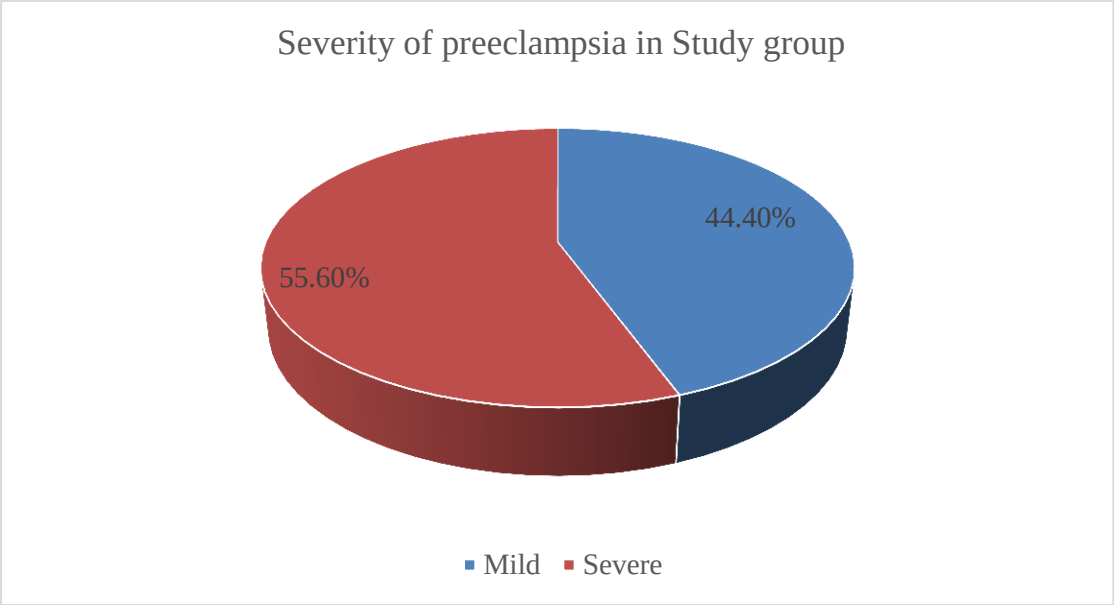


Chart 4: Pie diagram showing Severity comparison between two groups

Table 6 : Comparison of Serum Homocysteine levels between two groups

		Serum Homocysteine(µmol/l)	P value
		Mean ± SD	
Groups	Control group	7.9 ± 1.3	<0.01*
	Study group	17.3 ± 4.4	

Mean Homocysteine levels in controls was 7.9 ± 1.3µmol/l and among cases was 17.3 ± 4.4µmol/l.. This difference in mean homocysteine levels was statistically significant. Higher homocysteine levels was observed in cases than controls.

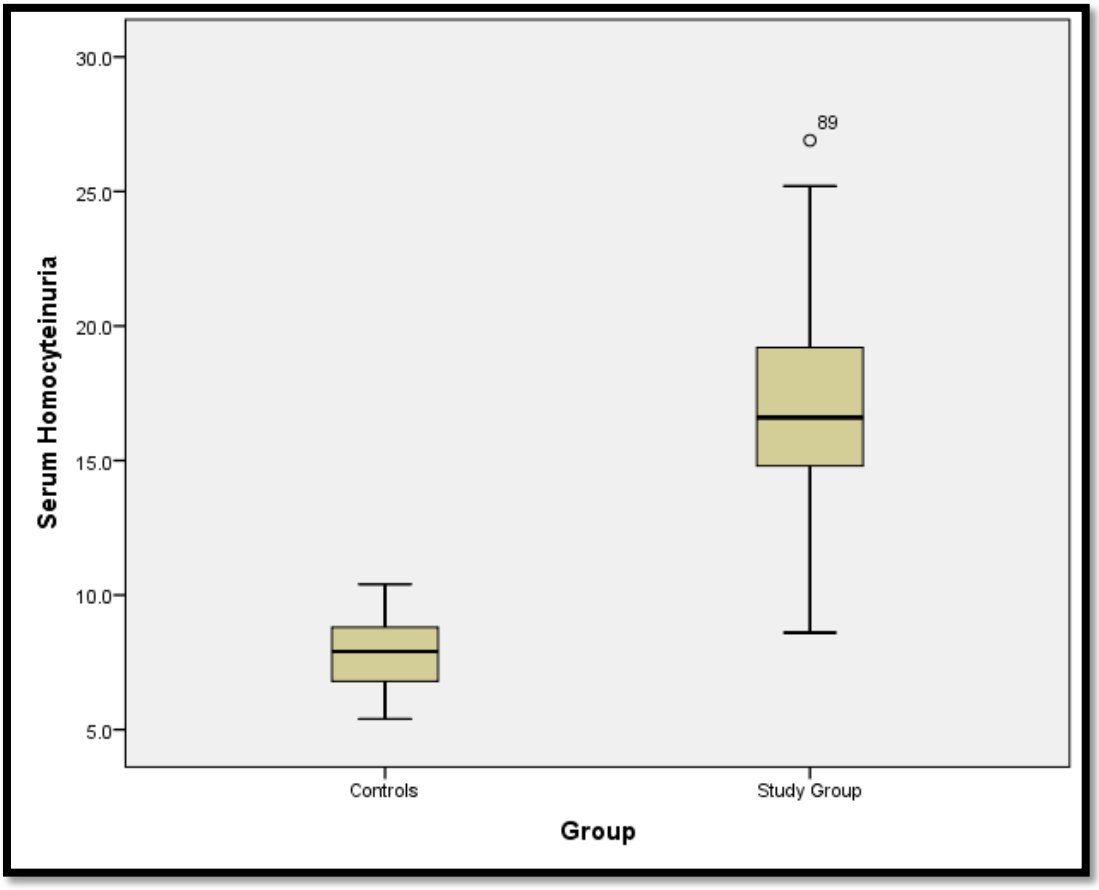


Chart 5: Box plot showing Serum Homocysteine levels comparison between two groups

Table 7: Comparison Mean serum Homocysteine levels between controls, mild and severe preeclampsia

GROUP	Serum Homocysteine levels (µmol/l)
	Mean ± SD
Control	7.9±1.3
Mild preeclampsia	14.5±3.2
Severe preeclampsia	19.6±3.9
p value	**< 0.001**

Table 7 and Chart 6 shows :Mean Homocysteine among controls was 7.9 ± 1.3µmol/l, among subjects with mild preeclampsia 14.5 ± 3.2µmol/l and among subjects with severe preeclampsia 19.6 ± 3.9µmol/l This difference in mean serum Homocysteine levels was statistically significant.

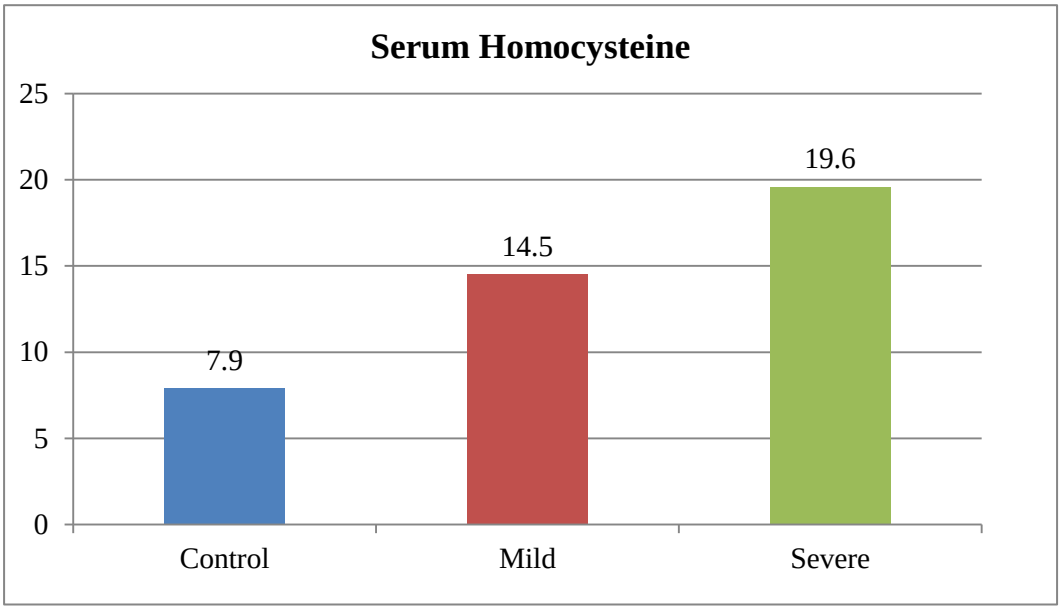


Chart 6: Bar diagram showing Mean serum Homocysteine levels in control group, mild and severe preeclampsia groups

Table 8: Correlation between Serum Homocysteine levels and Period of gestation, SBP and DBP in controls

Control Group		Serum Homocysteine	Period of gestation	SBP	DBP
Serum Homocysteine	Pearson Correlation	1	-0.272	-0.149	0.072
	P value		0.071	0.328	0.638
	N	45	45	45	45

There was negative correlation between Homocysteine and SBP among controls, and slight positive correlation was observed between homocysteine and DBP. However there was no significant correlation between them in control group.

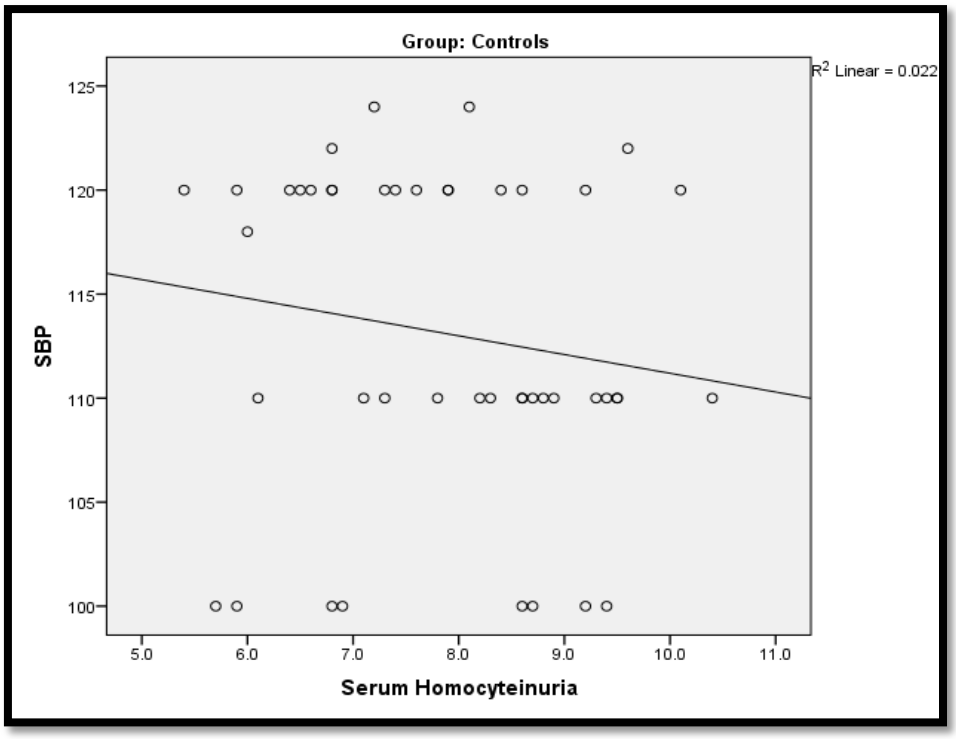


Chart 7: Scatter plot showing correlation between serum homocysteine and SBP in controls

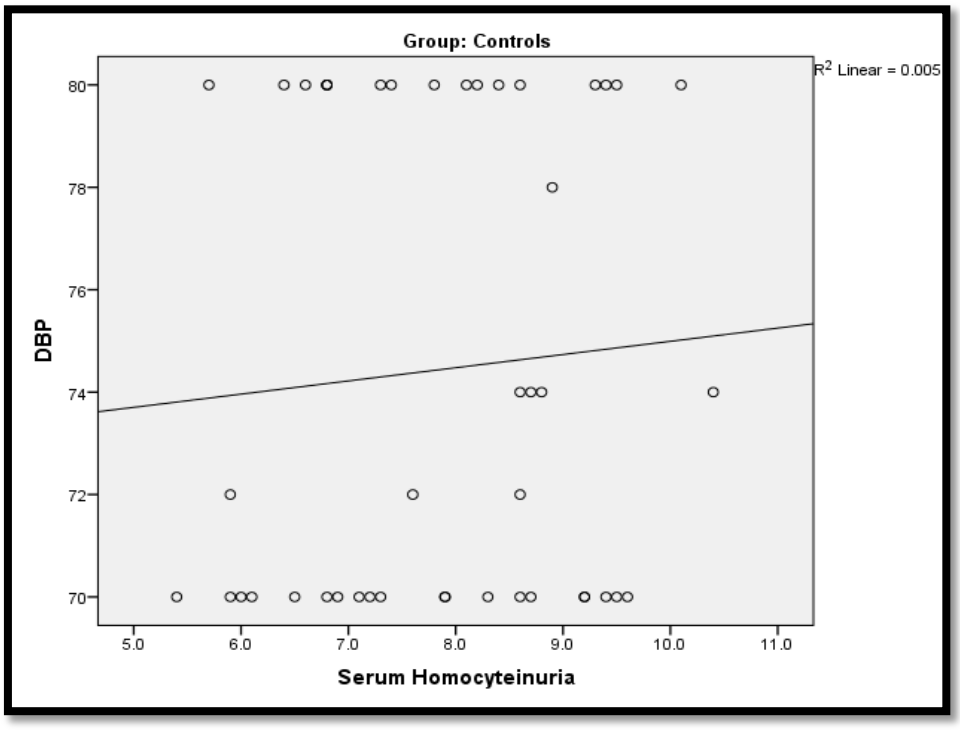


Chart 8: Scatter plot showing correlation between serum homocysteine and DBP in controls

Table 9: Correlation between Serum Homocysteine levels and Period of gestation, SBP and DBP in Study group

Study Group		Serum Homocysteine	Period of gestation	SBP	DBP
Serum Homocysteine	Pearson Correlation	1	0.023	0.454**	0.544**
	P value		0.878	0.002*	<0.001*
	N	45	45	45	45

There was significant positive correlation between Homocysteine and SBP and DBP among cases. i.e. with increase in SBP and DBP there was significant increase in Serum homocysteine levels in study group.

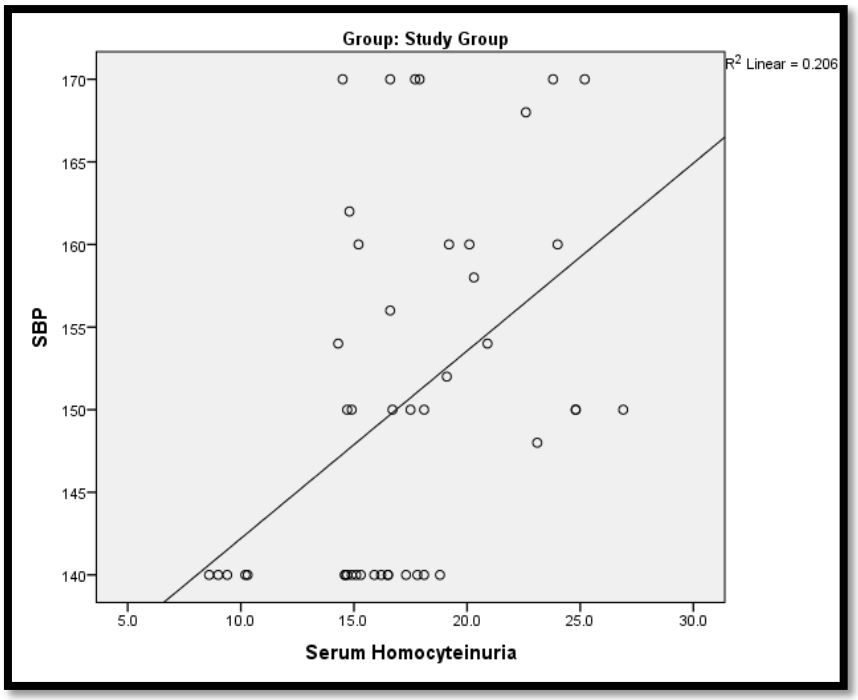


Chart 9: Scatter plot showing correlation between serum homocysteine and SBP in Study group

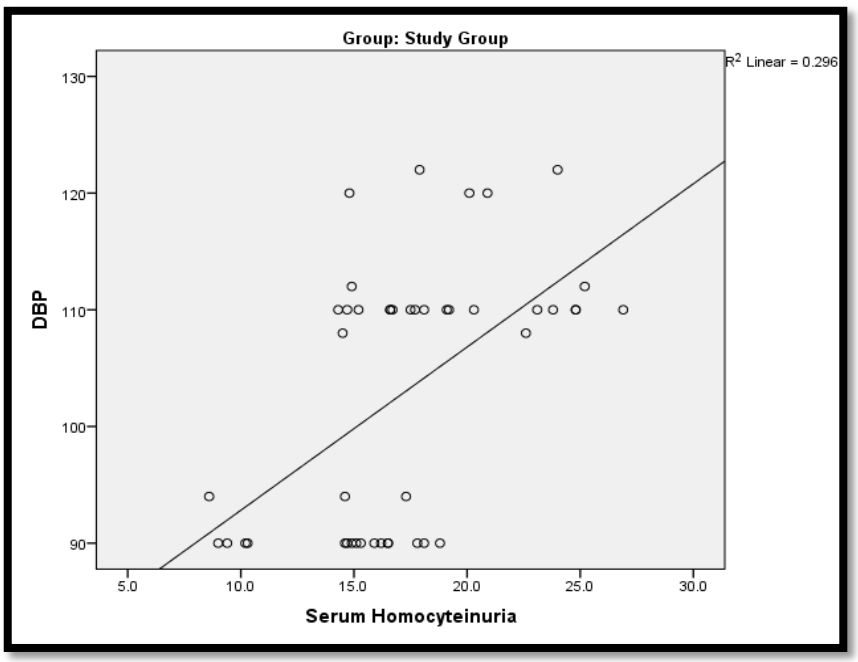


Chart 10: Scatter plot showing correlation between serum homocysteine and DBP in Study group

Table 10: Comparison of Proteinuria with Mean Homocysteine levels

Estimation Proteinuria on Dipstick		Serum Homocysteine(µmol/l)
		Mean ± SD
Proteinuria	1+	14.3 ± 3.5
	2+	19.8 ± 4.2
	3+	18.1 ± 3.2
	4+	20.7 ± 4.5
	TRACES	14.5 ± 3.1
P value		0.001*

Mean serum Homocysteine levels were high with increase in albumin levels in the urine. There was significant difference observed in mean Homocysteine levels in comparison with urine albumin levels.

Table 11: Comparison of Mean Homocysteine levels with mode of delivery between two groups

				Serum Homocysteine(µmol/l) Mean ±SD	P value
Group	Control	Mode of Delivery	Vaginal	7.8 ± 1.3	0.655
			LSCS	8.1 ± 1.3	
	Study	Mode of Delivery	Vaginal	17.3 ± 4.6	0.919
			LSCS	17.1 ± 2.4	

In this study, among controls mean homocysteine levels in vaginal delivery subjects was 7.8 ± 1.3µmol/l and in LSCS subjects was 8.1 ± 1.3µmol/l. There was no significant difference in mean Homocysteine levels between modes of delivery in controls.

In this study, among cases mean Homocysteine levels in vaginal delivery subjects was 17.3 ± 4.6µmol/l and in LSCS subjects was 17.1 ± 2.4µmol/l. There was no significant difference in mean Homocysteine levels between modes of delivery in cases.

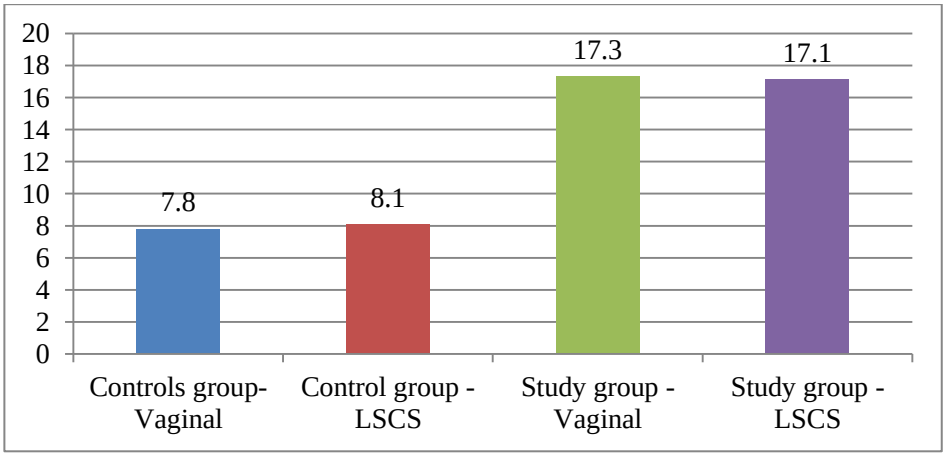


Chart 11: Bar diagram showing Comparison of Mean Homocysteine levels with mode of delivery between two groups

Table 12: Comparison of Mean Serum Homocysteine levels and Perinatal outcome in control group and study group

		Severity					
		Control		Cases			
				Mild		Severe	
		No	MeanSerum Homocysteine	No	MeanSerum Homocysteine	No	MeanSerum Homocysteine
Perinatal outcomes	Normal	38 (84.4%)	7.8	9 (45%)	12.0	7 (28%)	17.4
	IUGR	2 (4.4%)	8.5	7 (35%)	16.8	15 (60%)	21.1
	SGA	5 (11.1%)	8.1	4 (20%)	15.8	3 (12%)	16.8
	Prematurity	8 (17.8%)	8.9	6 (30%)	14.3	7 (28%)	20.2
P value			<0.001*		<0.001*		<0.001*

In the study mean Homocysteine levels were highest in IUGR subjects among controls and subjects with mild and severe preeclampsia. This difference was statistically significant.

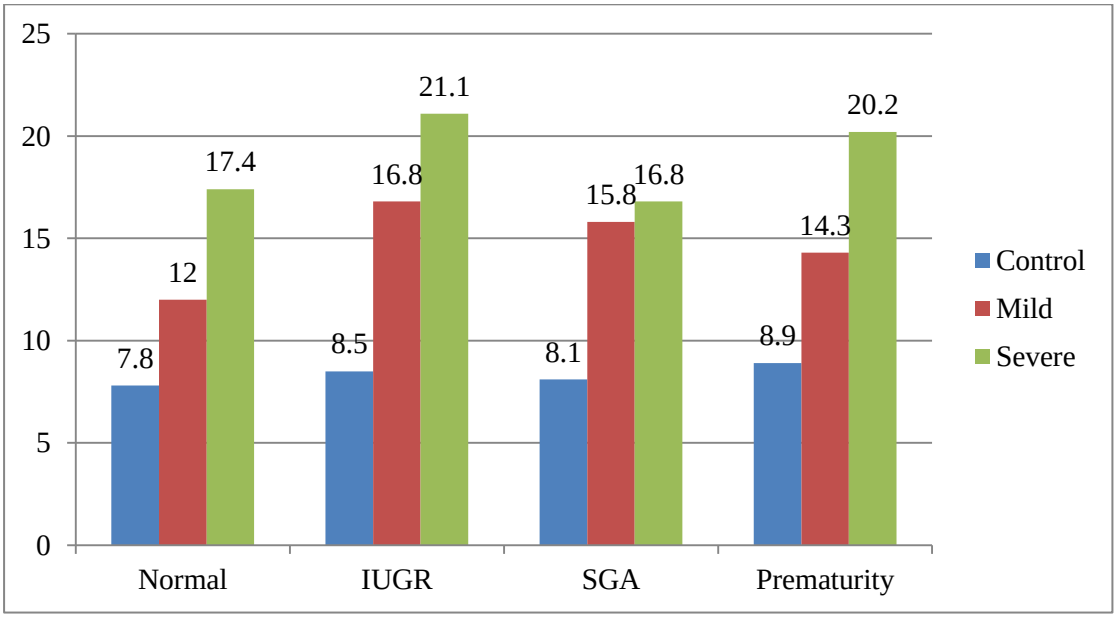


Chart 12: Bar diagram showing Comparison of Mean Serum Homocysteine with Mode of delivery and severity of preeclampsia

DISCUSSION

Pre-eclampsia is a leading cause of maternal and fetal morbidity. Although, the exact cause of pre-eclampsia is still unknown, it is known that in pre-eclampsia the basic pathology is endothelial dysfunction and intense vasospasm. Recently homocysteine, a metabolite of essential aminoacid methionine, has been postulated to produce oxidative stress and endothelial cell dysfunction.

Elevated plasma homocysteine concentration is an independent risk factor for peripheral vascular diseases and for coronary artery diseases.

Serum homocysteine may prove to be the missing link in the etiology of pre-eclampsia.

The most relevant findings in the present study were (i) elevation of maternal serum homocysteine levels in preeclampsia compared to normal pregnant women, (ii) a significant increase in the maternal serum homocysteine levels in both mild and severe preeclampsia groups than in control group, (iii) a significant positive correlation between serum homocysteine levels and systolic and diastolic blood pressure and proteinuria (iv) a significant correlation between serum homocysteine levels and perinatal outcome.

In the present study, the mean serum homocysteine levels in the control is 7.9±1.3µmol/l. Our study is supported by various other studies viz., Singh Urmila et al[105]., showed that the value in the normotensive pregnant women is 11.5±4 µmol/l, Karg et al(9.39±1.3µmol/l), Rajkovic et al[121](9.93 ± 1.3 µmol/l), Hoque et al[122](6.86±2.47µmol/l), Georgios Makedos et al[123](6.40 µmol/l).

Levels of homocysteine are generally lowered during pregnancy either due to physiological response to pregnancy like hemodilution, increased glomerular filtration rate, hormonal changes or increased demand for methionine by both mother and the fetus.

In our study , 45 subjects were diagnosed PE and the mean serum homocysteine level was 17.3 ± 4.4 µmol/l in them which was statistically highly significant ($p<0.01$) compared to control group.This observation conforms to other similar studies done by Rajkovic et al, Khosrowbeygi etal[118], Hoque et al, Karunashree et al[120].

This shows that the decrease in homocysteine levels which occurs in normal pregnancy do not occur in pre-eclampsia. So it is possible that the increase in homocysteine concentration in pre-eclampsia is related to the defect in the mechanism that usually decreases homocysteine during normal pregnancy.

In our study group (diagnosed PE cases), 20 cases were mild PE, 25 cases were severe PE. The mean serum homocysteine levels in mild PE cases was 14.5 ± 3.2µmol/l, which when compared to normotensive pregnant women is elevated and is highly statistically significant ($p<0.001$).These results were consistent with results of Cotter et al and Khosrowbeygi et al; however this findings differ from Metin Ingec et al[117] showed that serum homocysteine levels were not significantly different between mild preeclampsia(7.7± 2.4µmol/l) and controls(6.7±1.6µmol/l) and other study conducted by Hasanzadeh et al[124] detected no significant difference of homocysteine levels among mild preeclampsia and control groups(10.4± 2.3µmol/l and 8.8±2.8µmol/l respectively).

In the present study, the mean serum homocysteine levels in severe PE cases is 19.6 ± 3.9µmol/l, which is highly statistically significant ($p<0.001$) than those women without preeclampsia. Similar studies done by Ingec et al, Cotter et al and Khosrowbeygi et al found same results.

In present study, the mean serum homocysteine levels were significantly higher in severe PE cases (19.6 ± 3.9 µmol/l) than in mild PE cases (14.5 ± 3.2µmol/l) and $p< 0.001$ which is highly statistically significant. This suggests that homocysteine levels are directly correlated with the severity of pre-eclampsia.

Our study is supported by Singh Urmila et al., who found that the mean value in pre-eclamptic pregnant women was 13.6±3.5 µmol/l in mild PE and 16.69±4.18 µmol/l in severe PE group. In other studies conducted by Rajovic

et al., mean serum homocysteine levels in pre-eclamptic pregnant women was 13.9±4.8 µmol/l, Asmitha Kulkarni et al[125] found it in the range 14.8±7.3 µmol/l. However, Salikan F et al found that there is no significant difference statistically in homocysteine levels between mild and severe preeclampsia groups($p>0.05$).

Our study showed significant positive correlation between Homocysteine and SBP and DBP among cases. i.e. with increase in SBP and DBP there was significant increase in Serum homocysteine in cases. This positive correlation was also seen in a study conducted by Ferdusai et al[119]. Positive correlation was noticed between serum homocysteine levels and urinary total protein (p value is 0.001). These findings were consistent with findings of Ingec etal and Ferdusai et al.

In our study, maternal serum homocysteine levels did not show any correlation with mode of delivery(LSCS and vaginal delivery).A study by Jian Van, in which majority of the subjects were delivered by LSCS not related to homocysteine level. But Karunashree et al demonstrated that majority of the subjects who underwent LSCS had hyperhomocysteinemia.

In present study, we found that hyperhomocysteinemia has increased incidence of preeclampsia with poor pregnancy outcome. Our study showed pregnancy outcome like IUGR(48.9%), Preterm(28.9%) and SGA(15.6%).Studies done by Rajkovic and Stein Emil et al[126] found elevated homocysteine levels associated with preterm delivery. Leida et al found correlation between homocysteine and IUGR. Homocysteine has atherogenic property and proved to be independent risk factor for atherosclerosis and atherothrombosis. The reason for this poor pregnancy outcome is due to hyperhomocysteinemia causing thrombosis in the placental blood vessels leading to ischemia and infarction of placenta resulting in fetoplacental insufficiency.

It is possible that in pre-eclampsia, the elevated homocysteine level injures the vascular endothelium which contribute to the pathogenesis of PE. In addition vascular endothelium in pregnant women may be more sensitive to injury. Therefore, elevation in homocysteine levels may lead to endothelial injury with subsequent activation of various factors that eventually results in pre-eclampsia.

CONCLUSION

In present study, maternal serum Hcy levels were significantly increased in preeclampsia compared to normotensive subjects. Serum Hcy was significantly rised in severe pre-eclampsia than in mild preeclampsia. This shows association between serum Hcy and severity of preeclampsia. Our findings suggested that hyperhomocysteinemia is related to poor pregnancy outcome like IUGR, prematurity and SGA.

The exact mechanism how hyperhomocysteinemia promotes endothelial dysfunction is still unclear, but involves both cytotoxic and oxidative stress mechanism to promote endothelial dysfunction in preeclampsia. Therefore, further cohort or case control studies with large sample should be carried out to evaluate the association of serum Hcy with preeclampsia.

Elevated levels of homocysteine can be due to genetic or nutritional deficit or a combination of both. Nutritional defects involve inadequate intake of folic acid, vitamin B12 and vitamin B6.All these vitamins are involved in metabolism of homocysteine. Hyperhomocysteinemia is a marker of low B-vitamin status or decreased methylation capacity of cells.

Further studies are required to know the cause of hyperhomocysteinemia (whether nutritional or genetic) observed in pregnant women with pre-eclampsia, which may help in pharmacological management of pregnant women at risk for PET.

Elevated levels of homocysteine can be reduced by administering vitamins which help by increasing the metabolism of homocysteine. The internationally accepted treatment for hyperhomocysteinemia is using a combination of 3 vitamins viz., folic acid 400µg, vitamin B12 500 µg and pyridoxine 10 mg initiating from conception.

Continuing these agents in the therapeutic dose in second and third trimester would help to reduce increased levels of homocysteine and might help substantially to reduce the adverse pregnancy outcome.

SUMMARY

- The objective of the study was to find out the changes in homocysteine levels in pre-eclampsia and to find out correlation between homocysteine concentration and severity of PE and pregnancy outcome.
- 45 normotensive pregnant women and 45 diagnosed PE cases were selected consecutively with the application of inclusion and exclusion criteria as and when they presented.
- Enzyme linked immunosorbent assay was used for determining total homocysteine levels in blood.
- Statistical analysis was done by applying :
    - Independent t test or Mann Whitney U test was used as test of significance to identify the mean difference between two quantitative variables and qualitative variables respectively.
    - Pearson correlation or Spearman's correlation was done to find the correlation between concentration of homocysteine and severity of PE.
- Results of the study revealed statistically significant elevation in the level of homocysteine in pre-eclamptic women compared to normotensive women (mean 17.3± 4.4µmol/l v/s mean 7.9 ± 1.3µmol/l).
- The study showed a strong association between increased BP and homocysteine levels. The mean homocysteine levels in severe PE cases (19.6 ± 3.9 µmol/l) were significantly higher than in cases with mild PE (14.5± 3.2 µmol/l).
- The perinatal outcome in women with higher levels of homocysteine was poor with increased incidence of IUGR (48.9%), prematurity (28.9%), SGA (15.6%) and the need for NICU care was increased in such cases.

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ANNEXURES

SRI DEVARAJ URS MEDICAL COLLEGE & RESEARCH CENTRE, TAMAKA, KOLAR

PATIENT CONSENT FORM

Case No:

Title: STUDY OF SERUM HOMOCYSTEINE LEVELS IN PREECLAMPSIA AND RELATION TO ITS SEVERITY AND OBSTETRIC OUTCOME

Name of the investigator:

Name of the participant: _____

I _____ d/o, w/o _____

give my full, free and voluntary consent to participate in the study entitled "STUDY OF SERUM HOMOCYSTEINE LEVELS IN PREECLAMPSIA AND RELATION TO ITS SEVERITY AND OBSTETRIC OUTCOME". I have read (or it has been read to me) and understood this consent form. I have understood that I have the right to refuse consent or withdraw it at any time of the study. I have been explained the procedures involved or non invasive and there are no potential risks or discomforts.

I had oppurtunity to ask any questions regarding various aspects of this study and my questions are been answered to my satisfaction. I have been explained about the intent of the study.

Signature/Thumb impression of the Participant

Name: _____ Date: _____ Time: _____

PROFORMA

NAME : IP NO. :

AGE :

SEX : D.O.A :

ADDRESS : DOD :

Socio-Economic Status :

Occupation :

Diagnosis :

H/O AMENORRHOEA: Imminent signs :

Appreciates fetal Movements: Yes / No

Labour Pain : Yes/ No No. of convulsion :

Bleeding P.V : Yes/ No Other symptom :

Swellings of feet : Yes/ No Urine output :

Immunization :

No.of ANC :

OBSTETRIC HISTORY:

Married Life: Consanguinous / NCM

GRAVIDITY : PARITY: ABORTION : LIVING:

H/o PREVIOUS PREGNANCIES/PP :

MENSTRUAL HISTORY :

AOM: LMP: PMC :

EDD:

USG EDD:

Medical history: Renal disease/Diabetes /epilepsy /T.B /Heart Disease Chronic HTN/ Bronchial asthma

Past h/o : Blood Transfusion

Surgery

Family h/o : TB / HTN / DM /Twin Pregnancy / Pre-eclampsia

Congenital anomalies

Personal h/o : Diet:

Appetite:

Sleep:

Bowel habits:

Bladder habits:

GENERAL PHYSICAL EXAMINATION:

Built : Nourishment :

Height (cm) : Weight (kg) :

Pallor : ICTERUS:

CYANOSIS : CLUBBING:

LYMPHADENOPATHY: OEDEMA :

KOILONYCHIA:

Pulse rate(bpm) : Breast :

Blood pressure(mmHg): Thyroid :

Temperature : Spine :

SYSTEMIC EXAMINATION:

Cardio-Vascular System:

Respiratory System:

Central Nervous System:

PER-ABDOMEN:

Abdominal Wall edema: Presence of ascites :

Uterus size (in weeks) Fundal ht (in cms)

Relaxed / Acting & relaxing/ tense Abdominal girth (in cms)

Lie :

Presentation :

Engaged/Not engaged :

Position :

FHS :

Per Speculum Any leak: Clear/Meconium Stained/Blood stained

Per Vaginum :

Cervix -Position : Posterior / Anterior / Middle

-Effacement :

-Consistency : Firm / Medium / Soft

-Dilatation :

Membranes : Intact / Absent

Station :

Moulding/Caput :

Pelvis : Adequate / not adequate

Cephalo-pelvic disproportion: present/Absent

Modified Bishops score :

INVESTIGATIONS:

Hemoglobin (gm/dl) : RBS:

Blood Grouping : Blood Urea:

Rh typing : S. Creatinine:

HIV : S.Uric acid:

HBsAg : LFT:

VDRL : Platelet Count

S. LDH :

Urine- Albumin :

Serum Homocysteine:

TREATMENT GIVEN :

Anti-hypertensives :

Anti -Epileptics :

MODE OF DELIVERY Vaginal:

Caesarean:

Indication :

DETAILS OF THE BABY:

Number :

Alive/dead :

Term/ preterm:

Male/Female :

Weight (kg) :

Time of Delivery :

Date of Delivery :

APGAR:

Score :

IUGR :

Morbidity:

REMARKS:

KEY TO MASTER CHART

FTND- Full term normal delivery

DBP- Diastolic blood pressure

IUGR- Intrauterine growth restriction

LSCS- Lower segment cesarean section

POG- Period of gestation

PRIMI- Primigravida

PT- Preterm

SBP- Systolic blood pressure

S.Hcy- Serum Homocyateine

SGA- Small for gestational age

T- Term

VD- Vaginal delivery

Wks- Weeks

WNL- With in normal limits

Yrs- Years

MASTER CHART- CONTROLS

SL.NO	Name	Age (Yrs)	IP.NO	POG (Wks)	Gravidity	SBP (mmHg)	DBP (mmHg)	Urine Albumin	S.Hcy (µmol/L)	Remarks	Perinatal Outcome	Term/ Preterm	Mode of Delivery
1	Uma	20	328881	38	PRIMI	120	70	NIL	9.2	WNL	SGA	T	FTND
2	Roja	22	328842	36	PRIMI	110	80	NIL	8.2	WNL	SGA	PT	VD
3	Asha	22	328925	39	PRIMI	110	70	NIL	7.1	WNL	SGA	T	FTND
4	Nabeela	20	329206	37	PRIMI	110	70	NIL	8.3	WNL	SGA	T	FTND
5	Sumithra	30	329222	36	PRIMI	120	70	NIL	7.9	WNL	SGA	PT	LSCS
6	Naveena	25	329412	34	PRIMI	120	80	NIL	10.1	WNL	IUGR	PT	LSCS
7	Radhika	21	329343	37	PRIMI	120	70	NIL	6.8	WNL	IUGR	T	LSCS
8	Bhavani	23	249992	37	PRIMI	110	70	NIL	8.6	WNL	N	T	FTND
9	Mamatha	22	329911	38	PRIMI	120	80	NIL	8.4	WNL	N	T	FTND
10	Tejaswini	26	297302	39	PRIMI	110	80	NIL	9.5	WNL	N	T	FTND
11	Premavathi	23	328787	38	PRIMI	100	70	NIL	5.9	WNL	N	T	FTND
12	Sowmya	27	329094	39	PRIMI	110	70	NIL	8.7	WNL	N	T	FTND
13	Apoorva	24	330257	39	PRIMI	120	70	NIL	7.9	WNL	N	T	FTND
14	Sukanya	26	288981	38	PRIMI	110	80	NIL	9.3	WNL	N	T	FTND
15	Jayalakshmi	23	264705	39	PRIMI	120	80	NIL	6.8	WNL	N	T	FTND
16	Parvathi	25	279052	38	PRIMI	110	70	NIL	9.5	WNL	N	T	FTND
17	Tabassum	21	329878	39	PRIMI	100	74	NIL	8.7	WNL	N	T	FTND
18	Latha	29	305110	38	PRIMI	100	80	NIL	8.6	WNL	N	T	FTND
19	Ambika	27	278124	34	PRIMI	110	78	NIL	8.9	WNL	N	PT	VD
20	Mamatha	20	330478	39	PRIMI	120	72	NIL	5.9	WNL	N	T	FTND

MASTER CHART- CONTROLS

SL.NO	Name	Age (Yrs)	IP.NO	POG (Wks)	Gravidity	SBP (mmHg)	DBP (mmHg)	Urine Albumin	S.Hcy (µmol/L)	Remarks	Perinatal Outcome	Term/ Preterm	Mode of Delivery
21	Mallika	22	330951	34	PRIMI	124	80	NIL	8.1	WNL	N	PT	VD
22	Anitha	24	325808	39	PRIMI	118	70	NIL	6	WNL	N	T	FTND
23	Durga	27	330381	37	PRIMI	120	80	NIL	6.6	WNL	N	T	FTND
24	Sukanya	29	331026	38	PRIMI	110	74	NIL	8.8	WNL	N	T	FTND
25	Uma devi	22	331483	39	PRIMI	124	70	NIL	7.2	WNL	N	T	LSCS
26	Sharada	26	331477	37	PRIMI	120	80	NIL	7.4	WNL	N	T	FTND
27	Shilpa	23	331931	38	PRIMI	120	72	NIL	8.6	WNL	N	T	FTND
28	Geetha	22	331175	38	PRIMI	110	74	NIL	10.4	WNL	N	T	FTND
29	Nandini	21	331942	38	PRIMI	100	70	NIL	6.9	WNL	N	T	FTND
30	Gowramma	30	331944	37	PRIMI	122	80	NIL	6.8	WNL	N	T	FTND
31	Varalakshmi	27	332000	38	PRIMI	110	80	NIL	7.3	WNL	N	T	FTND
32	Naziya	23	313342	38	PRIMI	120	72	NIL	7.6	WNL	N	T	FTND
33	Ranjitha	20	332072	39	PRIMI	110	70	NIL	6.1	WNL	N	T	FTND
34	Lakshmi	30	329880	34	PRIMI	100	70	NIL	9.4	WNL	N	PT	VD
35	Nethravathi	28	132027	38	PRIMI	100	80	NIL	5.7	WNL	N	T	FTND
36	Salma	27	332590	37	PRIMI	120	80	NIL	6.4	WNL	N	T	FTND
37	Radhika	24	331797	38	PRIMI	110	80	NIL	7.8	WNL	N	T	FTND
38	Kavya	26	319401	38	PRIMI	100	70	NIL	9.2	WNL	N	T	FTND
39	Ramadevi	20	325705	37	PRIMI	120	70	NIL	6.5	WNL	N	T	FTND
40	Sirisha	25	328168	36	PRIMI	110	74	NIL	8.6	WNL	N	PT	LSCS

MASTER CHART- CONTROLS

SL.NO	Name	Age (Yrs)	IP.NO	POG (Wks)	Gravidity	SBP (mmHg)	DBP (mmHg)	Urine Albumin	S.Hcy (µmol/L)	Remarks	Perinatal Outcome	Term/ Preterm	Mode of Delivery
41	Ayesha	27	327909	38	PRIMI	110	80	NIL	9.4	WNL	N	T	FTND
42	Asha	28	243604	36	PRIMI	122	70	NIL	9.6	WNL	N	PT	VD
43	Asma	30	333033	37	PRIMI	120	70	NIL	5.4	WNL	N	T	FTND
44	Purnima	30	317782	37	PRIMI	120	70	NIL	7.3	WNL	N	T	FTND
45	Radha	27	332591	38	PRIMI	100	80	NIL	6.8	WNL	N	T	FTND

MASTER CHART- CASES

SL.NO	Name	Age (Yrs)	IP.No	POG (Wks)	Gravidity	SBP (mmHg)	DBP (mmHg)	Urine Albumin	S.Hcy µmol/L	Remarks	Perinatal outcome	Term/ Preterm	Mode of delivery	Severity
1	Swetha	22	329709	38	PRIMI	140	90	1+	14.9	RAISED	N	T	FTVD	Mild
2	Nethravathi	20	330350	36	PRIMI	140	90	TRACES	17.8	RAISED	IUGR	PT	VD	Mild
3	Narayanamma	30	330663	38	PRIMI	140	90	TRACES	15.3	RAISED	SGA	T	FTVD	Mild
4	Shashikala	20	330986	39	PRIMI	140	90	1+	15.9	RAISED	SGA	T	FTVD	Mild
5	Chandana	23	330883	34	PRIMI	140	94	TRACES	17.3	RAISED	SGA	PT	LSCS	Mild
6	Amreen taj	25	331722	36	PRIMI	140	90	TRACES	16.5	RAISED	IUGR	PT	LSCS	Mild
7	Kalpana	24	323657	38	PRIMI	140	90	1+	16.5	RAISED	IUGR	T	LSCS	Mild
8	Narayanamma	27	332589	37	PRIMI	140	90	1+	18.1	RAISED	IUGR	T	FTND	Mild
9	Gayathri	20	330019	37	PRIMI	140	90	1+	18.8	RAISED	IUGR	T	FTND	Mild
10	Chandrakala	24	333013	38	PRIMI	140	90	TRACES	14.6	RAISED	SGA	T	FTND	Mild
11	Veena	25	332688	34	PRIMI	140	90	TRACES	9.35	N	N	PT	VD	Mild
12	Sahistha banu	25	333391	39	PRIMI	140	90	1+	8.95	N	N	T	FTND	Mild
13	Redamma	22	333491	38	PRIMI	140	94	1+	8.6	N	N	T	FTND	Mild
14	Radhamma	20	335382	38	PRIMI	140	94	1+	14.6	RAISED	N	T	FTND	Mild
15	Jamuna	22	333881	38	PRIMI	140	90	TRACES	15.1	RAISED	N	T	FTND	Mild
16	Chinapillamma	25	336288	38	PRIMI	140	90	TRACES	10.2	N	N	T	FTND	Mild
17	Salma	23	337638	39	PRIMI	140	90	1+	16.2	RAISED	N	T	FTND	Mild
18	Aruna	22	339504	36	PRIMI	140	90	1+	14.7	RAISED	IUGR	PT	VD	Mild
19	Mukambika	20	340522	36	PRIMI	140	90	1+	10.3	N	N	PT	VD	Mild
20	Manjula	24	341160	38	PRIMI	160	110	3+	15.2	RAISED	IUGR	T	FTND	Mild

MASTER CHART- CASES

SL.NO	Name	Age (Yrs)	IP.No	POG (Wks)	Gravidity	SBP (mmHg)	DBP (mmHg)	Urine Albumin	S.Hcy µmol/L	Remarks	Perinatal outcome	Term/ Preterm	Mode of delivery	Severity
21	Sowmya	22	341695	36	PRIMI	150	110	4+	24.8	RAISED	IUGR	PT	VD	Severe
22	Najma	22	341666	36	PRIMI	150	110	2+	26.9	RAISED	IUGR	PT	VD	Severe
23	Nagarathna	27	341630	39	PRIMI	168	108	4+	22.6	RAISED	IUGR	T	FTND	Severe
24	Sashikala	25	342178	38	PRIMI	170	110	3+	23.8	RAISED	IUGR	T	FTND	Severe
25	Pooja	22	343126	36	PRIMI	154	120	4+	20.9	RAISED	IUGR	PT	LSCS	Severe
26	Niha begum	20	343158	38	PRIMI	170	110	2+	17.7	RAISED	IUGR	T	FTND	Severe
27	Sulthana	20	342647	37	PRIMI	170	108	2+	14.5	RAISED	SGA	T	FTND	Severe
28	Prema	21	343593	38	PRIMI	170	110	2+	16.6	RAISED	SGA	T	FTND	Severe
29	Krishnamma	28	343606	38	PRIMI	150	112	2+	14.9	RAISED	IUGR	T	FTND	Severe
30	Suma bhanu	30	291164	39	PRIMI	152	110	2+	19.1	RAISED	N	T	FTND	Severe
31	Geetha	28	343737	38	PRIMI	158	110	3+	20.3	RAISED	IUGR	T	FTND	Severe
32	Sumaya	22	131187	38	PRIMI	150	110	2+	24.8	RAISED	IUGR	T	FTND	Severe
33	Asha	24	344851	38	PRIMI	148	110	2+	23.1	RAISED	IUGR	T	FTND	Severe
34	Papitha	20	345342	36	PRIMI	150	110	3+	17.5	RAISED	N	PT	VD	Severe
35	Sunitha	20	345617	36	PRIMI	156	110	2+	16.6	RAISED	IUGR	PT	VD	Severe
36	Indramma	20	345820	37	PRIMI	162	120	3+	14.8	RAISED	IUGR	T	FTND	Severe
37	Ashida	24	346220	38	PRIMI	170	122	2+	17.9	RAISED	N	T	FTND	Severe
38	Vanajakshi	20	346388	38	PRIMI	160	110	2+	19.2	RAISED	SGA	T	FTND	Severe
39	Nandini	29	168871	37	PRIMI	150	110	3+	18.1	RAISED	N	T	FTND	Severe
40	Shilpa	25	70965	36	PRIMI	154	110	4+	14.3	RAISED	N	PT	LSCS	Severe

MASTER CHART- CASES

SL.NO	Name	Age (Yrs)	IP.No	POG (Wks)	Gravidity	SBP (mmHg)	DBP (mmHg)	Urine Albumin	S.Hcy µmol/L	Remarks	Perinatal outcome	Term/ Preterm	Mode of delivery	Severity
41	Nadiya	27	167572	38	PRIMI	150	110	2+	16.7	RAISED	IUGR	T	FTND	Severe
42	Lakshmi	28	168303	36	PRIMI	160	120	3+	20.1	RAISED	N	PT	VD	Severe
43	Chaithra	30	166246	37	PRIMI	160	122	2+	24	RAISED	IUGR	T	FTND	Severe
44	Kavitha	30	163331	37	PRIMI	150	110	3+	14.7	RAISED	N	T	FTND	Severe
45	Susheelamma	27	163308	38	PRIMI	170	112	2+	25.2	RAISED	IUGR	T	FTND	Severe