VALIDATION OF SERUM BASED BIOMARKERS FOR PREECLAMPSIA

By

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CERTIFICATE

This is to certify that **DR. SWETHA REDDY VIZA** postgraduate student (2009-2012) in the department of Obstetrics and Gynaecology, PSG INSTITUTE OF MEDICAL SCIENCES AND RESEARCH, Coimbatore has done this dissertation titled "**VALIDATION OF SERUM BASED BIOMARKERS FOR PREECLAMPSIA**" under the direct guidance and supervision of **Prof .DR.KANCHANAMALAI** in partial fulfillment of the regulations laid down by the Tamilnadu Dr.M.G.R. medical university, Chennai, for M.D. Obstetrics and Gynaecology degree examination.

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I, **Dr. SWETHA REDDY VIZA**, solemnly declare that this dissertation **"VALIDATION OF SERUM BASED BIOMARKERS FOR PREECLAMPSIA"** is a bonafide and genuine work done in the Department of Obstetrics and Gynaecology, PSG institute of medical sciences and research, under the guidance of **Prof. DR.KANCHANAMALAI**.

This dissertation is submitted to the Tamilnadu Dr.M.G.R. Medical University, Chennai in partial fulfillment of the University regulations for the award of M.D degree in Obstetrics and Gynaecology. This dissertation has not been submitted earlier for the award of any degree.

Place: Coimbatore **(Dr. SWETHA REDDY VIZA)**

Date:

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ABBREVATIONS

PE – Pre Eclampsia.

PIH - Pregnancy Induced Hypertension

PIGF – Placental Induced Growth Factor

VEGF – Vaso Endothelial Growth Factor

PTX3 – Pentraxin3

GA – Gestational Age

OBS.SCR – Obstetrics Score

GA-SC – Gestational Score

SEV – PE – Severity of Preeclampsia.

SEV – Severe

Urine –ALB – Urine Albumin.

LFT – Liver Function Test

PLAT – Platelet count

LDH – Lactate Dehydrogenase

P.S – Peripheral Smear

Creat – Creatinine

UMA-Dop – Umblical Artery Doppler

NOR – Normal

R – Raised

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INTRODUCTION

Preeclampsia is a multi-system disorder of pregnancy, which is characterized by new onset hypertension in pregnancy, that develop after 20 weeks of gestation in previously normotensive pregnant women. Dependening on the systemic involvement, several other signs and symptoms, such as pedal edema, epigastric pain, vomiting, blurring of vision, oliguria, HELLP syndrome (hemolysis, elevated liver enzymes, and low platelet counts) and renal failure also complicate the clinical picture[1]. Preeclampsia can have an early onset (preeclampsia starting before 34 weeks of gestation) or late onset (preeclampsia starting after 34 weeks of gestation), can show mild or severe symptoms (systolic blood pressure ≥ 160 mmHg or diastolic blood pressure ≥ 110 mmHg, proteinuria >5 g/24 hours, oliguria, neurological symptoms, other clinical symptoms such as deranged liver function, thrombocytopenia < 100 000 mm3, HELLP syndrome), and can evolve into eclampsia in the most severe cases. In addition, it can manifest as a maternal disorder only, with an appropriate fetal growing, or it can present itself with a growth restricted fetus (intrauterine growth restriction (IUGR)) or sudden fetal distress[2].

Preeclampsia is one of the most important complications of pregnancy and is the leading cause of maternal and perinatal morbidity and mortality in the world. With

the development of the medical technology and maternal/child health care system, complications of illegal abortions, infections and mortality and morbidity from postpartum hemorrhage are effectively routed out in recent years. The maternal and perinatal mortality rates have declined dramatically in many countries. However, hypertensive disorder of pregnancy and their complications are a major cause of maternal mortality, still complicating approximately 0.34 - 11.5% of pregnancies. These variations depend on age distribution, socioeconomic status, and number of previous deliveries. In addition, hypertensive disorders of pregnancy are also found to be strongly associated with intrauterine growth restriction and prematurity, contributing largely to perinatal morbidity and mortality. Preeclampsia occurs in 2–5% of pregnancies in the Occident, but it complicates up to 10% of pregnancies in the developing countries, where emergency care is often inadequate or lacking[3]. Therefore we are in need of a widely applicable and affordable test that could permit presymptomatic diagnosis in order to identify and monitor the patients at risk and thus provide the best prenatal care for these women and their children. Such a test would also be of benefit to confirm a confounding clinical diagnosis and for future studies investigating prophylactic treatments or temporizing therapies[3].

This disorder has a higher incidence among nulliparous women, in women who conceive with assisted reproduction techniques, and in women affected by

autoimmune disorders, reflecting the probable influence of an "inexperienced" or dysregulated maternal immune system in its emergence[4, 5]. On the other hand, women with preexisting metabolic, vascular or renal disease are especially at increased risk for superimposed preeclampsia, possibly due to their elevated sensitivity to the mere normal physiological changes imposed by pregnancy itself [6].

Despite decades of research into the condition, predicting which women are at increased risk of developing preeclampsia remains problematic. Identifying “at-risk women” is an important aim; because modern obstetric care places emphasis upon the primary care setting for expectant women, a marker which identified high-risk women would allow for closer supervision in secondary care. Such a marker would also facilitate recruitment for trials of potential therapeutic agents, for accurate diagnosis, and for timely intervention whenever problems develop. Furthermore, predicting preeclampsia in women with underlying conditions such as diabetes and chronic hypertension would be of great clinical value. Clinicians have traditionally relied on maternal risk factors, such as increased maternal age, family history, and preexisting diseases, for determining which women are at increased risk. The problem with using these risk factors is that millions of women worldwide have these risk factors but do not develop preeclampsia. Moreover, the majority of them are nonmodifiable. There have been many screening tests such as

evaluated in the literature over the years for predicting preeclampsia; these have been comprehensively reviewed in a World Health Organization publication[7].

Irrespective of the lack of existing prophylactic and therapeutic means against preeclampsia, the search for noninvasive, blood-borne or urinary biomarkers that could predict the development or assist in the detection of this life-threatening pregnancy disorder is still of utmost importance. The availability of such markers could have decisive impact on the medical management of pregnant women and their perinatal outcome but also on the health costs associated with this poor medical condition. Since many years, different biophysical and biochemical markers have been investigated, based on pathophysiological observations that have been noted in case of preeclampsia, such as Placental Induced Growth Factor and Pentraxin - Biochemical markers which might allow the stratification of preeclamptic patients in different categories according to symptoms, severity and/or perinatal outcome and thus improve in its clinical management[7]. So here considering the validation and the sensitivity of serum biomarkers in the prediction of preeclampsia.

Doppler sonography is used for non-invasive assessment of circulation in many clinical conditions. This technique has been used for studying most of the major fetal circulatory systems, including the umbilical artery (UA), umbilical vein,

aorta, heart and middle cerebral artery .Assuming that defective placental circulation results in adverse pregnancy outcome, Doppler ultrasonography has been used as a modality to evaluate placental circulation and fetal well being for about three decades. Abnormal development of placental vasculature is considered as the pathophysiological basis for development of preeclampsia and this could be reflected in abnormal umbilical Doppler velocimetry[8].

In normal pregnancies, the feto-placental circulation acts as a low resistance system unit. Thus, the blood velocity waveforms in umbilical artery (UA) show continuous forward flow throughout the cardiac cycle .The imaging technique that has so far been most widely used for predicting preeclampsia has been uteroplacental Doppler ultrasound. Impaired placental perfusion, one of the hallmarks of preeclampsia, can be assessed by measuring flow waveform ratios or by detecting diastolic notching of the uterine arcuate vessels and umbilical artery[7]

Despite extensive clinical trials, no therapeutic approaches are available for prevention of preeclampsia like anti-hypertensive drugs, corticosteroids for lung maturation or magnesium sulfate to prevent from eclampsia are given to manage (or prevent the worsening of) the symptoms and can thus temporize over the short term to allow for safe delivery with a good perinatal outcome. However, the maternal risks must be carefully weighed against the possible fetal benefits in the

management, as the risk of fatal deterioration of the maternal and perinatal outcome in these conditions is high. Several prophylactic therapies (anti-oxidant, vitamins, folic acid supplementation and Aspirin) have so far failed to prove its efficacy in the prevention of preeclampsia in healthy nulliparous, although some benefit has been shown in preeclampsia groups [9]. As a consequence, the sole management of preeclampsia is the removal of the placenta, and in case of prematurity, with the adverse consequence of delivering a pre-term baby. Therefore, preeclampsia, with or without IUGR, remains a major cause of maternal and neonatal mortality and morbidity worldwide [9].

AIMS AND OBJECTIVES

1. To study the clinical presentations and determinants of pre eclampsia among clinically diagnosed preeclampsia in a tertiary care hospital
2. To compare and validate serum based biomarkers (Placental Induced Growth Factor (PIGF) & Pentraxin-3 (PTX3) values in normal pregnancy and pre eclampsia.
3. To correlate the levels of these biomarkers with the pregnancy outcomes.

REVIEW OF LITERATURE

Eclampsia was first identified in the pre Roman times. In mid 19th century doctors realized that fits that were seen in pregnancy was a collection of circulatory disturbances due to preeclampsia. Eclampsia is a Greek word meaning "bolt from the blue" or lightening strike and so preeclampsia is called before lightening strike. Rayers landmark observation (1839-1841) provided evidence for renal involvement with observation of protein in urine of pregnant edematous women. Lever in 1843 reported proteinuria occurs in eclampsia and noted that there is disappearance of proteinuria after delivery of the child [10].

Many classifications focus on diastolic blood pressure, or changes in diastolic blood pressure. Because of the great variation in clinical expression of the syndrome and the inability to distinguish symptoms induced by pregnancy from underlying (but often latent) maternal disorders, we have difficulty in defining the symptoms, the various clinical forms, and the pathophysiology that becomes more complex every time new evidence is found. In fact, the criteria used to identify the disorder remain a subject of confusion and controversy. Obviously, a comprehensive and easily obtainable classification is needed to improve prognostics and decision making and to enable comparison of research work. American College of Obstetricians and Gynecologists (ACOG) has recommended

a classification to define hypertensive disorders of pregnancy. This classification is widely accepted and consists of four terms as follow [11].

- **Chronic hypertension**
- **Pre-eclampsia / Eclampsia**
- **Pre-eclampsia superimposed upon chronic hypertension**
- **Gestational hypertension**:

It is a transient hypertension of pregnancy if pre-eclampsia is not present at the time of delivery and blood pressure returns to normal by 12 weeks postpartum or Chronic hypertension if the elevation persists.

Definition of chronic hypertension

Chronic hypertension is defined as hypertension that is present before pregnancy or diagnosed before the 20th week of gestation. Hypertension is defined as a blood pressure equal to or greater than 140 mm Hg systolic or 90 mm Hg diastolic. During pregnancy the hypertension remains without proteinuria. Women who develop hypertension during pregnancy, without proteinuria or seizures, and blood pressure remains elevated after pregnancy are also diagnosed with chronic hypertension. Hypertension that is diagnosed for the first time during pregnancy and that does not resolve postpartum is classified as chronic hypertension too.

Definition of preeclampsia/eclampsia

Preeclampsia is characterized by blood pressure greater than or equal to 140 mm Hg systolic or 90 mm Hg diastolic occurring after midpregnancy (20 weeks gestation), and accompanied by proteinuria. Preeclampsia may be further categorized as mild or severe. In the absence of proteinuria the disease is highly suspected when increased blood pressure appears accompanied by the symptoms of headache, blurred vision, and abdominal pain, or with abnormal laboratory tests, specifically, low platelet counts and abnormal liver enzymes. It is recommended that gestational blood pressure elevation is defined on the basis of at least two determinations.

Proteinuria is defined as the urinary excretion of 0.3 g protein or greater in a 24-hour specimen, with 30 mg/dL ("1+ dipstick") or greater in a random urine determination with no evidence of urinary tract infection. However, because of the discrepancy between random protein determinations and 24-hour urine protein in pre-eclampsia, it is recommended that the diagnosis be based on a 24-hour urine if at all possible or a timed collection corrected for creatinine excretion if this is not feasible. Pre-eclampsia always presents potential danger to mother and baby. Other conditions may increase blood pressure and even result in proteinuria; thus, as the certainty of the diagnosis increases, the requirements for careful assessment and

consideration for delivery also increase. According to the severity of syndrome, ACOG made three categories for pre-eclampsia:

a. Mild preeclampsia BP 140/90, 300mg of proteinuria in 24hrs

b. Severe preeclampsia (any of these)

- BP 160/110mm of Hg
- 5g of proteinuria in 24hrs
- Oliguria or <500 ml in 24hrs
- Cerebral or visual disturbances
- Pulmonary edema or cyanosis
- Persistent pain of right-upper quadrant of abdomen
- Fetal growth restriction
- Thrombocytopenia (Platelet count is less than 100,000 cells/mm3 and/or evidence of microangiopathic hemolytic anemia, with increased lactic acid dehydrogenase >600IU/ml).
- Impaired liver function (Elevated hepatic enzymes -alanine aminotransferase [ALT] or aspartate aminotransferase [AST]).

c. Eclampsia-

The onset of convulsions in a woman with preeclampsia that cannot be attributed to other causes is termed as eclampsia. The seizures are generalized and may appear before, during, or after labor. Typically occurs during or after the 20th week of gestation or in the postpartum period. The clinical manifestations of maternal preeclampsia are hypertension and proteinuria with or without coexisting systemic abnormalities involving the kidneys, liver, or blood. There is also a fetal manifestation of preeclampsia involving fetal growth restriction, reduced amniotic fluid, and abnormal fetal oxygenation. Preeclampsia/eclampsia produces multiple systemic derangements that can involve a diversity of organ systems including hematologic, hepatic, renal, and cardiovascular systems as well as the central nervous system. The severity of these derangements often correlates with maternal medical (preexisting renal or vascular pathology) or obstetric factors (multifetal gestations or molar pregnancy) [1].

INDICATORS OF SEVERITY OF GESTATIONAL HYPERTENSIVE DISORDERS

Abnormality	Mild	Severe
Diastolic blood pressure	<110mm Hg	>110mm Hg
Systolic blood pressure	<160 mm Hg	>160 mm Hg
Proteinuria	<2+	>3+
Headache	Absent	Present
Visual disturbances	Absent	Present
Upper abdominal pain	Absent	Present
Oliguria	Absent	Present
Convulsions	Absent	Present
Serum creatinine	Normal	Elevated
Thrombocytopenia	Absent	Present
Fetal growth restriction	Absent	Obvious
Pulmonary oedema	Absent	present

Definition of pre-eclampsia superimposed upon chronic hypertension

Pre-eclampsia may occur in pregnant women who are already hypertensive (chronic hypertensives) and the prognosis of maternal and fetal outcome is much worse than with either condition alone. High suspicion is needed to distinguish superimposed preeclampsia from worsening chronic hypertension. The principle of high sensitivity over diagnosis should be appropriate in order to facilitate clinical management. Close observation, with delivery indicated for the overall assessment of maternal-fetal wellbeing should be provided at suspicion of superimposed pre-eclampsia. The diagnosis of superimposed pre-eclampsia is highly associated with the following findings:

• In women who is hypertensive but with no proteinuria in early pregnancy (<20 weeks) or who had recent onset of proteinuria, which is defined as the urinary excretion of 0.3 g protein or greater in a 24-hour specimen.

• In women with hypertension and proteinuria before 20 weeks' gestation.

• Sudden increase in proteinuria.

• A sudden increase in blood pressure in a woman whose hypertension has previously been well controlled.

• Thrombocytopenia (platelet count <100,000 cells/mm3).

Pathogenesis of Preeclampsia

The mechanisms by which preeclampsia occurs is not certain, and numerous maternal, paternal, and fetal factors have been implicated in its development. The factors currently considered to be the most important include the following:

- defective placentation,
- placental ischemia
- endothelial cell dysfunction

Immunologic factors have long been considered to be major factors in preeclampsia. One important component is a poorly understood dysregulation of maternal tolerance to paternally derived placental and fetal antigens. This maternal-fetal immune maladaptation is characterized by defective cooperation between uterine natural killer (NK) cells and fetal human leukocyte antigen (HLA)-C, and results in histological changes similar to those seen in acute graft rejection.

The endothelial cell dysfunction that is characteristic of preeclampsia may be partially due to an extreme activation of leukocytes in the maternal circulation, as evidenced by an up regulation of type 1 helper T cells[12]. (Figure -1)

Placentation in Preeclampsia

Normal placental development requires that cytotrophoblastic invasion in to the maternal spiral arterioles. This remodeling of the spiral arterioles into large-capacitance, low-resistance vessels starts in the late first trimester and ends by 18 to 20 weeks of gestation resulting in replacement of the endothelium and muscular tunica media[13]. Trophoblast invasion/ differentiation entail changes in the expression of certain cytokines, adhesion molecules, extracellular matrix molecules, metalloproteinase, and the class Ib major histocompatibility complex molecule and histocompatibility leukocyte antigen (HLA-G) [14,15]. Pseudovasculogenesis is the change representing a transformation from epithelial (adhesion molecule expression of integrinS and E-cadherin) to endothelial cell adhesion molecule, and VE-cadherin) [16].

Placental implantation with abnormal trophoblastic invasion of uterine vessels is a major cause of pregnancy induced hypertension associated with preeclampsia .In fact, studies have shown that the degree of incomplete trophoblastic invasion of the spiral arteries is directly associated with the severity of pregnancy induced hypertension. This is because the placental hypoperfusion results due to incomplete trophoblastic invasion leads to an unclear pathway to the release of systemic vasoactive compounds that cause an exaggerated inflammatory response,

vasoconstriction, endothelial damage, hypercoagulability, capillary leak and platelet dysfunction all of which contribute to various clinical features ,organ dysfunction ,and end organ damage in preeclamptic women.

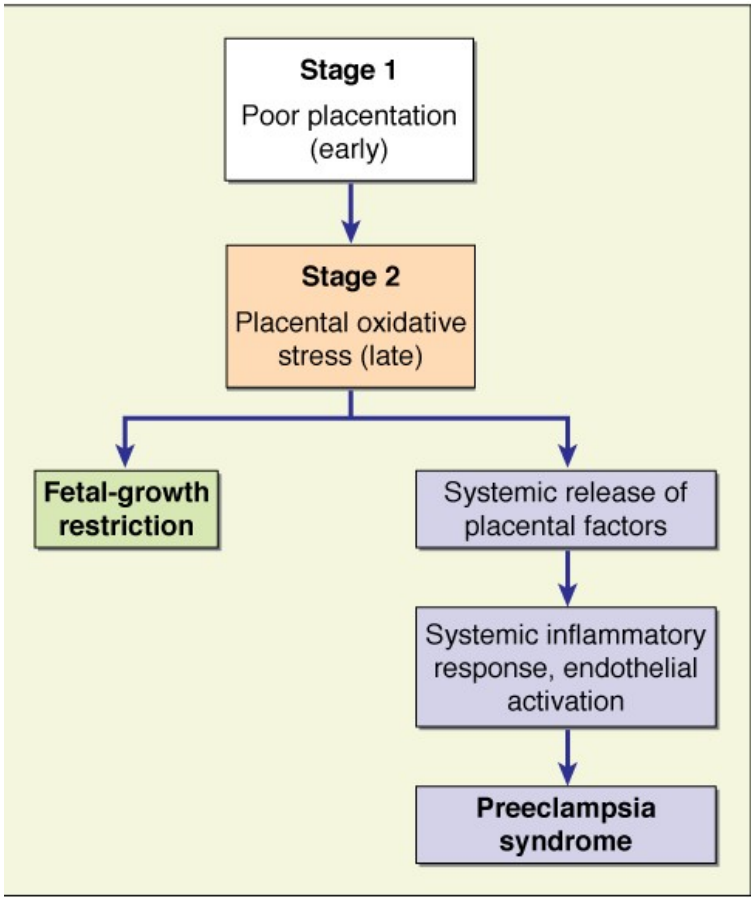


Figure -2A Normal pregnancy

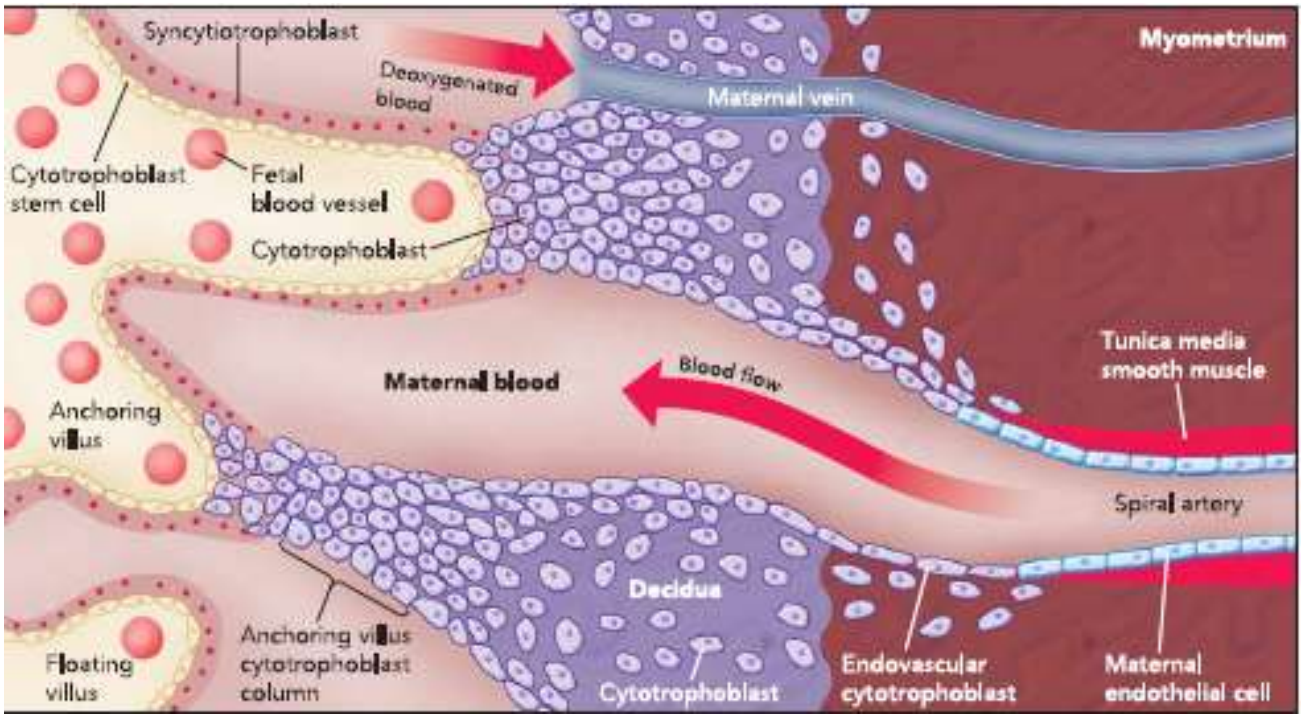
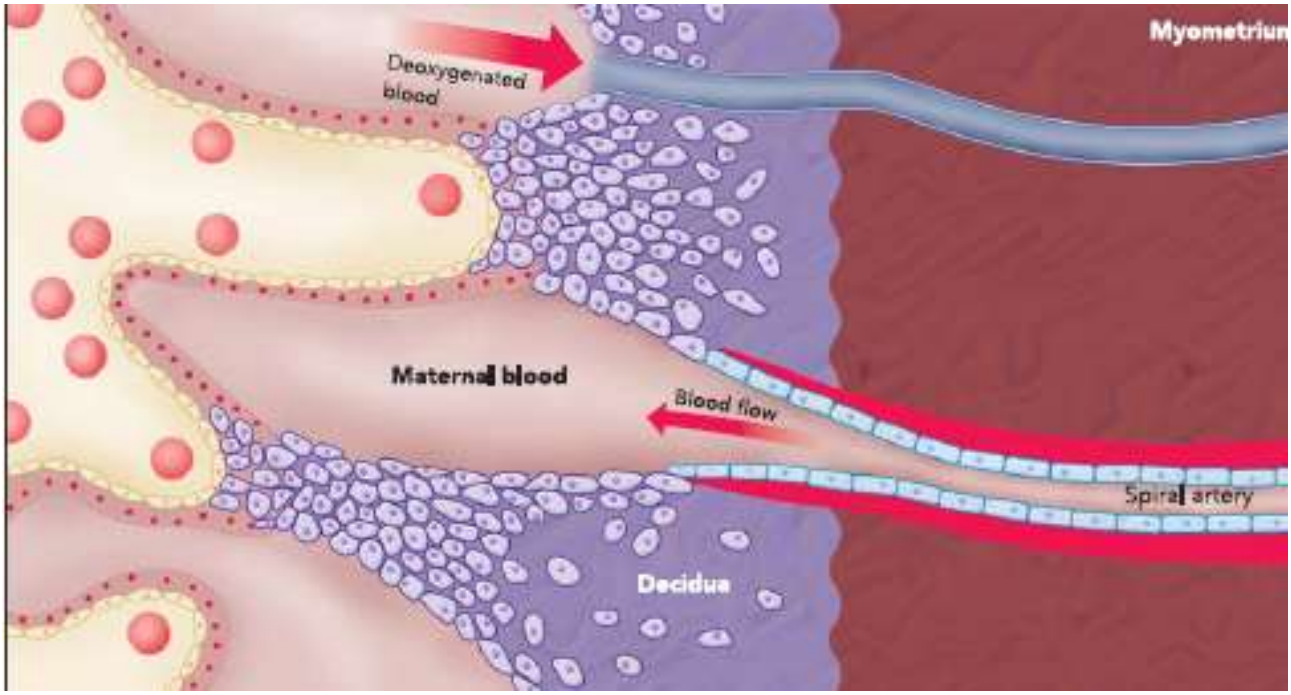


Figure-2B Preeclampsia



In normal pregnancies, a subset of cytotrophoblasts called invasive cytotrophoblasts migrate through the implantation site and invade decidua tunica media of maternal spiral arteries and replace its endothelium in a process called pseudovascularization. It involves a considerable number of transcription factors, growth factors, and cytokines. In women destined to develop preeclampsia, cytotrophoblast endovascular invasion remains shallow, leading to a defective uteroplacental circulation and subsequent placental ischemia. Biopsy specimens from preeclamptic placentas also show narrow and constricted vessels as a result of insufficient trophoblast invasion of maternal decidual arterioles [17]. Imbalance of proangiogenic and antiangiogenic factors produced by the placenta may play a major role in mediating endothelial dysfunction. Angiogenesis is critical for successful placentation and the normal interaction between trophoblasts and endothelium. (Figure 3A and 3B).

Several circulating markers of endothelial cell injury have been shown to be elevated in women who develop preeclampsia before they became symptomatic. These include endothelin, plasminogen activator inhibitor-1 and cellular fibronectin with an altered prostacyclin/thromboxane. Evidence also suggests that oxidative stress, circulatory maladaptation, inflammation, and humoral, mineral, and metabolic abnormalities contribute to the endothelial dysfunction and pathogenesis of preeclampsia [17].

Figure -3A

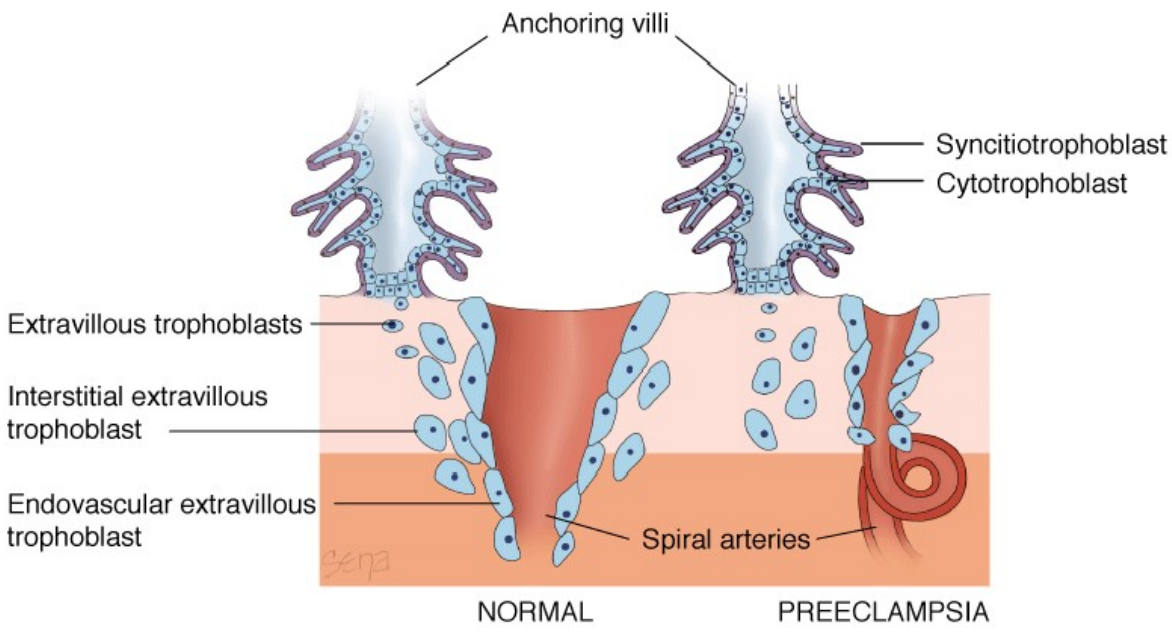
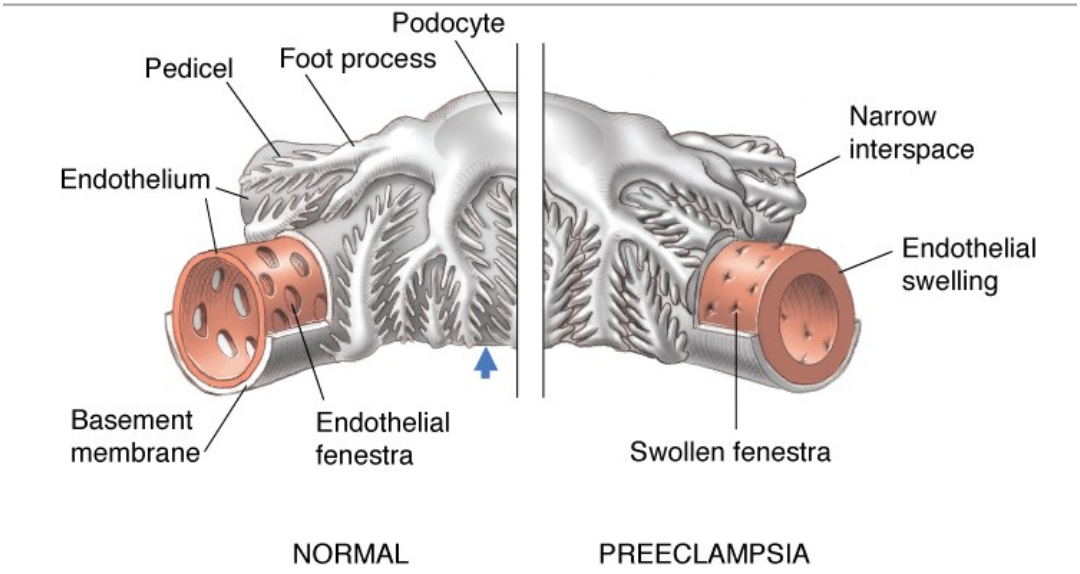


Figure-3B



Circulating Angiogenic Factors

As research in the field of preeclampsia is progressing, much of the attention in recent years has been focused on peptides related to angiogenesis. Angiogenesis, the development of new blood vessels from existing endothelium, is essential for normal placental development. Two of the angiogenic growth factors, vascular endothelial growth factor (VEGF) and placental induced growth factor (PlGF) are thought to contribute to normal trophoblastic proliferation and implantation. It has been hypothesized that an imbalance in levels of these growth factors has a crucial role in preeclampsia [18].

A soluble and endogenously secreted form of sFlt-1 is produced by alternative splicing and contains the extracellular ligand-binding domain but not the transmembrane and cytoplasmic portions [19]. sFlt-1 is able to block the effects of VEGF by inhibiting interaction with its receptors. Similarly, sFlt-1 also inhibits placental growth factor (PlGF), a member of the VEGF family of growth factors, which is produced chiefly by the placenta. Increased sFlt-1 during preeclampsia is associated with decreased PlGF in the blood. In vitro studies have indicated that the antiangiogenic state in preeclampsia induced by excess placental production of sFlt-1 could be “rescued” by giving PlGF [20].

PlGF

Placenta Induced Growth Factor, a member of the vascular endothelial growth factor family, is a 132-aminoacid residue, 50-kDa dimeric glycoprotein, shares a number of biochemical and functional features with other members of the vascular endothelial growth factor family. Placental induced growth factor is an important local mediator of angiogenesis. Prominent expression of placental induced growth factor has been identified in placenta, human umbilical vein endothelial cells, and choriocarcinoma cell lines. In the placenta, placenta induced growth factor is produced mainly by the cytotrophoblast, syncytiotrophoblast, and extravillous trophoblast. Previously, decreased levels of placental induced growth factor in preeclamptic patients after the clinical manifestations occurred had been reported, mostly in the third trimester [21]. PlGF has been demonstrated to be decreased in preeclamptic serum. This is most likely because of its binding with elevated levels of circulating sFlt-18 rather than decreased production of PlGF by the preeclamptic placenta [22].

The expected trend of PlGF concentrations in normal pregnancy is a steady increase during the first 2 trimesters, a peak at 29 to 32 weeks, and a consistent decline thereafter. The decrease of PIGF is thought to be a result of increasing sFlt-1 concentrations from 33 to 36 weeks of gestation through the end of pregnancy

and indeed, is the reciprocal of sFlt-1. That is the higher the sFlt-1 concentration, the lower the PlGF level. Numerous studies have documented that beginning in the early second trimester and as early as 10 to 11 weeks of gestation, PlGF concentrations in women who develop preeclampsia are lower than those of normotensive controls. Taylor et al in his study quoted serum PlGF levels at 21 to 32 weeks of gestation were lower in earlier-onset preeclampsia (37 weeks) versus later onset; in severe versus mild preeclampsia; and in preeclampsia associated with Small for Gestational Age (SGA) rather than an appropriate-size-for-gestational-age (AGA) infant [23].

In preeclampsia, PlGF concentrations begin to decrease 9 to 11 weeks before the appearance of hypertension and proteinuria, with considerable diminution during the 5 weeks before the onset of disease. More than 5 weeks preceding occurrence of the maternal syndrome, the difference in PlGF levels between normotensive controls and those who later developed preeclampsia was less marked [23].

PENTRAXIN-3

Pentraxin (PTX3) is a recently described inflammatory molecule that belongs to the same family of the well known C-reactive protein (CRP) [24]. PTX3 differs from CRP in terms of cellular origin, molecular inducers, and kinetic of production. It is expressed by different cells like endothelial cells, macrophages, monocytes and

fibroblasts exposed to inflammatory stimuli. PTX3 plasma levels increase dramatically during endotoxic shock, sepsis and other inflammatory conditions. PTX3 plays an important role in innate immunity, female fertility, and inflammatory processes. PTX3 is produced at high levels by vessel wall elements, binds to the angiogenic growth factor fibroblast growth factor 2 and tunes its action in vitro and in vivo. PTX3 plasma levels are increased in vascular disorders including myocardial infarction and small vessel vasculitis and correlate with outcome or disease activity. These biological and clinical properties of PTX3 prompted us to investigate this molecule in preeclampsia, a syndrome characterized by a prominent vascular component [25].

The long pentraxin 3 (PTX3) was first identified as a TNF- stimulated gene in fibroblasts16 and as an interleukin (IL-1) inducible gene in endothelial C ells. Elevated serum PTX3 levels have been described in some infectious and inflammatory conditions. Experimental models indicate a no redundant role of PTX3 in immunity to fungi and in female fertility. Smooth muscle cells, adipocytes, mononuclear phagocytes produce PTX3 as well. PTX3 binds to dying cells and restricts the cross-presentation of antigens derived from the processing of the dying cell, possibly limiting their immunogenicity. PTX3 could reveal higher extents of tissue remodeling during preeclampsia, and therefore, that higher circulating levels of this protein could identify patients with preeclampsia [25].

Umbilical Artery Doppler-

Assuming that defective placental circulation results in adverse pregnancy outcome, Doppler ultrasonography has been used as a modality to evaluate placental circulation and fetal well being for about three decades [26]. Abnormal development of placental vasculature is considered as the pathophysiological basis for development of preeclampsia and this could be reflected in abnormal umbilical Doppler velocimetry. In normal pregnancies, the feto-placental circulation acts as a low resistance system unit. Thus, the blood velocity waveforms in umbilical artery (UA) shows a continuous forward flow throughout the cardiac cycle. Goldkrank et al documented a steady increase in the blood flow of the umbilical artery as pregnancy progresses. The diameter of the umbilical artery increases until reaching a plateau at 32-34 weeks' gestation, whereas the systolic/diastolic (S/D) ratio, resistance index (RI) decrease throughout pregnancy. An abnormally elevated impedance to blood flow in the umbilical artery is an indirect reflection of placental pathology. Studies of placentas obtained from pregnancies with abnormal umbilical artery velocity waveforms end-diastolic flow in the umbilical artery show vascular sclerosis with obliteration of tertiary stem villi. The results of Seyam et al [27] study revealed that fetuses with abnormal UA velocity waveforms are at a significantly increased risk for early delivery, decreased birth weight, and neonatal intensive care unit admissions. The association between abnormal UA

Doppler indices and lower arterial and venous pH values, shows a increased likelihood of intrapartum fetal distress, and a higher incidence of respiratory distress syndrome. Acharya et al also showed that the frequency of preeclampsia, intrauterine growth retardation, oligohydramnios and nicotine abuse were significantly higher in a group of patients with reverse flow of umbilical artery compared to the control group [28].

LDH (Lactate Dehydrogenase)

Preeclampsia is a syndrome which affects almost all maternal organ systems. There is in increasing evidence that endothelial cell and altered endothelial cell function play an important role in the pathogenesis of preeclampsia. LDH is most often measured to evaluate the presence of tissue damage. The enzyme LDH is in many body tissues, especially heart, liver, kidney, skeletal muscle, brain, blood cells and lungs. Dysfunction of endothelial cell can contribute to inappropriate vasoconstriction and platelet aggregation which are early signs of atherosclerosis, hypertension and coronary vasospasm. Acute clinical symptoms that danger life of fetus in preeclampsia correlate with distinct activity of AST and LDH [29].

Intra Uterine Growth Restriction

The theory of abnormal placental implantation or reduced trophoblast invasion continues to link preeclampsia and intrauterine growth restriction (IUGR) as

pregnancy disorders with a common pathogenesis. In fact, fetal IUGR has been traditionally included in the diagnostic criteria of severe preeclampsia regardless of other maternal manifestations of the disease. The impact of diagnosing severe preeclampsia after a certain gestational age is iatrogenic delivery, which often is preterm. Given the wide range of clinical phenotypes in preeclampsia, this raises the question of whether all the criteria that determine severity of preeclampsia should be managed similarly to optimize maternal and fetal outcome. Further confounding the relationship between preeclampsia and IUGR is that preexisting maternal co-morbid conditions, such as chronic hypertension (CHTN), have been associated with the development of IUGR, independent of preeclampsia. Current theories suggest that the leading insult for IUGR in preeclampsia and in maternal preexisting CHTN is abnormal placental trophoblast invasion [30].

METHODS AND METHODOLOGY

- **Type of study**- Prospective
- **Period of study** – September 2009 to September 2011
- **Sample size-** 35 confirmed cases of pre eclampsia and 35 normal pregnant women.
- **Place of study** – PSG Institute of Medical Science and Research, Coimbatore.
- The study was approved by Institutional Ethical Committee.
- **Plan of study** – Patients who are diagnosed as cases of Pregnancy Induced Hypertension are included under Group A and Normotensive Pregnant women are included under Group B.
- **Inclusion Criteria**

  Pregnant women under-

  Any age group

  Any parity

  Gestational age > 20 weeks

- **Exclusion Criteria**

  Pregnant women with following morbidity

Chronic renal disease

Hypertension

Systemic Lupus Erythematosus (SLE)

Multiple pregnancy

Diabetes

Epilepsy

Sepsis and endotoxic shock.

- The plasma samples will be analyzed for various biomarkers (PlGF, and PTX3) using ELISA kits developed by R&D system. The assay will be performed according to manufacturer's instruction. The experiments will be carried out in duplicates.
- **Statistical Analysis**

  Analysis of variance (ANOVA) has been used to find the significance of study parameters between three or more groups of patients, Student t test has been used to find the significance of study parameters. Leven1s test for homogeneity of variance has been performed to assess the homogeneity of variance. Chi-square/ Fisher Exact test has been used to find the significance of study parameters on categorical scale between two or more groups. ROC curve analysis was performed to find the diagnostic markers for early

diagnosing the pre-eclampsia. Pearson correlation was used to establish the correlation of LDH and Uric acid with ng/mL and PlGF Pg/mL.

Significant figures

+ Suggestive significance (P value: $0.05<P<0.10$)

* Moderately significant (P value:$0.01<P \leq 0.05$)

** Strongly significant (P value : $P \leq 0.01$)

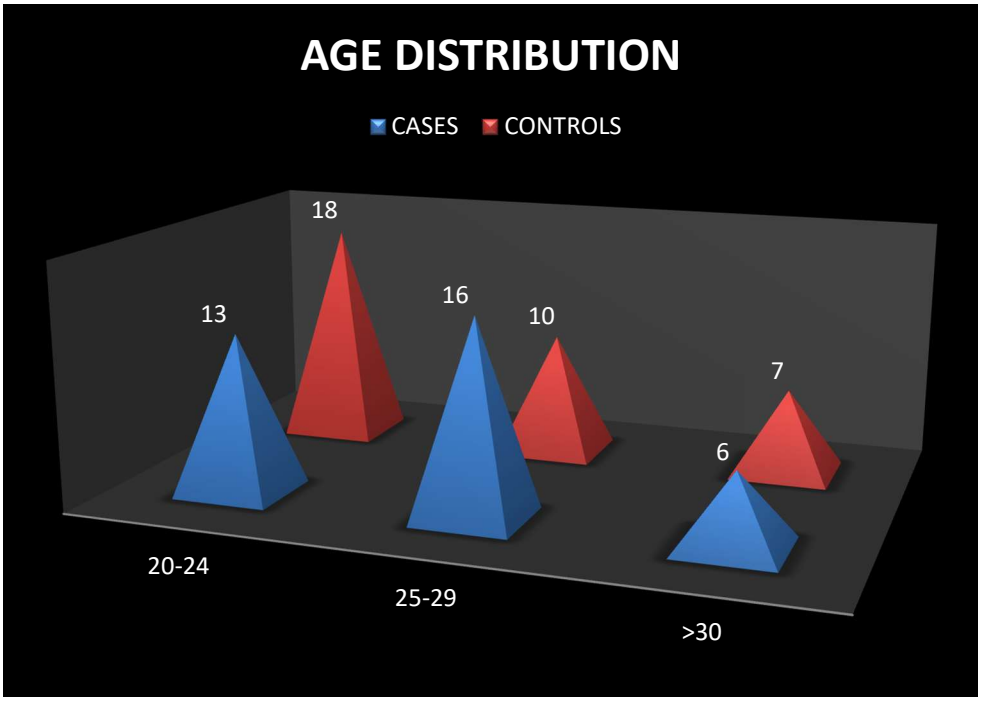
Statistical software: The Statistical software namely

- SAS 9.2
- SPSS 15.0,
- Stata 10.1,
- MedCalc 9.0.1 ,
- Systat 12.0 and
- R environment ver.2.11.1 was used**.**

OBSERVATION AND RESULTS

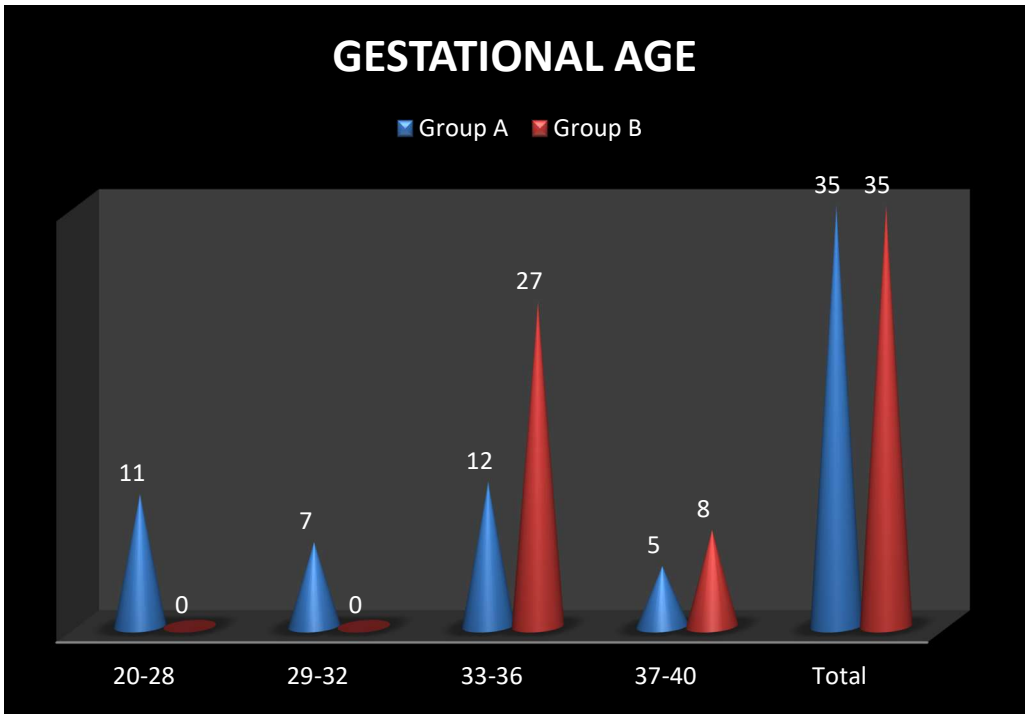
1. Age Distribution

Age in years	Group A		Group B	
	No	%	No	%
20-24	13	37.1	18	51.4
25-29	16	45.7	10	28.6
30 & above	6	17.1	7	20.0
Total	35	100.0	35	100.0
Mean ± SD	25.77±4.01		25.08±4.34	



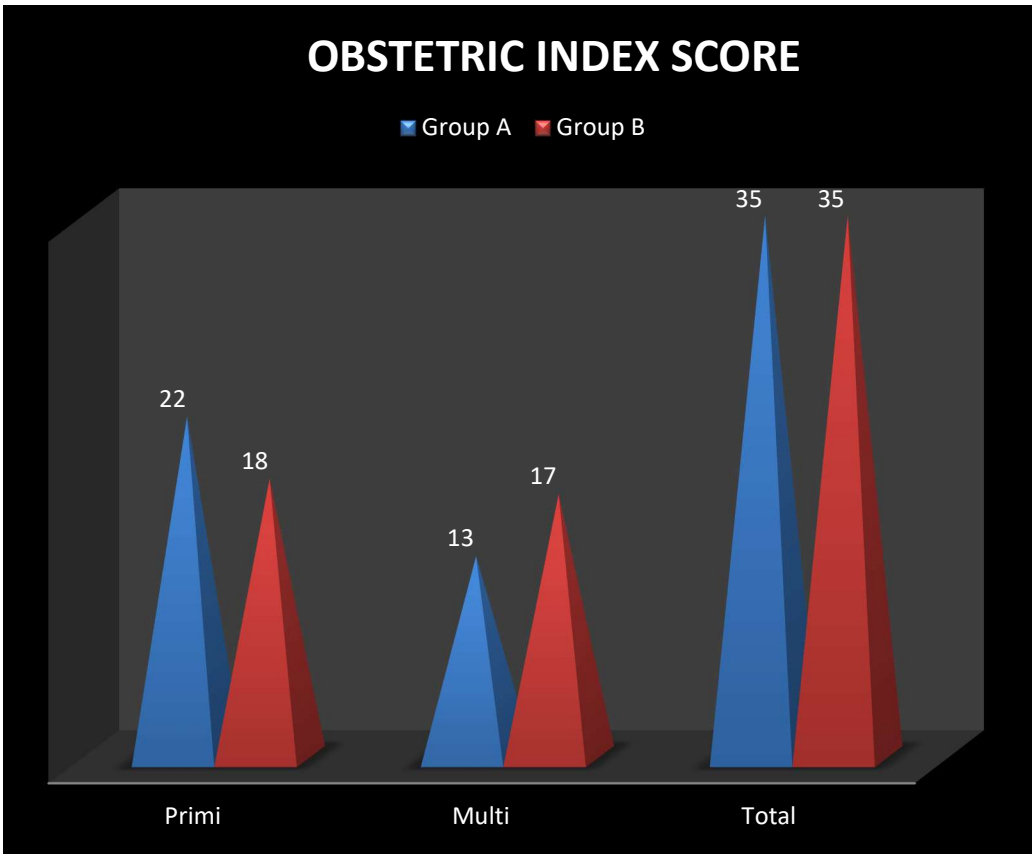
2. Distribution of study population based on Gestational Age

Gestational age in weeks	Group A		Group B	
	No	%	No	%
20-28	11	31.4	0	0.0
29-32	7	20.0	0	0.0
33-36	12	34.3	27	77.1
37-40	5	14.3	8	22.9
Total	35	100.0	35	100.0
Mean ± SD	29.74±8.67		35.77±0.94	



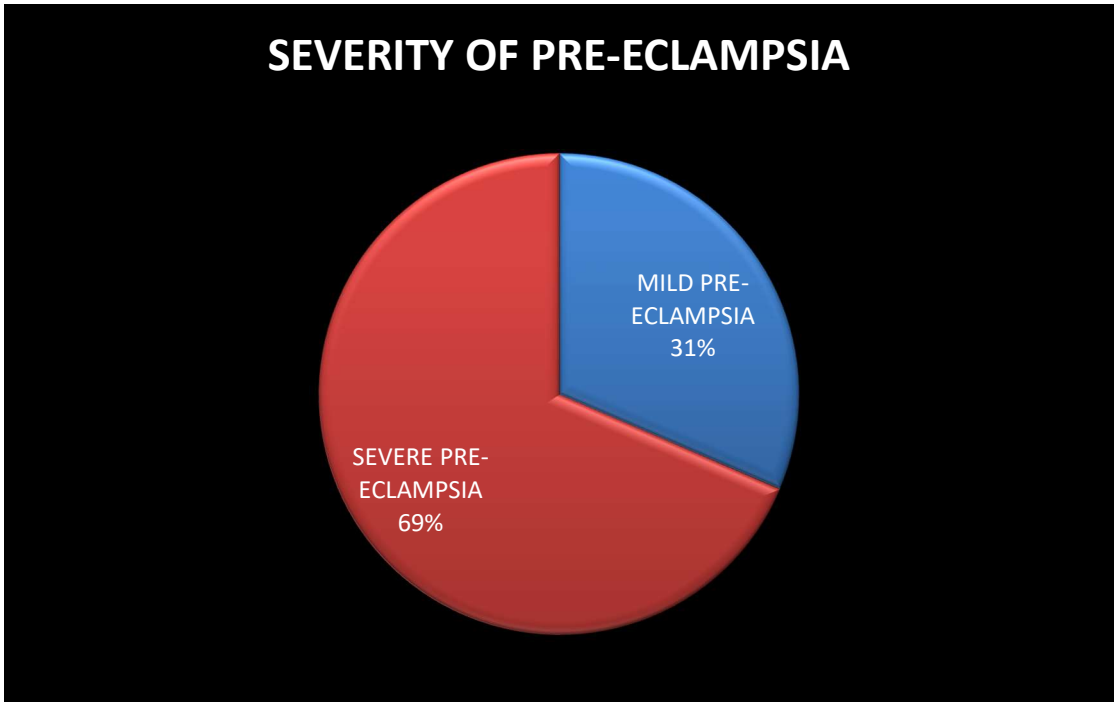
3. Obstetric index score

Obstetric Index score	Group A		Group B	
	No	%	No	%
Primi	22	62.9	18	51.4
Multi	13	37.1	17	48.6
Total	35	100.0	35	100.0



4. No. of patients under mild and severe preeclampsia

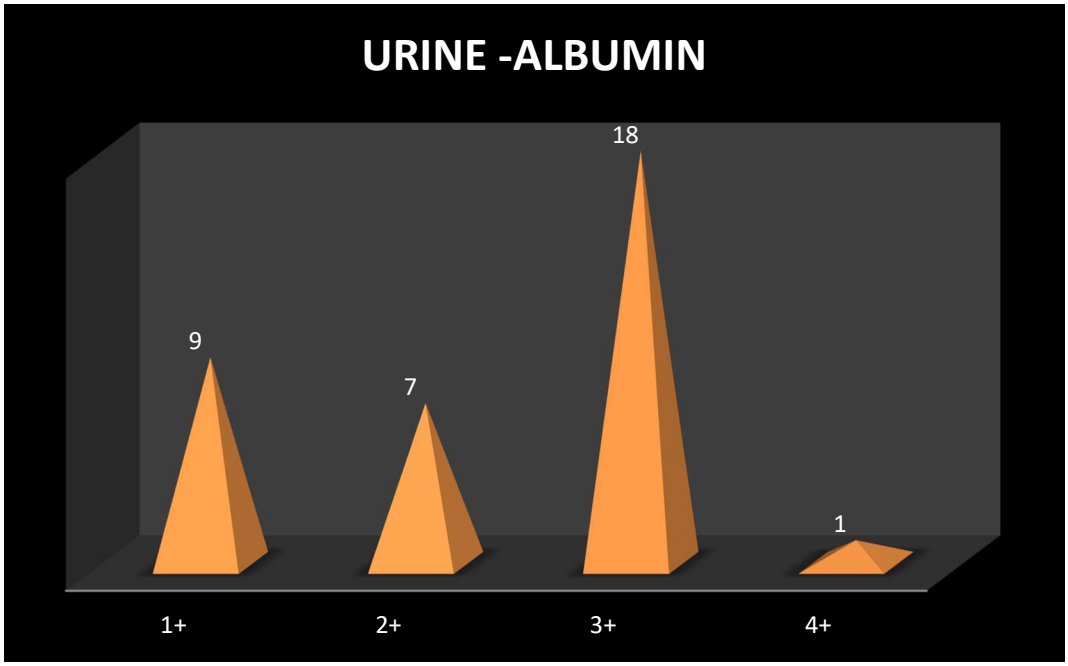
PRE-ECLAMPSIA	PTS NO
Mild pre-eclampsia	11
Severe pre-eclampsia	24
Total	35



Cases presented with severe eclampsia is 68.5%

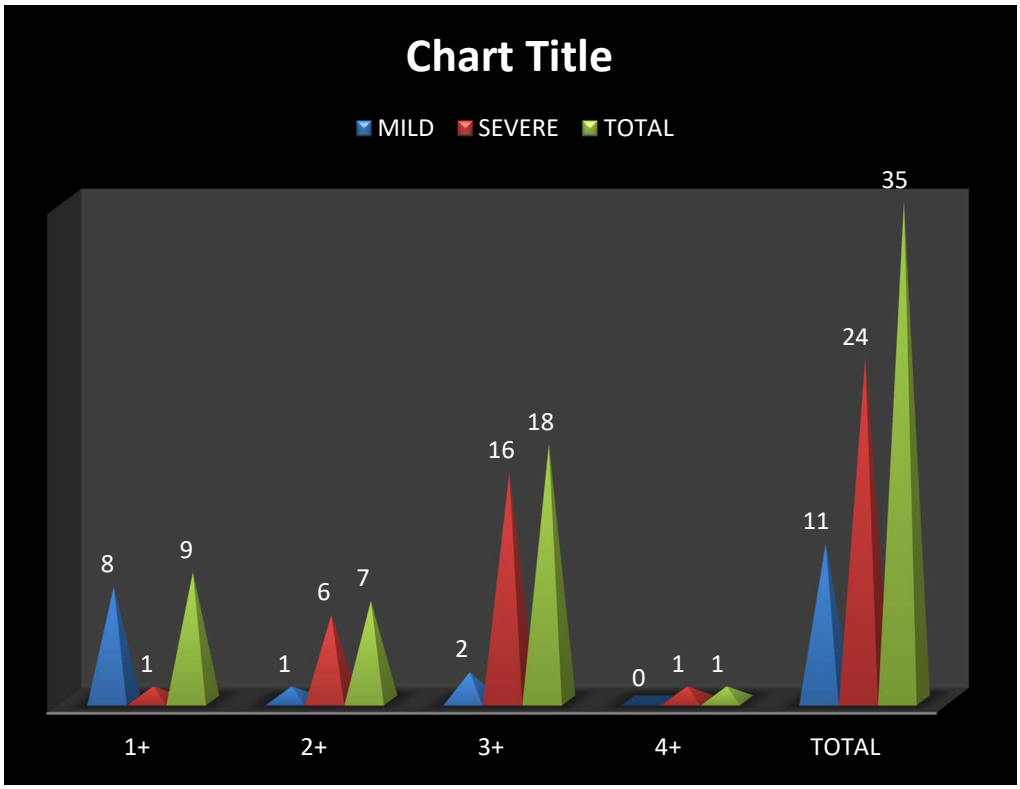
5. Urine Albumin in Group A

URINE ALBUMIN	PATIENTS
1+	9
2+	7
3+	18
4+	1



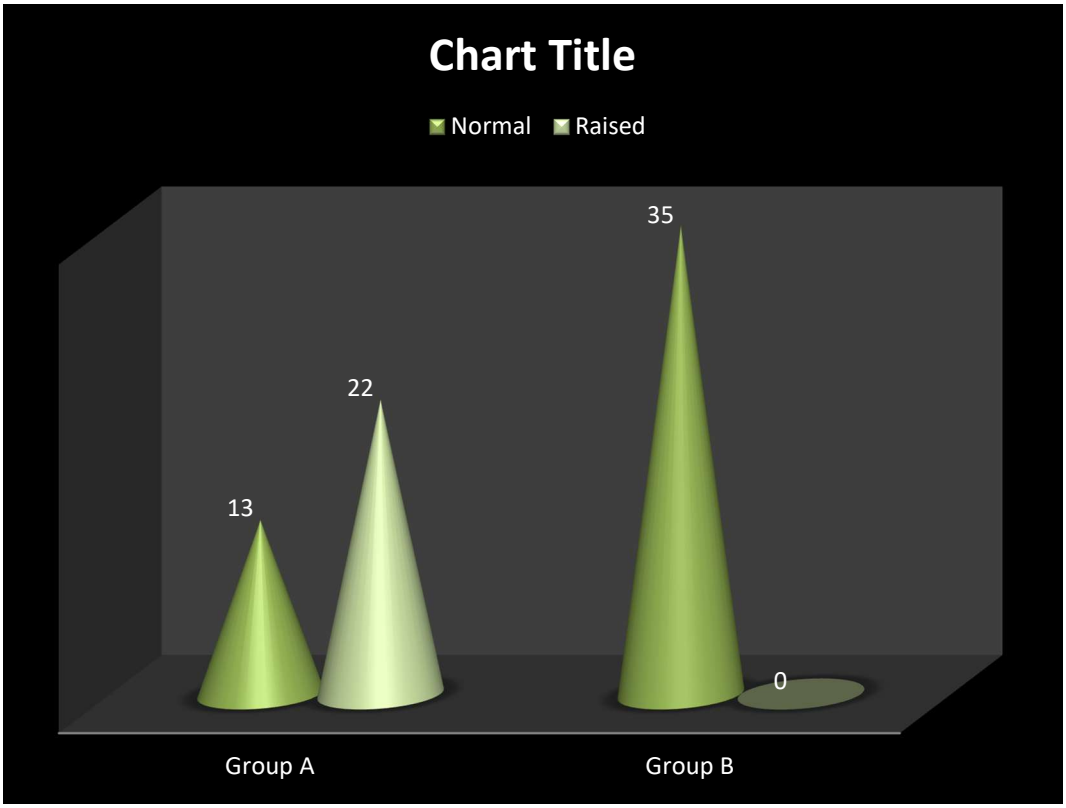
6. Urine Albumin with preeclampsia of varying severity

PE	1+	2+	3+	4+	TOTAL
MILD	8	1	2	0	11
SEVERE	1	6	16	1	24
TOTAL	9	7	18	1	35



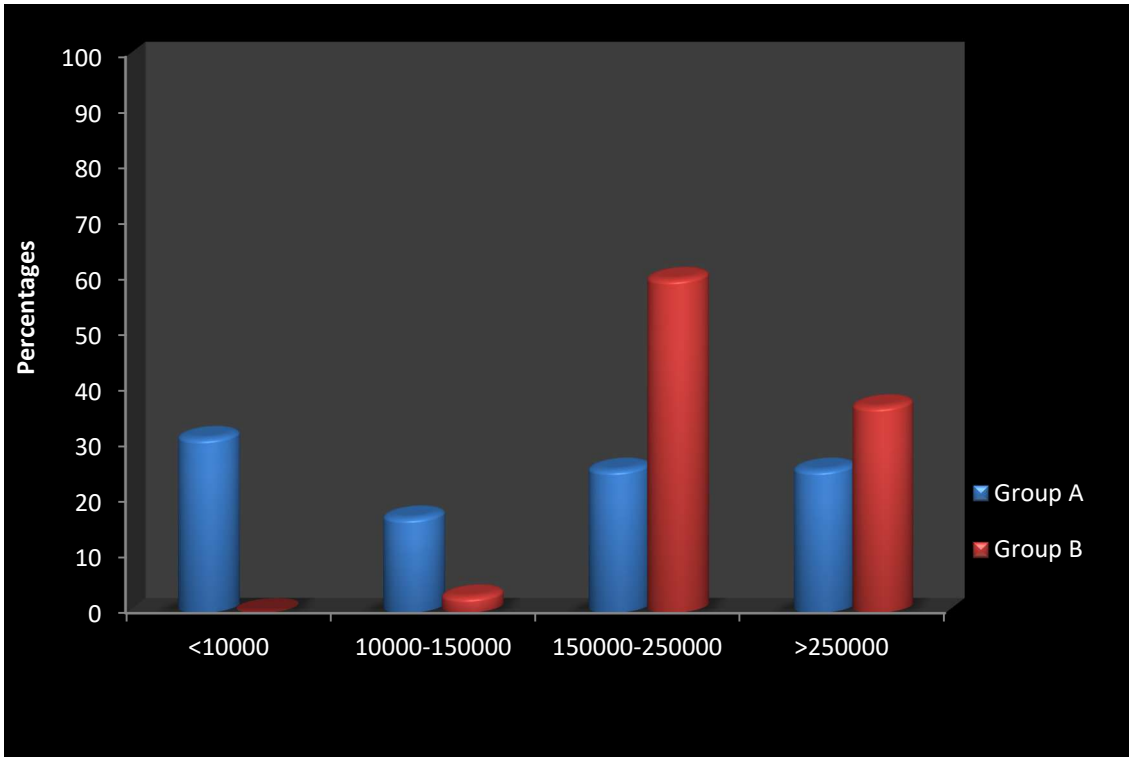
7. Liver Function test

LFT	Group A (n=35)		Group B (n=35)	
	No	%	No	%
Normal	13	37.1	35	100.0
Raised	22	62.9	0	0.0
Inference	Raised LFT is significantly more associated with Group A with P<0.001**			



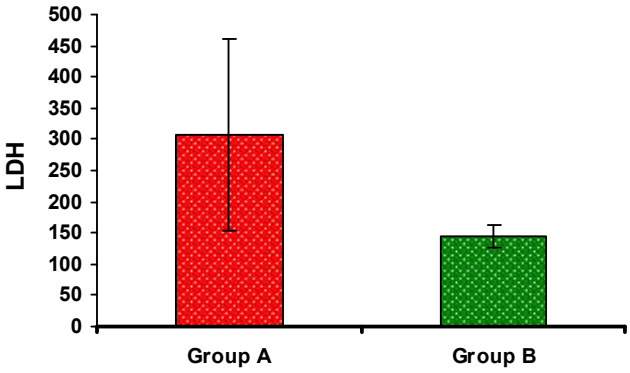
8. Platelet count

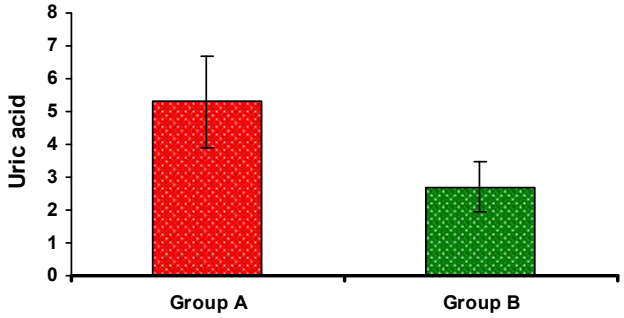
Platelet count	Group A (n=35)		Group B (n=35)	
	No	%	No	%
<10000	11	31.4	0	0.0
10000-150000	6	17.1	1	2.9
150000-250000	9	25.7	21	60.0
>250000	9	25.7	13	37.1
Mean ± SD	176428.6±97239.3		239385.7±58027.63	
Inference	Mean platelet count is less in Group A when compared to Group B with P=0.002**			

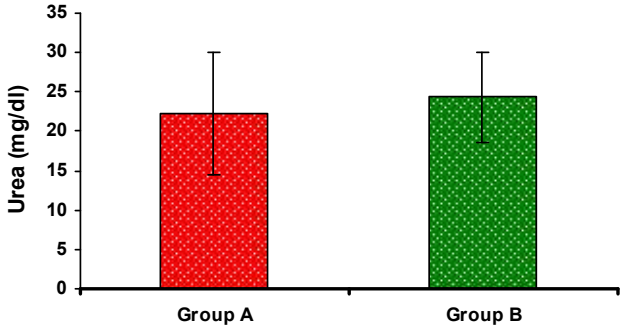


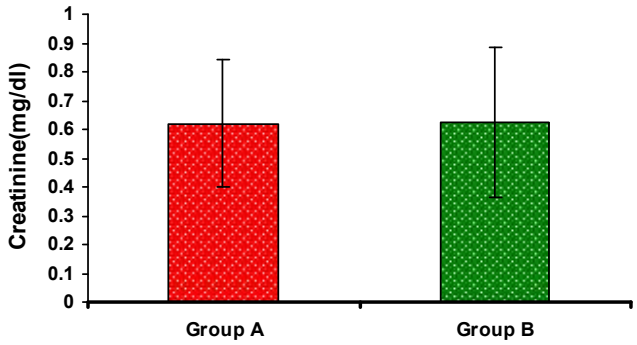
9. Uric acid, Urea And creatinine

Components	Group A	Group B	P value
LDH	308.31±153.21	145.08±17.98	<0.001**
Uric acid	5.29±1.40	2.71±0.74	<0.001**
Urea (mg/dl)	22.17±7.77	24.34±5.73	0.188
Creatinine(mg/dl)	0.62±0.22	0.623±0.26	0.968



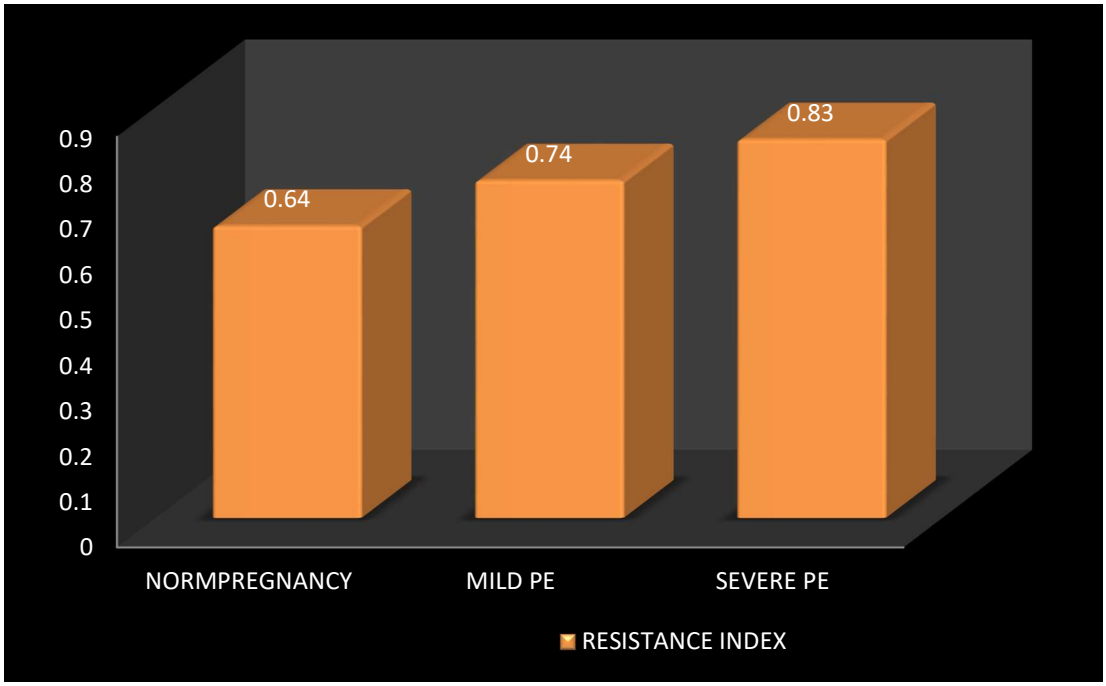






10.Sensitivity of umbilical artery Doppler

Index	Normal Pregnancy	Mild Preeclampsia	Severe Preeclampsia
RESISTANCE INDEX	0.64	0.74	0.83



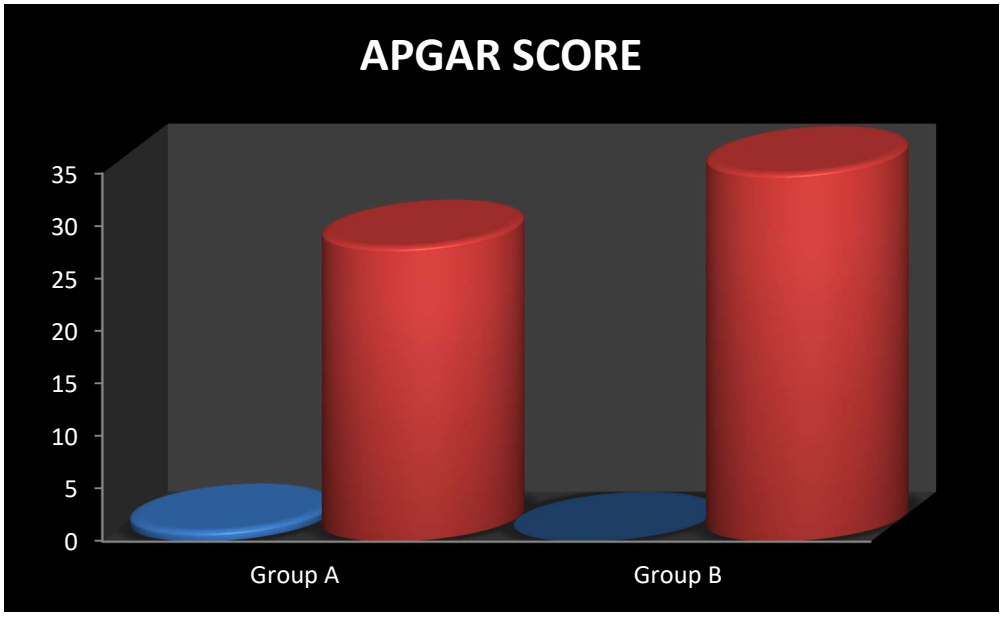
Chi-square test, p value is 0.001 which is clinically significant.

11. Outcome: Apgar score

Outcome Apgar score	Group A (n=29)		Group B (n=35)	
	No	%	No	%
<7	1	3.6	0	0.0
>7	27	96.6	35	100.0

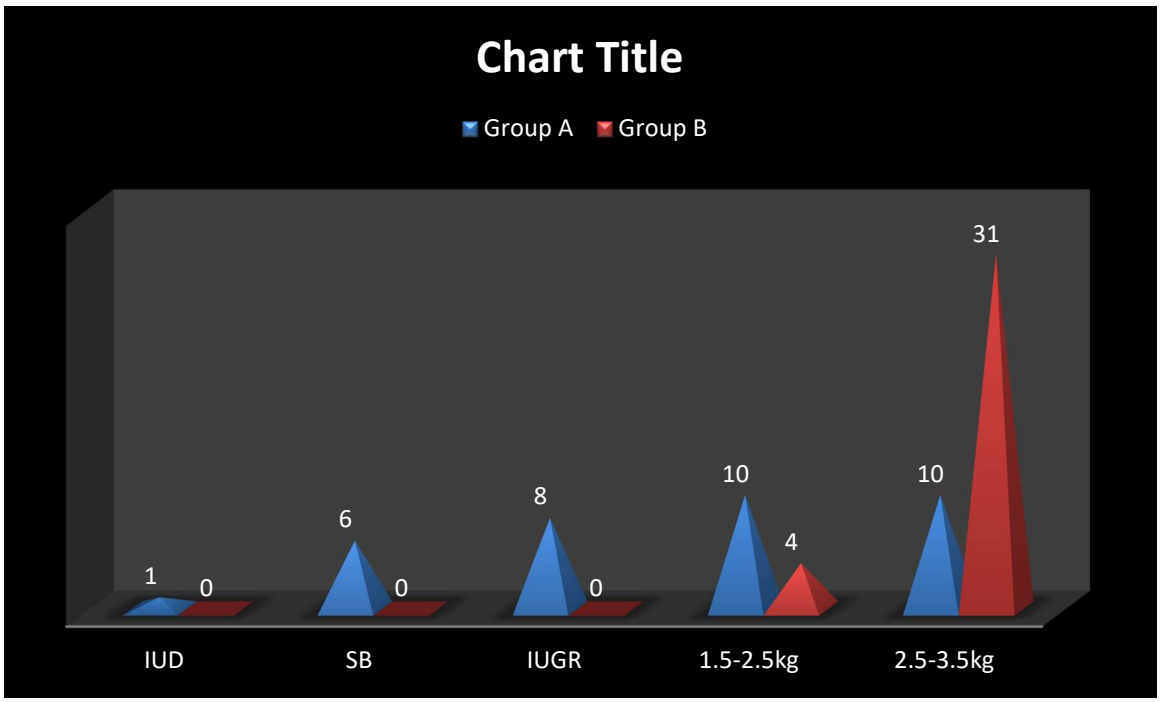
SB-1

IUD - 6



12. Pregnancy outcomes among women with pre eclampsia

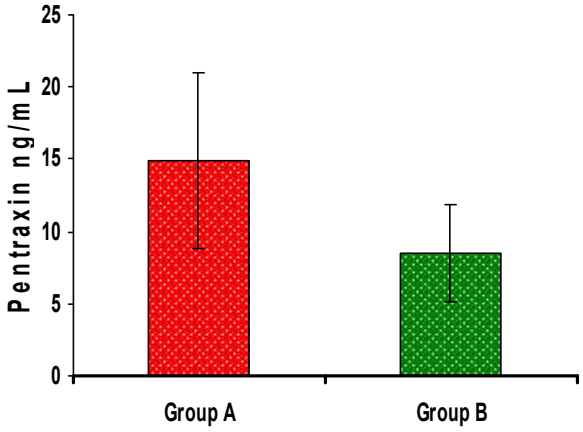
Outcome Birth weight	Group A (n=35)		Group B (n=35)	
	No	%	No	%
IUD	1	2.9	0	0.0
SB	6	17.1	0	0.0
IUGR	8	22.9	0	0.0
1.5-2.5 kg	10	28.6	4	11.4
2.5-3.5 kg	10	28.6	31	88.6
Mean ± SD	2.09±0.70		2.69±0.22	

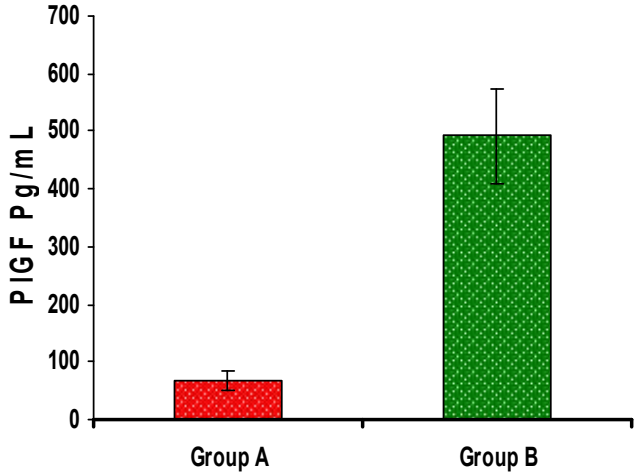


13.Comparison of Pentraxin ng/mL and PlGF Pg/mL

Biomarkers	Group A	Group B	P value
Pentraxin ng/mL	20.85±6.12	11.83±3.42	<0.001
PlGF Pg/mL	33.26±17.01	570.80±81.53	<0.001

Biomarkers		Group A	Group B
PENTRAXIN ng/ml	Highest	103.3	68
	lowest	0.113087961	0.0156
PIGF pg/ml	Highest	410	1486
	lowest	4.815041215	65.578048





14. PIGF

Cases	PlGF Pg/mL	Controls	PIGF Pg/ml
PRC 1	27.8228577	PRC 1	412
PRC 2	67.11989443	PRC 2	1486
PRC 3	410.5925958	PRC 3	759.1595508
PRC 4	14.74797714	PRC 4	243.0758657
PRC 5	171.8670962	PRC 5	451.7836888
PRC 6	4.815041215	PRC 6	139.751984
PRC 7	126.6407375	PRC 7	652.8903696
PRC 8	80.03988328	PRC 8	257.8761052
PRC 9	nil	PRC 9	1378
PRC 10	43.56861008	PRC 10	636.0576815
PRC11	52.06071638	PRC11	328.1935738
PRC12	126.0905352	PRC12	430.7857606
PRC13	67.47816569	PRC13	1183
PRC14	174.9693078	PRC14	345.9152057
PRC15	50.98623933	PRC15	96.85708223
PRC16	74.27690155	PRC16	647.2671271
PRC17	77.68687619	PRC17	210.374522
PRC18	8.606857595	PRC18	93.80773589
PRC19	44.60537437	PRC19	65.57804848
PRC20	Nil	PRC20	147.1059729
PRC21	8.910916754	PRC21	932.9
PRC22	125.740682	PRC22	656.7
PRC23	13.05	PRC23	766
PRC24	26.69	PRC24	1462
PRC25	218.3	PRC25	1321
PRC26	98.12	PRC26	116.8
PRC27	5.58	PRC27	1278
PRC28	147	PRC28	817.8
PRC29	124.1	PRC29	109.3
PRC30	179.4	PRC30	648.4
PRC31	32.34	PRC31	296.345
PRC32	18.07	PRC32	356.1243
PRC33	27.01	PRC33	197.329
PRC34	50.13	PRC34	598
PRC35	89.98	PRC35	456

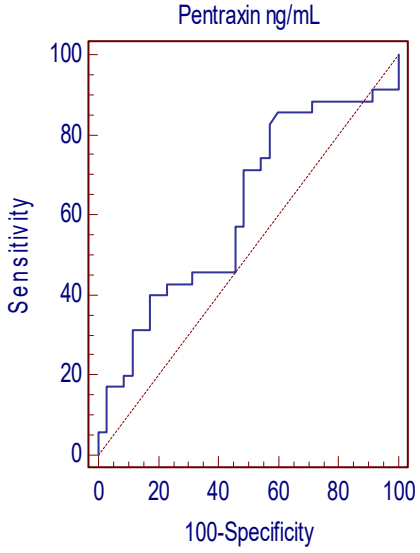
15.PENTRAXIN

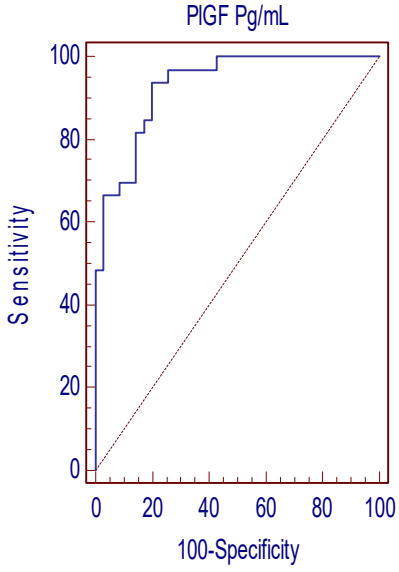
Controls	Pentraxin ng/mL	Cases	Pentraxin ng/mL
PRCC1	5.608229111	PRC1	3.531079468
PRCC2	3.526736621	PRC2	11.66698887
PRCC3	6.341609128	PRC3	11.49
PRCC4	3.273046032	PRC4	3.554306381
PRCC5	2.911467408	PRC5	13.94
PRCC6	2.455541649	PRC6	6.990352421
PRCC7	14.65	PRC7	61.36
PRCC8	68.89	PRC8	11.63422233
PRCC9	0.625421936	PRC9	4.000326544
PRCC10	42.99	PRC10	51.79
PRCC11	0.620639925	PRC11	0.2763107
PRCC12	2.319083536	PRC12	4.499192814
PRCC13	4.203806013	PRC13	49.61
PRCC14	13.84	PRC14	0.113087961
PRCC15	0.558107805	PRC15	0.57552799
PRCC16	13.14	PRC16	2.510632374
PRCC17	14.52	PRC17	12.17
PRCC18	0.857983842	PRC18	8.456380515
PRCC19	45.35	PRC19	7.910133294
PRCC20	12.97	PRC20	8.413586392
PRCC21	13.57	PRC21	0.146537643
PRCC22	11.34	PRC22	3.891096619
PRCC23	26.95	PRC23	7.611965119
PRCC24	12.63	PRC24	16.14295162
PRCC25	18.58	PRC25	25.06
PRCC26	21.63	PRC26	13.15
PRCC27	13.39	PRC27	24.18
PRCC28	13.07	PRC28	14.75
PRCC29	2.1345	PRC29	15.72
PRCC30	3.21	PRC30	102.26
PRCC31	1.667	PRC31	34.51
PRCC32	0.4756	PRC32	103.3
PRCC33	12.326	PRC33	22.17
PRCC34	0.5632	PRC34	24.62
PRCC35	2.9891	PRC35	47.83

16. ROC curve analysis for assessing the diagnostic markers of pre-eclmapsia of pregnant women

Markers	Cut-off value	Accuracy	Specificity	P value	AUC
Pentraxin ng/mL	>3.27	62.86	40.00	<0.001	0.616
PlGF Pg/mL	<179.4	84.29	80.00	<0.001	0.931
LDH (Lab cut-off)	>250	77.14	100.00	<0.001	-
Uric acid (Lab cut-off)	>6.0	61.11	100.0	0.001	-

LDH and Uric acid have low sensitivity but high specificity, while the PIGH is good diagnostic marker with high sensitivity and specificity (>80.0%) while Pentraxin ng/mL has high sensitivity but low specificity





DISCUSSION

The aim of this study was first to define whether pregnancy itself, a condition associated with relevant involvement of inflammatory molecules at the implantation site, is associated with changes in maternal circulating PTX3 levels and PIGF compared with the normal pregnant condition. Most of the patients in our study in cases are aged 20 - 24 years which is 37.1%, 25-29 years is 45.7% and more than 30 years is 17% and as per controls 20-24 years is 51.4%, 25-29 is 28.6% and more than 30 years is 20% which is very much identical to Ranjit Akolekar et al [31]. Primigravida constitutes of 57% and G2 of 24% which is almost identical to Assi F et al [32] and Ranjit Akolekar et al [31].

The cases presented with severe preeclampsia in our study is 24/35 (68.6%) and the primigravida presented with preeclampsia is 62.9% which is identical to Sivalingam N et al [33], Sharma AK et al [34], Robillard P et al [35], Ranjit Akolekar et al [31] and Adel F et al [36].

Pentraxins, in particular, are involved in their response against microbes. Recent data, however indicate that they are recruited at sites of tissue damage and repair and influence female fertility [24]. PTX3, which is generated in peripheral tissues under the control of inflammatory stimuli, edits the cross presentation of epitopes expressed by apoptotic cells to T lymphocytes[37]. Extensive cell death/tissue

necrosis represents a challenge for the immune system because autoantigens are released in a context in which antigen presentation is favored [38]. This is particularly important at the maternal/embryonic interface. Regulatory mechanisms exist at this level: apoptosis is a normal event during pregnancy, and accordingly, PTX3 levels rise in preeclamptic patients, whose tissues cope with a substantially higher load of placental debris [39].

The findings of our study confirm the association between PE(PreEclampsia) and increased maternal plasma concentration of PTX3 which shows PTX3 level is very much high in severe preeclampsia women with an average value of 20.8524194 ng/ml and where as for women who are controls the PTX3 is 11.8336307 ng/ml. In Group A the highest value of PTX3 is 103.3ng/ml and the lowest value is 0.113087961ng/ml, where as in Group B the highest value obtained is 68ng/ml and the lowest is 0.0156ng/ml, PTX3 levels in mild PE is 12.87ng/ml, in severe PE is 24.50ng/ml which shows PTX3 is further more increased in severe PE cases than mild PE, which is statistically significant ($p=<0.001$) and the similar results were seen in the study done by Cetin et al [40] and Rovere-Querini et al [41] . Impaired perfusion of the placenta is thought to cause hypoxia-related trophoblastic cell death and the release of inflammatory factors, which in turn cause endothelial dysfunction and the development of the clinical symptoms of the disease The

extent to which PTX3, which is expressed in vascular endothelial cells is involved in the pathogenesis of PE.

Women with preeclampsia have a significantly higher (6 to 10-fold) median serum/plasma PTX3 concentration than women with uncomplicated pregnancies[41]. Moreover, it has been reported that serum PTX3 concentrations correlate with the severity of preeclampsia [42]. Since PTX3 is expressed in endothelial cells it was proposed that elevated circulating concentrations of PTX3 in women with preeclampsia may represent a state of endothelial dysfunction that characterizes this obstetrical syndrome [43,44]. Robert et al in his study reported on maternal circulating PTX3 concentrations in women with preterm delivery. Assi et al[31]reported that, regardless of the clinical presentation women with a preterm delivery had a significantly higher maternal plasma PTX3 concentration than normal pregnant women.

PIGF

We also observed a significant decrease in the concentrations of PIGF in the sera of women with pre-eclampsia compared with the sera of normotensive, non-proteinuric pregnant women by the sandwich ELISA technique as been observed by betsy et al [45]. The patients with preeclamptic pregnancies showed a significant

lower serum concentration of PIGF compared to the patients with non-preeclamptic pregnancies [46].

The fetoplacental angiogenesis during gestation is biphasic, the first trimester is characterized by a branching of the small vessels. From the beginning of the second trimester, the gain of size of the placenta is a consequence of a proliferation of endothelial cells. In contrast to the branching angiogenesis of the first trimester, the angiogenesis of the second and third trimester is mainly characterized as non-branching angiogenesis. The underlying hypothesis that preeclampsia is caused by altered angiogenesis is supported by the fact, that the PIGF serum levels in patients with preeclampsia are significantly lower than the levels in patients with non-preeclamptic pregnancies [47].

Placental Induced Growth Factor measured for both Groups showed that the PIGF in Group B is 570.805 pg/ml, where as PIGF measured in Group A is 33.2692pg/ml, highest value of PIGF in Group A is 410pg/ml and lowest is 4.815041215pg/ml, where as the highest value in Group B is 1486pg/ml and the lowest is 65.578048pg/ml, so average PIGF values in mild PE is 95.64pg/ml, severe PE is 58.84pg/ml and in Group B is 570.805pg/ml which shows that PIGF is very much decreased in preeclamptic patient which is statistically significant ($p=<0.001$) The similar findings is seen in Seu et al [48], the risk of developing

preeclampsia subsequently was increased upto 2.5-fold. Angiogenesis, the sprouting of new capillaries from existing vessels, is an essential component of placenta development. In humans, vascular transformation occurs in the placental bed where extravillous trophoblast cells within the maternal decidual tissue transform the spiral arteries into high-flow, low-resistance vessels that are more efficient at supplying the placenta with maternal blood [49]. The placenta is a relatively rich source of angiogenic growth factors, and the regulation of vascular development in the placenta is thought to be highly associated with such factors. In recent years, great emphasis has been focused on placental growth factor, a key regulator of angiogenesis [50]. Placental induced growth factor, based on its 50% overall amino acid residual similarity with vascular endothelial growth factor, has been classified as a member of the vascular endothelial growth factor family of growth factors. It also shares a number of biochemical and functional similarities with other members of the vascular endothelial growth factor family. Moreover, it has the distinctive characteristic of being highly expressed only in the placenta, especially in the villous cytotrophoblast, syncytiotrophoblast, and extravillous trophoblast. These features suggest that placenta growth factor could play a significant role in the regulation of the vascular development in the placenta. Shore et al[51] reported that placenta growth factor expression decreased in trophoblasts cultured under hypoxic conditions. This finding suggests that the hypoxic

trophoblast will downregulate placenta growth factor expression. This finding was consistent with the observation of reduced maternal serum levels of placenta growth factor in patients with the clinical appearance of preeclampsia during the third trimester reported by Torry et al[21] and Reuvekamp et al [52]. In vitro, hypoxia had been found to induce significant morphologic changes in trophoblasts and reduce cytotrophoblast differentiation with less syncytial formation. These morphologic changes including limited cytotrophoblast invasion to the superficial decidua and few breached arterioles were also observed in the placenta of preeclamptic patients[53]. The phenomenon of poor vascular formation is possibly the response to hypoxia mediated through the activity of a hypoxia-regulated factor such as placenta growth factor. It seems that hypoxia will lead to poor vascular development of the placenta, and the poor vascular formation will in turn cause more severe hypoxia. This cascade of events will finally present the clinical manifestations of preeclampsia.

Moreover, the proper function of placenta growth factor in the physiologic and pathologic development of placentation needs further elucidation. But we did prove that lowered maternal serum placenta growth factor was strongly associated with the subsequent occurrence of preeclampsia. Accordingly, we can say that maternal serum placenta growth factor might be useful in screening the high-risk group of preeclampsia before the clinical appearance of maternal disease

(hypertension, proteinuria, and edema). Because of the lack of a proven prophylaxis for preeclampsia, prediction of risk to identify patients for more intensive care and possible intervention is attractive. Although placenta growth factor has been proved to be associated with preeclampsia and could be a potential marker for the early prediction of preeclampsia before the clinical manifestations are identified, further prospective, largescale, longitudinal studies are essential to determine the usefulness of placenta growth factor in predicting preeclampsia. These similar findings are obtained from the studies done by the Muna Noori et al[54], Alexander et al[55], Foidart et al[56], Simon Grill et al[57].

Platelet count in Group A patients with count less than 10,000 is 31.4% which is statistically significant compared to Group B in which there are no such variables. Platelet counts are decreased in Group A compared to Group B with p= <0.001, which is statistically significant.

LDH is most often measured to evaluate the presence of tissue damage. The enzyme LDH is in many body tissues, especially heart, liver, kidney, skeletal muscle, brain, blood cells, and lungs. LDH is a useful biochemical marker that reflects the severity of the occurrence of preeclampsia[58]. LDH levels in our study in mild preeclampsia women is 200.9IU/ml, in preeclamptic women is 357IU/ml, with HELLP is >600IU/ml and where as in controls is 145IU/ml with p<0.001,

which is statistically significant. The elevated LDH levels in preeclampsia are also found in many other studies such as Malarewicz A et al[59], Bayhan G et al[60]. Preeclampsia complicates 6% to 8% of all pregnancies with the majority of cases (75%) occurring during first pregnancies. The prevalence, complications as well as correlation of maternal and fetal outcome in a women in this study showed high incidence of preeclampsia. The multi organ dysfunction in severe preeclampsia caused by vascular endothelial damage, including maternal liver, kidney, lungs, nervous system, blood and coagulation system will lead to excessive LDH leakage and elevated levels in serum due to cellular dysfunction, which may cause the occurrence of preeclampsia these results are also supported by H S Qublan et al[58]. LDH values in our study had shown low sensitivity but high specificity.

Umbilical artery Doppler done in all the women in our study had Resistence Index which showed a range of 0.4-0.6 in normal women , 0.7 in mild preeclampsia and >0.8 in severe preeclampsia. Goldkrank et al[61] documented a steady increase in the blood flow of the umbilical artery as pregnancy progresses. The diameter of the umbilical artery increases until reaching a plateau at 32-34 weeks' gestation, whereas the systolic/diastolic (S/D) ratio, resistance index and pulsatility index (PI) decrease throughout pregnancy. Ertan et al[62]also showed that the frequency of preeclampsia, intrauterine growth retardation. In Arauz et al[63] study abnormal umbilical artery Doppler velocimetry was present in 52% of preeclamptic patients

and they suffered more from adverse neonatal outcomes than those with normal Doppler indices.

Regarding malplacentation as the main pathological event in preeclampsia, alteration in umbilical Doppler velocimetry is expected. However, considering the fact that preeclampsia is a disease of unknown etiology and can present with various degree of different organ dysfunction, the present study was designed to investigate the extent of changes in umbilical artery Doppler indices (resistance index)

Our results showed higher resistance indices in patients with preeclampsia than normal pregnancies. This confirms a state of high resistance in placental circulation in preeclampsia. Our findings are in line with the study performed by Goldker et al[61] which showed not only a higher pulsatility index in preeclamptic patients but also a significantly greater PI in severe cases of preeclampsia. In conclusion, S/D and RI as very safe and noninvasive tests seems to be more appropriate in excluding preeclampsia rather than confirming.

IUGR cases present in our study are 22.8% which is associated with increased risk of perinatal mortality and morbidity and both short-term and long-term maternal complications. The similar reports are observed in study of Witlin et al[64].

CONCLUSIONS

Despite there exists many different potential markers for preeclampsia, the reliability of these markers in predicting preeclampsia has been inconsistent between different studies. Furthermore, preeclampsia is a multifaceted disorder, certain say it is not one but several diseases. Although improvements in obstetric and neonatal care have led to a reduction in morbidity and mortality from preeclampsia, our ability to predict the condition has not improved significantly. We currently rely on “secondary prevention” of preeclampsia: women who have previously had the disease are closely monitored throughout pregnancy. The majority of women who develop preeclampsia, however, are only diagnosed once they have developed the full-blown manifestations of the condition, by which time treatment options are limited. Rather than being a separate condition, preeclampsia has been previously described as the extreme end of a maternal systemic response engendered by pregnancy itself. We have seen that many of the proposed biomarkers for preeclampsia are raised to a lesser extent in normal pregnancy, which will make discovery of accurate biomarkers for preeclampsia. From a diagnostic standpoint, the discovery of soluble angiogenic markers used either alone or in combination with other markers offers tremendous promise in the

diagnosis and screening of preeclampsia. Although there is a significant and growing body of evidence supporting the diagnostic use of such markers, virtually all of the data are retrospective. Nevertheless, if signs of abnormal placental and endothelial dysfunction could be detected prior to the onset of clinical disease, they would represent an extremely attractive target for emerging therapeutic strategies. In addition, any such treatments would be most likely to be effective if they could be started in early gestation. In routine clinical practice, we currently screen for several conditions in early pregnancy, most of which have a far lower incidence than preeclampsia. The result of this study suggests a role whether causal or consequential of PTX3 in pregnancy complications. The elevated maternal plasma levels of PTX3 in preeclamptic versus normal pregnancies might be a marker of altered endothelial function, typical of preeclampsia. PIGF is more specific than pentraxin 3, accordingly, we like to conclude that maternal serum PIGF might be useful in screening the high-risk group of preeclampsia before the clinical appearance of maternal disease (hypertension, proteinuria, and edema). As the new developed PIGF ELISA is authorized for routine diagnostic testing, it may offer new possibilities in the prediction of preeclampsia in clinical routine. So, serum biomarkers such as PIGF and pentraxin3 can be routinely used for the prediction of the preeclampsia.

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SUMMARY

INTRODUCTION

Preeclampsia is one of the most important complications of pregnancy and is the leading cause of maternal and perinatal morbidity and mortality in the world. Hypertensive disorder of pregnancy and their complications, a major cause of maternal mortality, still complicate approximately 0.34- 11.5% of pregnancies. Preeclampsia is a multi-system disorder of pregnancy, which is characterized by new onset hypertension (systolic and diastolic blood pressure of ≥ 140 and 90 mm Hg, respectively, on two occasions, at least 6 hours apart) and proteinuria (protein excretion of ≥ 300 mg in a 24 h urine collection, or a dipstick of ≥ 2+), that develop after 20 weeks of gestation in previously normotensive women.

AIMS AND OBJECTIVES-

1. To study the clinical presentations and determinants of pre eclampsia among clinically diagnosed preeclampsia in a tertiary care hospital

2. To compare and validate serum based biomarkers (Placental Induced Growth Factor (PIGF) & Pentraxin-3 (PTX3) values in normal pregnancy and pre eclampsia.

3. To correlate the levels of these biomarkers with the pregnancy outcomes.

MATERIALS AND METHODS

This is a prospective study carried out in a teritiary health centre of 70 patients which were divided into 2 groups- Group A (35 confirmed cases of preeclampsia) and Group B (35 normal pregnant women). The study was approved by Institutional Ethics Committee. The plasma samples will be analyzed for various biomarkers (PlGF, and PTX3) using ELISA kits developed by R&D systems (www.rndsystems.com). The assay will be performed according to manufacturer's instruction. The experiments will be carried out in duplicates.

OBSERVATIONS AND RESULTS

The age distribution in Group A is 27.77 ±4 and in Group B is 25± 4. Which are almost identical. The samples are matched with p= 0.494. The mean gestational age diagnosed PIH is 29.74± . Primigravida women in Group A constitute 62.9% Gestational age at which most of the Group A women presented is between 33 – 40 weeks, which constitutes 65.7%. In groupA women presented with mild preeclampsia is 31.4% and with severe preeclampsia is 68.6%. Outcome in Group A, Intra Uterine deaths were 2.9%, still birth were 17.1% , Intra Uterine Growth Retardation are 22.9%, children with weight > 1.5kg are 57.2%. Urine albumin with 3+ is seen in 51.4% in Group A is 51.4%. Liver Function test are raised in

62.9% in Group A. Platelet count with <10000 is 31.4% in Group A. Mean value of Lactate dehydrogenase is 308±153.21 in Group A and 145.08±17.98. Pentraxin value in Group A women 20.85± 6.12 and in Group B women is 10.83±3.42 which is statistically significant. PIGF value in Group A women is 28.02±17.01 and in Group B is 570.61±81.53 which is statistically significant.

DISCUSSION

The aim of this study was first to define whether pregnancy itself, a condition associated with relevant involvement of inflammatory molecules at the implantation site, is associated with changes in maternal circulating PTX3 levels and PIGF compared with the non pregnant condition. Women with preeclampsia have a significantly higher (6 to 10-fold) median serum/plasma PTX3 concentration than women with uncomplicated pregnancies. The findings of our study confirm the association between PE(PreEclampsia) and increased maternal plasma concentration of PTX3 which shows PTX3 level is very much high in preeclamptic women with an average value of 20.8524194 ng/ml and where as for women who are controls the PTX3 is 11.8336307 ng/ml. In Group A the highest value of PTX3 is 103.3ng/ml and the lowest value is 0.113087961ng/ml, where as in Group B the highest value obtained is 68ng/ml and the lowest is 0.0156ng/ml, average PTX3 levels in mild PE is 12.87ng/ml, in severe PE is 24.50ng/ml which

shows PTX3 is further more increased in severe PE cases than mild PE, which is statistically significant (p=<0.001). Placental Induced Growth Factor measured for both Groups showed that the PIGF in Group B is 570.805 pg/ml, where as PIGF measured in Group A is 33.2692pg/ml, highest value of PIGF in Group A is 410pg/ml and lowest is 4.815041215pg/ml, where as the highest value in Group B is 1486pg/ml and the lowest is 65.578048pg/ml, so average PIGF values in mild PE is 95.64pg/ml, severe PE is 58.84pg/ml and in Group B is 570.805pg/ml which shows that PIGF is very much decreased in preeclamptic patient which is statistically significant (p=<0.001). Umbilical artery Doppler done in all the women in our study had Resistence Index which showed a range of 0.4-0.6 in normal women , 0.7 in mild preeclampsia and >0.8 in severe preeclampsia, IUGR is associated with increased risk of perinatal mortality and morbidity and both short-term and long-term maternal complications.

CONCLUSIONS

Despite there exists many different potential markers for preeclampsia, the reliability of these markers in predicting preeclampsia has been inconsistent between different studies. The result of this study suggests a role whether causal or consequential of PTX3 in pregnancy complications. The elevated maternal plasma levels of PTX3 in preeclamptic versus normal pregnancies might be a marker of

altered endothelial function, typical of preeclampsia. PIGF is more specific than Pentraxin 3, according we like to conclude that maternal serum placental induced growth factor is useful in screening the high-risk group of preeclampsia before the clinical appearance of maternal disease (hypertension, proteinuria, and edema). As the new developed PIGF ELISA is authorized for routine diagnostic testing, it may offer new possibilities in the prediction of preeclampsia in clinical routine. So, serum biomarkers such as PIGF and pentraxin3 can be routinely used for the prediction of the preeclampsia.

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Sr.NO	NAME	IPD	AGE	SEX	Group	GA	OBS.SCR	PIH-GA	GA-SC	SEV-PE	BABY WT	APGAR	Urine-ALB	LFT	PLAT	LDH	Uric acid	P.Smr	Urea	Creat	UMA-Dop	Pentraxin	PlGF
1	SA	I10042631	26	F	A	36 WKS	G3P1L0A1	36WKS	36WKS	SEV	1.45KG	6	1	R	1,09,000	275	6.4	NN	19	0.64	S/D-2.20,RI-.8	3.531079468	27.8228577
2	ME	I10042709	25	F	A	35 WKS	G4P1L1A2	35WKS	37WKS	SEV	1.84KG	8	2	R	76,000	204	6.3	NN	20	0.71	S/D-2.24,RI-.8	11.66698887	67.11989443
3	KV	I10043780	22	F	A	27WKS	G1	27WKS	27WKS	SEV	SB	-	2+	R	89,000	228	5.6	NN	14	0.52	NIL	11.49	410.5925958
4	FA	I10044124	26	F	A	36WKS	G1	36WKS	39WKS	MILD	3.38KG	9	1+	NOR	2,26,000	218	4.6	NN	21	0.54	S/D-2.41,RI-.6	3.554306381	14.74797714
5	VM	I10045077	26	F	A	29WKS	G1	29WKS	29WKS	SEV	SB	-	3+	R	4,05,000	260	5.4	NM	13	0.73	NIL	13.94	171.8670962
6	KM	I10046079	27	F	A	31WKS	G1	30WKS	31WKS	SEV	1.6KG	8	3+	R	1,41,000	329	8	NH	43	1.1	S/D-3.5,RI-.8	6.990352421	4.815041215
7	JD	I10046059	21	F	A	28WKS	G2A1	27WKS	29WKS	SEV	SB	-	3+	R	2,30,000	265	5.7	NN	18	0.66	S/D-2.6	61.36	126.6407375
8	KV	I10046453	27	F	A	32WKS	G2P1L1	32WKS	32WKS	SEV	1.29KG	8	4+	R	61,000	456	5.4	MN	42	1	S/D-2.3,RI-.8	11.63422233	80.03988328
9	PM	I10047275	26	F	A	39WKS	G1	39WKS	39WKS	MILD	2.75KG	8	2+	NOR	2,69,000	201	4.2	NN	21	0.15	S/D-2.4,RI-.7	4.000326544	nil
10	DS	I10048285	29	F	A	36WKS	G2P1L1	36WKS	36WKS	SEV	IUD	-	2+	R	2,05,000	247	4.1	NH	23	0.65	NIL	51.79	43.56861008
11	MG	I10049267	23	F	A	20WKS	G1	32WKS	32WKS	MILD	1.46KG	8	3+	NOR	2,65,000	201	6.9	NN	24	0.7	S/D-3.5,RI-.6	0.2763107	52.06071638
12	JV	I10049400	29	F	A	39WKS	G3P1L1A1	39WKS	39WKS	SEV	2.7KG	8	2+	R	17,000	281	4	NHT	21	0.4	S/D-2.4,RI-.8	4.499192814	126.0905352
13	SM	I10049703	22	F	A	40WKS	G1	39WKS	40WKS	MILD	2.6KG	9	3+	NOR	2,35,000	256	4.4	NN	12	0.7	S/D-2.5,RI-.7	49.61	67.47816569
14	MM	I10050373	25	F	A	35WKS	G2P1L1	34WKS	35WKS	SEV	2.6KG	8	2+	NOR	1,60,000	191	4.7	NN	9	0.6	S/D-2.3,RI-.6	0.113087961	174.9693078
15	HM	I10050301	35	F	A	35WKS	G2P1L1	35WKS	35WKS	MILD	2.5KG	9	1+	NOR	3,35,000	193	4	NN	9	0.6	S/D-2.4,RI-.5	0.57552799	50.98623933
16	MW	I10051267	20	F	A	28WKS	G1	28WKS	28WKS	SEV	SB	8	3+	R	2,70,000	299	5.3	NN	23	0.8	NIL	2.510632374	74.27690155
17	JE	I10000651	19	F	A	38WKS	G1	38WKS	38WKS	MILD	3.38KG	8	1+	NOR	3,00,000	194	4.3	NN	20	0.6	S/D-2.2,RI-.6	12.17	77.68687619
18	JP	I11001043	30	F	A	26WKS	G2P1L1	26WKS	26WKS	SEV	SB	-	3+	NOR	3,00,000	289	4	NN	23	0.8	NIL	8.456380515	8.606857595
19	AV	I11000958	32	F	A	12WKS	G1	37WKS	37WKS	MILD	2.46KG	8	1+	NOR	2,75,000	182	5.5	NN	19	0.6	S/D-2.2,RI-.5	7.910133294	44.60537437
20	UM	I11002217	25	F	A	36WKS	G1	36wks	36wks	SEV	1.36KG	8	3+	R	56,000	600	4	NN	20	0.6	S/D-2.4,RI-.8	8.413586392	nil
21	GJ	I11003748	24	F	A	12WKS	G1	37WKS	37WKS	MILD	3.09KG	9	1+	NOR	1,56,000	191	5.4	NN	21	0.6	S/D-2.3,RI-.7	0.146537643	8.910916754
22	KR	I11004061	24	F	A	36WKS	G2P1L1	36WKS	36WKS	SEV	1.3KG	8	3+	R	64,000	744	3.4	NN	20	0.5	S/D-2.5,RI-.8	3.891096619	125.740682
23	RJ	I11004586	21	F	A	33WKS	G1	33WKS	33WKS	SEV	1.1KG	7	3+	R	2,40,000	212	6	NH	26	0.7	ABSENT DF	7.611965119	13.05
24	PR	I11004575	23	F	A	12WKS	G1	36WKS	38WKS	MILD	2.2KG	8	1+	NOR	1,48,000	180	5	NN	24	0.5	S/D-2.2,RI-.5	16.14295162	26.69
25	RE	I11006049	28	F	A	12WKS	G1	38WKS	38WKS	MILD	2.2KG	8	1+	NOR	1,63,000	189	6.3	NN	25	0.8	S/D-2.2,RI-.5	25.06	218.3
26	RL	I10047253	34	F	A	35WKS	G2P1L1	35WKS	35WKS	SEV	2KG	8	3+	R	96,000	253	5.2	NN	25	0.9	S/D-3.2,RI-.8	13.15	98.12
27	AN	I11009684	29	F	A	22WKS	G1	22WKS	22WKS	SEV	SB	-	3+	R	3,39,000	223	3.8	NH	12	0.2	NIL	24.18	5.58
28	SM	I1100	21	F	A	32WKS	G1	32WKS	32WKS	SEV	1.5KG	7	3+	R	80,000	739	9.2	NNT	35	0.7	ABSENT DF	14.75	147
29	VF	I11016743	21	F	A	31WKS	G2A1	31WKS	31WKS	SEV	960GM	8	3+	R	1,45,000	325	4.8	NN	19	0.5	S/D-4,RI-.7	15.72	124.1
30	CH	I11016753	26	F	A	33WKS	G1	31WKS	33WKS	SEV	1.9KG	8	2+	R	1,40,000	455	6.4	NNL	36	1.1	S/D-3,RI-.7	102.26	179.4
31	DP	I11018959	30	F	A	35WKS	G1	35WKS	35WKS	SEV	2.4KG	8	3+	R	88,000	598	5.4	NN	19	0.6	S/D-2.5,RI-.6	34.51	32.34
32	SR	I11018213	21	F	A	39WKS	G1	37WKS	37WKS	SEV	2.7KG	8	3+	R	1,03,000	433	6.3	NH	27	0.3	S/D-2.4,RI-.6	103.3	18.07
33	JF	I11019255	28	F	A	12WKS	G3A2	32WKS	36WKS	MILD	2.7KG	8	1+	NOR	2,10,000	205	2.7	NN	26	0.4	S/D-2.2,RI-.5	22.17	27.01
34	TN	I11022828	31	F	A	30WKS	G1	28WKS	31WKS	SEV	1.1KG	7	3+	R	89,000	330	8.3	NH	22	0.4	S/D-3.1,RI-.6	24.62	50.13
35	LX	I11023419	26	F	A	29WKS	G1	37WKS	37WKS	SEV	2.2KG	7	3+	R	90,000	345	4	NH	25	0.4	S/D-2.3,RI-.5	47.83	89.98
36	JL	I11004218	29	F	B	35WKS	G3P1L1A1	NO	35WKS	NO	2.7KG	9	NIL	NOR	2,30,000	180	5	NN	21	0.3	S/D-1.8,RI-.5	5.608229111	1486
37	SR	I11002150	24	F	B	36WKS	G1	NO	36WKS	NO	3.0KG	9	NIL	NOR	1,98,000	160	2.3	NN	18	0.4	S/D-1.9,RI-.5	3.526736621	759.1595508
38	NA	I11004036	26	F	B	37WKS	G2A1	NO	37WKS	NO	2.8KG	9	NIL	NOR	2,37,300	180	3	NN	16	0.5	S/D-2,RI-.5	6.341609128	243.0758657
39	BA	I11016040	21	F	B	36WKS	G1	NO	36WKS	NO	2.5KG	9	NIL	NOR	1,54,000	160	3.2	NN	15	0.3	S/D-1.8,RI-.4	3.273046032	451.7836888
40	RE	I11010458	24	F	B	35WKS	G3P1L1A1	NO	35WKS	NO	3.0KG	8	NIL	NOR	2,32,500	156	4	NN	21	0.7	S/D-2,RI-.5	2.911467408	139.751984
41	PY	I11010525	22	F	B	36WKS	G1	NO	36WKS	NO	2.7KG	9	NIL	NOR	1,45,000	161	3	NH	20	0.2	S/D-1.8,RI-.5	2.455541649	652.8903696

Sr.NO	NAME	IPD	AGE	SEX	Group	GA	OBS.SCR	PIH-GA	GA-SC	SEV-PE	BABY WT	APGAR	Urine-ALB	LFT	PLAT	LDH	Uric acid	P.Smr	Urea	Creat	UMA-Dop	Pentraxin	PIGF
42	VB	I11004099	20	F	B	37WKS	G1	NO	37WKS	NO	2.5KG	9	NIL	NOR	2,00,000	122	3	NN	23	0.7	S/D-1.8,RI-.4	14.65	257.8761052
43	SB	I11002127	19	F	B	36WKS	G1	NO	36WKS	NO	3.0KG	9	NIL	NOR	1,67,000	133	4	NN	15	0.2	S/D-1.8,RI-.5	68.89	1378
44	GM	I10039208	36	F	B	35WKS	G5P3L1A1	NO	35WKS	NO	2.6KG	8	NIL	NOR	2,90,000	126	2	NN	25	0.3	S/D-2,RI-.5	0.625421936	636.0576815
45	RK	I10049669	21	F	B	36WKS	G2A1	NO	36WKS	NO	2.5KG	8	NIL	NOR	2,15,000	138	3.4	NN	31	0.6	S/D-2.1,RI-.4	42.99	328.1935738
46	KS	I10042849	23	F	B	35WKS	G2P1L1	NO	35WKS	NO	2.7KG	9	NIL	NOR	1,98,000	121	2.2	NH	21	0.6	S/D-2,RI-.5	0.620639925	430.7857606
47	SB	I10042730	28	F	B	36WKS	G2P1L1	NO	36WKS	NO	3.0KG	8	NIL	NOR	2,56,500	151	2.6	NN	24	0.3	S/D-1.8,RI-.4	2.319083536	1183
48	KP	I10042865	31	F	B	38WKS	G1	NO	38WKS	NO	2.8KG	9	NIL	NOR	2,76,000	141	3.2	NN	31	1	S/D-2,RI-.5	4.203806013	345.9152057
49	SU	I10038261	23	F	B	37WKS	G2P1L1	NO	37WKS	NO	2.9KG	8	NIL	NOR	1,70,000	128	3.3	NN	17	0.9	S/D-2.1,RI-.4	13.84	96.85708223
50	YS	I11002989	29	F	B	35WKS	G3P1L1A1	NO	35WKS	NO	2.7KG	9	NIL	NOR	2,23,100	149	3.5	NN	15	0.8	S/D-1.8,RI-.5	0.558107805	647.2671271
51	ZF	I10039092	23	F	B	36WKS	G3P1L1A1	NO	36WKS	NO	3.0KG	8	NIL	NOR	2,15,000	135	2.9	NH	19	0.3	S/D-2,RI-.5	13.14	210.374522
52	JR	I10039097	19	F	B	35WKS	G1	NO	35WKS	NO	2.7KG	9	NIL	NOR	1,89,000	165	3	NN	26	0.5	S/D-2.2,RI-.5	14.52	93.80773589
53	JW	I10039072	20	F	B	36WKS	G1	NO	36WKS	NO	2.5KG	9	NIL	NOR	2,45,000	143	3.1	NH	29	0.5	S/D-2,RI-.5	0.857983842	65.57804848
54	UH	I10042571	21	F	B	35WKS	G1	NO	35WKS	NO	2.6KG	9	NIL	NOR	2,65,500	145	2.6	NN	21	0.6	S/D-1.9,RI-.5	45.35	147.1059729
55	MU	I10039140	25	F	B	35WKS	G3P1L1A1	NO	35WKS	NO	2.9KG	8	NIL	NOR	1,76,600	132	2	NN	22	0.8	S/D-1.8,RI.5	12.97	932.9
56	KT	I10045576	30	F	B	36WKS	G3P1L1A1	NO	36WKS	NO	2.3KG	9	NIL	NOR	3,65,000	103	2.2	NN	21	0.4	S/D-1.5,RI-.4	13.57	656.7
57	RU	I10046554	25	F	B	35WKS	G2P1L1	NO	35WKS	NO	3.0KG	9	NIL	NOR	2,43,000	123	1.8	NH	23	0.8	S/D-2,RI-.5	11.34	766
58	TY	I10046987	21	F	B	34WKS	G1	NO	34WKS	NO	2.4KG	9	NIL	NOR	1,99,000	143	2	NN	21	1	S/D-1.8,RI.5	26.95	1462
59	GH	I10049768	23	F	B	37WKS	G1	NO	37WKS	NO	2.6KG	8	NIL	NOR	3,56,000	151	1.8	NH	25	0.6	S/D-2.1,RI-.4	12.63	1321
60	KC	I10051256	29	F	B	36WKS	G2P1L1	NO	36WKS	NO	2.7KG	9	NIL	NOR	2,39,000	121	2.7	NH	32	0.5	S/D-2.2,RI-.4	18.58	116.8
61	AB	I10056321	30	F	B	35WKS	G3P1L1A1	NO	35WKS	NO	2.4KG	9	NIL	NOR	2,90,000	165	3.1	NN	35	0.78	S/D-2,RI-.5	21.63	1278
62	NM	I10062900	31	F	B	37WKS	G1	NO	37WKS	NO	2.7KG	9	NIL	NOR	2,23,000	170	2.9	NN	31	0.6	S/D-2.2,RI-.4	13.39	817.8
63	MU	I10065783	21	F	B	36WKS	G1	NO	36WKS	NO	2.6KG	8	NIL	NOR	1,78,000	149	2.2	NN	29	0.9	S/D-2,RI-.5	13.07	109.3
64	NK	I10069344	24	F	B	35WKS	G1	NO	35WKS	NO	2.3KG	9	NIL	NOR	3,08,000	153	2	NN	30	1	S/D-2.2,RI-.4	3.21	648.4
65	LP	I10072997	25	F	B	34WKS	G1	NO	34WKS	NO	3.1KG	9	NIL	NOR	2,87,000	169	1.8	NN	28	0.6	S/D-2.1,RI-.4	1.667	296.345
66	PA	I10076100	27	F	B	37WKS	G2P1L1	NO	37WKS	NO	2.5KG	8	NIL	NOR	2,76,000	155	1.7	NH	25	0.5	S/D-1.8,RI-.3	0.4756	356.1243
67	TU	I10082821	19	F	B	36WKS	G1	NO	36WKS	NO	2.7KG	9	NIL	NOR	3,76,000	147	2.3	NN	28	0.6	S/D-2.2,RI-.5	12.326	197.329
68	JD	I10083453	28	F	B	35WKS	G3P1L1A1	NO	35WKS	NO	2.6KG	9	NIL	NOR	3,01,000	129	2.5	NN	29	1.1	S/D-2.2,RI-.4	0.05632	598
69	ST	I10089219	30	F	B	37WKS	G1	NO	37WKS	NO	2.9KG	9	NIL	NOR	2,55,000	136	1.8	NN	32	0.8	S/D-2,RI-.5	2.9891	456
70	TI	I10094635	31	F	B	35WKS	G1	NO	35WKS	NO	2.5KG	8	NIL	NOR	1,99,000	138	2.6	NN	33	01-Jan	S/D-2.3,RI-.5	2.1345	412