SERUM BIOMARKERS AND FLUORIDE ESTIMATION WITH HISTOPATHOLOGICAL CHANGES IN PLACENTAE OF PRE-ECLAMPSIA

By

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DEPARTMENT OF ANATOMY

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Dedication.....

To my beloved parents and grandparents: For whatever I am for today with your boundless affection, love, encouragement words, undefeatable with constant support, be with all my ups and downs as a backbone and prayers of day and night make me able to get success and honour. This what a small present from my side dedicating to you all with my whole heart.

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I am whatever I am grateful to god to give all these people to me in form of gods.

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I hereby declare that the thesis titled **"Serum Biomarkers and Fluoride Estimation with Histopathological Changes in Placentae of Pre-Eclampsia"** is bonafide and genuine research work which I have carried out, under the guidance of Dr Raghuveer C.V, Professor, Department of Pathology, Sri Devaraj Urs Academy of higher Education and Research, Former Vice-Chancellor.

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LIST OF ABBREVIATIONS

Sl. No.	ABBREVIATED	EXPANSION
1.	PE	Pre-eclampsia
2.	BP	Blood Pressure
3.	ACOG	American College of Obstetrics and Gynecologist
4.	ISSHP	International Society for the Study of Hypertension in Pregnancy
5.	RCOG	Royal College of Obstetricians and Gynaecologists
6.	SOGC	Society of Obstetricians and Gynaecologists of Canada
7.	SOMANZ	Society of Obstetric Medicine of Australia and New Zealand
8.	HT	Hypertension
9.	WK	Week
10.	HELLP	Hemolysis Elevated Liver enzymes, and Low Platelet count
11.	SBP	Systolic Blood Pressure
12.	DBP	Diastolic Blood Pressure
13.	EOPE	Early Onset Pre- Eclampsia
14.	LOPE	Late Onset Pre- Eclampsia
15.	ABPM	Ambulatory BP monitoring/Automated home BP monitoring
16.	BMI	Body Mass Index
17.	VEGF	Vascular Endothelial Growth Factor
18.	PIGF	Placental Inhibitor Growth Factor
19.	sFlt1	soluble Feline McDonough Sarcoma(fms) like tyrosine kinase-1
20.	sEng	Soluble Endoglin
21.	VEGFR-1	Vascular Endothelial Growth Factor Receptor-1
22.	F	Fluoride
23.	H&E	Haematoxylin & Eosin
	PE P A	Preeclamptic Peripheral A
24.	PE P C	abnormal peripheral areas
25.	PE C D	central normal area of the placenta
26.	PE C B	Preeclamptic Center B
27.	A C P A	Abnormal areas Control Peripheral part
28.	C C P C	Control Peripheral abnormal area
29.	C C B	Control Center normal area part B
30.	C C D	Center abnormal area D
31.	DVH	Distal villous hypoplasia
32.	N	Necrosis
33.	HV	Hyper Vascularity
34.	MV	Matured Villi
35.	IV	Immature villi
36.	AV	Avascular Villi
37.	SK	Syncytial Knots
38.	CV	Crowding of Villi
39.	IUGR	Intrauterine Growth Restriction/Retardation
40.	IUD	Intra Uterine Death
41.	NK	Natural Killer cells
42.	TGF-β	Transforming Growth Factor-beta
43.	eNOS	Endothelial Nitric Oxide Synthase
44.	WHO	World Health Organisation
45.	UtA- PI	Uterine Artery Pulsatility Index

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1. INTRODUCTION

1.1 Definition of Pre-eclampsia: Pre-eclampsia (PE) is defined as pregnancy particular systemic vascular disorder ends with vascular endothelial malfunction and characterised by new-onset of hypertension, proteinuria which is evident after the $\geq 20^{th}$ week of gestation. [1]

The necessity to modernise the definition of PE: Due to extensive research in PE, the clinical outcomes are not similar in each individual and are different for each pregnancy.So the diagnosis part makes it tricky day by day. Hence there was a necessity to modify the diagnosis and management of hypertensive disorders in pregnancy according to time and situation. Many biomarkers were moving in the direction of modernization from conventional approaches to new ways to make use of routine investigation methods.In that move measuring blood pressure by using mercury sphygmomanometer to automated blood pressure devices was developed. The outcome of pregnancy should be a healthy full-term developing fetus to be delivered.[2] To provide better diagnosis the knowledge to distinguish between true chronic hypertension and PE subtypes is highly essential. [2] A broader definition of PE for clinical practice ensures specificity of the diagnosis.[3] According to ACOG's guidelines old definition was based on the degree of proteinuria. PE was classified as mild & severe PE. But in all the PE cases the proteinuria was not diagnosed. So the ACOG's hypertension 2013 task force has revised the criteria for PE diagnosis. These criteria basis ACOG's practice guidelines latest definition on PE (Table1). Still, a majority of women progress with general symptoms of PE such as low platelets or elevated liver enzymes which was observed before the proteinuria itself. So the consequences become complicated for diagnoses.[4] The major factor of whether proteinuria should be retained or not for the diagnosis of PE remains a question. So for the betterment in the diagnosis the society recommended a broad definition. Without proteinuria could be applied for the clinical definition of PE, with the inclusion of proteinuria it would ensure more specificity in diagnosis as clinical criteria for patients.[2] Yet, the guidelines approved by the International Society for the Study of Hypertension in Pregnancy (ISSHP), American College of Obstetricians and Gynecologists (ACOG), Society of Obstetricians and Gynaecologists of Canada (SOGC), Society of Obstetric Medicine of Australia and New Zealand (SOMANZ), Royal College of Obstetricians and Gynaecologists (RCOG) for PE definitions by different societies (Table 1).

Table 1: Definitions of Pre-eclampsia by different societies

Category	ACOG	SOGC	RCOG	SOMANZ	ISSHP
Chronic Hypertension (HT)/ essential HT	With Systolic Blood Pressure (SBP) ≥140 mm Hg and/or Diastolic Blood Pressure (DBP) ≥90 mmHg before 20 wk of gestation, with no underlying cause	SBP≥140 mmHg and/or DBP≥90 mmHg that develops at pre-pregnancy or at <20+0 wk of gestation. Pre-existing hypertension with comorbid conditions/super imposed PE.	HT that is present at the booking visit or before 20 wk or if the woman is already taking antihypertensive medication when referred to maternity services.	SBP≥140 mmHg and/or DBP≥90 mmHg confirmed before pregnancy or before 20 completed wk of gestation without a known cause.	High BP predating the pregnancy before 20 wk of gestation
Gestational HT PE /Eclampsia	New-onset elevations of BP after 20 wk of gestation, in the absence of proteinuria. Associated with proteinuria (24-h excretion ≥300 mg), diagnosed after 20 wk of uneventful gestation up to 2 wk postpartum.In the absence of proteinuria, new-onset HT with the following: Platelet count <100,000/μl, serum creatinine >1.1 mg/dl, or doubling of concentration in absence of other renal disease Transaminitis to twice normal concentration Pulmonary edema Cerebral/visual symptoms	HT that develops for the first time at ≥20+0 wk of gestation Gestational HT with comorbid conditions and leads to PE. New proteinuria with adverse conditions and severe complications	New HT after 20 wk without significant proteinuria.PE with significant proteinuria. **Eclampsia** is a convulsive condition with PE Hemolysis, elevated liver enzymes, and low platelet count syndrome **Severe PE:** PE with severe HT and/or symptoms and/or biochemical and/or hematologic impairment	New onset of HT after 20 wk of gestation without any maternal or fetal features of PE followed by return of BP to normal within 3 months postpartum Multisystem disorder unique to human pregnancy characterized by HT and involvement of one or more other organ	When *de novo* HT is present after 20 wk of gestation in the absence of proteinuria and uteroplacental dysfunction When *de novo* HT is present after 20 wk of gestation in the presence of proteinuria and uteroplacental dysfunction

PE/eclampsia superimposed on chronic HT	HT diagnosed before or in early gestation and with proteinuria	HT along with the development of one or more of the following at ≥20 wk Resistant HT New or worsening proteinuria, with other severe complications.	None specified	Woman with chronic HT developing one or more of the systemic features of PE after 20 wk of gestation	One or more of the above features of PE (*i.e.*, proteinuria and maternal organ/uteroplacental dysfunction) occur in addition to HT
White coat HT& other types of HT	**White coat HT** elevated BP primarily in the presence of health care providers	**White coat HT** BP that is elevated in the office but consistently normal outside of the office (<135/85 mmHg) by ABPM or HBPM **Transient hypertensive effect** elevated BP may be caused by environmental stimuli. **Masked hypertensive effect** BP is consistently normal in the office (SBP<140 mmHg or DBP<90 mmHg) BP outside the office (≥135/85 mmHg) by ABPM/ HBPM	None specified	**White coat HT** raised BP in the presence of a clinical attendant but normal BP otherwise as assessed by ABPM or HBPM **Secondary HT** raised BP in the presence of an inciting factor such as CKD (*e.g.*, reflux nephropathy, and adult polycystic kidney disease) Renal artery stenosis, Diabetes mellitus Endocrine disorders Coarctation of aorta	**White coat HT** normal BP using 24-h ABPM in the first half of pregnancy

ACOG: American College of Obstetrics and Gynecology; SOGC: Society of Obstetricians and Gynecologists of Canada; RCOG: Royal College of Obstetricians and Gynecologists; SOMANZ : Society of Obstetric Medicine of Australia and New Zealand; ISSHP: International Society for the Study of Hypertension in Pregnancy; HT: hypertension; BP: Blood pressure; SBP: systolic BP; DBP: diastolic BP; ABPM: ambulatory BP monitoring; HBPM: home BP monitoring.

1.2 Classification of PE

PE is classified based on the degree of hypertension, proteinuria, and the systemic/organ involvement into mild and severe PE (Table 2). [5] The most recent revised classification for hypertensive disorders in pregnancy is by the International Society for the Study of Hypertension in Pregnancy (ISSHP) in 2014:The previous history of hypertension, hypertension in pregnancy, pre-eclampsia – de novo or superimposed on chronic hypertension, White coat hypertension.

Table 2: Classification of PE based on the degree of Hypertension, Proteinuria [5]

CRITERIA	MILD PE	SEVERE PE
Blood Pressure	≥ 140/90—2 occasions 6 h apart (not more than 1 wk apart)	≥ 160/110—2 occasion at least 6 h apart (not more than 1 wk apart)
Proteinuria	≥ 300mg/24-h sample or ≥ 1 + on 2 urine samples 6 hrs apart (not more than 1 wk apart)	≥ 5g/24-h sample or ≥ 3+ on 2 urine samples 6 hrs apart (not more than 1 wk)
Other Clinical conditions	NA	Oliguria—<500mL/24 h, Thrombocytopenia—<100,000/mm,Epigastric or right upper quadrant pain, Pulmonary edema, Persistent cerebral or visual disturbances

1.3 Epidemiology of PE: Incidence rate, mortality, morbidity, and prevalence:

PE incidence worldwide is 5% to 8% of all hypertensive disorders of pregnancy and is expressed by an increase in morbidity and mortality rate to the mother and the fetus.[6] According to the latest American College of Obstetricians and Gynecologists Bulletin the maternal mortality rate due to pregnancy hypertensive disorders, globally responsible for 16% of maternal death and which is high in developing countries. In Central & South America, due to pregnancy hypertensive disorders, 26% of maternal deaths, whereas in Africo-Asian 9% mortality was noticed. In the United States, the rate of PE was increased by 25% in 17 years along with a 6.7 fold increased risk of severe PE. [7] In India, the overall incidence of PE is about 28.7 % whereas in South India particularly in Karnataka it is reported to be 19.8%. [8]

1.4 Risk factors

Rather than focuss on the pathology of PE which is not easy to understand, better is focussing on risk factors. This may help to understand the pathophysiology in a better way.

Major Risk Factors: Nulliparity, Family history of PE, Obesity, Multifetal gestation, PE/eclampsia in a previous pregnancy, poor outcome in a previous pregnancy like intrauterine growth retardation, abrupt placentae, and fetal death. [6]

Minor Risk Factors: Pre-existing medical conditions, chronic hypertension, renal disease, diabetes mellitus, thrombophilias, antiphospholipid antibody syndrome, protein C, S, or antithrombin deficiency, Factor V Leiden, Methyltetrahydrofolate, family history of genetic disorders.Abnormal uterine artery, Doppler studies with S/D ratio >2.6, Resistance index of >0.58, Presence of a notch (uterine artery).[6]

Added Risk Factors: The added risk factors are ethnicity, depends on parity, gestation, increased maternal age of >35 years, and maternal weight with a body mass index (BMI) of<19.8, and 13.3 with BMI of >35 kg/m. [9] Along with the above risk factors seasons, temperature, and humidity also play a role in PE. PE will be elevated in summer, and less affected in winter. [10] Women who were pregnant in sunny times had the highest risk for PE and early-onset PE with an odds ratio of 1.81.[11]

Dietary habits: Consuming less vegetarian diet along with fruits, eggs daily, fish weekly, are at high risk of PE. Underweight women, consuming alcohol, aged 30-39 years in the southern region of India who consume dairy products like milk (excluding coffee or tea), green leafy vegetables daily or weekly, pulses or beans at least weekly are less prone to PE. Occasionally consuming fish or chicken/meat are at increased risk for PE.[7] At a glance, by comparing the vegetarian and non-vegetarian diet, which includes vegetables in their diet are less prone to develop PE.[12]

1.5 Need for the study/Lacunae

PE is a pregnancy-specific syndrome with multi-systemic and multi-factorial disorder that often affects the mother and the fetus alike. The etiopathogenesis of PE still remains imprecise.[13,14,15] The risk factors differ with ethnicity, maternal age, and dietary habits, so each subject is different. As such still there is no specific, predictive biomarker for diagnosis of PE. The sFlt1, sEng/PIGF ratio has been shown to be of a diagnostic value to predict PE as the specificity and sensitivity are high.[14] For ≤34 weeks sensitivity is 72.9 % and specificity is 94%, for 30-34 weeks, sensitivity is 89% and specificity is 94%.The ratio of sFlt1:sEng/PIGF was significant in the prediction of late and severe PE. [16] Although sFlt1: sEng /PIGF ratio has been demonstrated to have diagnostic value in PE, whether it has a casual association needs to be considered. In Kolar, the incidence is 21% which is still higher (in R L Jalapa Hospital and Research Center).[8] There was a corelation between serum F levels and liver damage as evidenced by elevated liver enzymes as seen in PE. [17] To correlate the higher incidence of PE in the Kolar population the estimation of fluoride (F) could be a valuable parameter. The changes in placentae of PE exposed to excessive F intake can be noticed histopathologically.The study was aimed to determine whether high serum F levels were added risk factor for the increasing incidence of PE in the Kolar community, and can serum F levels be a predictive diagnostic tool for PE. There are hardly any studies on the effect of F on pregnancy and none of the studies was on effect of F in PE particularly in F endemic areas to find out the increasing incidence of PE. Thus we were focussed to take up this study to find out the association between serum F levels, serum biomarkers, and histopathological changes in PE women by comparing with normotensive pregnant women. Can high serum F levels could be an added risk factor for a higher incidence of PE in F endemic areas.

2.1 AIM

To find out the association between 3 serum biomarkers: soluble Feline McDonough Sarcoma- (fms-) like tyrosine kinase-1(sFlt1), Soluble Endoglin (sEng), and Placental Inhibiting Growth Factor (PIGF) by comparing with the serum F levels along with histopathological changes in placenta of PE women versus normotensive pregnant women.

2.2 OBJECTIVES OF THE STUDY

- To determine and compare the sFlt1, sEng and PIGF levels in PE women with normotensive pregnant women.
- To determine and compare the serum F levels in PE women with normotensive pregnant women.
- To observe and evaluate the histopathological changes in placenta of PE women along with serum F levels by comparing with placenta of normotensive pregnant women placenta and their serum F levels.

2.3 RESEARCH QUESTIONS/HYPOTHESIS

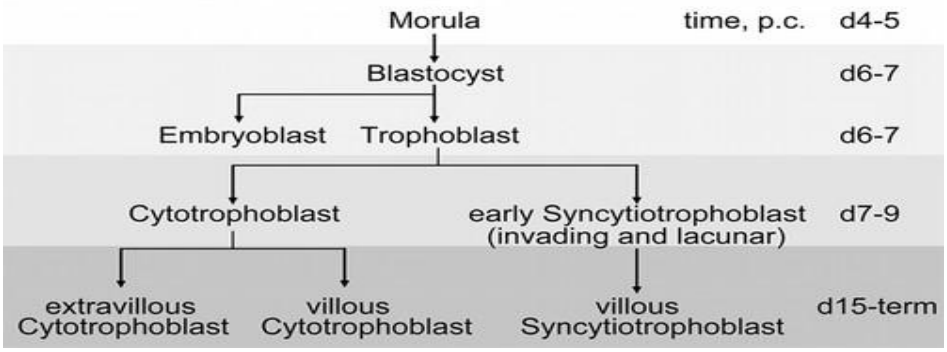
- Can serum sFlt1, sEng/ PIGF ratios and serum F levels be a predictor or a diagnostic marker in PE of south eastern Kolar population?
- Do PE women with high serum F levels show histopathological changes in placentae of south eastern Kolar population?

3. REVIEW OF THE LITERATURE

3.1 ANATOMY OF THE NORMAL PLACENTA: The placenta is an essential organ that helps for the survival of the fetus. [18] It is the most authentic evidence for fetal health. Placenta documents the anatomical to pathological structural events that occur in gestation. [19] It has induced immeasurable curiosity among obstetricians and pathologists, as there is a paucity of data to appreciate the "exclusively anatomical condition" of this complex organ in PE pregnancy.[20]

3.2 DEVELOPMENT OF THE PLACENTA: The placenta begins its function from the early third week of fetal intrauterine life. It has 2 surfaces: maternal, and fetal surfaces (Fig.1) with a dark reddish-blue, discoid organ, measuring 15-25 cm in diameter, weighing 400-600 gms, 2-3 cms in thickness and with 15 –20 cotyledons are present on the maternal surface (Fig.1) .[21] The maternal part develops from maternal uterine tissue decidua basalis. The fetal part develops from the same blastocyst as the chorion frondosum. Chorion frondosum contains villi, involved in the formation of the placenta (Fig.2). The placenta starts to develop in the maternal endometrium by implanting the blastocyst the division of the blastocyst was explained with days of gestation (Flowchart.1). The outer layer of the blastocyst becomes the trophoblast of the placenta. The trophoblast is further divided into 2 layers as an outer layer of cytotrophoblast and inner layer of syncytiotrophoblast.The underlying cytotrophoblast cells continue throughout placental development.[21]

Flow Chart: 1. Representing Stages of blastocyst



During normal pregnancy, cytotrophoblast migrates from chorionic villi to reach the inner layer of the uterine myometrium. Within the uterine wall, the cytotrophoblast deeply invades the spiral arterioles. Cytotrophoblast invades the uterine spiral arterioles to replace the maternal endothelial lining. During this process, uterine spiral arterioles lose the endothelial lining and musculo-elastic tissue (Fig.2).The cells microscopically look like small, round mononuclear cells with distinct cell borders, eosinophilic cytoplasm, and single vesicular nuclei. As a result, the spiral arteries attain the functional property by making the placenta rich in blood supply (Fig.3 & 4). This process of aggression for placental vascular remodeling starts at the early stages of implantation.[22] Cytotrophoblast function is to implant the blastocyst. Blastocyst comes in contact with the uterine endometrium the trophoblast begins to multiply. By the secretions of cytotrophoblast, proteolytic enzymes will release to collapse the endometrial cells and develop villi from the trophoblast. The villi of cytotrophoblast and syncytiotrophoblast push the embryo into the endometrium.Cytotrophoblasts frequently differentiate into syncytiotrophoblasts during villous formation and development. Cytotrophoblastic cells are stem cells for syncytiotrophoblasts. [23] The syncytiotrophoblast is a multinucleated cell layer that covers the superficial part of the placenta. The syncytiotrophoblast/syncytium acts as the barrier of the placenta (Fig.5). To make a reminder to the mother at initial stages of pregnancy these tiny syncytiotrophoblast cells initiate the secretions: Human Chorionic Gonadotropin, Human Placental Lactogen (HPL)/ Human Chorionic Somatomammotropin (HCS), Corticotropin-Releasing Hormone (CRH).[24]

Figure 1: Surfaces of placenta

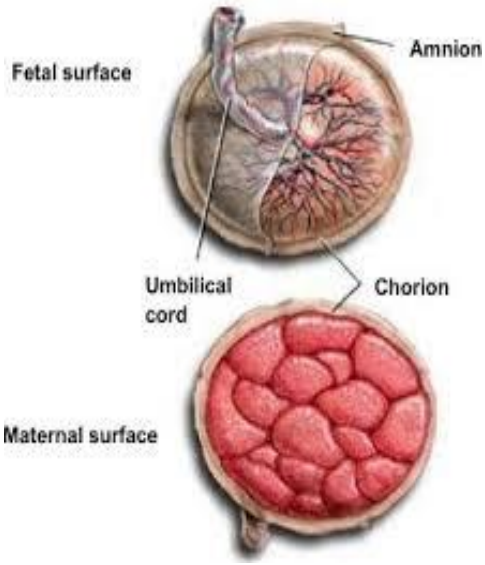


Figure 2: Blood Supply of Uterus & Placenta

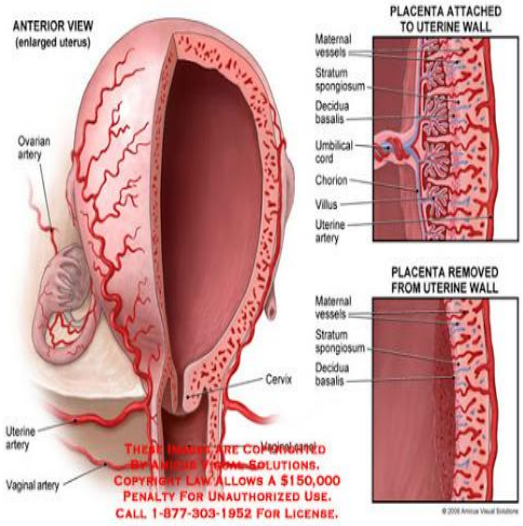
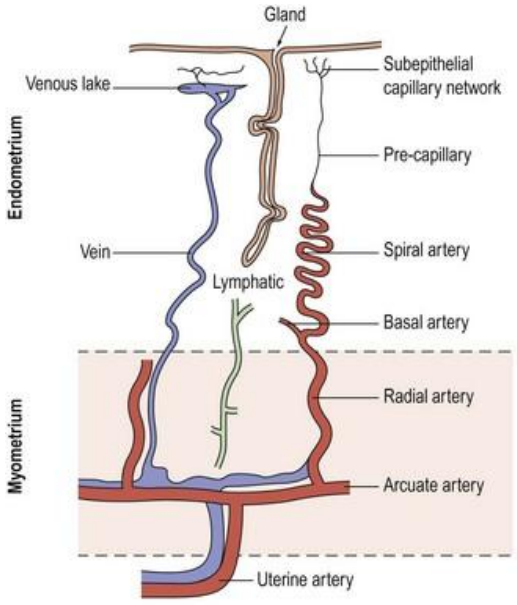


Figure 3: Arterial branches of Uterus

Figure 4: Blood supply to Placenta



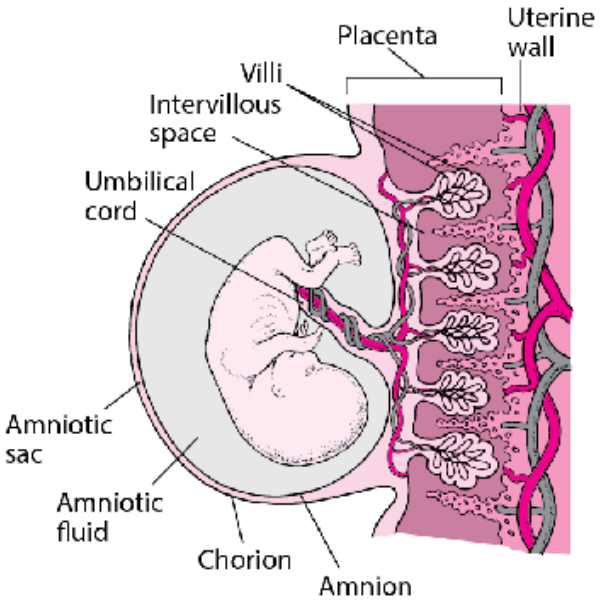
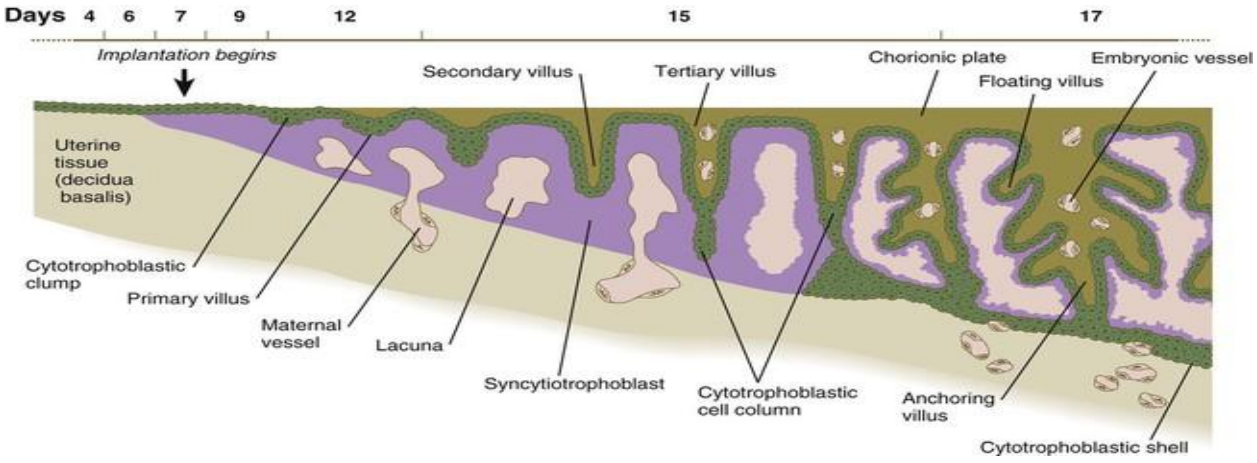


Figure 5: Extra Embryonic membranes of placenta



3.3 HISTOLOGY OF THE PLACENTAE

The functional units of the placentae are chorionic villi. The fetal part is connected to the maternal part by decidua basalis and by the cytotrophoblastic shell to fix the chorionic sac to the decidua basalis. Endometrial arteries and veins pass freely through gaps in the cytotrophoblastic shell and open into the intervillous spaces Figure 5.These chorionic villi are filled with maternal blood within which fetal blood is separated from maternal blood with intervillous spaces present in the decidual plate.[25] Villi connected with decidua basalis divide extensively and further assist in villous maturation of mesenchymal villus as primary, secondary, tertiary, stem/anchoring villi, and terminal villi. The cytotrophoblast in the villi further invades syncytiotrophoblast by attaching with decidua basalis further to form stem villi /anchoring villi (Fig.6).Terminal villi that develop from sides of stem villi.[26]

Mesenchymal Villi: These are primary villi, established at the initial phases of pregnancy. [27,28] Mesenchymal villi are rich in mesenchymal cells, poor in capillaries (Fig.6).[29] With weakly organized loose stroma. The stroma is enclosed by two trophoblastic layers– the cytotrophoblast (inner layer) and the syncytiotrophoblast (outer layer). During the process of pregnancy, the cytotrophoblastic cells slowly disappear and further develop along with syncytiotrophoblast as "islets" of cytotrophoblast. The main functional aspect of these villi at the initial weeks of pregnancy is hormones secretion and initiates further development of villi. [28]

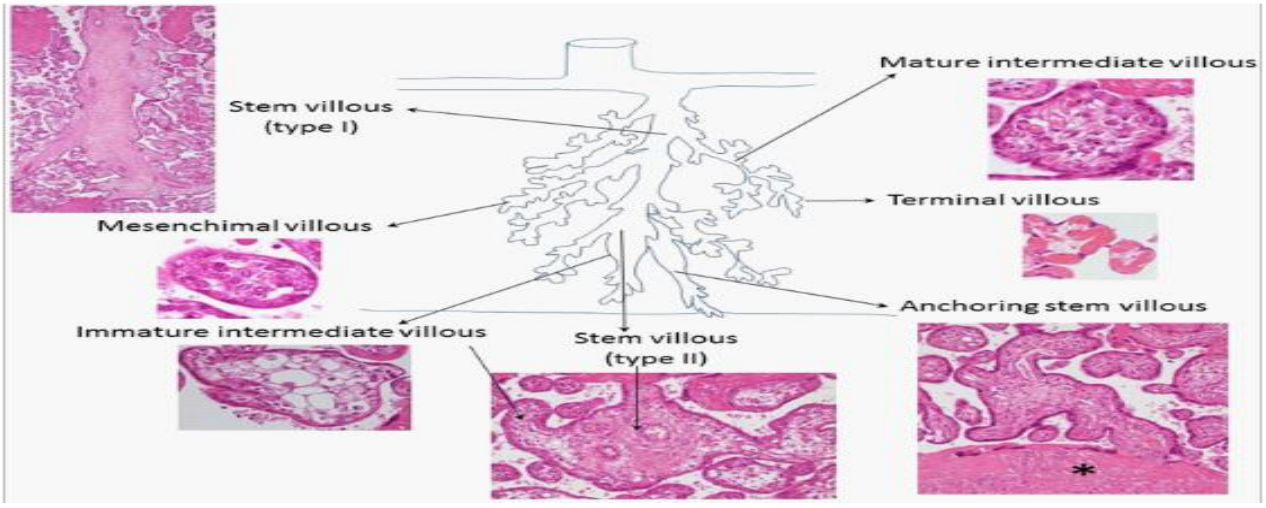
Stem villi: These villi are the largest villi in the placental villous tree further divides into mature intermediate villi and terminal villi Figure 5&6. Stem villi support the villous tree by a condensed fibrous stroma with central arteries and veins with a thick smooth muscle wall. [27, 28, 29]

Terminal Villi: Terminal villi are arranged in groups like grape bunches with highly dilated sinusoids and the thin vasculo-syncytial membrane developed from mature intermediate villi Figure 5&6. [29, 30, 31] These are the ending twigs of the placental villous tree.[29,32] The fetal capillaries of the terminal villi have a very thin syncytiotrophoblastic layer forming the vasculo-syncytial membranes, it is a site for physiological fetal-maternal exchanges.[30,31] In the normal full-term placenta, the terminal villi occupy 40% of the placental villous tree. [28] In a full-term placenta, 60–70 fetal lobules arise from the chorionic plate.[29] The majorities of villi float freely in the intervillous space, other villi were associated with decidua, providing structural stability for the placenta.[33] (Table 3).

Table 3: Histological Features of Placental villi

Type of villous	Diameter *(μm)*	Stroma	Vessels	Time of comparison	Percentage at full-term
Mesenchymal villi	100-250	Loosely arranged collagen fibers enmeshing mesenchymal and some Hofbauer cells	Poorly developed	Early first trimester	1
Immature intermediate villi	100-400	Reticular structure with numerous fluid-filled channels containing macrophages (Houfbauer cells)	Small arterioles, venules located between the stromal channels	Mid-first trimester (8 weeks of gestation)	0-5
Stem villi	100-3000	Condensed, fibrous, containing collagen fibers, occasional fibroblast and rare macrophages	Muscularized arteries and veins in the center of the villous	Mid-first trimester (8 weeks of gestation)	20-25
Mature intermediate villi	80-150	Loose bundles of connective tissues fibers, fixed connective tissue cells	Peripheral numerous capillaries, small terminal arterioles and collecting venules	Mid-gestation	25
Terminal villi	30-80	Absent	High degree of sinusoidal capillarization, involving more than 35% of the villous volume	Late second trimester-early third trimester	40-45

Figure 6: Placental Villous tree a histological view



4. MATERIALS & METHODS:

4.1. Study design and Study subjects selection: In this case-control study, 150 pregnant women who were diagnosed with PE based on the American College of Obstetrics and Gynecology in the Department of Obstetrics & Gynecology in R. L. Jalappa Hospital & Research Center. Remaining 150 were controls considered as normotensive pregnant women. Further it was analysed based on the gestational weeks as early and late PE.

4.2. Diagnostic Criteria of PE: According to the American College of Obstetrics and Gynecology PE was defined as new onset of hypertension (Blood Pressure ≥ 140/90mmHg) and proteinuria (urine dipstick of ≥ 1+) after 20 weeks of gestation. In the absence of proteinuria, PE was characterised by severe hypertension (≥160/110 mm Hg) and hemolysis, low platelet count, and elevated liver enzymes (HELLP syndrome) or symptoms (i.e. headache, visual changes, and right upper quadrant pain). Renal insufficiency was defined as creatinine ≥ 1.1 mg/dl. [34] Early gestation is defined as delivery before 34 weeks and late gestation as delivery at or after 34 weeks (34-37 weeks). [35] The PE women with ≥20th week of gestation were included as cases. Another 150 normotensive pregnant women with the ≥20th week of gestation without any complications till delivery were controls.

4.3.Inclusion Criteria: Pregnant women who were diagnosed with PE after the 20th week of gestation, both primigravida and multigravida were included as PE women and who were healthy normotensive pregnant women after the 20th week of gestation without any complications till delivery

4.4. Exclusion Criteria: The pregnant women with gestational hypertension, chronic hypertension, gestational diabetes, previous history of more than 2 abortions, previous pregnancy with an anomalous fetus, and thrombophilia like disorders.

Cases/Group-I: These are diagnosed as pre-eclamptic women by the obstetrician of OBG Department, primigravida or multigravida but.≥ 20 weeks of gestation with age group of 18-45 years.The methodology and techniques employed was explained in table 1.

Controls/Group-II: These are healthy normotensive pregnant women with no complications till delivery, may be primigravida or multigravida but.≥ 20 weeks of gestation with age group of 18-45 years.

4.5 Avoiding bias: Strictly following the inclusion & exclusion criteria, cases were selected for the study. Cases were all pooled after assigning a code number by an external observer who alone knew the codes. All the cases were subjected to estimation of all the selected parameters. After the reports of the parameters were known, the codes were de-coded and samples were assigned to designated groups. The encoded data were strictly kept under lock and key until all the estimations were carried out. Then the decoding process was done by another external observer selected exclusively for decoding purpose. The decoded data were arranged into different groups and thus bias was totally avoided.

5. RESULTS

Out of 300 subjects, according to gravida status primigravida were 149 and multigravida was 151. The severe PE were 68% and the remaining were mild PE. Out of 150 PE subjects 52.6% were primigravida and also remaining 150 controls 46.6% were primigravida. So this reveals PE is more prevalent to primigravidae. The pregnant women with early gestational weeks were 46 and late gestation was 254 participants Table 4.

Table 4: Gravida status and gestational weeks of subjects who participated in the (Case– Control) study

	Number of PE subjects (n = 150) Group-I	**Number of Normotensive subjects(n=150) Group-II**
Primigravida	79 (52.6%)	70 (46.6%)
Multigravida	71 (47.3%)	80 (53.3%)
Early Gestation	36 (24%)	10 (6.6%)
Late Gestation	114 (76%)	140 (93.3%)
Severe PE	102 (68%)	----------
Mild PE	48 (32%)	----------

n- Number of subjects, Group-I/ Cases- Pre-eclamptic pregnant women and Group-II/ Controls – Normotensive pregnant women,

The clinical outcomes in 150 cases were as follows: Due to severe PE intra uterine growth retardation (IUGR) were 33% out of 102 severe PE and preterm delivery were 15% and 15% intra uterine deaths (IUD) (Tables 5 & 6). Due to PE the clinical complications out of 150 were as follows (1) total IUGR in PE were 46% showed (2)19% preterm delivery, (3)15% anemia, (4) 8% hypothyroidism, (5) 6% were PE in their second pregnancy, and (6) 3% t**w**in pregnancies.

Table 5: Clinical Outcomes

Sl No	Clinical outcome due to PE	No. of PE in cases -150	Percentage (%)
1.	IUGR	69	46
2.	Preterm Delivery	28	19
3.	Anemia with PE	22	15
4.	Hypothyroidism with PE	12	08
5.	Primigravida repeated PE in second pregnancy	09	06
6.	Twin Pregnancy	04	03

PE- Pre-eclampsia

Table 6: Clinical outcome due to Severe PE

Severe PE and Clinical Outcomes	Number of Cases (n = 150)
Severe PE+ Intra Uterine Growth Retardation / Restriction (IUGR)	50 (33%)
Severe PE + Premature birth	23 (15%)
Severe PE + Intra Uterine Death(IUD)	23(15%)

The PE was classified into early and late gestational weeks. Based on the gestational weeks in both cases and controls the maternal age, gestational weeks, systolic, and diastolic blood pressures in cases, and controls were analyzed by the Mann-Whitney U test (Table 7).Due to non-distribution in data, the statistical significance in cases and controls were analyzed by the non-parametric test Mann-Whitney U test. When compared to the median values of maternal age in cases and controls there was not much difference between early and late gestation, but in gestational age, we found a statistical significance in early & late PE. In late PE the gestational weeks were 37 and in subjects with early PE was 30 weeks only and if you compare in controls of early gestation is same as in PE and in controls late gestation was extended to 39 weeks. So this proves that due to PE there were more chances of pre-term delivery which affects the fetal growth.

Table 7: Based on the gestational weeks in group I& II statical outcomes by Mann-Whitney U Test and P values.

Content	Gestational Weeks	Median± SE	P value
Maternal Age	Early Gestational Weeks in group-I	25 ± 0.616	0.1336
	Early Gestational Weeks in group-II	23 ± 1.13	
	Late Gestational Weeks in group-I	24 ± 0.40	0.1141
	Late Gestational Weeks in group-II	24 ± 0.267	
	Late Gestational Weeks in group-II	195± 65.8	
Systolic Blood Pressure	Early Gestational Weeks in group-I	160±4.18	
	Early Gestational Weeks in group-II	115±4.216	0.00001
	Late Gestational Weeks in group-I	110±3.39	0.00001
	Late Gestational Weeks in group-II	120±0.757	
Diastolic Blood Pressure	Early Gestational Weeks in group-I	110±3.39	0.00001
	Early Gestational Weeks in group-II	70 ± 2.62	
	Late Gestational Weeks in group-I	100 ± 1.44	0.00001
	Late Gestational Weeks in group-II	70 ± 0.586	
Gestational Age	Early Gestational Weeks in group-I	30 ±0.384	0.8807
	Early Gestational Weeks in group-II	29.5 ±0.87	
	Late Gestational Weeks in group-I	37 ±0.180	0.00001
	Late Gestational Weeks in group-II	39±0.128	

P value significant at ≥0.00001.

CHAPTER-I

ANGIOGENIC FACTORS AND PREECLAMPSIA

6. REVIEW OF THE LITERATURE

Angiogenesis is defined as the development of new blood vessels from vasculogenesis (pre-existing blood vessels generated from angioblast precursor cells). During pregnancy, the placenta undergoes vasculogenesis and angiogenesis to accommodate fetal demands. Pseudovasculogenesis and imbalance in secretions of placental angiogenesis during placental development leads to defective angiogenesis and endothelial vascular disorders, hypertension, and proteinuria. [36] Our understanding of angiogenic factors in contributing to PE assists in the early diagnosis of PE.The complication of PE pregnancy affects organs specific to the kidney, liver, and brain caused by circulating toxins. Further it affects the health of PE women, causes cardiovascular and renal consequences, especially with early and severe subtypes.[37] For the maintenance of placental vasculature, angiogenic factors and receptors play a major role. Although improper implantation occurs, placental perfusion still occurs and it leads to shedding of syncytiotrophoblasts which leads to vascular endothelial damage. As in PE women, there is an imbalance between pro-angiogenic and anti-angiogenic factors, viz; Proangiogenic - Vascular Endothelial Growth Factor (VEGF), Placental Inhibitor Growth Factor (PIGF), and Antiangiogenic-Soluble fms like tyrosine kinase (sFlt1), and Soluble Endoglin (sEng) in the maternal blood distribution. [34]

6.1 Soluble Endoglin: sEng is an antiangiogenic, endothelial cell impairment indicator and PE marker with a molecular weight of 180 KD. Endoglin is expressed by placental endothelial cells - chorionic villi (syncytiotrophoblasts) at 11 weeks of gestation. During gravid status, the vital performance of endoglin is to control the multiplication, differentiation, incursion of trophoblastic cells to regulate endothelial cell proliferation, and blastocyst implantation. Any troubleshoots in this progression clues to PE. [38] The

pathogenesis of PE is known to be associated with abnormalities of placental vascular remodeling by aggregation of trophoblastic cells into the spiral artery. This uterine spiral artery remodeling impairment will cause vasoconstriction, resulting in decreased blood flow, hints to placental hypoxia leading to placental ischemia. The placental ischemia stimulates to release sEng like factors.[38] The over-expressed placental endoglin in the placenta will be relieved by freeing sEng into the maternal blood flow. The released sEng in maternal blood binds with transforming growth factor-beta TGF-β (family), by inhibiting the stimulation of endothelial nitric oxide synthase (eNOS), decreases nitric oxide, and results in vascular inflammation, and vasoconstriction due to hypoxic conditions in the placenta. This increased sEng levels tempted to affect typical vascular functioning to increase vascular permeability, endothelial dysfunction, and induce hypertension, which is a cardinal feature in PE and relatively related to the severity of PE. [39,40] In plasma of normal pregnancy ≤ 34 weeks: median 5.6 ng/ml, with a range of 3.5-117.1 ng/ml; normal pregnancy ≥ 34 weeks: median 8.0 ng/ml range 3.8-30.5 ng/ml.[41]

6.2 Soluble Feline McDonough Sarcoma- (fms-) like tyrosine kinase-1(sFlt1): sFlt1 is a glycosylate anti-angiogenic protein. In human placenta available form of the Flt1 gene is in soluble form. [42] Due to lack of cytoplasmic transmembrane protein, sFlt1 moves freely in maternal circulation, and the presence of an extracellular domain binds with Vascular Endothelial Growth Factor (VEGF) and Placental Inhibitor Growth Factor (PIGF). [43,44] Apart from genetic factors, the other risk factors like environmental susceptibility, racial difference, and socio-economic factors it is evident that there was a great variation in elevated levels of sFlt1. In the early stages of a normal pregnancy, the uterine endometrial, and myometrial spiral arteries invade the cytotrophoblast for effective placental vascular remodeling. This process is initiated at 12 weeks of gestation and by 18 to 20 weeks this remodeling will be completed.[1] In humans, the physiological function of sFlt1 is to

maintain placental vasculature and to regulate the formation of new blood vessels (at the embryonic stage) especially in tissues like the kidney, cornea, and uterus. [43,44] In PE, cytotrophoblast cells fail to transform the arteries which lead to failure of placental vascular remodeling and affects the blood supply/nutrition to the fetus. At 20 weeks of gestation, the angiogenesis is completed. This might be a reason for ≥ 20 weeks PE is evident.[45] This is evidence by defective angiogenesis leads to placental ischemia in PE results in defective placental vascular remodeling.[44] Due to this, the function of sFlt1 is impaired and directly affects on maternal endothelium and counter part in the initiation of PE.[46] The mean value of sFlt1 levels in serum of normotensive pregnant women is (90% CI) of sFlt-1 (pg/ml) and at 10, 18, 28, and 37 weeks of gestation were 413 (174–981), 296 (125–704), 413 (174–982), and1,130 (477–2,690). [47]

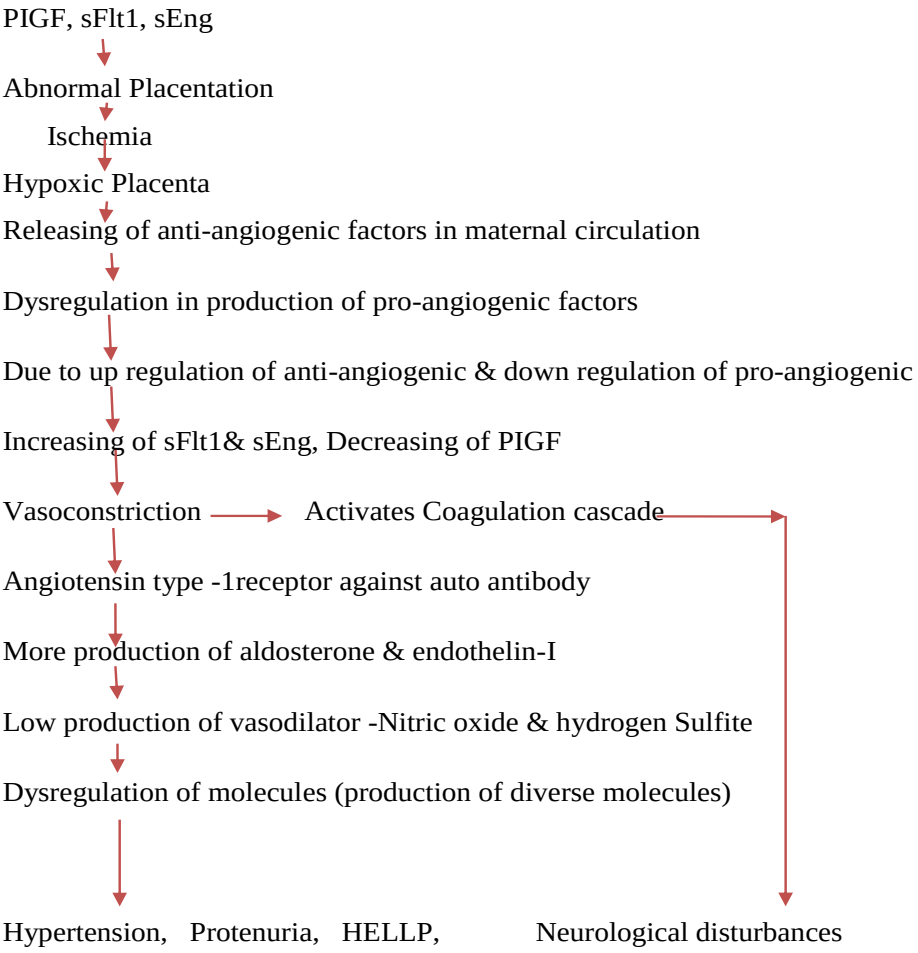
6.3 Phosphatidyl inositol-glycan class F Placental Inhibitor Growth Factor (PIGF)

Phosphatidyl inositol-glycan biosynthesis class F protein belongs to VEGF (Vascular Endothelial Growth Factor) family, a key molecule in angiogenesis and placental vasculature, particularly during embryogenesis. The major source of PIGF is the placental trophoblast. [48, 49] PlGF is one of the main factors that play a key role in the remodeling process of maternal arteries in normal pregnancy. This growth factor is released into the bloodstream by the migration of trophoblasts.PIGF/ PLGF/PGF belongs to the cysteine-knot superfamily.[50] Placental protein binds to form a heterodimer with VEGFR-1/FLT-1/soluble variant sFLT-1 (soluble fms-like tyrosine kinase-1). It also binds to neuropilin receptor1 and 2, recognized in the placenta but its role is still to be highlighted in detail. [51]PIGF is expressed in trophoblastic cells of placenta, neurons and in pathological conditions like colon and breast carcinomas. It signals through the vascular endothelial growth factor receptor-1 VEGFR1/FLT1 (fms-related tyrosine kinase-1) receptor and stimulates endothelial cell proliferation and migration.[50] During normal pregnancy, PIGF

levels continuously increase as gestation week increases, peaks at 26 to 30 weeks, and then decreases. Serum PIGF level is significantly reduced in PE patients. [52] The mean values (90% CI) of free PlGF (pg/ml) at 10, 18, 28, and 37 weeks of gestation were 36 (14–89), 206 (83–515), 518 (207–1,290), and 354 (142–884).[47]

6.4 ROLE AND MECHANISMS INVOLVED IN PRE-ECLAMPSIA

Flow Chart 2: Mechanism of Angiogenic and pro-angiogenic factors in PE



6.5 MATERIALS & METHODS:

Methodology employed: After collecting written informed consent from the 300 pregnant women 5ml of venous blood was collected in plain (red-capped) vacutainer before delivery in labor ward. The vacutainer was left for room temperature for 1hr and the blood was allowed to clot. After 1 hr the serum was separated by centrifuging at 3000 rpm for 10 minutes. Supernatant serum was separated and stored at -80˚C in Eppendorf tubes for further analysis.

Estimation of serum biomarkers: The angiogenic markers sFlt1, sEng, PIGF estimation in serum was followed by ELISA method (commercial kits). The stored serum at -80˚C gradually brought to -20˚C then to 4˚C and then to room temperature. As it is not done routinely in our laboratory, by purchasing (self-financing) commercially available Enzyme-Linked Immuno Sorbent Assay (ELISA) kits from Chongqing Biospes Co., Ltd., (suppliers: Infobio Company, New Delhi) the serum samples were measured by following strictly as per the protocol. The maternal serum analysis was further estimated with an ELISA microplate reader at 450nm (Merilyzer Eiaquant Company).

Flow Chart: 3 Procedure for serum markers sEng, sFlt-1 and PIGF

ELISA kit components were left for 15-30 min at room temperature.

↓

Set standard, test sample and control (zero) wells on the pre-coated plate

↓

Added 50µl of Standard diluent buffer (Kit Component 3) into the control (zero) well

↓

Do not add sample and HRP conjugated antibody into the control (zero) well.

↓

For test sample wells, added 40µl of Sample diluent buffer

↓

Shaken the plates moderately to get the components mixed up thoroughly

↓

Covered the plates with plate sealer and incubated at 37℃

↓

Discarded the solution in the plate without touching the side walls. Clapped the plate on absorbent filter papers. Filled each well completely with Wash Buffer (1×) and mixed manually for 2.30 min.

↓

Added 50µl of HRP conjugated anti- sFlt-1, anti-sEng, anti-PIGF antibodies

↓

Covered the plate with plate sealer and incubated at 37℃

↓

Removed the sealer, and washed the plate and added 50µl of TMB substrate A, B

↓

Shaken the plates on ELISA shaker for 30 seconds and incubated in dark at 37°C for 15 min .Added 50µl of stop solution

↓

After 15 min readings at absorbance at O.D $_{450}$ nm in microplate reader readings were taken.

6.6 RESULTS:

sEndoglin Levels: By comparing group-I and II, the maternal serum sEng levels were significantly higher in PE cases group I (z value =8.71).The p-value is significant with ≤0.0001 especially in late gestational weeks the sEng levels were highly significant (Table 8) . The AUC value was 0.87, sensitivity 89% and specificity 83% were observed with a cut off value of 1290.7± 50.66 pg/ml (Table 9 and Fig.8). In early gestational weeks of PE the serum sEng levels were high (Table 8).

sFlt1 Levels: By comparing median in cases /group-I and controls/group-II the maternal serum sFlt1 levels were significantly higher in PE cases/ group –I (z value =2.96, U= 9021). The p-value is significant with (≤0.05) <.00298 especially if we compare between early and late gestational weeks, in late gestational weeks the sFlt1 levels were highly significant (Table 8). The AUC value is 0.82, 91% sensitivity, and 79% specificity (Table 9 and Fig.7).Due to non-distribution in data, the cut-off value was calculated based on the median by SPSS (version 22.0; SPSS Inc, Chicago, IL, USA). The cut-off value in controls (median) was 320 pg/ml (Interquartile range 67.62-10). In PE the median cut-off value was 1535.1 ± 237.5 pg/ml (Interquartile range 958.1-160 = 798.1) (Table 9).

PIGF Levels: The maternal serum PIGF levels were significantly higher in PE (z value =2.65) with a significant p-value of ≤0.0001. In late gestational weeks the PIGF levels were highly significant (Table 6). The AUC value is 0.93 with sensitivity 92% and specificity77% (Table 9 and Fig 9).The cut-off value in controls (median) was 99.25±6.5pg/ml (Interquartile range186.5-53). In PE the median cut-off value was 82.5 ± 10.2pg/ml (Interquartile range 142-39.5= 102.5) (Table 9). PIGF levels in early gestational weeks of PE were high (Table 8).

sEng/PIGF ratio:AUC value was 0.98,sensitivity(98%)and specificity(80%) (Table 9 & Fig.10).

sFlt1/PIGF ratio: AUC value was 0.94,sensitivity(92%) and specificity(79%) (Table 9 & Fig.11).

Table 8: Statistical Outcome of Serum Biomarkers (sEng, sFlt-1, PIGF) in Early and Late Pre-eclamptic Women (Cases) versus Early and Late Normotensive Pregnant Women (Controls)

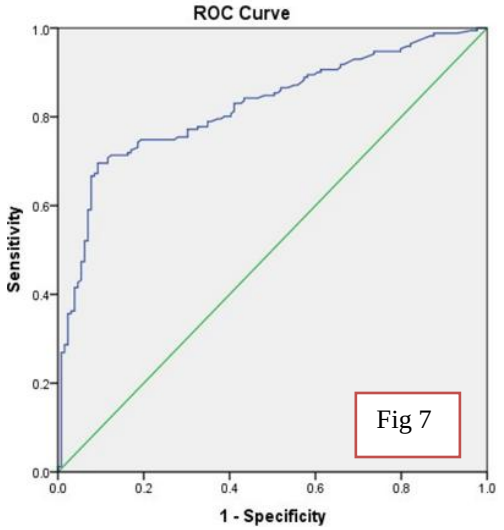
Content	Gestational Weeks	Median± SE	P value
Soluble Endoglin Levels	Early Gestational Weeks in group-I	1340 ±1118	0.00386
	Early Gestational Weeks in group-II	640 ± 195	
	Late Gestational Weeks in group-I	1270± 660	0.00001
	Late Gestational Weeks in group-II	480 ±85	
Soluble Flt1 levels	Early Gestational Weeks in group-I	1595.0 ± 549.5	.00022
	Early Gestational Weeks in group-II	200 ± 170	
	Late Gestational Weeks in group-I	1501 ± 3360	0.00001
	Late Gestational Weeks in group-II	320± 80.8	
PIGF Levels	Early Gestational Weeks in group-I	139±18	0.3572
	Early Gestational Weeks in group-II	140.3±62	
	Late Gestational Weeks in group-I	615±128	0.00001
	Late Gestational Weeks in group-II	195± 65.8	

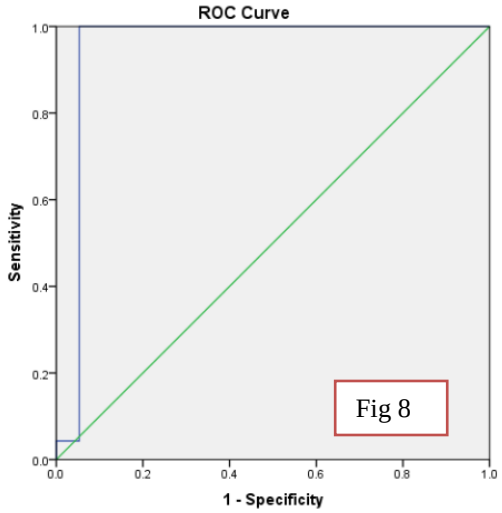
Significant with p value ≤ 0.0001, n- Number of subjects, SD -Standard Deviation, SE-Standard Error, Numbers of subjects involved in the study were as follows: Early Gestational Weeks in PE cases n=36; Early Gestational Weeks in controls n=10; Late Gestational Weeks in PE cases n=114; Late Gestational Weeks in controls n=140

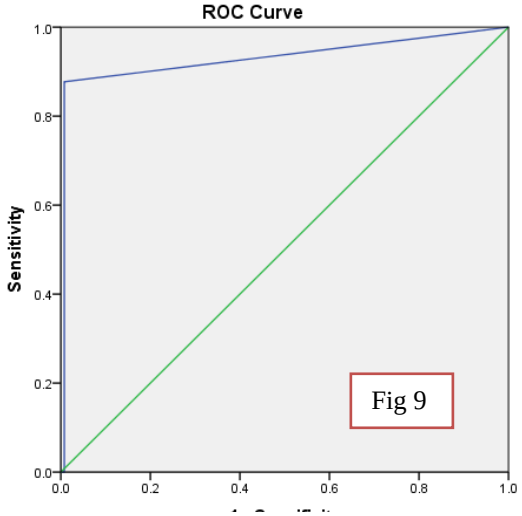
Table.9: Statistical values for sFlt-1, sEng, PIGF with their ratios

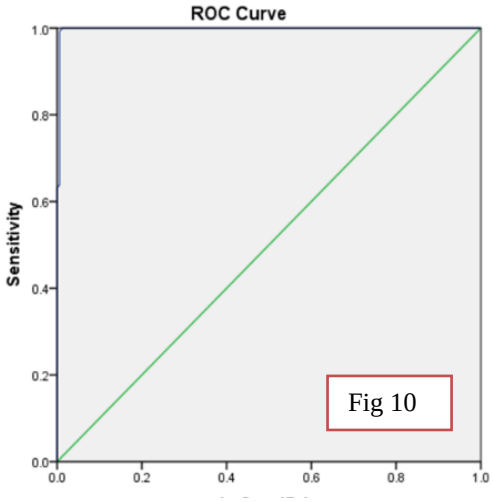
Angiogenic marker	AUC	Sensitivity	Specificity	Median Cut-off Value in PE	Inter Quartile Range
sFlt-1	0.82	91%	79%	1535.1± 237.5 pg/ml	958.1-160 =798.1
sEng	0.87	80%	72%	1290.7± 50.66 pg/ml	1600.3-1020 = 580.37
PIGF	0.93	92%	77%	82.5± 10.2 pg/ml	142-39.5 = 102.5
sEng/PIGF	0.98	98%	80%	------	------
sFlt1/PIGF	0.94	92%	79%	------	------

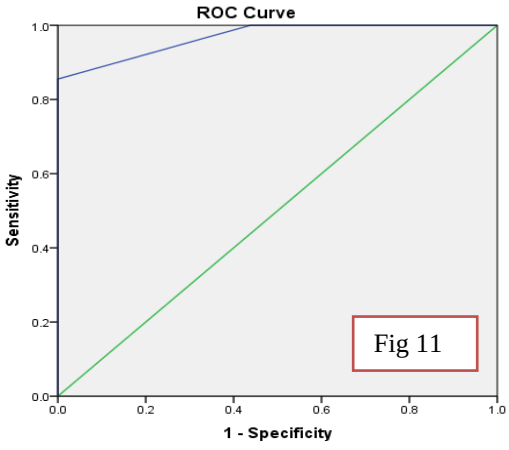
Fig7: The AUC curve value of sFlt-1 is 0.82, 91% sensitivity, and 79% specificity
Fig8: The AUC Curve value of sEng is 0.874,80% sensitivity, and 72% specificity
Fig 9: The AUC curve value of PIGF is 0.93, 92% sensitivity, and 77% specificity
Fig 10: The AUC curve value of sEng/PIGF is 0.98, 98% sensitivity, and 80% specificity
Fig 11: The AUC curve value of sFlt-1/PIGF is 0.94, 92% sensitivity, and 79% specificity











6.7 DISCUSSION

For a healthy fetal growth there should be a proper uterine establishment with healthy placental angiogenesis.This plays a vital role in placental perfusion. Due to disproportional ratio of pro-angiogenic factors and anti-angiogenic factors there will be defect in the remodeling of uterine spiral arteries and angiogenesis which can be one of the explanation for pathogenesis of PE.[53,54] To reduce PE-related mortality the non invasive biochemical markers should be emphasized for the pre symptomatic screening and diagnosis for the prediction of PE. So far, a number of biomarkers have been proposed for predicting PE, as there was inconsistency in their results.[55]

6.7.1 sFlt1: In Albania, a study was conducted on 95 PE plasma samples of women whose sFlt1 levels were highly significant. [56] By Tang et al., on 105 PE subjects, a study was piloted, they found a marked increase in sFlt1levels in PE and complicated PE pregnancies, especially in intra uterine fetal growth and development.[57] In a cohort study, in California on 97 pregnant women tested sFlt1 levels by comparing nulliparous and multiparous women, in first and second pregnancies and observed an increase in their sFlt1 levels during first pregnancies than second pregnancies and could be a predictor marker in primigravida.It is a well-known fact that primigravida women were at high risk for PE. This matches with the present study. [58] The sFlt1 levels were increased with increased maternal age, early gestational weeks and obesity, invitro fertilization (IVF). In the present study also the sFlt1 levels were high in late gestational weeks but not affected by maternal age.[59] Along with maternal age, early gestational weeks, obesity, IVF like risk factors, even ethnicity also play a role. The sFlt1 levels and PE were also associated with ethnicities. There are studies done on Hispanic women, Caucasian women, and African-Caribbean pregnant women. In Caucasian PE women, sFlt1 levels were elevated significantly when compared with Hispanic PE women. [1] Another study was performed by Lai et al., on

African-Caribbean and Caucasian populations, in the African-Caribbean population, the possibility for occurrence of PE was higher with increased sFlt1 levels in serum than Caucasian PE women, based on this ethinicity is also a risk factor in PE. [59] In an animal-designed study by Maynard et al, by inducing sFlt1 in pregnant rats, the findings were reflected with hypertension, proteinuria, and glomerular endotheliosis. [43] But regarding abnormal implantation of the placenta and excess sFlt1 production were left as hypothetical. The mean serum sFlt1 value of 7.6 ng/ml in humans was almost five times risk in severe PE than in normotensive pregnant women. If sFlt1 overexpression occurs early in pregnancy, it might serve as a diagnostic marker in patients at high risk due to PE. [43] By exhaustive research it was observed that sFlt1 cannot be a predictive diagnostic marker in the first trimester of PE pregnancy.[1] Fan and colleagues in a human and animal model studies proved that placental trophoblast cells overexpress sFlt1 in self-defense against excessive VEGFA. This study ensures that the maternal decidual cells were responsible for increased placental expression of sFlt1. [60] In 20 PE subjects studied by Chielie et al., the sFlt1 levels are significantly increased with mean ranges from 1.6 to 6.7 ng/ml whereas in controls it ranges from 0.95 to 3.0 ng/ml. [42] In an animal (mouse) designed study the administration of sFlt1 lead to cardiac dysfunction. This suggested that there was an association between PE and sFlt1 levels to cardiac dysfunction. There was a much stronger relationship between early-onset PE and later-life cardiovascular diseases in their first pregnancies. Increased sFlt1 levels are correlated with acute myocardial infarction even after postnatal delivery from 4-6 weeks to 5 to 8 years. The history of PE is a predictable cardiovascular marker in later life. [1] Out of a few studies in India especially from Southern India part, this is the first study. From Northern India, a total of 6 studies were performed on the sFlt1 levels and PE. From Lucknow, a study was focussed on maternal sera sFlt1 levels, which were higher in PE women. [61] In a study of the Delhi

population the mean maternal serum levels of sFlt1 in the PE subjects ranging from 6785.25 ± 1677.01 pg/ml and in controls were 3030.29 ±956.35 pg/ml (mean ±SD). The maternal serum levels of sFlt1 were higher in PE Women. [42] A twin study from the Delhi population evaluated the serum levels with median ranging from 2932.81; 1802.33–5760.46 pg/ml PE against controls with 1114.94; 655.03–2694.35 and found increased sFlt1 levels in PE women along with the association of sFlt1gene polymorphism.[62,63] From West Bengal, a study was evaluated and found increased serum sFlt1 levels in PE compared to the control group.[64] Another twin study from New Delhi was evaluated on 80 pregnant women and their sFlt1 levels were found significantly higher with a median of 11295.25 - 2936.2 pg/ml in PE than those in the sera of normotensive median 2893.20 pg/ml, range 1180.43–6706.6 pg/ml. [65] In the present study, we found a significant increase in the sFlt1 levels in PE women compared to the control group with a median ± standard error was 1535.1 ± 237.5 and in the control group it was 320 ± 80.2. So this study matches with the above the higher levels of serum sFlt1 in PE women.

6.7.2 sEndoglin

The function of Endoglin is to maintain vascular tone. Due to PE there will be increased levels of circulating endoglin levels which are associated with vasoconstriction, resulting in decreased blood flow, resulting in to placental hypoxia and placental ischemia which stimulate to release sEng into the maternal blood flow. Due to its imbalance in the pathway, it shows harmful effects like loss of control over the trophoblastic invasion of cells and leads to discrepancy which can be visible in form of PE. Whereas sEng is generated by the cleavage of the trans-membrane bound endoglin receptor, by damaging placental vessels and by desquamation of membrane-bound transforming growth factor-beta (TGF β), so it inhibits TGF β-mediated cell signaling and endothelial function and passes into the maternal circulation from the placentae.[38,66,67] The increased levels of sEng released into the maternal circulation leads to placental ischemia eventually leads to endothelial damage. According to Akbar et al., sEng levels increased in 12-16 weeks before the onset of PE.[38] According to the Kosinska-Kaczynska et al., in a review increased levels sEng were significant in 31 to 35 gestational weeks in late onset of PE. [68] According to Gaber et al ., sEng is a diagnostic marker to predict PE at 15– 18 weeks with a cutoff value of >7 ng/ ml as 94% and 119 times chances they are more likely to develop PE. [69]In a review piloted by Chen sEng cut off value ranges from 4.1 to 33 ng/ml. [70] The Gaber and Chen studies showed cutoff values were comparable to the present study population. In a study performed by Ali et al., sEng levels were significant in PE group with the mean value of 9.1+4 4.3-17.[36] A study performed in Romania stated that sEng levels were not so relevant for diagnostic use in PE.[71]A study with 43 PE women was performed in Brazil found that sEng levels were significantly increased in severe PE, in early and late gestation it was not specifically significant but overall sEng levels were significant in PE pregnancy.[72] But in the present study compared to late PE, in early PE the sEng median ±

standard error (SE) levels were more than late PE, but definitely there was a statistically significant correlation in increased serum sEng levels between normotensive and PE pregnant women. A study conducted by Hirashima et al on 56 PE women, in the early onset of PE the sEng levels were high compared to late onset of PE. In our study also sEng levels (median ± SE) in late onset was 127± 6.6 and in early onset (median ± SE) was 134 ±11.18 which matches with the present study but with a sample size of 300 pregnant women.[73] In a study performed by Khalil et al it was shown that plasma levels of sEng are a diagnostic marker in late gestational PE. [74] Another study performed in Indonesian population found that serum sEng levels were a diagnostic marker in early onset PE.[38] Sachan et al ., studied on non severe and severe PE with cut–off value of >7.76 ng/ml, sEng had 68.75% sensitivity, 85.3% specificity, positive predictive value 68.7% and negative predictive value 85.3%. sEng is a predictable biomarker in second trimester (13-20 weeks) of PE pregnancy.[75] The sEng levels were significantly increased in types of PE with AUC of 0.94, a cut-off value of 20.4 and is a notable diagnostic marker in Iranian population for PE.[76] In the Italian population, a study was performed which states that sEng levels were significant with AUC 0.88 and no difference in sEng levels in early & late PE. [77] In the Egyptian population at 13 weeks of gestation, sEng levels were increased in high exposure of PE subjects along with early onset PE. In the study by Levine et al it was shown that sEng levels can be a predictive early diagnostic marker in the Egyptian and American populations.[78] A study conducted by Lai et al., showed that sEng levels measured at early gestational weeks were more significant at early gestational weeks so it can be an early prognostic marker. [79] In the Japanese population also sEng was a diagnostic marker in PE with mean significant levels of 60.9 ± 28.8. [80] Cui et al., piloted a study in 110 PE and 62 normotensive pregnant women sEng levels were studied. Cui and Venkatesha et al study observations were increasing sEng levels are matching with the severity of PE which

matches with the present study observations. [81, 82] From Indian original studies, Northern part of India 3 studies were performed and only one study was piloted from southern part of India. Present study is initial study in south eastern part of India with 300 sample size. The details of these studies were as follows: In North India, Sachan et al., from Lucknow performed on 30 subjects the study revealed that sEng is a unique marker to evaluate diagnostic and prognostic accuracy in preeclamptic women with cut-off value ≥ 6.26 ng/mL. This study also matches with present study with a similar cut off value of ≥ 8 ng/ml with a 300 sample size. [83] Duhan et al from Haryana conducted on 100 subjects in PE and normotensive pregnant women. The sEng levels range from 2.54 ng/ml to 7.06 ng/ml and the levels were significant in PE. This study matches with present study but the cut off value is 8ng/ ml and with more sample size. [84] In south India, Archana et al., from Kanchi Tamil Nadu a study was analyzed on 35 pregnant women with increased serum sEng levels in late-onset PE.[85]A study from Chandigarh by Agarwal et al., conducted on sEng levels ranges from 84.9±38.8 vs 13.2 ± 6.3 ngml–1. [86] In Plasma sEng levels can be a useful as predictive & diagnostic marker.[87] Based on all these studies the present study states, sEng levels were statistically significant in severe PE and late PE. And if the serum levels are ≥8 ng/ml are prone to PE. The Northern Indian studies from Lucknow & Haryana, and Southern Indian study from Tamil Nadu states that sEng is a diagnostic marker in PE. This is the first study in south-eastern part of India.

6.7.3 PIGF

In humans, 4 different PlGF splice variants are found. PlGF may respond to hypoxia or ischemia and other co-morbidities influence the relationship between PE and PlGF. For example, there is an association between low PlGF levels and PE in obese women. [50] In a mouse-transgene designed model by Chau et al .,revealed that PIGF promotes angiogenesis for the feto-placental circulation and supports trophoblast development but the decidual invasion is not influenced. Consequently, abnormal spiral artery remodeling in mice does not lead to placental insufficiency or abnormal blood pressure regulation.[51]In a biomarker study conducted by Giardini et al., it was suggested that PlGF as a biochemical marker not only for PE, but also for placental dysfunction. [88] In a multi-centric study, PIGF levels ranged from 12 to 3000 pg/mL can be a preferably an early clinical marker of adverse suspected PE pregnancy. [89] In other studies by Lai et al, Myatt et al and Youssef et al.,AUC ranged from 0.61 and 0.734. In a cohort study it was mentioned that PIGF can be a diagnostic marker for PE. PIGF levels were significantly lower in first trimester serum samples of subjects who later developed PE. [90,91,92] A study performed by El- Deen et al., on 74 pregnant women and 50 PE Egyptian women for the maternal serum PIGF levels compared between severe and mild PE. They found that there was no statistically significant difference between severe PE and mild PE groups. But from controls to PE women the PIGF levels were significant.[93] The Indian studies on the role of PIGF or in combinations for prediction of PE availability has been very limited.[94] From India a total of 3 studies (original studies) were available, from north India (all from Delhi) 3 studies and from south India 1 study was available and present study is the 2nd from southern India. From northern India, in Delhi by Agarwal et al., a study was performed on 17 PE cases, the levels of PIGF were compared between early and late PE which was not statistically significant. When compared to PE and controls the PIGF levels were significant. The cut-

off values in early PE were <30 pg/ml with 88.2% sensitivity and 71.4% and in late PE it was <32 pg/ml with a sensitivity of 86.7% and specificity of 74.3%. [52] In a cohort study by Ghosh et al., from Delhi, they measured maternal serum PlGF levels at 11–14 weeks and 22–24 weeks of gestation.It showed that PIGF is an early second trimester marker.As maternal serum PIGF levels were <144 pg/ml at 22–24 weeks of gestation were much higher than those of serum PlGF <228 pg/ml at 11–14 weeks of gestation.[95] In a case control study by Varughese et al., from Delhi with 80 study subjects they measured the maternal PIGF levels. The mean PlGF levels were 236.77 pg/ml in PE where as in controls the mean PIGF levels were 744.98 pg/ml.[65] Another twin study by Agarwal et al., studied on PIGF and biophysical markers in late first trimester. They deliberated on 291 singleton pregnant women at 11–14 weeks of gestation who were primigravida of age group <40 years. PIGF alone had detection rate of 40% in PE.[94] The reduced levels of PlGF were evident from the first trimester, before 5 weeks of the onset of PE. It was evident that PIGF levels will be lower in early gestational weeks especially in suspected PE. So the maternal serum PlGF, would identify about 90% and 50% of patients developing early PE and late PE. There was a significant association between PlGF and the severity of PE also. [95] The present study results matche with that study.Chappell et al., in their study observed that PlGF has a high sensitivity and predictive value for PE within 14 days in early onset suspected PE. So PIGF alone has a better predictive value. [96] Treatment of PE with PlGF is an promising but many uncertainties remain.[51] In a multicentric, cluster-randomised controlled assessment, where 11 maternity units in UK were included, 1023 suspected PE women were enrolled, in that 576 (56%) women were exposed testing group, and 447 (44%) were in hidden testing group. When compared with hidden group 24 (5%) of 447 versus exposed testing group as 22 (4%) of 573 women were affected with PE. PlGF is useful as a diagnostic biomarker in women with suspected PE. [97]

6.7.4 sFlt1 / PIGF Ratio

In a cohort study by Perry et al.. the sFlt1/PIGF ratio used as a prognostic marker in late PE. In that study, a total of 302 hypertensive pregnant women were involved. The statistical value was as AUC of 0.83 - 0.88 with P value was 0.025 and in 2 weeks before delivery, the AUC value was 0.86 to 0.93 with P value of 0.001 in late onset PE. So this study showed that the sFlt1/PIGF ratio was statistically significant in late PE compared to the early- onset of PE.[98] A study by Nzelu et al., was done in the first trimester of pregnant women to estimate the serum levels of PIGF/sFlt1 ratio. The participants were 650 first trimester pregnant women with chronic hypertension, 202 were with super imposed PE, 448 women with chronic hypertension and who did not develop PE were compared with 142 normotensive first trimester pregnant women as controls. The findings were that the PIGF and sFlt1 were statistically significant in all the 3 groups of pregnant women when compared to 2 other groups. So that study concluded that during first-trimester there will be lower concentrations of PIGF/sFlt1 that will be prone to PE. The study proved that there was not much difference in the PIGF /sFlt1 ratios among chronic hypertension and PE. But in women with superimposed PE the serum PIGF/sFlt1 levels have the least predictive value. [99] For predicting the onset of PE, sFlt1/PlGF ratios have shown significant accuracy at second trimester of PE pregnancy. [100] A study was conducted in Iran on sFlt1/PlGF ratio by Nikuei et al., with 38 PE women and 20 normotensive pregnant women. The ratio was highly significant in PE women compared to controls with the mean in PE subjects 91.33 ng/ml with AUC of 0.90 with a cut-off value of 24.96, the sensitivity and specificity were 84.2 and 85.0% and in severe PE, the mean was 153.44, the cutoff value was 40.9% with AUC 0.80% and in early-onset PE the mean was 72.06, AUC was same as in severe PE with a cut off value of 39.7%. In normotensive pregnant women, the mean value was 17.62. So in this study it was observed that it was not statistically significant based on the

severity of PE, but when compared to normotensive pregnant women the ratio was highly significant.[101] Angiogenic factors (sFlt1/PlGF ratio; PlGF alone) with or without clinical characteristics was also a reliable tool for prediction of early-onset and late-onset PE along with pregnancy-related other hypertensive disorders, including superimposed PE and gestational hypertension. [102] Suzuki et al., (2018) reported in their study on sFlt1, PlGF or the sFlt1/PlGF ratios at 19-38 weeks. In early onset the levels were highly significant than late onset but in late onset at different weeks the ratios were the same.[103] Birdir et al ., (2018) studied on maternal serum levels of sFlt1, PlGF at third trimester of pregnancy measuring the maternal serum levels of sFlt1, PlGF at 32–37 weeks of pregnancy. sFlt1, PlGF and sFlt1/PlGF-ratio showed a prognostic capability to differentiate pregnancies developing PE and IUGR from controls. [104] Lokossou et al., (2018) in their study stated that sFlt1 and PlGF plasma levels help predict the occurrence of PE in pregnant women. The sFlt1/PlGF ratio was significantly higher in women with PE compared to normal pregnancies. Due to imbalance in these placental proteins like sFlt1 and PlGF in maternal serum leads to impaired placentation.[105]The sFlt1/PlGF ratio is a diagnostic marker for those who are at risk of placenta-related disorders, in the second half of pregnancy, and also helps to predict who develop adverse fetal outcomes. The diagnostic strategy for PE is based on a dual cut-off. The suggested cut-offs between 20+0 and 33+6 weeks are ≤33 and ≥85 to rule-out and rule-in PE respectively, and the values for 34+0 weeks and beyond are ≤33 and ≥110 respectively. A study in India revealed that the usage of sFlt-1/PlGF ratio lead to extension of pregnancies beyond 37 weeks without increase in perinatal mortality. Implementation of sFlt-1/PlGF ratio in the diagnostic process is a promising marker.[106] The sFlt1/PIGF ratio is a precisely predictive diagnostic marker able to predict within 4 weeks before disease gets established**.** The ratio is also able to predict other complications like Fetal Growth Retardation, and Intra Uterine Death.[107] A cohort study on Asian pregnant

women in Singapore was conducted which involved 934 women with different ethnicities which are from Malay, Indian & Chinese population. They have seen the major variance in sFlt1& PIGF levels among diverse ethnicities, as it helps for supplementary information for the researchers.[108] A study was done on sFlt1/PIGF ratios and their levels were compared before and delivery by using antihypertensive drugs for 3 days (after delivery with antihypertensive drugs), in 50 cases of severe PE and 90 normotensive pregnant women were recruited in this study. Then the serum sFlt1/PIGF ratios were considerable predictive diagnostic marker for before delivery rather than post-delivery in severe PE.[109] In a retrospective case control study 164 pregnant women were compared with sFlt1/PlGF ratios at delivery time. So the study proved that, those pregnant women who had increased levels of sFlt1/PlGF ratios had prolonged delivery period.[110] In a prospective case-control study of 96 pregnant women, they observed sFlt-1/ PlGF ratio between 34 + 0 and 36 + 6 gestational weeks. These ratios were not significant to predict the preterm deliveries especially in the occurrence of PE in late gestational weeks.[111]

6.7.5 sEndoglin /PIGF Ratio

Cui et al., (2018) included 110 PE and 62 normotensive pregnant women with eleven serum proteins (VEGF, sFlt-1, sEndoglin, PlGF, sEGFR, prolactin, PTX3, PAI-1, NGAL, IL-27, COX-2) were studied. Grouping of serum protein markers (VEGF, sEndoglin, PlGF) and other parameters (serum creatinine, platelet count) assists in diagnosis of PE in a better way. By utilizing of these two combinations PlGF and Uterine Artery Pulsatility Index (UtA- PI) helps in early diagnosis of PE. [81] They are different studies in individual and combination of markers (Table 10).

Table 10: Biomarker studies in sFlt1, sEng, ratios of PIGF/ sEng, ratios of sFlt1 /sEng, sFlt1 /PIGF, sFlt1+sEng/ PIGF

MARKER	NAME OF THE AUTHOR	LEVELS IN PREGNANCY
sFlt1	Shaikh 2020 [64]	Increased in PE cases
	Arora 2019[112]	Increased in PE cases along with sFlt-1 gene polymorphism
	Tang 2019[113]	In IUGR due to PE their levels are significantly high
	Tomimatsee 2019 [1]	Not a 1st trimester marker
	Mochan 2018[63]	Increased in PE cases
	Portelli 2018 [114]	Increased in PE cases
	Helmo 2017[115]	Increased in PE cases
	Sahai 2016 [116]	Increased and good II nd Trimester Marker
	Daka 2015[117]	Increased in PE cases
	Adamson 2014 [60]	Cardiovascular marker and ethnicity plays a role in increased levels of sFlt-1
	Kar 2014 [34]	Increased with severity of disease
	Lai 2014 [59]	Increased in early gestational weeks
	Rana 2014 [118]	Increases in multiple gestation, trisomy 13 &Molar pregnancy
	Petla 2013 [119]	Increases before 5 weeks of onset of the disease
	Yadav 2013[120]	Increased in PE cases
	Bates 2011 [121]	Increased in PE cases and effects endothelial activation
	Maynard 2011 [122]	Increased in PE leads to placental dysfunction
	Powers 2010 [123]	Increased in PE before 2to 5 weeks of before onset
	Noori 2010 [124]	Increases before 5 to 10 weeks of onset of the disease
	Varughese 2010 [65]	Increased in PE cases
	Wolf 2005 [125]	In Primigravida of PE cases there was increased levels
sEng	Kosinska-Kaezynska 2020 [126]	A good marker at 31 to 35getational weeks
	Akbar 2018[38]	Predictive marker in early onsetPE before 12-16weeks
	Archana 2018[85]	Diagnostic marker in late onset PE
	Cui 2018 [81]	Increased in PE cases
	Portelli 2018 [114]	Increased in PE cases
	Helmo 2017[115]	Increased in PE cases
	Nikuei 2017 [76]	Diagnostic marker in PE in severe PE
	Sachan 2016 [83]	Increased in PE cases
	Sahai 2016 [116]	II nd Trimester Marker
	Radulescu 2016 [127]	Diagnostic marker in PE
	Kar 2014 [34]	Increased in II nd& III rd trimester in severe PE
	Khalil 2014 [74]	Diagnostic marker in late onset PE
	Rana 2014 [118]	Increased in endothelial dysfunction,
	Lai 2013[79]	Prognostic marker in PE
	Petla 2013 [119]	9-11weeks before onset increased in small gestation
	Agarwal 2012 [86]	Increased in PE cases
	Elhawary 2012 [78]	Diagnostic marker in early onset PE
	Duhan 2011[84]	Increased in PE cases

sEng	Maynard 2011[122]	Increases in Uterine Perfusion
	Ali 2010 [39]	Increased in PE cases
	Gaber 2010[69]	15-18 gestational weeks as predictive marker
	Powers 2010[123]	Increases but not for high risk PE cases
	Noori 2010 [124]	Increases before 5 to 10 weeks of before onset of PE
	Devivo 2008 [128]	Increased in PE cases
	Hirashima 2008 [73]	Early onset PEmarker
	Lopez-Novoa 2007[87]	It is a predictive and diagnostic marker
	Masuyama 2007 [80]	It is a diagnostic marker
	Levine 2006[129]	Increases before 2 to 3 months
	Venkatesha 2006 [82]	In severe PE significantly increases
PIGF	Hurrell 2020 [130]	It is a diagnostic marker in adverse maternal outcomes
	Parchem 2020 [131]	It is an indicator for fetal and maternal outcomes
	Kahnamouei-aghdam 2018 [132]	Non-invasive marker at prenatal care for suspected PE
	Agarwal 2017 [52]	PLGF had better detection rate especially in late PE
	Chau 2017 [51]	marker for abnormal placentation
	Sivakumar 2016 [133]	Strong predictor of PE
	Chappell 2013 [96]	Predictive marker in early gestational weeks within 14 days
	Gosh 2013 [95]	Early II trimester marker than first trimester
	Varughese 2010 [65]	PlGF levels in non-proteinuric had significant imbalance.
	Akolekar 2008 [134]	combination of maternal characteristic & serum biomarkers have effective screening
PIGF/sEng	Sahai 2017[116]	Increased and good II nd Trimester Marker
	Myers 2013[91]	PIGF is a Predictive marker at 14-16 weeks
sFlt1/PIGF	Dragon 2016 [135]	Levels Increased at 4 wks before onset of PE
	Sahai 2017[116]	Diagnostic Marker
	Zeisler 2016 [136]	Levels Increased at 4 wks before onset of PE
	Kar 2014 [34]	Predictive Marker
	Ohkuchi 2013 [137]	Levels Increased at 4 wks before onset of PE
	Petla 2013 [119]	Before 5 weeks of onset of PE
	Verlohran2011 [138]	Increases in ratio is significant so novel marker
	Levine 2006 [129]	Before 5 weeks of onset of PE
sFlt1,sEng/ PIGF	Sahai 2017 [116]	Novel marker at 2nd trimester
	Meyers 2013 [91]	sEng and PIGF increased before 15 and 20 weeks of gestation. sFlt1 Not differ at any gestation
	Petla 2013 [119]	More specific and stronger marker
	Powers 2010 [123]	Predictive marker for developing PE
sFlt-1	Present Study	Increases at 3rd trimester and significant
sEng	Present Study	Increases at 3rd trimester and significant
PIGF	Present Study	Decreases at 3rd trimester and significant
sEng/PIGF	Present Study	It can be novel diagnostic marker
sFlt1/PIGF	Present Study	It can be a novel diagnostic marker

6.8 CONCLUSION

The risk factors like maternal characteristics such as maternal age, nulliparity, pre-existing medical conditions, and history of PE, family history of cardiovascular disease, can predict 30% of PE in pregnant women.[139, 44] The other predictors like uterine artery doppler scan, angiogenic markers like sEng, PIGF, sFlt1, genetic markers, unknown environmental hazards like high altitudes, O^{+ve} blood group may be responsible in the progress of PE but unfortunately, none of them are focussed markers.[44]

But none of the studies have confirmed the sFlt1 or any other markers to be a predictive or diagnostic marker in PE. Along with biophysical markers, serum biochemical, genetic markers individually or in combination cannot predict PE adequately.[140] The prediction rate of PE with maternal history like BP readings of 62.7%, 69.5–82.9% with combination of any two markers, 86.5% with any three markers and 91.4% with four markers. [141] By various studies from literature, it was observed that sFlt1 alone is competent for vascular endothelial dysfunction/damage.[142,143] Endothelial dysfunction is one of the cardinal features in PE. This encourages the use of sFlt1 levels in serum to diagnose the PE condition but still, cohort studies have to be done to predict the disease condition and to know if it can be an early diagnostic marker. In future studies on drug intervention for restoration of sFlt1, sEng, PIGF should be focused so prolonged of PE Pregnancy can be planned and also can reduce the endothelial impairment. [44]

CHAPTER-II
FLUORIDE AND PRE-ECLAMPSIA

7. REVIEW OF THE LITERATURE

F is derived from fluorine. F which is easilyavailable in nature, highly vulnerable, and unstable element in the environment.[144,145] F bonds with other chemical and establishes a strong bond due to its gretest sensitivity and negativity.So F forms a strong bond like magnesium fluoride, calcium fluoride, hydrogen fluoride, sodium fluoride, aluminum fluoride. [146] In this sodium fluoride (Na F) is the most common and most toxic. [14] NaF is immensely toxic, and 3–5 gms is enough to kill an adult.[147] In drinking, water F is in inorganic form as sodium fluoride, which the human body absorbs easily. [145] In addition to this, the local people mainly depend on ground water for drinking and domestic purpose, this can be a possible reason for increasing F toxicity. [147] As F is highly susceptible in the environment, each person receives F from different sources with different quantities and the amount of absorption by intestines varies in each individual. So it is challenging to assess the amount of F intake in each person. Some appear to be highly sensitive to F. According to the Agency for Toxic Substances and Disease Registry (1993), certain subsets of the population may be particularly vulnerable to toxic effects of F. At low levels of F, ingested F binds with hydrogen and inhibits numerous enzymes in the body.[147] The majority of F is absorbed rapidly and passively by digestive system and enters into the bloodstream. [146] Absorbed F will get in contact with HCl in the stomach and form hydrofluoric acid and crosses cell membranes bind with calcium, various enzymes and distributed throughout the body. So F accumulates in the body especially in calcium-rich areas like bone & teeth. Nearly 50% will be accumulated in the body remaining will be excreted. F consumption limits are determined by the human body capacity by exterminating from the body through urine.[148] Hence, it is evident that the analysis of F in body fluids is highly relevant and helps us for better understanding and the possibility for

further health implications. [149] F toxicity depends on the consumption and absorption of F by the human body and would depend upon the ethnicity, age, and sex of the individual, hormonal status, and nutritional standard. [150] Fluorine in form of fludrocortisone a common component used in medicine to increase blood pressure but with side effects.[151] According to WHO, permissible F concentration in drinking water is at 1.5 mg/L and as per Bureau of Indian Standards, it is 1.0 mg/L.[17] Singer and Armstrong in their study mentioned that the serum F is 8 µM (0.15 p.p.m.), despite the concentration of F in drinking water is up to 130 µM (2.5 p.p.m.) [152] Fluorosis is predominant in 19 states of the country (India). The incidence is tremendously more in Karnataka, including Kolar. [153] Kolar is 16th in fluorosis prevalent districts of Karnataka.[154]Nearly 25 million people have been affected by F toxicity and 66 million people are at risk.[155]Kolar is a fluorosis prevalent district of Karnataka and the incidence of PE is quite high. The ground water F concentration in Kolar ranges from 2.8 to 4.3 mg/L, which is high. [154] Whereas F concentration in drinking water in Kolar ranges from 0.2- 0.36 mg/L to 3.34-7.79 mg/L which is higher than the permissible limits.[156,157] Saxena in his study mentioned about National Database Jalsat (regarding F-affected areas in India); in Karnataka 20-50% are F-affected areas, in that Kolar was noticed as a highly affected area.[157] Higher-level leads to fluorosis, associated with dental, skeletal, and non-skeletal fluorosis. Still higher levels lead to fluoride toxicity which affects organs like the liver, kidney, and endocrine system resulting in hypothyroidism.[17,158] Based on a literature search, in PE the Hemolysis Elevated Liver Enzymes Low Platelet count (HELLP) and hypothyroidism are risk factors added to that, the F levels worsen the condition of PE.

7.1 The adverse effects of F on PE pregnancy: The higher levels lead to F toxicity which affects the systems in the body. In pregnancy, the placenta permits F to enter into the bloodstream after the 20thweek of gestation. [159] F crosses the placental membrane and is associated with perinatal risks such as premature rupture of membranes, prematurity, low birth weight, PE, and fetal death. These are mainly due to neurotoxic effects, interference with placental function, increased oxidative stress, inflammation, endothelial dysfunction, and down-regulation of nitric oxide. [160] Oxidative stress has been related through prolonged diseases (BP). Oxidative stress may not be the individual reason for increased BP. The pro-hypertensive factors i.e. salt intake, sympathetic hyperactivity also directly or indirectly play a role. As a result, oxidative stress is present in F toxicity and it tends to increase BP. [161]

7.2 MATERIALS and METHODS

Estimation of serum Fluoride levels: The stored serum samples from -80 ˚C were kept in -20˚C before the day of analysis and samples were thawed for 1-2 hrs to the room temperature during the day of analysis. Serum F analysis was done in the department of Biochemistry using F Ion-selective electrode of Thermofisher's scientific-ORION 96-09 (Fig.12). Standardization of instrument was done using different standard solutions of 0.01ppm, 0.1ppm, 1ppm and 10 ppm & after every 20 samples the calibration was verified. [66] Before estimation of the serum F levels, the instrument was calibrated to zero.The series of F standards were prepared and followed the company protocol given.The samples were calculated by adding equivalent TISAB –II buffer. The maternal serum F was measured with the electrode, and the electrode remained in the solution till the readings were recorded.

Figure 12: F Ion-selective electrode Thermofisher's scientific-ORION 96-09 with Buffers and standard solutions

7.3 RESULTS

Maternal Serum Fluoride levels: The statistical significance of the maternal serum F values in cases and controls were analyzed by the Mann-Whitney U test (Table 11). If the mean and SD of serum F levels were 1.8±0.8or more it leads IUGR, Preterm delivery or IUD (Table 11). Out of 300, 150 were cases and150 were controls, the pregnant women from Karnataka were (cases-143, controls-144). A few cases and controls were included from the neighboring states of Andhra Pradesh (cases-04 controls-03) and Tamil Nadu (cases-03controls-03) were included. The maternal serum F levels in cases and controls from 3 states, the median, and interquartile range, were calculated (Table 13). By comparing cases and controls the maternal serum F levels were significantly higher in PE cases (z value =14.4).The mean and standard deviation (SD) of complications due to PE and serum F (Table 12) with their interquartile ranges.

Table 11: Mean of serum F in samples with P- value calculated by Mann-Whitney U test

Mean ±SD of serum F^-in cases/Group-I (n= 150)	**Mean ±SD of serum F^-in controls/Group-II (n=150)**	**P -Value**
1.8 ± 0.6	0.18 ± 0.3	< .05

SD- standard deviation, n= number of subjects, P value is significant at $p < .05$.The z-score14.44

Table 12: Mean±SD, median and interquartile range of serum F in PE cases with its clinical outcomes

PE Clinical outcomes	**Mean±SD of serum F**	**Median of serum F**	**Upper (x U) Lower (x L) Quartile**	**Interquartile Range**
IUGR	1.9±0.8	2.0	2-1	1
IUD	1.8±0.8	1.6	2-1	1
Preterm Delivery	2.0±1.1	2.1	3-1	2

Table 13. Shows number (No.) of pregnant women in both samples (cases and controls) from different places /districts (3 states) in India and their serum fluoride levels from Karnataka, Andhra Pradesh, and Tamil Nadu [median, upper and lower quartile, and Interquartile range of samples (cases and controls) NA- not applicable

Name of the places	**No. of Pregnant women**	**Serum F levels**	**Median**	**Upper (xU) - Lower (xL) Quartile**	**Interquartile Range (xU-xL)**
KARNATAKA	143 144	Cases Controls	2.04 0.06	1-1 0.16-0.04	1 0.12
Kolar	62 70	Cases Controls	2.03 0.05	2-1 0.13-0.04	1 0.09
Mulbagal	28 20	Cases Controls	2.04 0.05	2-1 0.21-0.04	1 0.17
Bangarpet	17 16	Cases Controls	2.04 0.07	2.5-1 0.15-0.03	1.5 0.12
Malur	09 16	Cases Controls	2.03 0.08	2-1 0.16-0.04	1 0.12
KGF	06 01	Cases Controls	1.58 0.45	2-1 NA	1 NA
Srinivasapura Narsapura Domasandra Dinahalli Devanahalli Gouribidanne Gaddur Siddalapet	3,0,0,0,0,1,1,0 5,1,1,1,1,0,0,2	Cases Controls	2.05 0.15	NA 0.68- 0.15, NA, NA, NA, NA, NA,NA, 0.23	1 0.53
Bangalore Hoskote Chintamani Chikballapur Muthyalapet	1,7,7,1,0 1,8,0,0,1	Cases Controls	2.05 0.12	NA, 3-1, 2-1, NA, NA 0.28-0.05	NA, 2, 1, NA, NA 0.23
ANDHRA PRADESH Chittor Mehaboobnagar Kadiri, Kuppam Madanapally	4- 2,1,0,0,1 3-1,0,1,1,0	Cases Controls	2.06 0.032	3.5-1.25, NA	2.25 0.12
TAMIL NADU Krishnagiri	3 3	Cases Controls	1.73 0.3	NA NA	2.5 0.12

7.4 DISCUSSION

7.4.1 Fluoride and PE: In an exhaustive work by Susheela et al., in treating anemia in pregnant women with F toxicity to prevent the complication of low birth weight babies, despite several investigations showing the effect of F consumption on various organs and systems, there was a dearth of procedures currently in place to prevent or treat F toxicity in pregnant women and children. Studies showed that F accumulates in the placenta and adversely and affects fetal growth.[160,162,163] Chlubek at al. found that increased levels of F were present in the placenta of healthy pregnant women after the intake of F, at low concentrations, from water.[164] The human placenta has been found to be a biomarker of exposure to environmental F. [165] Gardiner et al. found, in 1952, that pregnant women in Newburgh, New York, USA, who drank artificially fluoridated water with 1.0–1.2 ppm (mg/L) of F had a placental F level of 2.09 ppm, which was an almost three-fold increase compared to the placental level of 0.74 ppm in pregnant women from Rochester, New York, USA, who drank non-fluoridated water with approximately 0.06 ppm. [166] The maternal blood F levels in the pregnant women in fluoridated Newburgh (mean 0.040ppm) were approximately three times higher than the levels in the pregnant women in non-fluoridated Rochester (mean 0.014 ppm). [166] MacArthur reported, in 2015, that from 1996 to 2004, the prevalence of PE was 19% higher (31.7 cases per 1000 deliveries) in the two most fluoridated regions of the USA (South and Northeast) than in the two least fluoridated regions (Midwest and West, 26.6 cases per 1000 deliveries).[160] The PE rate averaged 40% higher in the South (34.1 cases per 1000 deliveries) than in the West (24.3 cases per 1000deliveries). In 2004, the average fluoridation rate in the South's 16 states was 81%, compared with 46% in the West's 13 states.[160] An increase in the incidence of PE in fluoridated areas is evident and this is likely to be due to F toxicity. The prevalence of PE is higher in populations in

areas where calcium consumption is lower, and lower in populations given calcium supplementation during pregnancy. [167] The role of calcium in the pathogenesis of PE is unclear but numerous studies have specified that there was a reduced occurrence of PE where calcium supplementation was included in the diet during pregnancy. [168,169] Hypocalciuria and hypophosphaturia are significant in severe PE. In rats a high intake of NaF was associated with increased abortions and intra-uterine death (IUD). [170] Chlubek et al. found that the mean fluoride concentration in the maternal plasma of 30 healthy pregnant women at full term delivery was 4.27 μM/L while the levels in the marginal and central parts of the placenta were 42.1 μg/g of ash and 33.7 μg/g of ash,respectively. [164] They concluded that most placental F was stored in the marginal part of the placenta, presumably as a result of the higher concentration of calcium found in that area. [164] Sastry et al. found, in a study of 200 healthy pregnant women, that the fluoride concentration on the peripheral side of the placenta(2.54±1.55 ppm) was two-fold higher than in the maternal serum (1.62±0.78 ppm)and six-fold higher than in the cord blood (0.45±0.35 ppm). [171] They deduced that the placenta accumulates fluoride, especially in the peripheral part, when women are exposed to relatively high fluoride concentrations in water and food. [171] The study also suggested that the placenta can act as a backstop or guard to stop the passage of fluoride to the fetus, thus protecting the developing fetus against neonatal fluoride-induced complications. [171] Sastry et al.38 found that increased serum F levels cause a high risk of preterm delivery and low birth weight babies. When the maternal serum F levels were >1 mg/L, there was a 10.58-fold increase in the risk of fetal complications and an 8.65-foldincrease in the risk of preterm delivery. If the cord serum F levels were >0.22 mg/L there was a 2.76-fold increase in the risk of low birth weight and a 4.6-fold increase in the risk of preterm delivery. [172] Sastry et al.37,38 studied the F levels in maternal serum

and cord blood. [171,172] In the present study the maternal serum F levels were examined in pregnant women with PE and in normotensive pregnant women. The values of maternal serum F levels can be used as a diagnostic biomarker for pregnancy complications like PE. If the mean±SD (standard deviation) of the maternal serum F levels are ≥1.8± 0.6 in F endemic areas the women will be prone to developing PE and related complications. Opydo-Szymaczek and Borysewicz-Lewicka, in a study of 30 pregnant women at the time of giving birth, found that the mean concentration of F in maternal plasma,3.54 μmol/L, (3.54 μM/L, 0.0673 mg/L) was significantly higher ($p<0.001$) than the level in venous cord blood 2.89 μmol/L, (2.89 μM/L, 0.0549 mg/L).[173] Similarly, Chlubek et al. found that the mean fluoride concentrations in maternal plasma of 30healthy pregnant women at full term delivery were 4.27 μM/L (4.27 μmol/L, 0.0811mg/L). [164] In the present study, while the mean serum F of 0.18 mg/L in the healthy women without PE was comparable to the values of 0.0673 and 0.811 mg/L found in healthy pregnant women at full-term by Opydo-Szymaczek and Borysewicz-Lewicka, and by Chlubek et al., respectively, the value found by us of 1.8 mg/L in the women with PE was approximately 10-fold higher than the value we found for the healthy pregnant women. [164,173] While a few studies have estimated F levels in maternal plasma or serum, cord serum, and placentae,22 none of the previous studies have examined the F levels in maternal serum in women with PE, especially in F endemic areas. Our study, in the Kolar district, an endemic area for fluorosis, is the first study of maternal serum F levels in PE. Based on all the relevant pieces of evidence, F appears to be a meaningful risk factor for PE. Our study results show that there is a strong association between the maternal serum F levels and PE ($p<0.05$). Based on our findings in the Kolar population, a maternal serum F level of ≥1.8±0.6 mg/L can be considered to be a diagnostic biomarker for predicting PE and the related pregnancy

outcomes of IUGR, preterm delivery, intra-uterine deaths (IUD), anemia, hypothyroidism, PE in a second pregnancy, and twin pregnancies.

7.5 CONCLUSION

The finding, of the present study especially in the F endemic areas like Kolar, in the southeastern part of Karnataka, there was a strong association between the presence of a maternal serum F level of ≥1.8 mg/L and PE suggests that high maternal serum F levels may cause PE. We conclude that an increased serum F level is an added risk factor for development of PE and suggests that the estimation of the maternal serum F levels can be used as a diagnostic marker for predicting of development of PE. We recommend that the maternal serum F level may be introduced as a routine laboratory/clinical test in areas which are endemic for fluorosis or when there are indications that the fluoride intake may be high.

CHAPTER –III

HISTOPATHOLOGY OF PRE-ECLAMPSIA

8. REVIEW OF THE LITERATURE

The placenta is an essential organ that helps in the survival of the fetus. [174] It is the most authentic evidence of fetal health. The placenta records the anatomical to pathological structural events that occur during gestation. [19] It has induced immeasurable curiosity among obstetricians and pathologists, as there is a paucity of data to appreciate the "exclusively anatomical condition" of this complex organ in PE pregnancy. [20]

8.1 PATHOPHYSIOLOGY OF PRE-ECLAMPSIA

PE is a multisystemic pregnancy related hypertensive syndrome that involves improper placental implantation, and vascular endothelial dysfunction. Hence PE is called a disease of theories. The pathogenesis of PE is indecisive and imprecise.[39] To understand the pathophysiology of PE, it was assumed to be a two stage disease: Early and Late. The early-stage-I: decrease in cytotrophoblastic invasion of uterine spiral arterioles which leads to utero-placental vascular insufficiency. Late-stage-II: Due to the delicate placenta there will be an imbalance in soluble angiogenic factors that leads to systemic endothelial dysfunction and clinical outcomes of PE. [175] Early-onset PE which occurs before 34 weeks of gestation is related to fetal and maternal consequences like improper implantation of placenta categorized by placental lesions and diminished fetal development, whereas late-onset PE occurs after 34 weeks and much related to maternal factors like obesity, metabolic syndrome, dyslipidemia but not appreciated with fetal development.[176] In normal pregnancy during the implantation process the trophoblast enters the uterus and encourages spiral arteriole remodeling. This process assists in accommodating proper nourishment by increasing blood flow to the developing fetus. But in PE, the placenta is not properly implanted which leads to poor placental perfusion. This process leads to the failure of

spiral arteriole remodeling and leads to hypoxic conditions in the placenta, oxidative stress, and further results in reduction of blood flow and affects the growth of developing fetus. [22]

8.2 HISTOPATHOLOGICAL CHANGES IN PLACENTAE OF PRE-ECLAMPSIA

The placenta has more impact on the origin of PE. The placenta is a villous structure and helps in nourishment to fetus by a rich source of the vasculature. In PE decidual vessels are invaded by endovascular trophoblasts further leading to placental ischemia, which is the basis for placental toxemia. The elementary changes in placental anatomy significantly affect the physiological functions of placentae which is evident pathologically like placental infarcts, syncytial knots, and acute atherosclerosis which can be observed histopathologically.[177,13] In the microscopic examination, common and significant features in PE placentae are distal villous hypoplasia, villous necrosis, fibrin deposition, and decidual arterial hypertrophy. [178] The gross placental changes like placental calcification, infarction, fibrin deposition, retroplacental haemorrhages are observed in PE placentae.

8.3 MATERIALS and METHODS:

Immediately after delivery placentae were collected in labor room and observed for gross features.

8.3.1 Gross Examination of maternal side of placenta: For gross lesions as abnormal areas were identified as placental infracts, retro-placental hematoma, and placental weight.

i. Placental Infracts- Firm and collapsed white or yellow colour. It represents dead villous tissue. Due to compromised intervillous (maternal) circulation the parenchymal infarcts are identified away from the placental margins and particularly when they are randomly distributed[180]. Most placental infarcts (Fig.13,14,16,17,18) are due to thrombotic occlusion of the maternal arteries, retroplacental hematoma, abnormalities of spiral arteries, IUGR, fetal hypoxia and fetal death. About 25% of normal placentas contain infarcts involving less than 5% of the placental parenchyma is common in the placentae. [181]

ii.Retro placental hematoma- A hematoma behind placenta. It is adherent to the maternal surface [180].They are usually soft, red and can be easily separated from the maternal placental surface (Fig.13,16). They can be associated with abruption placentae, pre-eclampsia, preterm delivery and placentae with large areas of infarction.[181]

iii.Calcification: Calcification is evident for placental degeneration at term (Fig.19) [13]. Histologically it is seen as basophilic material on H & E. Grossly identifiable calcification is seen in 14-37% of placentae at term .These appear as small, firm to hard, scattered, whitish plaques on the maternal surface and give a gritty feel while sectioning. [181]

The PE placentae also showed even irregular shape with irregular cotyledons on maternal surface (Fig:15).

Figure 13 to 16: with scale and green background images of PE placenta along with biopsy numbers and PE placentae with infarcts, hemorrhage & retro-placental hematoma

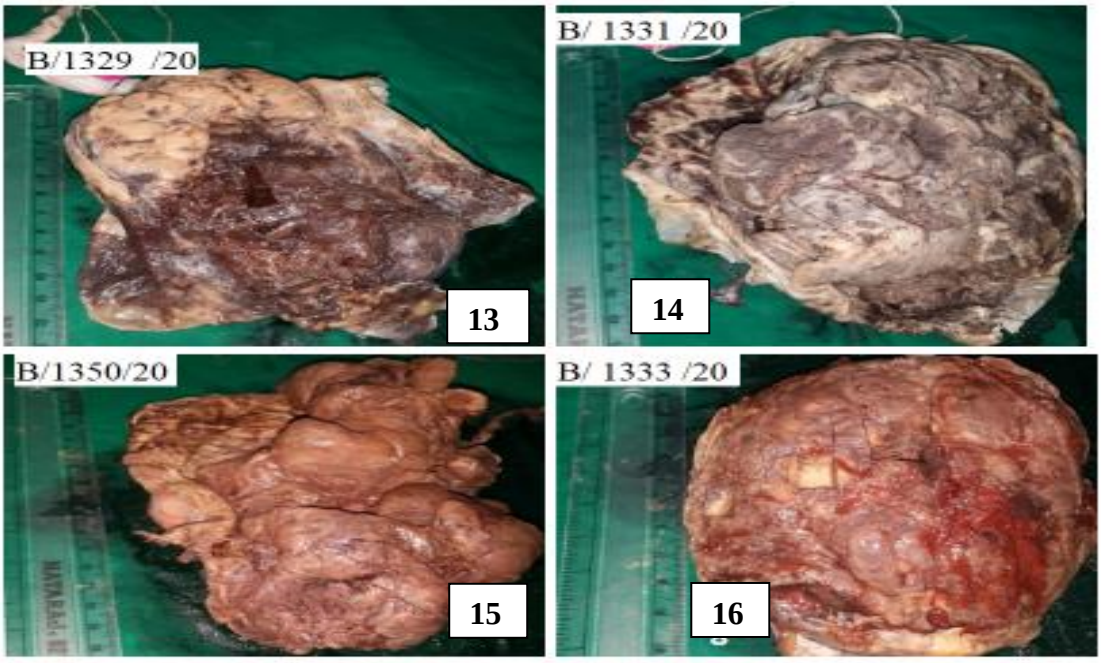


Figure 17 and 18: PE placenta with placental infracts

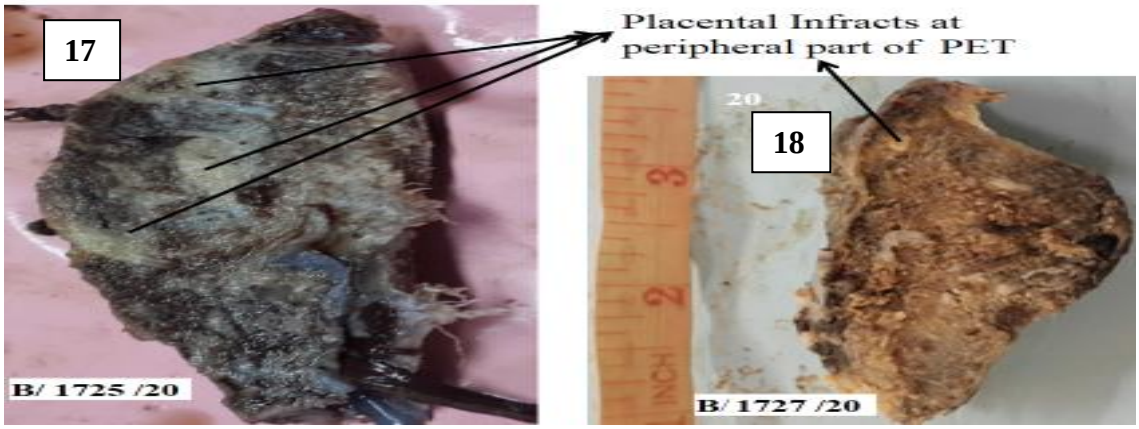


Figure 19: PE placenta with placental infracts and calcification
Calcification

Placental Infracts

8.3.2 Histopathological Examination of Placentae: After gross examination of the placenta, they were fixed in 10% formalin. After fixation paraffin blocks were prepared and sections of 5 microns were taken by rotary microtome (Leica). The placental tissue bits were processed routinely, sections were taken and stained with Haematoxylin & Eosin (H and E). [178] Tissue bits from placenta were divided into peripheral & central part. The peripheral normal areas were, labelled as Preeclamptic Peripheral A PE P A and abnormal peripheral areas were labelled as PE P C and central normal area of the placenta was labelled as Preeclamptic Center B PE C B and abnormal areas as PE C D in. Whereas in controls the tissues were taken from periphery normal area as Control Peripheral part A C P A, Control Peripheral abnormal area C C P C, Control Center normal area part B as C C B and Center abnormal area D C C D . The following parameters were considered for grading (Table 14).Tissue bits from the placenta were divided into peripheral and central part as follows:

In Preeclamptic cases:

Pre-eclamptic Peripheral Normal area PE P A,
Pre-eclamptic Abnormal Peripheral area PE P C
Pre-eclamptic Central Normal area PE C B,
Pre-eclamptic Abnormal Centre area PE C D

In Controls:

Control Periphery Normal area C P A,
Control Peripheral Abnormal area C P C,
Control Centre Normal area C C B,
Control Centre Abnormal area C C D

There is no specific gold standard for histopathological classification for placental lesions. But the placental lesions were explained in Amsterdam classification.[179] They are (Table17).

Distal Villous Hypoplasia (DVH), Hypervascularity (HYV),
Mature Villi (MV),
Immature Villi (IV),
Syncytial Knots (SK),
Necrosis (N)
Crowding of Villi (CV)
Avascular Villi (AV)

Table 14: Shows the method of grading under different Parameters

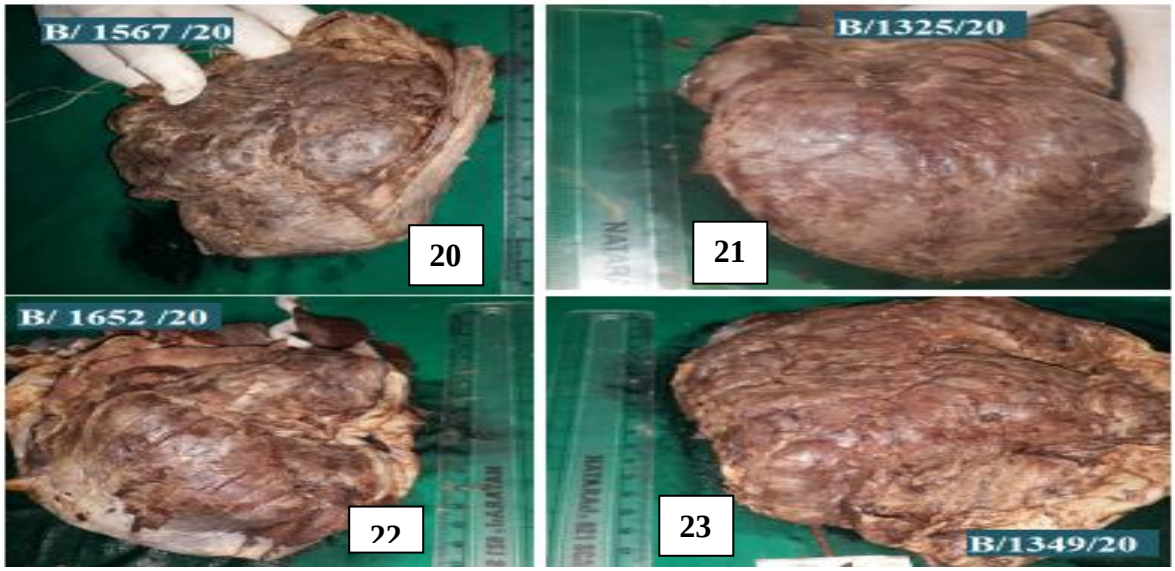
GRADE	DVH	HYV	MV	IV	SK	N	CV	AV
1	< 20%	< 10%	< 20%	< 20%	< 20%	<5%	P	P
2	20-50%	10-40%	20-50%	20-50%	20-50%	6-50%	A	A
3	>50%	>40%	>50%	>50%	>50%	> 50%		

DVH, HYV, MV, IV, SK, N grading was followed but for CV and AV present & absent was observed. P-Present , A-Absent.

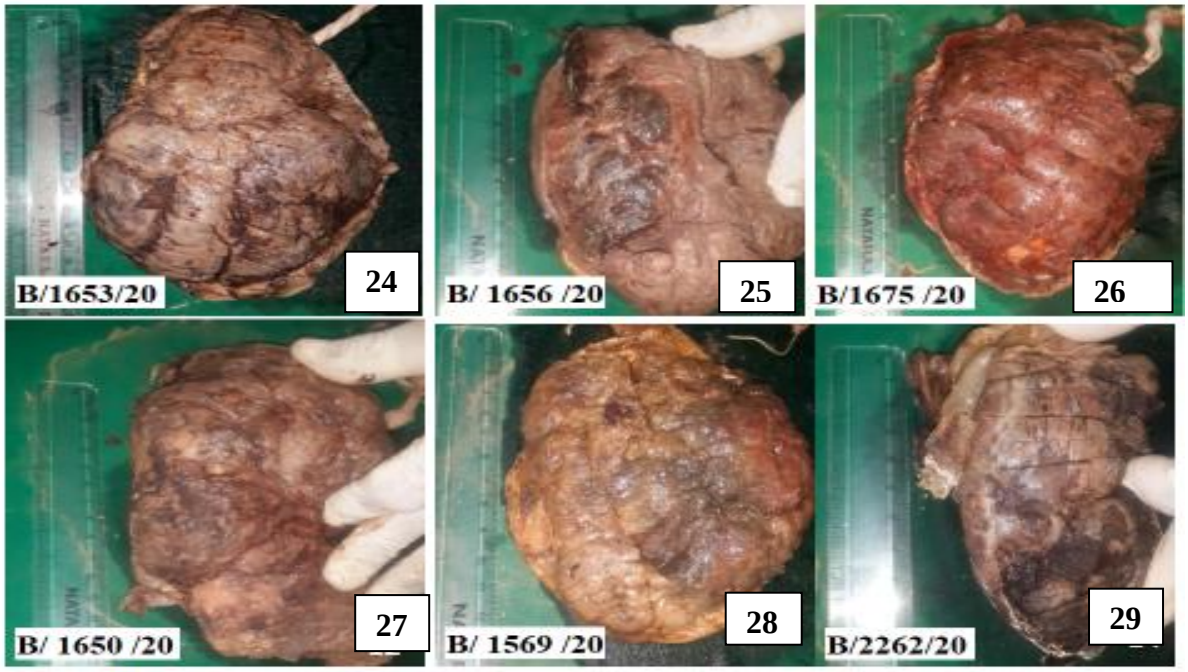
8.4 RESULTS HISTOPATHOLOGY OF PLACENTA:

8.4.1 The gross abnormal areas of PE placenta: The gross abnormal placental areas are: placental infarcts, retro-placental hematoma, and placental weight. The placental weight is more significant in group-I (PE) compared to group-II (controls). In early gestational weeks: placental weight is less than 350 gms. In late gestational weeks of PE 375 gms. In healthy controls 475 gms was observed. Out of 150 PE/ group-I, around 29%, 44 PE pregnant women were with the normal range of F levels. Out of 44 PE subjects 6 were below 1 the placentae of PE women (Fig: 20 to 23). The PE women whose serum F levels with median value below 1.0 - 1.2 (Inter quartile range) in F endemic areas are at safe zone. The 21 PE subjects were 1.0 to 1.2 and 17 subjects were 1.3 to 1.7 (Fig: 24 to 29).These placentae were evident with infarcts, hemorrhage and retro-placental hematoma. The placentae of PE women with maternal serum F levels are high with mean value ≥ 2 (Fig: 30 to 33) .The placentae of PE women with maternal serum F levels are ≥1.8± 0.6 in F endemic areas the women will be prone to developing PE and related complications and placentae showed significant infarcts, hemorrhage and retro-placental hematoma, calcification (Fig:30 to 34).

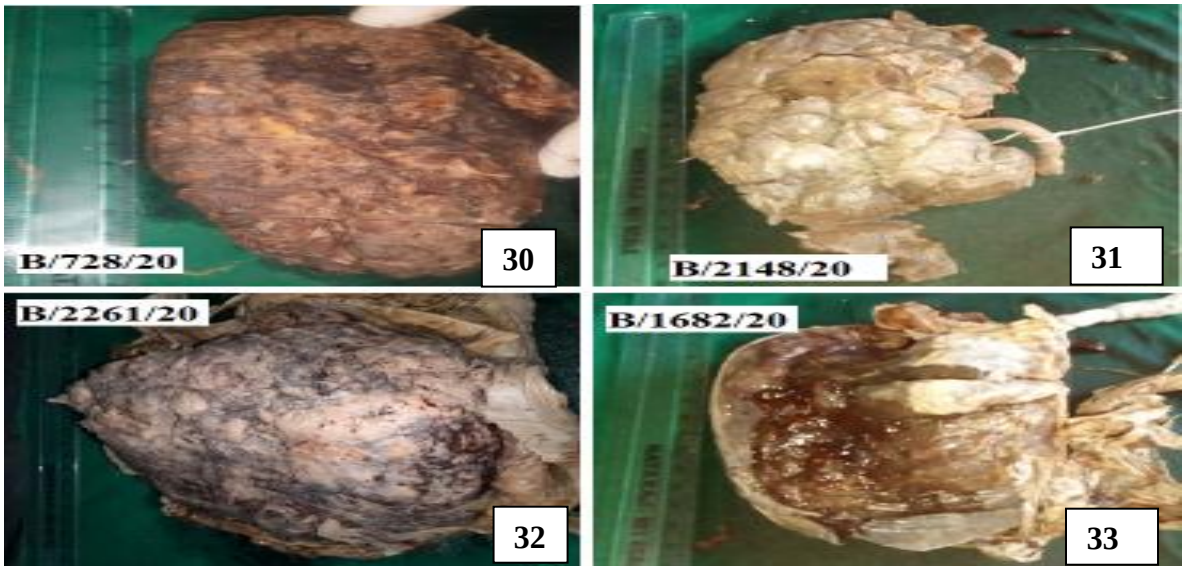
Figure 20 to 23: Placentae of normal serum F levels in PE group-I with incomplete cotyledons and shape.

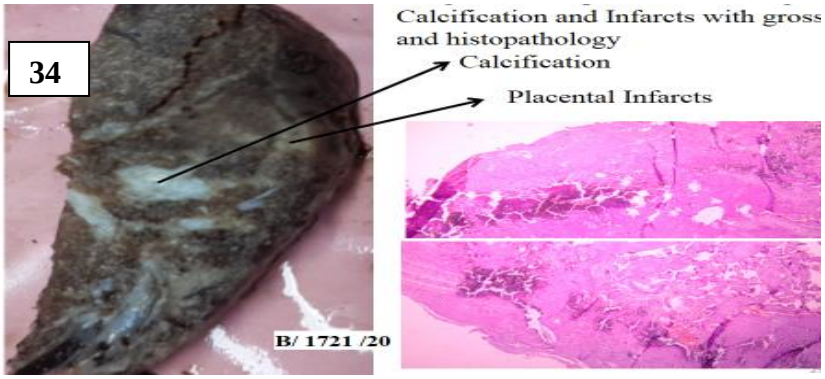


Figures 24 to 29: PE Placentae with serum F levels between 1 to 1.8 with infarcts, hemorrhage and retro-placental hematoma.



Figures 30 to 34: PE Placentae with high serum F levels (more than 2ppm) with infarcts, hemorrhage and Retro-placental Hematoma, calcification





8.4.2 Microscopy of PE: The placental tissue from the periphery and center of placenta was graded. The periphery &center part of placenta from cases & controls were compared by Mann Whitney U test. The Distal Villous Hypoplasia (DVH), Hypervascularity (HYV), Matured Villi (MV),Immature Villi (IV) were significant when compared with cases & controls as P value = 0.0001 but the Syncytial Knots(SK) and Necrosis (N) at center B of PE & SK at Periphery C of PE were not statistically significant (Table15).

DVH: Present study states that the DVH is statistically significant in group I than group II (Fig.35 to 37 and 61, 62).

HYV: Chorangiosis is showed in images 22 to 27 HYV is statistically significant in group I than group II (Fig:54 to59 and 63,64).

MV: The MV of villi was significant changes were observed at peripheral part to central part of placentae it is significant in PE group -I especiaaly the maturity is not completed in the villi (Fig: 65 and 66).

IV: The IV were significant when compared between group I and group II as P value = 0.0001(Fig: 67 and 68).

SK: The SK was significant in groupI when compared to group II except the SK at centre B & SK at Periphery C were not statistically significant when compared with groupII (Fig:44 to 46 and 69, 70).

N: The peripheral part of PE placenta showed more N and fibrin deposition also (Fig: 47 to 50 and 67,68).

CV and AV: CV in PE were 61% and in controls 21% was observed (Fig: 51 to 58 and 73,74). CV were statistically significant in group I than in group II. The AV (Fig: 59, 60 and 75,76) in group I AV were 45.2% and in group II 16.8%.

These grading and the histopathological parameters were validated by externals also the validation report attached in annexure and the differences in present validation and external validation was clearly explained (Table 16).

Table.15 : Shows Student Paired T Test Results of placental tissue from Periphery A & C of Cases & Controls, Center B& D for Cases & Controls with their P values

Sl.No.	DVH	N	HYV	MV	IV	SK
A	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
C	0.0001	0.0001	0.0001	0.0009	0.0001	**0.0603**
B	0.0478	**0.9074**	0.0012	0.0001	0.0001	**0.6763**
D	0.0001	0.0001	0.0001	0.0001	0.0001	0.0006

The parameters expect SK, FN (Centre B) & SK (Periphery C) bolded were not significant and all other parameters were highly statistically significant. As P value >0.05 is statistically significant. This change is measured to be enormously statistically significant as P=0.000

Table 16: Comparison of External Validation Observations and Previous Observations

Sl NO	Grading Criterias	New Findings by external validation	Comparison of External & Previous Observations (n=300 cases)	Percentage of matching to the criteria
1	DVH grading	No change	281 cases matched	94% (93.6%)
2	Avascular villi	In IUD cases the avascular villi were more evident	286	95.3%
3	Crowding of villi	No change	287	96% (95.6%)
4	Hypervascularity	No change	285	95%
5	Matured & immature Villi	Delayed in maturation, Decidual vasculopathy, Infarctions, Hematoma was observed in PE cases	289	96.3%
6	Necrosis	Previously observed is fibrosis & necrosis. Suggested is necrosis will be observed in PE cases. So modified accordingly	280	93.3%
7	Syncytial knots	No change	292	97.3%

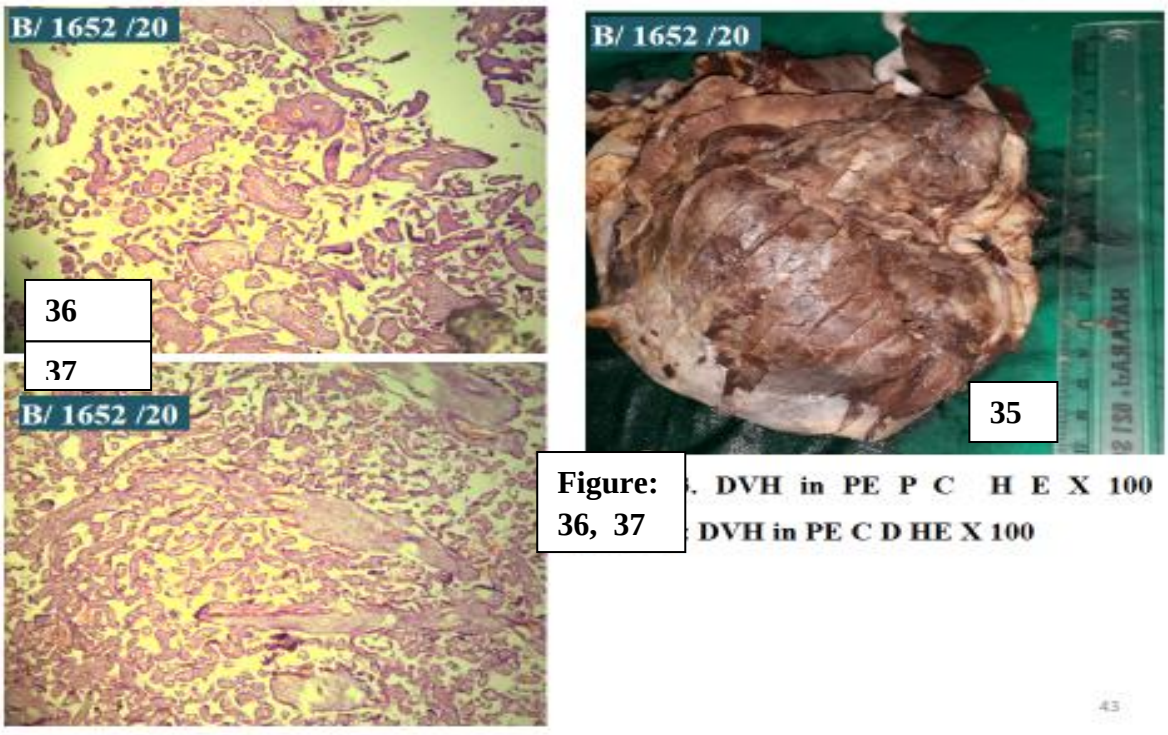


Figure: 36, 37

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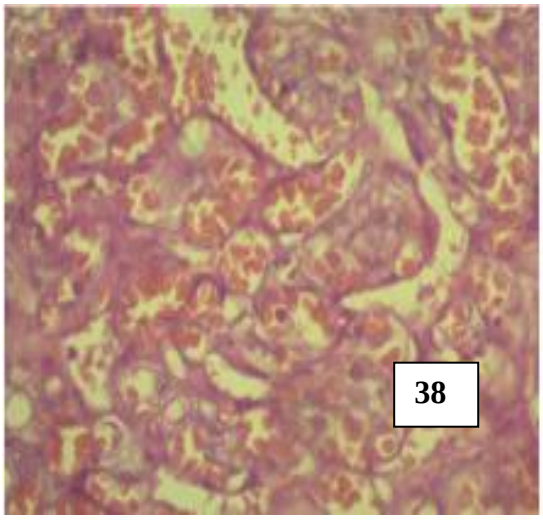
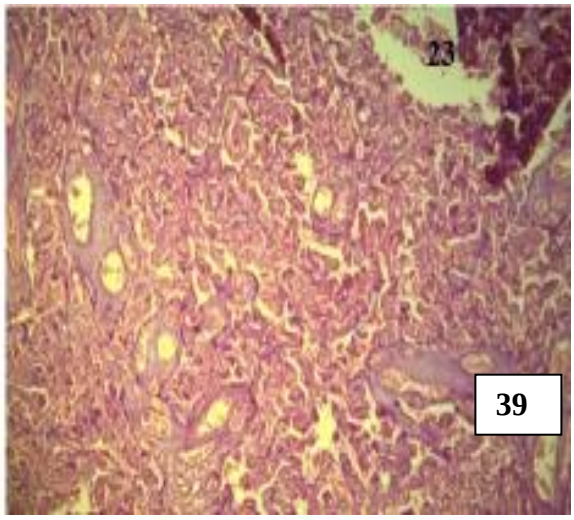
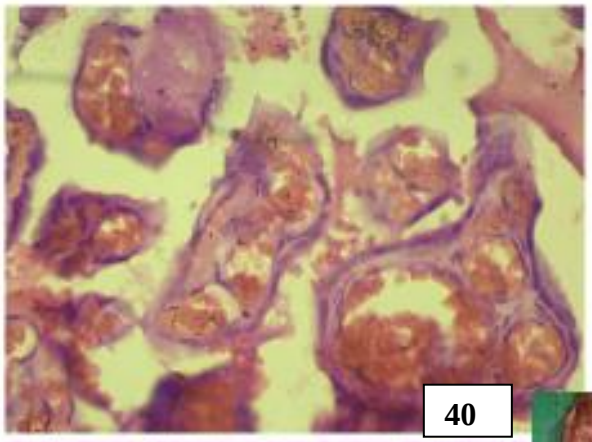
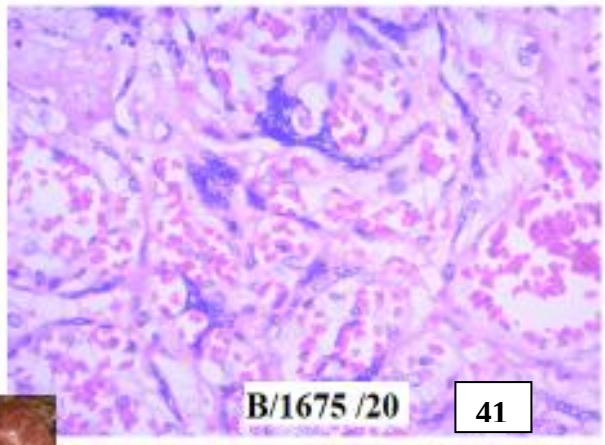
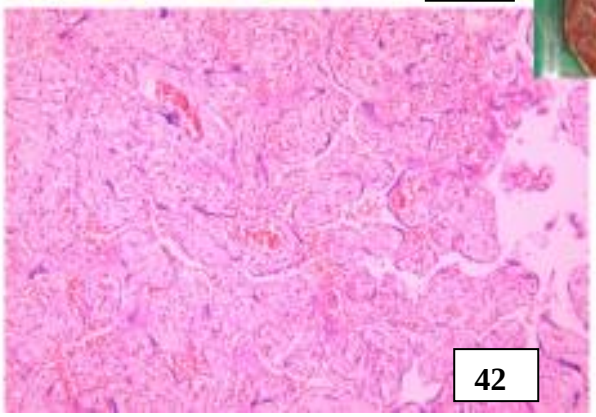
Figure 38 to 43: HYV H & E 400X, 100X

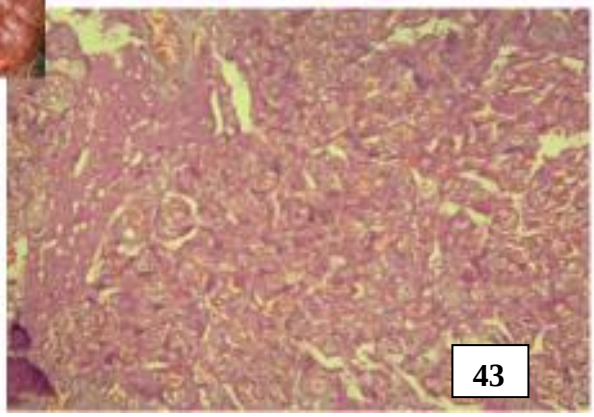


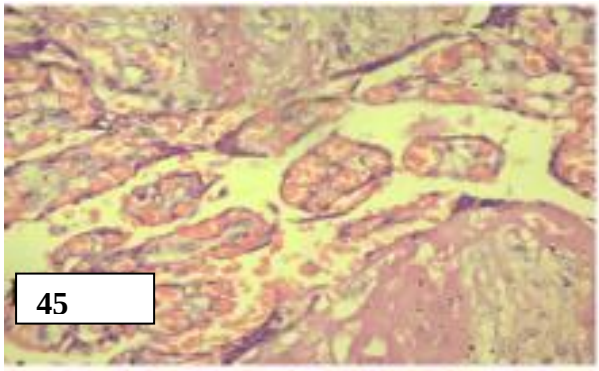












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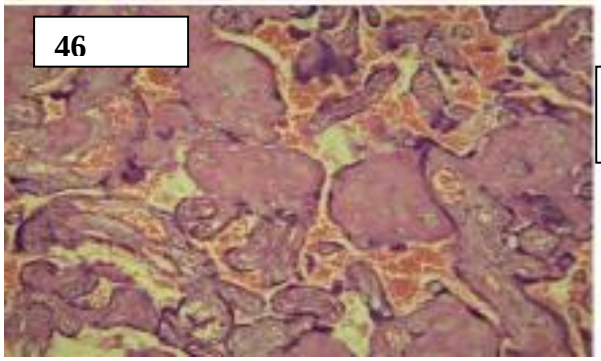

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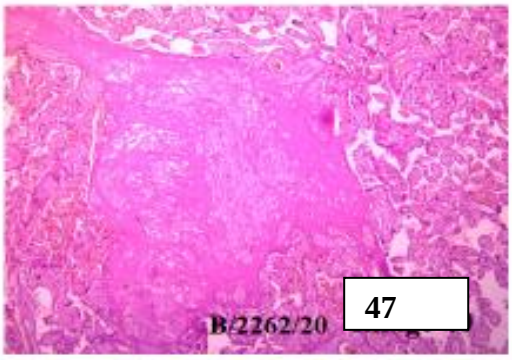
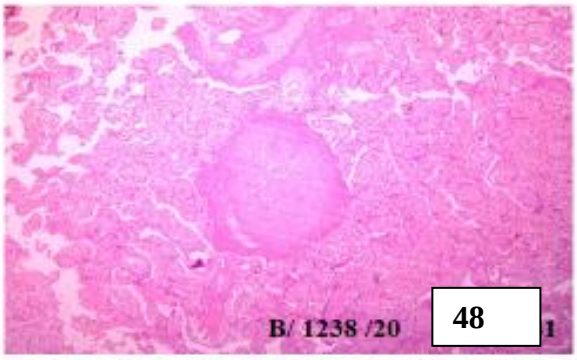
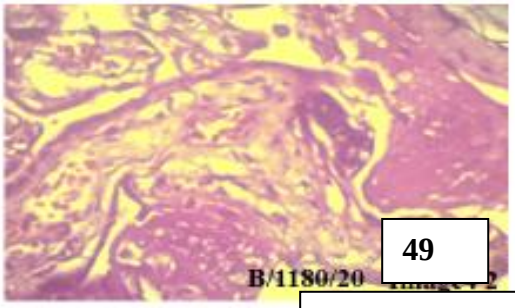
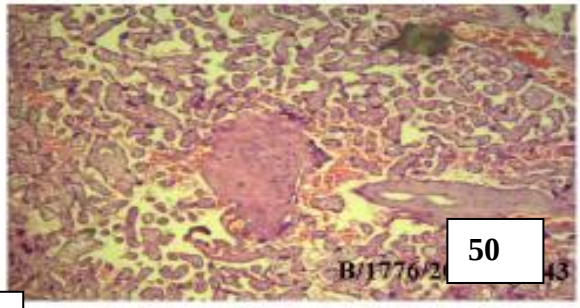
Figure 45,46: SK in PE P A HE X 400
SK in PE C B HE X 400





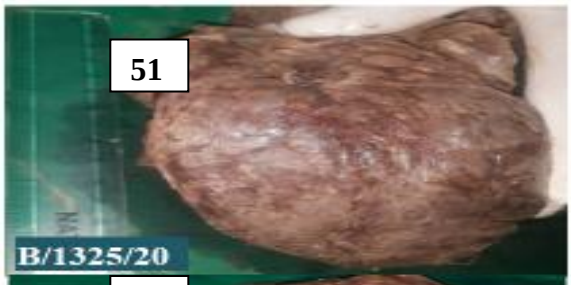




N in PE P A HE X 100
N in PE P A HE X 400

Figure: 47, 48, 49, 50

N in PE P C HE X100
N in PE C D HE X 100



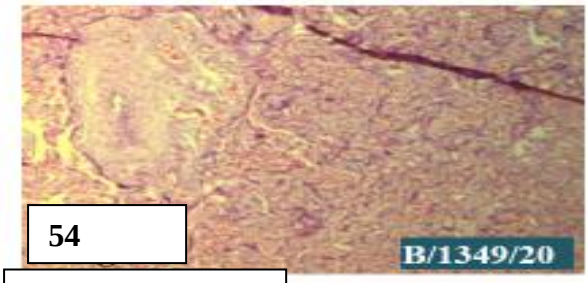
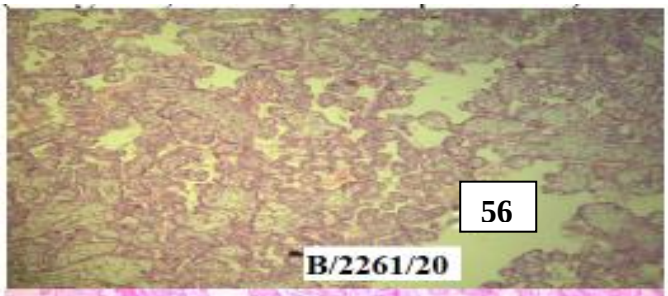


Figure 52 & 54 Crowding of Villi in HE X100



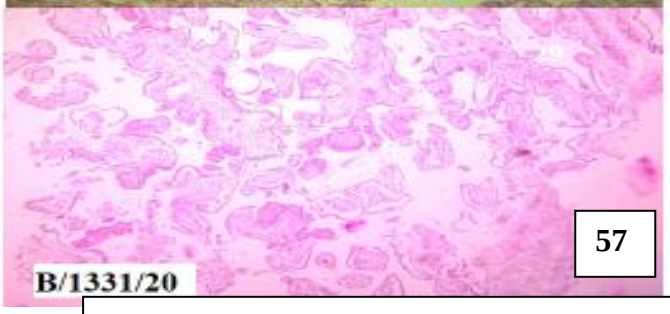
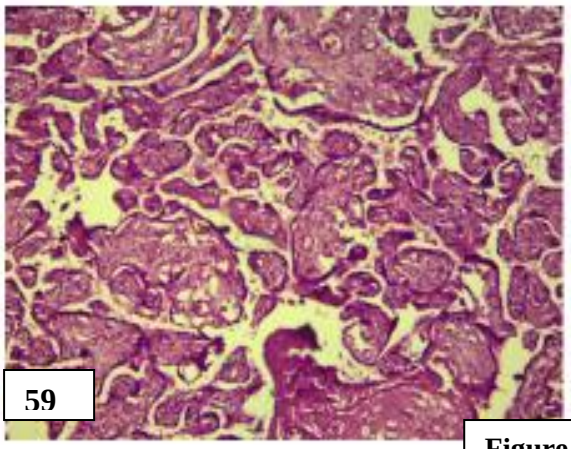
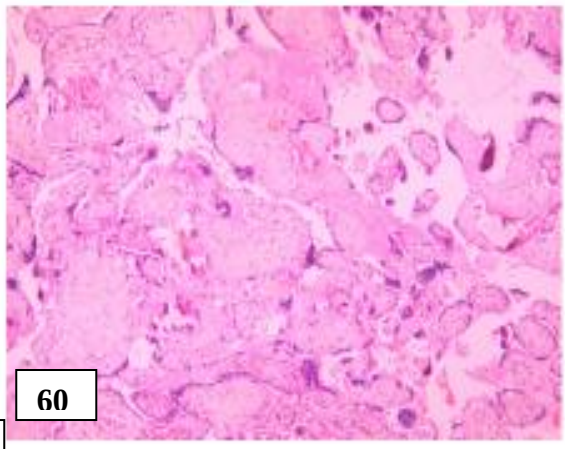


Figure 56 and 57: Crowding of Villi H E X 100





Avascular Villi in PE PA 400X H&E Figure :59, 60 Avascular Villi in PE PA 400X H&E

8.5 DISCUSSION

It is known fact that pathophysiology of PE is still unclear. As such no gold standard methods have been adopted to follow in PE but in 2014 Amsterdam Placental Workshop Group criteria a classification was initiated. Based on that classification it was grouped as placental vascular process, placental inflammatory-immune processes, and other placental processes. A few of them were considered in this review.[179]On gross examination loss of placental weight indicates presence of red infarcts. Microscopic examination reveals distal villous hypoplasia, villous necrosis, and decidual arterial hypertrophy which are common and significant findings especially in PE placentae.[178] On pregnancy consequences in relation to placental histopathology in PE women a review for 10 years was conducted. In that study, they selected the PE women who had first-time PE and repeated PE in their second pregnancy. The maternal vascular malperfusion lesions, placental weight, neonatal outcomes were compared. These features are more significant in repeated (second) PE pregnancy than the first pregnancy with PE.[179] In a case-control study on Babylon pregnant women, studied on morphological and histopathological features of placentae, the morphological features include placental weight and measurements of placentae (shape, size, length, etc.) which are significant when compared to normal women. The histopathological features like the number of syncytial knots, trophoblastic basement membrane thickening, cytotrophoblastic cell proliferation, areas of fibrinoid necrosis, hyalinisation, calcification, and areas of infarction were significant in PE.[182] There are a lot of differences in data because different parameters were followed, as well as the study design and methodology adopted, inclusion and exclusion criteria followed in their studies. By comparing with blinding and unblinding studies there is inconsistency in results. In an unblinded review, the villous lesions were 11.6% and 48.2% in normal and PE pregnancies respectively with an odds ratio (OR) of 7.59. In blinded studies, the villous lesions

observed were 18.5% and 42.0% in normal and PE pregnancies respectively with an OR of 4.28. In blinded studies, the incidence of both placental villous and vascular histopathological lesions was higher in PE than in normal pregnancies. Greater differences are reported in unblinded studies. Despite the higher probability (point prevalence) of finding abnormal placental pathology in pregnancies with PE, placental lesions are not specific to the diagnosis of PE.[183] In a single tertiary care hospital study including 10 years review performed by Bustan-Nahumson et al., on PE subjects by observing the maternal age groups along with maternal characteristics, fetal outcomes, and placental histopathology like syncytial knots, placental infracts, and calcification. Based on the maternal age the subjects were grouped into 3: group 1: < 27 years; group 2: 27-35 years; and group 3: >35 years. In the late-onset of PE that is >35 years of maternal age significant changes were observed in histopathology of placentae.[184] The histopathological assessment of placentae like infarcted areas, calcified areas, and marginal insertion of the umbilical cord in the PE women show a significant increase in value ($p>0.01$) The morphology of placentae also shows significant changes like smaller in size, weight, volume, area, thickness, diameter, circumference and fetoplacental ratio than normal placentae.[185] Based on mild or severe PE, ischemic placentae had shown significant changes in the degree of placental infarction which is inversely proportional to fetal birth weight. But with other parameters like placental calcifications, stromal oedema, stromal fibrosis, and syncytial knots were not statistically significant.[186] A original study performed in Japan by Tataeishu et al., on the pathological changes in the 107 placentae of early and late-onset PE. The observations include that hypoxic placentae show infarctions at different areas in placentae with an increase in DVH and SK.[187]

8.5.1 DVH: A study conducted by Tateishi et al., Gunasena et al., Stark et al., mentioned that in early-onset PE there was evidence of increase of distal villous hypoplasia especially in placental hypoxic conditions.[187,188,189] Stark et al., in their study observed that placental weight and DVH are inversely proportional to each other.[189] Decidual arteriopathy results in a reduction of the blood flow to the placental villus that in turn leads to distal villous hypoplasia.[178]

8.5.2 N: Kambale et al, Sahay et al., Nahar et al., Shams et al studied fibrinoid necrosis in PE placentae they found 47% to 80% necrosis is highly significant in PE mothers.[190,191,192,193] In the present study also we found N is significant in PE placentae than normotensive.

8.5.3 HYV: Kiran et al studied HYV in anaemic PE women. The study revealed that HYV is prominent with increased incidence of anaemia. [194] Chauhan et al., in his review revealed that 3% of HYV is evident in PE women. [195] Srinivasan et al., observed that in terminal villi HYV is more significant which matches with the present study.[196]

8.5.4 MV: Jaiman et al., and Ezeigwe et al., in their observations mentioned that accelerated villous maturation was significant in PE and especially in IUD cases and in severe PE. [186,197] A twin study piloted by Jaiman et al., observed that there was defective villous maturation due to hypoxia.[198] Morgan et al., in their study observed in terminal villi there was significant hyper maturity.[199] Egbor et al., in their study noticed about villous vasculature and morphology are significantly changed in the early onset PE. But in terminal villi no significant changes were noticed in early onset. In late onset PE villous vasculature is not at all affected. But in late onset PE complication is of fetal growth retardation were noticed that in intermediate and terminal villi more significant changes in villous vasculature was observed.[200] Schweikhart et al., in their study observed 33% of villous mal-development and in PE 60% of villi were evident with hyper-maturity.[201]

8.5.5 IV: Jaiman et al.,, Wang et al., Stoz et al., in that study observed that retarded maturation of villi is significant in PE.[197.202.203] Jaiman et al.,, also observed IV was 44% prominent in fetal death.[197]

8.5.6 SK: Ezeigwe et al., observed there is no significant difference in syncytial knots.[186] Gunasena et al, and Morgan et al., and Rogers et al., observed that there was significant increase in SK of PE placentae.[188,199,204] Tateishi et al., & Salam et al., in their interpretations that the early PE is associated with more aggressive histological changes and increased SK reflecting placental ischemia in early onset PE.[187,204,205] Stark et al., piloted a study and his research work revealed that there is an inversely proportion to placental weight and SK which was more evident in early-onset PE.[189] Increased syncytial knots are formed by an imbalance between the production and shedding of villous syncytiotrophoblast in PE placentae. Increased SK with increasing gestational age assist in evaluating villous maturity.[178]

8.5.6 AV: Kaur et al .,in their study observed significant avascular villi in PE.[206] Chauhan et al., in their findings mentioned 6% of AV villi were observed in PE placentae.[195] Mehendale et al., in their work found the reason for AV was due to placental ischemia. [207]

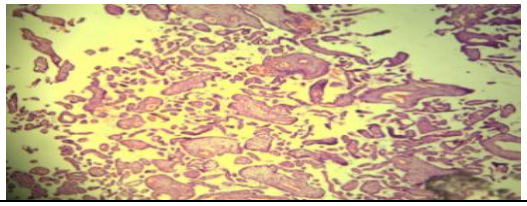
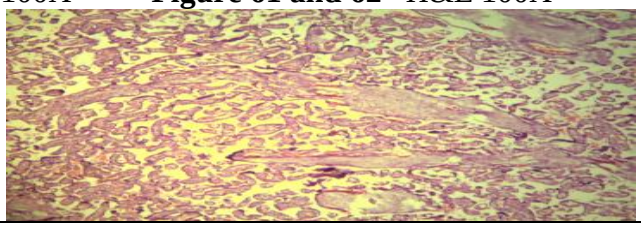
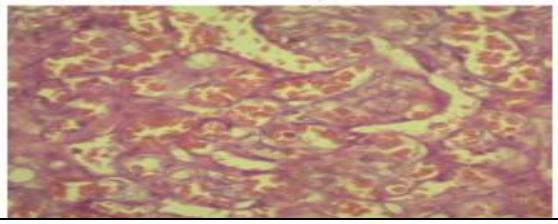
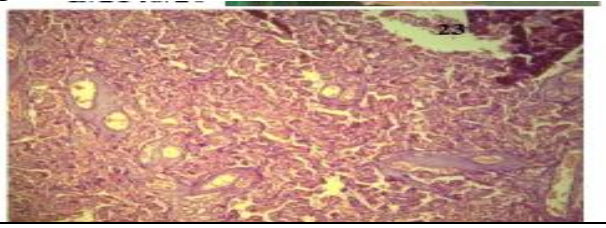
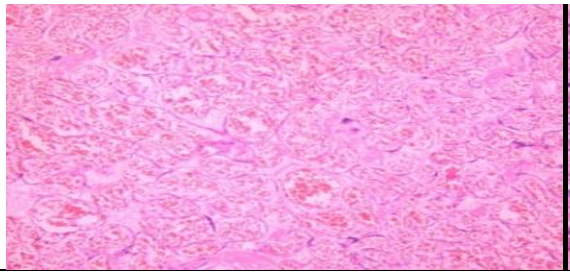
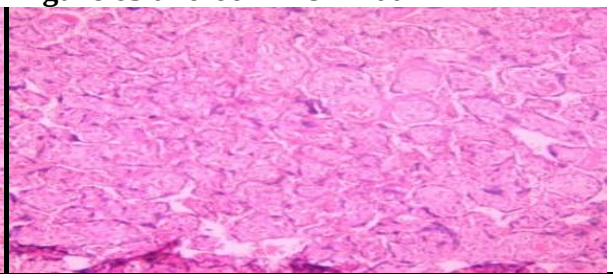
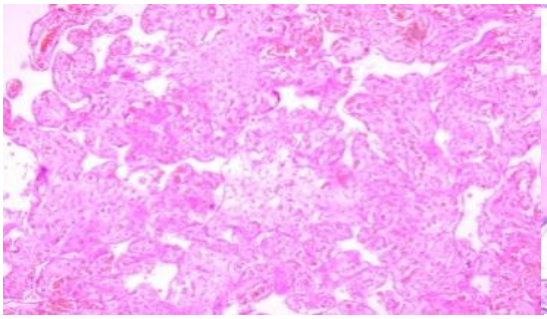
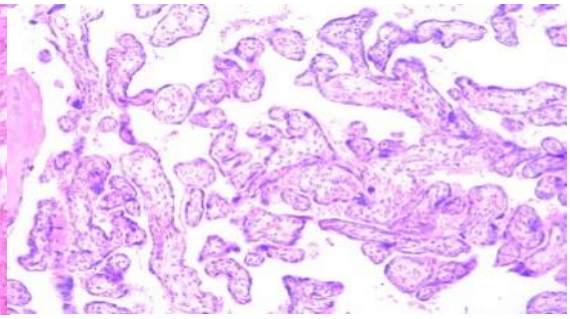
8.5.7 CONCLUSION: The PE placentae has shiwed significant changes with infracts, calcification, retroplacental hematoma .Even the histopathological changes in DVH, HYV, CV, and AV are significant at peripheral part of placenta.The placentae of PE women with maternal serum F levels are ≥1.8± 0.6 in F endemic areas the women will be prone to developing PE and related complications.Those placentae with normal and high serumF levels showed significant changes.

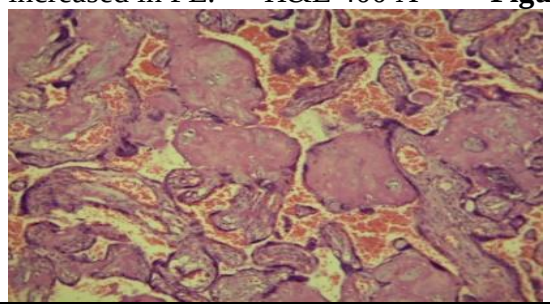
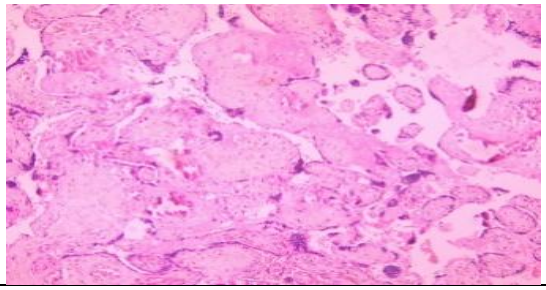
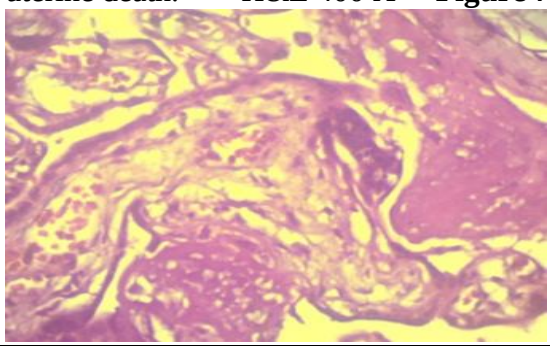
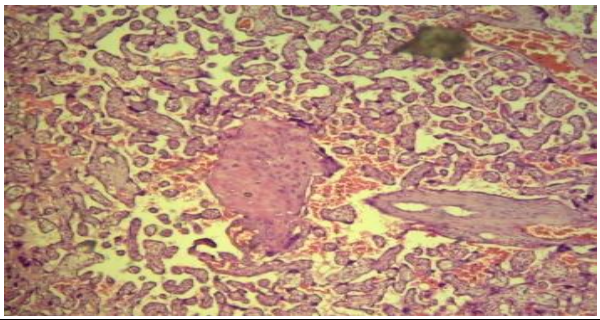
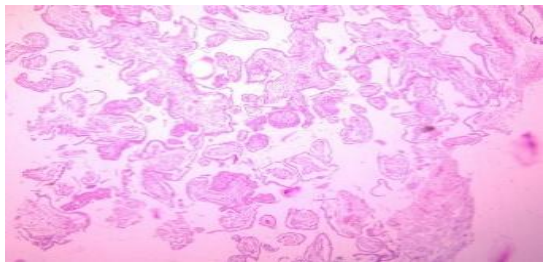
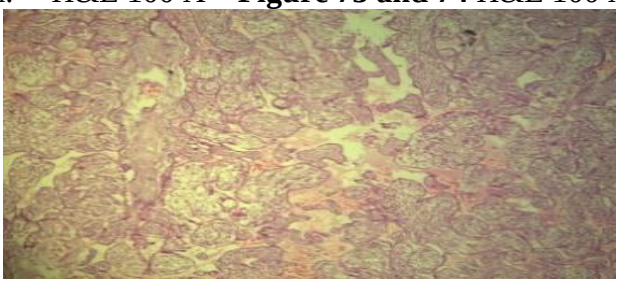
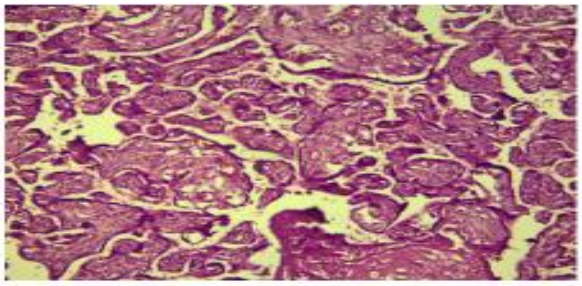
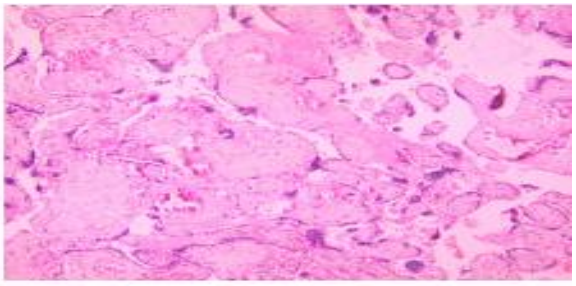
8.6 LIMITATIONS OF THE STUDY

Study was limited to Kolar F effected area only.

The screening of markers was estimated in early < 34 weeks and late ≥ 34 gestational weeks.

Table 17: Microscopic observations of PE, with definition and characteristic features

Sl. No.	Microscopic Observations of Placentae with specific definition & characteristic features
1	**Distal Villous Hypoplasia (DVH)**:A thin & broadly spaced out small distal villi. [208] Characterized by sparse, poorly developed distal villous tree with abnormally shaped, elongated, slender villi and widening of the intervillous space indicate that there is obstruction of decidual vessels.[209] H&E 100X **Figure 61 and 62** H&E 100X  
2	**Hypervascularity (HYV)** : HYV/ Chorangiosis is defined as villous hyper vascularity. In the terminal villi, excessive number of capillaries with intact basement membrane is observed. Altshuler defined as more than 10 capillaries per villous in 10 medium power fields in at least 3 non infarcted area..[210] H& E 400 X **Figure 63 and 64** H&E 100 X  
3	**Mature Villi (MV) :** MV are long, slender villi 80-150 μm in diameter with loose stroma, capillaries, and small vessels that can be differentiated from mid-gestation from peripheral ramifications of stem villi. [211] H&E 100 X **Figure 65 and 66** H&E 100 X  
4	**Immature Villi (IV)**: IV shows characteristic features like reticular arrangement of stroma with Hofbauer cells, arterioles, venules, and capillaries in the villous stroma are very small. The cytotrophoblastic layer is discontinuous whereas the outer syncytiotrophoblastic layer remains thick. [211] H&E 100 X **Figure 67 , 68** H&E 100 X  

5	**Syncytial Knots (SK):** Poorly developed villous tree, and widening of the intervillous space, evidence for poor syncytiotrophoblast development.So syncytial knots are generally increased in PE. [212] H&E 400 X **Figure 69 and 70** H&E 400 X  
6	**Necrosis (N):** Normally 3% of mature villi in placentae show N.More than 3% of the villi having N is seen in complicated pregnancies like PE. [209]Among Intermediate villi - mature & immature villi and stem villi the N is more evident.Small infarcts are insignificant.[213] N more than 10 to 15% of the placental parenchyma is associated with intra uterine death. [182] H&E 400 X **Figure 71 and 72** H&E 100 X  
7	**Crowding of villi (CV):** The CV is increased branching of villi that result in increased surface area for exchange. The increasing branching morphogenesis was observed in CV which is an indicator for hypoxic condition.[214] H&E 100 X **Figure 73 and 74** H&E 100 X  
8	**Avascular Villi (AV):** The AV shows following characteristics: 2.5% or more of parenchyma affected, Lesion measuring 0.25 cm - 2 in multiple sections, or single foci. AV was observed in (IUGR), acute and chronic abnormalities, oligohydramnios, and maternal coagulation disorders. [215] H&E 400 X **Figure 75 and 76** H&E 400 X  

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Sl. No.	PE Hosp No	Biopsy No	Age	Place	Mari Life	Cong	Sys BP	Dia BP	Bd grp	Gravida	Weeks	Gender of Fetus	Baby Wt	Type of Delivery	Others
1	457259	B/ 1319 /20	23	Srinivasapura	1	NCM	170	110	O+	Primi	39	M	2.8	LSCS	Mild Polyhro amniotis
2	457450	B/ 1320 /20	32	Mulbagal	7	NCM	200	120	B+	G4P3 L1A1	34	M IUD	1	LSCS	Anemia
3	459134	B/1321/20	28	Chintamani	2	NCM	200	120	A+	Primi	36	M IUGR	1.7	Normal	
4	459436	B/1322 /20	23	Hosakote	2	CM (II)	160	90	B+	Primi	38	F IUGR	1.4	LSCS	Throm Bocyto penia, Hydro amnios
5	459227	B/1323/20	24	Bangarpet	5	CM (II)	200	120	B+	G2P1L1	33	M Preterm	2.5	LSCS	
6	462241	B/1324/20	20	Kolar	2	CM (II)	150	100	A+	Primi	36	M IUGR	2.6	Normal	
7	462618	B/1325/20	22	Mulbagal	5	NCM	140	90	B+	Primi	37	M	2.54	Normal	Infertility treatment
8	466289	B/1329 /20	26	Srinivasapura	1.5	NCM	200	120	O+	Primi	32	F Preterm	2	Normal	
9	466251	B/ 1330 /20	26	Kolar	11	NCM	200	120	B+	G2P1L1	28	F Preterm, IUGR	1.02	LSCS	Anemia
10	464296	B/ 1331 /20	26	Mulbagal	5	NCM	150	100	O+	G2P1L1	37	F IUGR	2.6	LSCS	
11	468158	B/ 1332 /20	20	Mulbagal	1	NCM	140	90	O+	Primi	34	F IUGR	2.8	LSCS	Hypo thyroidism
12	467932	B/ 1333 /20	26	Mulbagal	2	NCM	150	110	A+	G2A1	39	M IUGR	2.8	LSCS	Anemia
13	469555	B/1345/19	25	Kolar	1	NCM	200	120	B+	G2A1	31	F Preterm	1.5	Normal	

												, IUD			
14	471458	B/ 1346/20	22	Kolar	4	CM (II)	180	120	O+	G2P1	38	F IUD	2.6	Normal	Anemia
15	472285	B/ 1347 /20	26	Bangarpet	1	CM (II)	200	120	B+	Primi	30	F IUGR,FPI, Preterm	1	LSCS	
16	480619	B/ 1348/20	24	Kolar	1	NCM	160	110	O+	Primi	34	M IUGR Preterm	1.42	LSCS	
17	481593	B/1349/20	24	Kolar	1	NCM	170	100	O+	Primi	40	F	2.6	LSCS	
18	488505	B/ 1350/20	28	Mulbagal	10	NCM	180	120	O+	G4P1L1A1	30	F IUD	1	Normal	Anamolous baby
19	482065	B/1351/20	22	Chintamani	6	NCM	170	110	O+	G2P1L1	33	F Preterm	1	LSCS	
20	488544	B/ 1567 /20	26	Mulbagal	2	NCM	120	80	O+	G2P1L1	38	M IUGR	2.8	Normal	Hypothyroidism
21	489868	B/ 1568 /20	24	Chikballapur	2	NCM	170	140	A+	Primi	37	F IUGR	2.32	Normal	
22	496102	B/ 1569 /20	29	Gouribidanne	3	NCM	170	100	o+	Primi	37	M IUGR	2.1	Normal	
23	502244	B/ 1570 /20	21	Kolar	3	NCM	170	110	B+	Primi	31	F Preterm , IUD	1	Normal	
24	506433	B/1571 /20	23	Bangarpet	1	NCM	160	110	B+	Primi	34	M Preterm, IUGR	1.48	LSCS	
25	425283	B/ 1572 /20	21	Mulbagal	1	CM (II)	180	110	O+	Primi	40	F IUGR	2.8	Normal	Hypothyroidism
26	501464	B/1573/20	24	Kolar	1	CM (II)	180	120	O+	Primi	38	M	2.6	LSCS	
27	508456	B/ 1650 /20	19	Bangarpet	1	NCM	150	90	AB +	Primi	34	F Preterm ,IUGR	1.48	LSCS	Hypothyroidism
28	508767	B/ 1651 /20	30	Kolar	11	NCM	150	100	A+	G3P2L2	33	F.M Twin	2.1/1.8	LSCS	
29	512833	B/ 1652 /20	20	Chittor	3	NCM	140	90	AB +	G2 A1	41	M	3.2	Normal	Anemia
30	513722	B/ 1653/20	22	Mulbagal	1	NCM	160	110	B+	Primi	37	M IUGR	2.65	LSCS	

31	451549	B/1654 /20	22	Kolar	1	NCM	170	120	A+	Primi	38	M IUGR	2.83	LSCS	
32	516056	B/ 1655 /20	32	Kolar	17	NCM	160	100	O+	G3P2L1D1	38	F	2.08	Normal	
33	523256	B/ 1656 /20	27	Malur	4	NCM	170	100	B+	G3P2L1A1	36	F IUGR	1.96	LSCS	Anemia
34	530540	B/ 1657 /20	38	Mulbagal	20	NCM	150	90	B+	G4P2L2A1	39	M IUGR	2.1	LSCS	
35	531383	B/ 1658 /20	24	Kolar	24	CM (II)	140	90	B+	Primi	39	M IUGR	2.4	LSCS	Hypo thyroidism
36	439909	B/ 1659 /20	24	Kolar	2	NCM	130	90	B+	G2A1	38	M	2.9	Normal	
37	532273	B/ 1660 /20	28	Kolar	10	NCM	150	90	B+	G4P3L2A1	32	M Preterm, IUGR	1.5	Normal	
38	534413	B/ 1674 /20	24	Banglore	5	NCM	160	110	B+	G3P1L1A1	36	F IUD	1.73	Normal	Anemia
39	534402	B/ 1675 /20	20	Mulbagal	2	NCM	150	110	A+	G2A1	38	F IUGR	1.62	LSCS	
40	534442	B/ 1676 /20	20	Kolar	1	NCM	150	100	o-	Primi	36	F	3.48	LSCS	
41	533644	B/2262/20	20	Hosakote	3	NCM	150	120	B+	G2A1	30	F Preterm	1	LSCS	Anemia
42	536764	B/ 1677/20	25	Mulbagal	5	NCM	150	110	A+	G3P1A2	35	M IUGR	2.6	Normal	
43	538121	B/ 1678 /20	20	Kolar	1	NCM	150	90	B+	Primi	30	F Preterm	1	Normal	
44	539216	B/ 1679 /20	40	Kolar	18	CM(III)	130	90	A+	G3A2	38	M IUGR	2.3	LSCS	Hypo thyroidism
45	542061	B/ 1680 /20	22	Kolar	3	CM(III)	120	70	A+	G2P1D1	37	M	2.4	Normal	
46	542035	B/1681 /20	32	Kolar	1.5	NCM	120	70	B+	G2A1	34	M IUD	1	Normal	Anemia
47	542314	B/2145/20	34	Bangarpet	1	NCM	140	80	o-	Primi	39	M IUGR	1	Normal	
48	581651	B/728/20	29	Kolar	1	CM(II)	140	90	A-	Primi	38	M	2.9	LSCS	
49	520251	B/730/20	31	Kolar	8	NCM	140	90	O+	G2P1L1	40	M	2.5	LSCS	Infertility treatment
50	533437	B/732/20	23	Kolar	2	CM (II)	140	90	O+	G2P1A1	39	M IUGR	3.8	LSCS	

51	544023	B/2148/20	25	Bangarpet	1	NCM	160	120	B+	Primi	35	M IUGR	2.08	LSCS	Anemia
52	544706	B/734/20	24	Kolar	5	NCM	140	70	B+	G2P1L1	36	F IUGR, IUD	1.77	LSCS	
53	544156	B/736/20	27	Kolar	1	CM (II)	140	90	O+	Primi	38	F IUGR	2.085	LSCS	
54	547141	B/737/20	21	Kolar	4	NCM	150	100	B+	Primi	29	M IUD	1	Normal	
55	553030	B/739/20	20	Kolar	1.5	NCM	170	110	O+	Primi	38	F IUGR	2.8	Normal	Anemia
56	557054	B/740/20	28	Bangarpet	5	CM(III)	150	100	O+	G4P3L3	36	F IUGR	2.6	Normal	
57	557048	B/747/20	25	Gadduru	2	NCM	150	100	O+	Primi	38	M IUGR	3	LSCS	Infertility treatment
58	524850	B/749/20	20	KGF	1	NCM	180	130	A+	Primi	30	F Preterm	1	Normal	
59	557065	B/753/20	26	Kolar	5	CM (II)	140	100	B+	G3P1L1A1	38	M IUGR	2.8	LSCS	
60	557000	B/757/20	25	Kolar	3	CM (II)	140	100	A+	G2P1L1	35	F IUGR	2	LSCS	
61	553974	B/756/20	27	Chintamani	1	NCM	160	130	B+	Primi	34	M Preterm	2.1	LSCS	
62	557067	B/759/20	21	Hoskote	1	NCM	160	100	A+	Primi	35	F	1.98	LSCS	Anemia
63	557594	B/2264/20	20	Bangarpet	1	CM (II)	150	100	B+	Primi	34	M IUGR	2.54	Normal	
64	566573	B/ 1682 /20	22	Kolar	1	NCM	150	90	AB+	Primi	37	F IUGR	2.44	Normal	
65	556730	B/2149/20	24	Kolar	1	CM (II)	170	120	O-	Primi	38	M IUGR	2.5	Normal	
66	559398	B/ 1723 /20	36	KGF	1	NCM	170	110	A+	Primi	24	M IUD	1.5	Normal	Anemia
67	484027	B/ 1724 /20	24	Kolar	5	NCM	130	100	A+	G3P2L2	37	F	3	LSCS	
68	558602	B/2261/20	21	Mulbagal	1	NCM	180	140	A+	Primi	35	F	2.62	Normal	Hypo thyroidism
69	554626	B/ 1725 /20	23	Kolar	3	CM (II)	180	140	B+	G2A1	35	M IUGR	1	Normal	
70	556294	B/ 1726 /20	25	Mulbagal	2	NCM	120	70	AB+	G2A1	39	M IUGR	3.05	LSCS	

71	562006	B/ 1727 /20	20	Malur	1	CM (II)	180	120	B+	G2A1	38	M	1.6	LSCS	Anemia
72	563270	B/ 1128 /20	30	Mehaboobnagar	9	NCM	180	130	B+	G5P3L3A1	28	M Preterm	1	LSCS	
73	565773	B/ 1721 /20	26	Bangarpet	6	NCM	200	180	O+	G3P1D1A1	32	F Preterm	1.5	LSCS	
74	566552	B/ 1722 /20	26	Kuppam	2	NCM	150	100	B+	G2P1A1	39	M IUGR	2.8	Normal	
75	568894	B/1728 /20	24	Hoskote	4	NCM	130	90	B+	G2P1L1	37	M	2.6	Normal	Anemia
76	569269	B/2263/20	21	Kolar	1	NCM	170	120	B+	Primi	34	M IUD	1.89	LSCS	Anemia
77	496124	B/ 1729 /20	23	Malur	1	NCM	104	70	O+	G2A1	37	M IUGR	2.81	LSCS	
78	569652	B/2146/20	30	Kolar	10	NCM	150	90	O+	G4P1L1A2	37	F IUGR	2.47	LSCS	
79	569817	B/1730 /20	22	Bangarpet	1	CM (II)	150	90	B+	G2A1	41	F	3.15	LSCS	
80	569804	B/ 1734 /20	25	TamilNadu	1	NCM	150	70	B+	Primi	31	M	1.26	LSCS	
81	568774	B/ 1735 /20	33	Mulbagal	14	CM(III)	140	110	O+	G3P1L1A1	36	M	3.32	LSCS	
82	571156	B/ 1736 /20	26	Kolar	2	NCM	160	100	O+	Primi	30	F IUGR	1.56	LSCS	
83	571882	B/ 1737 /20	22	Chintamani	2	CM (II)	140	90	B+	Primi	40	M	3.1	LSCS	Infertility treatment
84	467875	B/1738 /20	28	Kolar	10	NCM	160	100	B +	G3P3L3	35	F	3.3	Normal	
85	573862	B/ 1739 /20	27	Bangarpet	1	NCM	140	100	B+	G2P1A1	36	M IUGR	2.5	Normal	Hypo thyroidism
86	574113	B/ 1740 /20	36	Malur	4	NCM	150	90	O+	Primi	37	M	2.5	Normal	
87	522582	B/ 1741 /20	22	Mulbagal	11	NCM	160	110	O+	G4P1L0A3	32	M	2.1	Normal	
88	582184	B/ 1742 /20	28	Kolar	7	NCM	130	80	O+	G3P2L2	40	M IUD	2.8	Normal	Thrombo cytopenia
89	532994	B/1743 /20	25	Kolar	1	NCM	140	80	B+	G3P3L3	34	M	1.5	LSCS	
90	583365	B/ 1744 /20	25	Kolar	6	NCM	180	120	B-	Primi	37	M FPI	2.08	Normal	Severe

91	584222	B/1110/20	29	Kolar	5	NCM	150	120	O+	G3P1D1	34	M IUGR	1.22	LSCS	
92	585574	B/2147/20	21	Hoskote	7	NCM	150	120	B+	G2P1L1	39	F IUGR	3	LSCS	
93	586544	B/2303/20	28	Chintamani	1	CM (II)	150	100	AB +	Primi	38	M	1.72	LSCS	
94	510751	B/1112/20	25	Mulbagal	6	NCM	170	110	O+	Primi	37	F IUGR	2.29	Normal	
95	588657	B/1114/20	22	Chintamani	7	CM (II)	180	130	O+	G3A2	33	M Preterm	1.2	LSCS	Hypo thyroidism
96	585252	B/1115/20	27	Malur	2	NCM	190	140	O+	G2P1L1	33	F IUGR, Preterm	1.2	LSCS	
97	590392	B/1116/20	19	Malur	1	NCM	120	90	O+	Primi	35	F IUGR	1.8	LSCS	
98	590557	B/1117/20	21	Hoskote	1	NCM	160	110	B+	Primi	39	M	3.6	LSCS	
99	590991	B/1118/20	22	Mulbagal	1	NCM	160	110	A+	Primi	26	F IUD	1	LSCS	
100	592122	B/1119/20	24	Kolar	2	NCM	120	90	B+	Primi	38	F IUGR	2.6	LSCS	Hypo thyroidism
101	592470	B/1120/20	21	Krishnagiri	8	NCM	160	90	B+	Primi	32	F IUGR, Preterm	1.5	LSCS	Anemia
102	592564	B/1123/20	28	Kolar	5	NCM	150	110	o+	Primi	30	Twin 2 Males IUD	1-Jan	LSCS	
103	594428	B/1125/20	25	Mulbagal	1.5	NCM	150	110	B+	G4A3	29	M Preterm	1	LSCS	Anemia
104	596209	B/1126/20	21	Mulbagal	1	NCM	160	110	B+	Primi	34	F Preterm	1.66	LSCS	
105	596380	B/864/20	21	Mulbagal	3	CM (II)	140	90	O+	Primi	35	M IUGR	2.34	Normal	

106	600108	B/865/20	29	Kolar	4	NCM	150	110	AB +	G2P1L1	35	F IUGR	1.82	LSCS	
107	465692	B/866/20	22	Kolar	3	CM (II)	170	120	AB +	G3P2A2	35	F IUGR	2.6	Normal	Hypo thyroidism
108	600709	B/867/20	27	KGF	2	NCM	140	100	A+	Primi	28	F Preterm	1.2	LSCS	
109	595560	B/868/20	25	Bangarpet	2	NCM	140	100	B+	Primi	37	M IUGR	3.44	LSCS	
110	599605	B/1237 /20	26	Kolar	5	CM (II)	130	70	AB +	Primi	36	M IUGR	2.8	LSCS	
111	601391	B/869/20	24	Bangarpet	1	NCM	150	100	O+	G2P1L1	40	M IUGR	2.9	Normal	
112	599913	B/870/20	20	Kolar	6	NCM	140	90	O+	Primi	36	M IUGR	2.8	Normal	
113	601757	B/ 1238 /20	27	Bangarpet	1.5	NCM	180	120	O+	G2P1L1	39	F	2.6	Normal	Anemia
114	410386	B/ 1177 /20	25	Kolar	1	NCM	150	90	O+	Primi	40	M	3.36	LSCS	
115	603331	B/2304/20	20	Mulbagal	1	NCM	130	70	B+	Primi	31	M IUD	1	Normal	
116	559362	B/1178 /20	22	Mulbagal	1	CM (II)	130	70	AB +	Primi	36	F	2.8	LSCS	
117	603396	B/1775/20	26	Mulbagal	1	CM (II)	140	100	AB +	Primi	32	M Preterm	1.2	LSCS	
118	552713	B/ 1179/20	20	Bangarpet	1.5	NCM	140	90	A+	Primi	36	Twin 2 M	1.88/1.64	LSCS	Hypo thyroidism
119	601447	B/1180/20	20	Kolar	1	CM (II)	160	100	B+	Primi	37	M	2.58	Normal	
120	604970	B/1776/20	30	Kolar	3	NCM	160	120	A+	Primi	30	F IUD	1	Normal	
121	596030	B/1777/20	20	Kolar	1	NCM	160	110	A+	G2P1L1	40	F	3.2	LSCS	Anemia
122	605737	B/1778/20	26	Kolar	7	CM (II)	140	100	B+	G3P3L3	38	M	2.9	LSCS	
123	605457	B/ 1181 /20	27	Bangarpet	1	CM(III)	160	90	O+	G3P1L1A1	29	M IUD	1	Normal	
124	605454	B/1182 /20	23	Kolar	3	NCM	160	100	B+	Primi	32	F IUGR	1.01	LSCS	Growth lag

125	605813	B/ 1183/20	20	Kolar	2	NCM	150	100	A+	Primi	36	M	2.58	Normal	
126	606263	B/1779/20	25	Kolar	8	NCM	180	100	o+	Primi	34	M Preterm	1.84	LSCS	
127	607180	B/1801/20	28	Kolar	7	NCM	250	130	B+	G3P2L2	27	F IUD	1	Normal	Anemia
128	609009	B/ 1184 /20	36	Mulbagal	5	NCM	180	120	A+	G3P2L2	37	M IUGR	2.3	LSCS	
129	608892	B/ 1185 /20	24	Malur	1	NCM	130	80	O+	G2P1L1	37	M	3.2	LSCS	
130	609348	B/1191 /20	23	Mulbagal	5	NCM	150	110	O+	Primi	32	Twins M (IUD)&F	1.6	LSCS	Anemia
131	609355	B/1802/20	28	KGF	3	NCM	160	110	AB +	G2P1L1	34	Mother died / F IUD	1.3	LSCS	Thrombocytopeni
132	581651	B/ 1192 /20	23	Kolar	1	NCM	120	90	O+	Primi	36	F Preterm	2.8	LSCS	
133	609386	B/2266/20	21	Chittor	1	NCM	140	80	B+	Primi	38	F IUGR	3.76	LSCS	
134	561487	B/1803/20	19	Kolar	1	NCM	150	100	O+	Primi	27	M IUD	0.29	Normal	
135	610167	B/1804/20	18	Kolar	1	NCM	150	100	B+	Primi	35	F Preterm	1.88	LSCS	
136	610158	B/1805/20	30	Kolar	6	NCM	150	100	O+	G2P1L1	37	F	2.46	LSCS	
137	610190	B/793/20	29	chintamani	1	NCM	180	100	AB +	Primi	38	F IUGR	1.6	LSCS	Severe oligihydroamnios
138	610666	B/794/20	26	Mulbagal	1	NCM	190	110	O+	Primi	38	F IUGR	2.8	Normal	
139	610708	B/795/20	22	Bangarpet	2	NCM	170	80	O+	Primi	40	M IUGR	2.48	LSCS	
140	600026	B/802/20	25	Kolar	5	NCM	140	100	AB +	G3A1P1D1	37	F IUGR	2.3	Lscs	
141	611950	B/803/20	28	Mulbagal	1	NCM	160	120	B+	G2A1	38	M IUGR	2.62	Normal	
142	611970	B/804/20	24	Kolar	4	NCM	150	90	O+	G2P1L1	40	M	2.5	Normal	Severe oligihydroamnios
143	612558	B/805/20	31	Hoskote	4	NCM	200	120	O+	G2P1L1	39	F IUGR	2.4	LSCS	
144	604837	B/806/20	23	Krishnagiri	5	NCM	140	90	A+	G2P1L1	40	M	3.38	LSCS	oligihydroamnios

145	612812	B/807/20	27	KGF	1	NCM	150	90	O+	Primi	38	M IUGR	2.86	Normal	
146	612698	B/808/20	33	KGF	4	NCM	160	110	O+	G2P1L1	39	F IUGR	2.8	Normal	
147	613387	B/809/20	25	Malur	1	NCM	140	90	O+	Primi	26	M Preterm	1.92	LSCS	oligihydroamnios
148	612862	B/810/20	30	Kolar	7	NCM	130	90	A+	G2P1D1	39	M IUD	3.2	LSCS	Thrombocytopeni
149	613728	B/2308/20	20	Srinivasapura	1.5	NCM	140	90	A+	Primi	41	F	2.8	LSCS	
150	614227	B/2307/20	22	Malur	3	NCM	160	110	O+	Primi	39	F IUD	2.6	LSCS	HELLP
151	456052	B/116/20	25	Kolar	1	NCM	120	80	B+	Primi	40	M	2.9	Normal	
152	459897	B/117/20	23	Kolar	1	NCM	130	80	O+	Primi	36	M	2.5	Normal	
153	407302	B/118/20	23	Kolar	3	NCM	120	70	B+	Primi	40	F	2.6	Normal	
154	448234	B/119/20	27	Bangarpet	9	NCM	110	70	A+	G2P1L1	40	F	2.9	Normal	
155	461273	B/120/20	26	Mulbagal	5	NCM	110	70	O+	G2P1L1	40	F	2.8	Normal	
156	461797	B/183/20	20	Kolar	1	CM(III)	110	70	B+	Primi	37	M	2.6	Normal	
157	461823	B/184/20	28	Mulbagal	1	NCM	110	70	A+	Primi	38	M	3	Normal	Hypothroidism
158	462357	B/185/20	27	Bangarpet	4	CM (II)	120	80	B+	G2P1L2	40	M	2.5	Normal	
159	384434	B/186/20	24	Bangarpet	2	CM (II)	130	80	A+	Primi	39	M	2.8	Normal	
160	465197	B/187/20	21	Malur	3	CM (II)	140	90	B+	G2 P1 A1	41	F	2.7	Normal	
161	479330	B/248/20	20	Mulbagal	1	NCM	130	80	B+	Primi	39	F	2.5	Normal	
162	457688	B/249/20	24	Malur	1	NCM	120	70	A+	Primi	39	F	2.6	Normal	
163	470836	B/250/20	24	Kolar	1	NCM	120	70	O+	Primi	39	M	3.2	Normal	
164	478783	B/251/20	19	Kolar	3	NCM	120	70	B+	Primi	39	F	3.1	Normal	
165	514990	B/252/20	26	Hosakote	4	CM (II)	110	70	O+	G4 P1 L1 A2	38	F	2.6	Normal	
166	531138	B/337/20	19	Kolar	1	CM (II)	110	80	B+	Primi	40	M	2.9	Normal	
167	521603	B/338/20	19	Kolar	1	CM (II)	110	70	B+	Primi	40	M	3.2	Normal	
168	479555	B/339/20	29	Devanahalli	5	NCM	110	70	B+	G2P1L1	39	M	3.4	Normal	

169	470930	B/340/20	25	Kolar	5	CM (II)	130	80	A+	G2P1L1	38	M	3	Normal	
170	524850	B/341/20	20	Bangarpet	1	NCM	140	80	A+	Primi	30	M IUD	1	Normal	
171	559482	B/679/20	23	Mallur	5	CM (II)	120	70	AB +	G2P1L1	37	M Preterm	2.5	LSCS	
172	551720	B/680/20	23	Mulbagal	1	NCM	120	70	O+	Primi	39	M	2.9	Normal	Hypothroidism
173	468724	B/681/20	25	Kolar	1	NCM	120	70	A+	Primi	40	M	2.7	Normal	
174	493187	B/2265/20	27	Mulbagal	1	NCM	110	70	A+	Primi	40	M	2.8	Normal	
175	566561	B/683/20	22	Narsapura	1.5	NCM	130	80	O+	Primi	37	F	2.9	Normal	
176	567434	B/690/20	25	Domasandra	1	CM (II)	110	70	B+	Primi	38	M	3.6	Normal	
177	514049	B/691/20	28	Bangarpet	3	NCM	120	90	B+	G2P1L1	41	M	2.86	Normal	
178	567525	B/692/20	28	Mallur	5	NCM	120	80	A+	G2P1L1	40	M	2.87	Normal	
179	541173	B/693/20	25	Kolar	5	NCM	130	80	B+	Primi	39	F	3.5	Normal	
180	418111	B/694/20	30	Mulbagal	6	NCM	120	80	A+	G3P1L1A1	37	F	3.6	Normal	
181	567084	B/ 1272 /20	25	Kolar	2	NCM	120	80	O-	G2A1	39	M	3.2	Normal	
182	564786	B/1274 /20	25	Mulbagal	2	NCM	110	60	O+	Primi	38	M	2.4	Normal	
183	569855	B/ 1275 /20	22	Mallur	1	NCM	130	80	O+	Primi	40	M	3.13	Normal	
184	570383	B/727/20	20	Mulbagal	1	CM (II)	100	70	O-	Primi	38	M	2.8	Normal	
185	500342	B/729/20	21	Kolar	4	NCM	110	70	O+	G4P2L1A1	39	M	2.7	Normal	
186	491293	B/731/20	32	Mulbagal	12	NCM	110	70	B-	G4P1L1A1	38	M	3.3	Normal	
187	561948	B/733/20	27	Mulbagal	1.5	NCM	130	80	A+	Primi	38	M	3.9	Normal	
188	557594	B/735/20	20	Mallur	1	CM (II)	130	80	A+	Primi	41	F	3.3	Normal	
189	570400	B/ /20	24	Kolar	1.5	NCM	120	80	A+	Primi	38	F	2.4	Normal	
190	571032	B/738/20	21	Mallur	2	NCM	130	80	B+	Primi	40	M	2.7	Normal	
191	581596	B/741/20	22	Banglore	7	NCM	120	80	O+	Primi	40	M	3.1	Normal	
192	580700	B/746/20	25	Kadiri	6	NCM	120	90	A+	G2P1L1	37	M	2.9	Normal	

193	581724	B/748/20	21	Bangarpet	2	CM (II)	120	80	O+	G2P1L1	32	M	2.8	Normal	
194	558815	B/750/20	22	Kolar	4	CM (II)	110	70	A+	Primi	39	F	2.5	Normal	
195	515104	B/754/20	20	Kolar	1	NCM	130	70	A+	Primi	38	F	2.9	Normal	
196	585529	B/755/20	27	Bangarpet	10	NCM	120	70	B+	G4P2L2A1	38	M	2.6	Normal	
197	589491	B/ 1276 /20	25	Kolar	6	CM (II)	110	70	B+	G2P1L1	38	M	2.8	Normal	
198	538843	B/758/20	19	Malur	1	CM (II)	120	70	A+	Primi	39	F	2.7	Normal	
199	525857	B/760/20	24	Kolar	4	NCM	120	70	B+	G2P1L1	38	M	2.9	Normal	Thrombocytopeni
200	589128	B/1277/20	23	Kolar	6	NCM	120	80	A+	G3P3L3	29	M IUD	1	Normal	
201	581540	B/1278 /20	23	Kolar	1	CM (II)	120	70	A+	Primi	40	F	2.9	Normal	
202	594306	B/1279/20	21	Bangarpet	1	NCM	120	70	O-	Primi	39	F	3	Normal	
203	585905	B/682/20	23	Kolar	6	CM (II)	110	70	A+	G2P1L1	40	M	3.7	Normal	
204	593997	B/ 1280 /20	19	Kolar	3	CM (II)	120	70	O+	G2P1L1	36	M IUD	1	Normal	
205	581540	B/ 1281 /20	23	Kolar	1	CM (II)	120	80	A+	Primi	40	F	2.8	Normal	
206	564616	B/1290 /20	26	Hosakote	1	NCM	120	70	B+	Primi	40	M	2.9	Normal	
207	589878	B/ 1291 /20	24	Bangarpet	1	NCM	120	70	O+	Primi	40	M	2.6	Normal	
208	586705	B/1292 /20	25	Bangarpet	5	NCM	110	70	O+	G3P2L2	29	Twin 1F& 1M	2.4,2.5	Normal	
209	598892	B/ 1293 /20	21	Kolar	3	NCM	120	70	O+	G2L1A1	40	M	3.6	Normal	
210	553744	B/ 1890 /20	19	Kolar	1.5	NCM	120	70	O+	G2P2	26	M IUD	1	Normal	
211	600770	B/1130/20	30	Malur	11	NCM	110	80	A+	G2P1L1	27	F IUD	1	Normal	
212	565313	B/1294 /20	23	Kolar	3	NCM	110	70	B+	G2P1L1	37	F	2.5	Normal	
213	602288	B/1113/20	26	Kolar	5	NCM	100	60	B+	G2P1L1	36	M IUD	1	Normal	
214	452533	B/1122/20	25	Kolar	3	CM (II)	130	80	B+	G2P2L2	36	M	2.6	Normal	
215	601975	B/1124/20	25	Kolar	2	NCM	130	70	A+	Primi	40	F	3.6	Normal	
216	501464	B/ 1295 /20	25	Mulbagal	3	CM(II)	120	70	B+	G2P1D1	40	F	2.1	Normal	

217	520593	B/1127/20	28	Kolar	7	NCM	130	80	O+	G2P1L1	40	F	2.9	Normal	
218	566918	B/1129/20	21	Kolar	3	CM (II)	130	80	O+	G2P1L1	41	F	2.7	Normal	
219	603833	B/1296/20	20	Kolar	1	NCM	120	80	B+	Primi	34	F	2	Normal	
220	604172	B/ 1213 /20	28	Madanapally	5	NCM	110	60	A+	G3P2L2	38	M	2.9	Normal	
221	604248	B/833/20	29	Kolar	3	NCM	140	90	B+	Primi	35	M	2.68	Normal	
222	610168	B/ 1214 /20	25	Kolar	1	NCM	110	70	A+	Primi	37	M	2.8	Normal	
223	605486	B/ 1215 /20	24	Hossur	4	NCM	130	80	A+	G2P1L1	42	M	4	Normal	
224	605404	B/1216/20	25	Kolar	6	NCM	130	70	O+	G2P1L1	40	M	3.96	Normal	
225	605801	B/ 1217 /20	24	Kolar	5	CM (II)	110	60	B+	G2P1L1	36	M	3.1	Normal	
226	605896	B/788/20	23	Krishnagiri	1	NCM	110	70	A+	Primi	39	M	3.3	Normal	
227	605892	B/ 1219 /20	19	Malur	1	NCM	120	80	O+	Primi	41	M	2.9	Normal	
228	605898	B/ 1220 /20	26	KGF	5	NCM	110	80	A+	G2P1L1	38	M	3	Normal	
229	606756	B/ 1229 /20	19	Siddalaghatta	3	NCM	130	80	O+	Primi	40	M	2.9	Normal	
230	600644	B/1230/20	21	Muthyalapet	1.5	NCM	120	70	A+	Primi	39	M	3	Normal	
231	596077	B/1218/20	26	kolar	7	NCM	130	80	O+	G3P2L1D1	39	F	2.8	ELSCS	
232	606415	B/1231/20	22	Mulbagal	1	NCM	110	70	B+	Primi	40	M	3.5	Normal	
233	459252	B/ 1302 /20	22	Kolar	1	CM (II)	130	80	A+	Primi	40	F	3.8	Normal	
234	606402	B/ 1232 /20	21	Kolar	1	NCM	130	80	O+	Primi	40	M FD	2.1	Normal	
235	607203	B/ 1233 /20	22	Mulbagal	7	NCM	130	80	B+	Primi	36	Twin 1F& 1M	2.6, 2.5	LSCS	
236	598792	B/ 1234 /20	21	Hosakote	2	CM (II)	130	80	O+	Primi	41	M	2.8	Normal	
237	593261	B/1235/20	28	Srinivasapura	10	NCM	120	80	A+	G2P1L1	39	F	2.8	Normal	
238	608531	B/1236 /20	19	Mulbagal	2	CM (II)	130	80	B+	Primi	40	F	2.9	Normal	
239	608526	B/1097/20	22	Srinivasapura	5	NCM	120	70	B+	G4P1L1A2	38	M	3.3	Normal	
240	608516	B/ 1303 /20	23	Kolar	1	CM (II)	120	70	AB	Primi	39	M	2.4	Normal	

									+						
241	613184	B/ 1304 /20	24	Kolar	5	NCM	130	70	B+	G2P1L1	38	F	2.7	Normal	
242	608530	B/1098/20	20	Bangarpet	3	NCM	130	80	A+	G2P0L0A1	38	M	2.57	Normal	
243	556315	B/1099/20	26	Kolar	4	NCM	130	90	A+	G3P2L2	40	F	3	Normal	
244	550607	B/1100/20	27	Bangarpet	5	CM (II)	130	80	O+	G2P1L1	38	M	3	Normal	
245	457238	B/1101/20	27	Kolar	7	NCM	110	80	A+	G2E1	36	M	2.4	Normal	
246	612350	B/1305 /20	32	Kolar	15	CM (II)	130	70	A+	G2P1L1	40	M	3.4	LSCS	
247	527041	B/1102/20	21	Kolar	2	NCM	130	70	A+	G2P1L1A1	41	M	3.1	Normal	
248	599147	B/1103/20	22	Kolar	1	CM (II)	120	80	B+	Primi	36	M	3.2	Normal	
249	528781	B/1104/20	22	Kolar	1	NCM	120	70	O+	Primi	40	M	2.7	Normal	
250	609407	B/1105/20	24	Siddhalaghatta	4	NCM	120	70	B+	G2P1L1	40	M	2.9	Normal	
251	610074	B/1106/20	23	Kolar	10	NCM	100	60	A+	G3P2L2	28	1.5		Normal	
252	611587	B/ 1306 /20	21	Kolar	4	CM (II)	130	80	O+	G2P1L1	36	M IUD	1	Normal	
253	611573	B/1193 /20	29	Kolar	8	NCM	120	80	O+	G2P1L1	38	F	2.7	Normal	
254	610168	B/ 1307 /20	24	Kolar	5	CM (II)	120	70	A+	Primi	37	M	3	Normal	
255	570584	B/1194 /20	26	Mulbagal	7	NCM	120	70	O+	G2P1L1	41	M	2.9	Normal	
256	611107	B/ 1195 /20	24	Kolar	3	NCM	140	90	A+	G3P1L1A1	40	F	2.3	Normal	
257	611597	B/ 1196 /20	29	Kolar	4	NCM	120	70	A+	G2P1L1	38	M	3	Normal	
258	611926	B/ 1197 /20	23	Malur	4	NCM	120	70	A+	G2P1L1	40	M	3	Normal	
259	577870	B/ 1201 /20	22	Kolar	3	NCM	120	70	O+	G2P1L1	40	F	3.5	LSCS	
260	611976	B/1198 /20	24	Chittor	3	NCM	120	80	B+	Primi	40	F	3.6	Normal	
261	611656	B/ 1199 /20	22	Mulbagal	1	NCM	130	80	O+	Primi	39	F	2.6	Normal	
262	610234	B/ 1200 /20	22	Kolar	6	CM (II)	130	80	O+	G2P1L1	39	F	2.7	Normal	
263	600103	B/789/20	28	Hosakote	7	NCM	120	80	B+	G3P1L1A1	40	F	2.86	Normal	

264	612292	B/790/20	28	Bangarpet	8	NCM	110	70	O+	G3P1L2	40	F	3.2	Normal	Hypothroidism
265	612414	B/791/20	24	Malur	4	CM (II)	130	70	AB+	G3P2L2	33	F	2.8	Normal	
266	612404	B/792/20	18	Mulbagal	1	NCM	100	60	A+	Primi	33	M	2.66	Normal	
267	609998	B/828/20	22	Bangarpet	1	NCM	110	70	O-	Primi	37	M	2.8	Normal	
268	601298	B/829/20	19	Kolar	1.6	NCM	110	76	O+	Primi	40	M	3.2	Normal	
269	611975	B/830/20	25	Srinivasapura	2	CM (II)	110	70	B+	Primi	38	M	2.28	Normal	
270	612671	B/831/20	25	Mulbagal	8	CM (II)	130	80	AB+	G4P3L3	40	F	3.1	Normal	
271	602227	B/832/20	22	Kolar	1.5	NCM	130	90	O-	Primi	40	F	3	Normal	
272	605896	B/1787/20	23	Krishnagiri	1	NCM	110	70	A+	Primi	39	F	3	Normal	
273	604248	B/833/20	25	Kolar	1	NCM	140	90	B+	Primi	35	M	2.68	Normal	
274	530024	B/834/20	23	Srinivasapura	3	CM (II)	130	70	O+	G2 A1	40	M	3.1	Normal	
275	612822	B/827/20	24	Kolar	6	NCM	120	80	B+	G2 A1	38	F	2.68	Normal	Hypothroidism
276	612821	B/773/20	20	Kolar	1	NCM	150	90	O+	Primi	39	F	2.3	Normal	
277	612839	B/774/20	22	Dinahalli	4	CM (II)	110	70	B+	G2P1L1	39	F	2.3	Normal	
278	562329	B/775/20	24	Kolar	3	CM (II)	120	80	B+	G2P1L1	39	M	2.6	Normal	
279	543178	B/776/20	30	Hoskote	7	CM (II)	110	70	O+	G2P1L1	39	M	3	Normal	Hypothroidism
280	612874	B/777/20	20	Bangarpet	1	NCM	130	80	O+	Primi	40	M	3.7	LSCS	
281	613118	B/778/20	27	Mulbagal	5	NCM	130	80	A+	G2P1L1	40	M	3.5	LSCS	
282	525387	B/2542/20	23	Mallur	6	CM (II)	120	80	B+	G2P1L1	37	M	3	Normal	
283	613293	B/2541/20	20	Kolar	1	NCM	130	80	AB+	G2 A1	40	F	3.5	LSCS	
284	612948	B/2464/20	31	Hosakote	9	CM (II)	120	80	B-	G4P2L2D1	38	M	2.9	Normal	
285	475997	B/2463/20	22	Kolar	1	CM (II)	120	80	AB+	Primi	40	F	2.9	Normal	Hypothroidism

286	6137691	B/2462/20	21	Srinivasapura	2	CM (II)	120	80	A+	G2P1L1	40	F	2.9	Normal	
287	609991	B/2461/20	23	Hosakote	5	NCM	130	80	O+	G3P1L1A1	37	M	2.8	Normal	
288	457214	B/788/20	25	Kolar	3	NCM	110	70	A+	G2P1L1	38	M	3.1	LSCS	
289	613765	B/789/20	36	Mallur	8	CM (II)	120	70	B+	G3P1L1A1	40	F	2.5	Normal	
290	613795	B/2341/20	30	Mulbagal	10	NCM	120	70	O+	G3P2L2 A1	39	M	3	LSCS	
291	613794	B/2342/20	24	Kolar	4	NCM	120	80	B+	G2P1L1	40	F	3	LSCS	
292	613787	B/2459/20	21	Kolar	1	NCM	120	80	B+	Primi	35	M	3	LSCS	
293	592916	B/2460/20	26	Kolar	5	NCM	110	70	O+	G3 A2	33	M	2.6	Normal	
294	530013	B/779/20	23	Kolar	1	NCM	130	70	O+	Primi	39	M	3.2	Normal	
295	613963	B/2337/20	24	Kolar	2	NCM	120	80	O-	Primi	39	M	2.3	Normal	
296	619359	B/2338/20	25	Hosakote	3	CM (II)	120	70	O+	G2P1L1	36	M	3.2	Normal	
297	593470	B/2339/20	24	Kolar	3	NCM	110	70	B+	G2P1L1	40	F	3.2	Normal	
298	614664	B/2340/20	19	Malur	1	CM (II)	140	100	A+	Primi	38	F	2.7	Normal	
299	567420	B/ 1272 /20	23	Bangarpet	6	NCM	110	70	O+	G2P1L1	38	M	2.7	Normal	
300	530013	B/ 1308 /20	23	Mallur	1	NCM	130	70	O+	Primi	39	M	3.2	ELSCS	

13. PUBLICATIONS

Publication1: Serum Fluoride Levels as aBiomarker in Pre-Eclamptic Women: A Case-Control Tertiary Care Hospital Based Study.

Source: Fluoride.Jul-Sep2020; 53(3) Part 1: 425-435.

Author(s): Changalvala K.; Prabhavathi K.; Venkateshu K. V.; Sheela S. R.; Kalyani R.; Raghuveer C. V.; Kiranmayee P.

Abstract: The aim and objectives of the study were to determine (i) whether a high serum fluoride (F) level was an added risk factor for the development of pre-eclampsia in Kolar population, an area with endemic fluorosis, and (ii) whether the serum F levels could be used as a predictive diagnostic marker for pre-eclampsia. The study was designed as a case-control study in pregnant women after 20th week of gestation in the Department of Obstetrics and Gynecology of a tertiary care hospital. After obtaining ethics clearance and written informed consent from 300 pregnant women (150 cases and 150 controls), 5 mL of venous maternal blood was collected in a plain (red-capped) vacutainer and serum was separated for estimation of the serum F levels by using a fluoride ion-selective electrode. The serum F levels in the cases and controls were statistically analyzed by the Mann-Whitney U test. The mean±standard deviation in the cases and controls was 1.8±0.66 and 0.18±0.31 mg/L, respectively. The serum F levels were significantly higher in the pre-eclampsia cases with a z value of 14.4. We found that F is an added risk factor for pre-eclampsia and that a maternal serum F level of =1.8 mg/L can be considered as a diagnostic biomarker for predicting pre-eclampsia and the related pregnancy outcomes.

Publication 2: Association of sFlt-1 as a maternal serum biomarker in preeclampsia: A case-control tertiary care hospital based study.
Source: Indian Journal of Medical Sciences. Sep-Dec2021; 73(3): 311-316.
Author(s): Changalvala, Krishnaveni; Kiranmayee, P.; Raghuveer, C. V.; Sheela, S. R.; Venkateshu, K. V.; Kalyani, R.

Abstract: Preeclampsia (PE) is a multisystemic disorder portrayed by the new beginning of circulatory pressure more noteworthy than 140/90 mmHg and proteinuria with 0.3 g in a 24 h on dip stick emerging after 20 weeks of incubation. The hidden pathophysiology of PE includes endothelial brokenness and vasospasm beginning principally in the placenta. The unusual growth of blood vessels in placenta leads to poor perfusion. This relative hypoxic condition in placenta causes arrival of antiangiogenic factors into the maternal blood dissemination which prompts the modifications in maternal fundamental endothelial functions and causes hypertension. Soluble fms-like tyrosine kinase (sFlt) can form a heterodimer, binding with vascular endothelial growth Factor A and placental growth factor. In preeclamptic subjects, there will be an imbalance in anti-angiogenesis factors and there will be incomplete arterial transformation and cytotrophoblast cell division. Due to imbalance in sFlt levels in preeclamptic women it effects in the blood vessels by constriction and leads to endothelial dysfunction. This study aim is to compare the maternal serum concentration of sFlt levels in normotensive pregnant women to preeclamptic women in early and late gestational weeks. Material and Methods: Out of 300 participants in the case-control study, 150 were preeclamptic women as cases and 150 as normotensive pregnant women as controls participated in the present study. A 5 ml of maternal venous blood was collected; the serum was separated and stored at -800°C till the analysis. Using commercially available enzyme-linked immunosorbent assay (ELISA) kits from Chongqing Biospes Co., Ltd., (suppliers: Infobio Company, New Delhi) was measured with ELISA microplate reader at 450 nm (Merilyzer Eiaquant Company). Results: Out of 300 participants in the study, 46 pregnant women were early gestational weeks and 254 were late gestational weeks. The complications due to severe PE such as intrauterine death are 15%, intrauterine fetal growth retardation 33%, and premature 15%. The statistical analyses were performed by Statistical Packages for the Social Sciences Software 22. The area under the receiver operating characteristic curve is 0.82, with 91% sensitivity, and 79% specificity. The significance in the maternal serum sFlt levels was calculated by the Mann-Whitney U-test. By comparing the cases and controls, it was found that maternal serum sFlt1 were significantly higher in preeclamptic women with $Z = 2.96$ and $U = 9021$ with $P = 0.005$ significance. Conclusion: This is the first South Indian study. If we compare the sFlt1 levels in early and late gestational weeks, in late gestational weeks in controls and PE the levels were highly significant than early gestational weeks of PE and controls. Maternal serum sFlt1 can be used as a preeclamptic diagnostic marker in South Eastern Kolar population.

Publication 3: HISTOPATHOLOGY OF PLACENTAE IN PRE-ECLAMPSIA: A REVIEW

Source: Journal of Clinical and Biomedical Sciences ahead of print

Author(s): Changalvala Krishnaveni; Kiranmayee P.; Raghuveer C. V.; Sheela S. R.; Venkateshu K. V.; Kalyani R

Abstract

Preeclampsia (PE) is a pregnancy related complex multisystemic disorder with a triad of symptoms including increased blood pressure, oedema, and proteinuria phenotypically observed after 20 weeks of gestation. The disorder worsens amongst the early onset patients PE (< 34 weeks). The placenta shares more responsibility for the cause of PE. The placenta is an essential organ for pregnancy and assists in the development of the fetus. It shares the same stress and strain to which the fetus is exposed. Any disease which affects the mother has a great impact on the placenta and also placenta acts as a future evidence of mother and fetus health. Therefore careful observation of histopathological changes can provide clinically useful information to pave the way for pathophysiology of PE. So the histopathological parameters like distal villous hypoplasia, accelerated villous maturity, increase in syncytial knots, immature villi, chorangiosis, fibrin deposition, infarction, and calcification are evident in histopathological observations of PE placentae.

14. APPENDIX-I INSTITUTIONAL ETHICAL CLEARANCE

CENTRAL ETHICS COMMITTEE
Sri Devaraj Urs Academy of Higher Education & Researc
POST BOX NO.62, TAMAKA, KOLAR-563 101, KARNATAKA, INDIA
Ph:08152-210604, 210605, 243003, 243009, ,ext. 438. E-mail: co.rd@sduu.ac.in
SDUAHER

DCGI Registration NO. ECR/425/Ins/KA/2013/RR-16

No. SDUAHER/KLR/R&D/~~80~~35/2017-18 **Date07-07-2017**

Members

1.Dr.Kiran Katoch
Chairman, Central Ethics Committee SDUAHER, + Kolar Ex-Director, National, JALMA Institute for Leprosy & other Mycobacterial Diseases (ICMR),Tajganj, Agra(UP)

2. Mr . Subramani
Assistant Professor
Basaweshawara College of Law Kolar

3. Mr.B.Suresh
President - District Chamber of Commerce, Vice Chairman, Indian red Cross Society Reporter Press Trust of India BRM colony Kolar.

4.Dr. K.N.Srinivas
Dean, College of orticulture, Tamaka, Kolar.

5. Swami Chinmayananda Avadhuta
Co-ordinator, South India Ananda Marga Prachara Sangha Ananda Marga Ashram Kithandur, Kolar (T)

6 Dr.V.Lakshmaiah
Professor of Medicine
SDUMC, Kolar

7.Dr.N.Sarala
Prof. & HOD Pharmacology, SDUMC, Kolar.

8. Dr. Sharath B.
Ph.D
Assistant Professor
Dept. of Cellular Biology & Molecular Genetics
SDUAHER, Kolar

9.Dr.T.N.Suresh
Member Secretory
Professor of Pathology
Co-ordinator, Research & Development, SDUAHER, Kolar

Central Ethics Committee, SDUAHER, Kolar

To:

Mrs.Krishna Veni.C
Department of AnatomySri Devaraj Urs Medical College,
Tamaka, Kolar 563101

Subject: Central Ethics Committee clearance for the research proposal

The Central ethics Committee of Sri Devaraj Urs Academy of Higher Education and Research, Kolar has examined Ph.D synopsis titled "**Serum Biomarkers And Flouride Estimation With histopathological Changes In Placentae Of Pre-Eclampsia** " and the detailed work plan of the project.

The central ethics committee has unanimously decided to approve the project and grant permission to investigator to carry out the research work. The interim and final report has to be submitted to the ethics committee after completion of the project for the issue of Central Ethics Committee certificate. Principal investigator should maintain the records of the Project and consent form for not less than 5 year from the date of completion or termination of the project.

Member Secretary

T.N.Su

(Dr. TN Suresh)

MEMBER SECRETARY
CENTRAL ETHICS COMMITTEE
SRI DEVARAJ URS ACADEMY OF
HIGHER EDUCATION & RESEARCH
TAMAKA, KOLAR-563 101

Chairman

Kiran Katoch

(Dr. Kiran Katoch)

SRI DEVARAJ URS ACADEMY OF HIGHER EDUCATION & RESEARCH

SRI DEVARAJ URS MEDICAL COLLEGE

Tamaka, Kolar

INSTITUTIONAL ETHICS COMMITTEE

Members

1. **Dr. D.E.Gangadhar Rao, (Chairman)** Prof. & HOD of Zoology, Govt. Women's College, Kolar,
2. **Dr. Sujatha.M.P, (Member Secretary),** Assoc. Prof. of Anesthesia, SDUMC,
3. Dr. C.S.Babu Rajendra Prasad, Prof. of Pathology, SDUMC
4. Dr. Srinivasa Reddy.P, Prof. & HoD of Forensic Medicine, SDUMC
5. Dr. Prasad.K.C, Professor of ENT, SDUMC
6. Dr. Sumathi.M.E Prof. & HoD of Biochemistry, SDUMC.
7. Dr. Bhuvana.K, Prof. & HoD of Pharmacology, SDUMC
8. Dr. H.Mohan Kumar, Professor of Ophthalmology, SDUMC
9. Dr. Hariprasad, Assoc. Prof Department of Orthopedics, SDUMC
10. Dr. Pavan.K, Asst. Prof of Surgery, SDUMC
11. Dr. Mahendra.M , Asst. Prof. of Community Medicine, SDUMC

No. DMC/KLR/IEC/ 131 /2019-20 Date: 04-11-2019

CERTIFICATE

This is to certify that, the Institutional Ethics Committee of Sri Devaraj Urs Medical College, Tamaka, Kolar has examined and unanimously approved the study topic entitled **"Serum Biomarkers & Fluoride Estimation in Preeclamptic Women: A Case- Control Tertiary Care Hospital based Study"** for **Presentation & Publication** authored by **Mrs. Krishnaveni. C,** Dr. Kiranmayee. P[1], Dr. Sheela S R[2], Dr. Venkateshu K. V, Dr. Prabhavathi. K[3], Dr. Kalyani. R[4] & Dr. Raghuveer. C. V[4] in the Departments of Anatomy, Cell Biology & Molecular Genetics[1], OBG[2], Biochemistry[3] & Pathology[4] at Sri Devaraj Urs Medical College, Tamaka, Kolar.

Sujatha.M.P

Member Secretary

Member Secretary
Institutional Ethics Committee
Sri Devaraj Urs Medical College
Tamaka, Kolar.

Chairman

CHAIRMAN
Institutional Ethics Committe
Sri Devaraj Urs Medical College
Tamaka, Kolar

CENTRAL ETHICS COMMITTEE

Sri Devaraj Urs Academy of Higher Education & Research

POST BOX NO.62, TAMAKA, KOLAR-563 101, KARNATAKA, INDIA

Department of Research and Innovation

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Central Ethics Committee Re- registered under CDSCO -Registration No. ECR/425/Inst/KA/2013/RR-20 dated 28.4.2020

Central Ethics Committee registered under NECRBHR ,DHR --Registration No. EC/NEW/INST/2020/588 dated 28.5.2020

No: SDUAHER/KLR/CEC/ 62 /2020-21 Date: 03.11.2020

PERMISSION FOR PUBLICATION

The Central Ethics Committee of Sri Devaraj Urs Academy of Higher Education and Research, Tamaka, Kolar has examined and approved part of the Ph. D thesis work entitled: **"Serum Biomarkers and Fluoride Estimation with Histopathological Changes in Placentae of Pre-Eclampsia".**

In continuation CEC of SDUAHER is approving part of the Ph. D thesis work titled: **"Association of sflt-1 as a maternal serum biomarkers in preeclampsia: A case- control tertiary care hospital based study"** for publication.

This study is carried out by **Dr. Krishnaveni C[1]**, Dr. Kiranmayee P[2], Dr. Raguveer C.V[3], Dr. Sheela S.R[4], Dr. Venkateshu K.V[5], Dr. Kalyani R,[6] in the Department of Anatomy, Cell Biology and Molecular Genetics, Pathology and OBG at Sri Devaraj Urs Academy of Higher Education and Research Tamaka, Kolar.

Permission is granted for part of the Publication of the Ph. D thesis.

Corresponding author to mandatorily submit a copy of the published article with details of indexation, impact factor, quartiles, UGC recognition etc.

Affiliation has to be given to Sri Devaraj Urs Academy of Higher Education and Research, Tamaka, Kolar.

Member secretary
Central Ethics Committee

CENTRAL ETHICS COMMITTEE

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Central Ethics Committee Re- registered under CDSCO -Registration No. ECR/425/Inst/KA/2013/RR-20 dated 28.4.2020

Central Ethics Committee registered under NECRBHR ,DHR --Registration No. EC/NEW/INST/2020/588 dated 28.5.2020

No: SDUAHER/KLR/CEC/ 61 /2020-21 **Date: 03.11.2020**

PERMISSION FOR PUBLICATION

The Central Ethics Committee of Sri Devaraj Urs Academy of Higher Education and Research, Tamaka, Kolar has examined and approved part of the Ph. D thesis work entitled: **"Serum Biomarkers and Fluoride Estimation with Histopathological Changes in Placentae of Pre-Eclampsia"**

In continuation CEC of SDUAHER is approving part of the Ph. D thesis work titled: **"Maternal serum soluble endoglin levels as a biomarker in preeclampsia: A case control tertiary care Hospital based study** "for publication.

This study is carried out by **Dr. Krishnaveni C**[1], Dr. Kiranmayee P[2], Dr. Raguveer C.V[3], Dr. Sheela S.R[4], Dr. Venkateshu K.V[1], Dr. Kalyani R,[5] in the Department of Anatomy, Cell Biology and Molecular Genetics, Pathology and OBG at Sri Devaraj Urs Academy of Higher Education and Research Tamaka, Kolar.

Permission is granted for part of the Publication of the Ph. D thesis.

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Affiliation has to be given to Sri Devaraj Urs Academy of Higher Education and Research, Tamaka, Kolar.

Member secretary
Central Ethics Committee

MEMBER SECRETARY
CENTRAL ETHICS COMMITTEE
SRI DEVARAJ URS ACADEMY OF
HIGHER EDUCATION & RESEARCH
TAMAKA, KOLAR-563 101.

Sri Devaraj Urs Academy of Higher Education & Research
Comprising Sri Devaraj Urs Medical College
A DEEMED TO BE UNIVERSITY
Research and Innovation
Declared under Section 3 of UGC Act, 1956, MHRD GOI No.F.9-36/2006-U.3 (A) Dt. 25th May 2007
POST BOX NO.62, TAMAKA, KOLAR-563 101, KARNATAKA, INDIA
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Central Ethics Committee Re- registered under CDSCO -Registration No. ECR/425/Inst/KA/2013/RR-20 dated 28.4.2020
Central Ethics Committee registered under NECRBHR ,DHR --Registration No. EC/NEW/INST/2020/588 dated 28.5.2020

No. SDUAHER/KLR/ R&I/ /2021-22 **Date: 09.02.2022**

152

PERMISSION FOR PUBLICATION

The Core Committee of Research and Innovation of Sri Devaraj Urs Academy of Higher Education and Research, Tamaka, Kolar has examined and approved part of the Ph. D thesis work entitled: **"Serum Biomarkers and Fluoride Estimation with Histopathological Changes in Placentae of Pre-Eclampsia"**

In continuation CEC of SDUAHER is approving part of the Ph. D thesis work titled: "**Association of Maternal Serum PIGF Levels as a Biomarker in Preeclampsia: A Case Control Tertiary Care Hospital Based study**" for publication.

This study is carried out by Dr. Krishnaveni C[1], Dr. Kiranmayee P[2], Dr. Raghuveer C. V[3], Dr. Sheela S.R[4], Dr. Venkateshu K.V[1] and Dr Kalyani R[3] in the Department of Anatomy[1], Cell Biology and Molecular Genetics[2], Pathology[3], and OBG[4] at Sri Devaraj Urs Medical College, Tamaka, Kolar.

Permission is granted for part of the Publication of the Ph. D thesis.

Corresponding author to mandatorily submit a copy of the published article with details of indexation, impact factor, quartiles, UGC recognition etc.

Affiliation has to be given to Sri Devaraj Urs Academy of Higher Education and Research, Tamaka, Kolar.

9.2.2022

Member Secretary
Central Ethics Committee

MEMBER SECRETARY
CENTRAL ETHICS COMMITTEE
SRI DEVARAJ URS ACADEMY OF
HIGHER EDUCATION & RESEARCH
TAMAKA, KOLAR-563 101

Copy to:
Dr. Krishnaveni C
Lecturer
Department of Anatomy
SDUMC, Tamaka, Kolar

Sri Devaraj Urs Academy of Higher Education & Research
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No. SDUAHER/KLR/ R&I/ /2021-22 **Date: 28.01.2022**

137

PERMISSION FOR PUBLICATION

The Core Committee of Research and Innovation of Sri Devaraj Urs Academy of Higher Education and Research, Tamaka, Kolar has examined and approved part of the Ph. D thesis work entitled: **"Serum Biomarkers and Fluoride Estimation with Histopathological Changes in Placentae of Pre-Eclampsia"**

In continuation ~~CEC~~ of SDUAHER is approving part of the Ph. D thesis work titled: "**Review on Histopathology of Preeclampsia**" for publication.

This study is carried out by Dr. Krishnaveni C[1], Dr. Kiranmayee P[2], Dr. Raghuveer C.[3], Dr. Sheela S.R[4], and Dr. Venkateshu K.V[1] in the Department of Anatomy[1], Cell Biology and Molecular Genetics[2], Pathology[3], and OBG[4] at Sri Devaraj Urs Medical College, Tamaka, Kolar.

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28/1/2022

Member Secretary
Central Ethics Committee

MEMBER SECRETARY
CENTRAL ETHICS COMMITTEE
SRI DEVARAJ URS ACADEMY OF
HIGHER EDUCATION & RESEARCH
TAMAKA, KOLAR-563 101

Copy to:
Dr. Krishnaveni C
Lecturer
Department of Anatomy
SDUMC, Tamaka, Kolar

APPENDIX-II EXTERNAL VALIDATION

ST. JOHN'S MEDICAL COLLEGE

(A Unit of CBCI Society for Medical Education)
Koramangala, BENGALURU - 560 034.

Bangalore

20-4-2020

To,

Prof. C V Raghuveer. MBBS,MD, DCP
Pro Vice Chancellor,
Yenepoya Deemed to be University,
Deralakatte, Mangalore 575018
Mobile 9845383092

TO WHOMSOEVER IT MAY CONCERN

Respected Sir,

With reference to your letter dated 26 Feb 2020, requesting for validation of placental microscopy for your PhD student, Mrs.C Krishnaveni's PhD thesis, I hereby state that I have validated the slides given to me as per the criteria laid down in her thesis methodology. At the same instance evaluation of placental microscopy was also taught to her. She was further advised to carry out the evaluation of the rest of her thesis cases, based on the training given to her and record her observations under the guidance of her Guide/Co-guide.

Thanking you

Yours Truly,

R. Gayatri

Dr. Gayatri Ravikumar,MD,PhD
Assistant Professor
Department of Pathology
St.John's Medical College
Bangalore 560076

Julian Crasta

Dr.Julian Crasta MD,DNB
Professor and Head
Department of Pathology
St.John's Medical College
Bangalore 560076

Patient Information Sheet

This informed Consent form is for women who attending OBG department, and whom we are inviting to participate in research involving in pre-eclampsia. The title of our research project is "Serum Biomarkers and Fluoride Levels in Patients with Pre-Eclampsia and Correlation with Histopathological Changes in Placenta".

Name of the Principal Investigator : Mrs. C.Krishnaveni

Name of the Guide/Supervisor : Dr. C.V. Raghuveer

Name of Organization : Sri Devaraj Urs Academy of Higher Education and Research

Information Sheet

Introduction: I Mrs.C.Krishnaveni, doing project work on Pre-eclampsia, which is a hypertensive pregnancy disorder in this country. I am going to give you information and invite you to be a part of this research. You do not have to decide today whether or not you will participate in the research. Before you decide, you can talk to me and feel comfortable to ask questions about the research. There may be some words that you do not understand. Please ask me to stop as we go through the information and I will take time to explain. If you have questions later also, you can ask me or the duty doctor or the staff.

Purpose of the research: Pre-eclampsia is a condition involving abnormally increasing blood pressure during pregnancy. We are doing this research to find out marker and to know the incidence in this Kolar is high and which is the cause of it.

Participant selection: We are inviting women who are diagnosed with pre-eclampsia and also women with normal pregnancy (without pre-eclampsia and pregnancy complications).

Voluntary Participation: Your participation in this study is entirely voluntary. It is your choice whether to participate or not. Whether you choose to participate or not, all the services you receive at this clinic / hospital will continue and nothing will change. If you choose not to participate in this research project, you will offer the treatment that is routinely offered in this clinic / hospital for pre-eclampsia, and we will tell you more about it later. You may change your mind later and stop participating even if you agreed earlier.

- **Question to elucidate understanding:** If you decide not to take part in this research study, do you know what your options are? Do you know that you do not have to take part in this research study, if you do not wish to? Do you have any question?

Procedures and protocol: We will take blood from your arm using a syringe and needle. (Show a vial).Palmar prints also will be taken for analysis.At the end of the research, any leftover blood sample will be destroyed.

- **Description of the process**
  - A 10ml amount of blood will be taken from your arm with a syringe. The serum will be separated from the blood. The serum will be tested by ELISA Method to check for elevated levels of parameters which will be helpful in predicting as the markers in pre-eclampsia.
  - We will take the clinical investigations, radiological investigations for the requirement.
  - We will also ask you a few questions about your health.

Duration: The research takes place over 3 years in total

Risks: No drug will be tested on you. As there is no drug introducing to the patient, Adverse Effects (AE) and Serious Adverse Effects (SAE) are not involved. Blood sample will be collected with a sterile, single use and disposable syringe.

Benefits: This study enabled discovery of serum biomarkers of pre-eclampsia in Kolar population.

Reimbursements: You will not be given money or gifts to take part in this research.

Confidentiality: We will not be sharing the identity of participant. This information that we collect from you will be kept confidential and only the researchers involved in this project will have access to it.

Sharing the results: The results obtained from the study will be shared by publishing in scientific / medical journal. Results may also be presented in scientific/medical conferences.

Right to refuse or withdraw: You do not have to take part in this research if you do not wish to do so and refusing to participate will not affect your treatment at this clinic in any way. You will still have all the benefits that you would otherwise have at this clinic. You may stop participate in research at any time you wish without losing any of your rights as a patient here. Your treatment at this clinic will not be affected in any way.

Who to contact; If you have any questions you may ask us now or latter, even after study as started. If you wish to ask us any questions latter, you may contact me.

Mrs C. Krishna Veni,
Dept. of anatomy,
Sri Devaraj Urs Medical College,
Tamaka, Kolar.
Ph.No. 9972692715
Email- krishna.i@hotmail.com

Certificate of Consent

Patient identification number:

I have read the foregoing information, or it has been read to me. I have had the opportunity to ask questions about it and any questions that I have asked have been answered to my satisfaction. I consent voluntarily to participate as a participant in this research.

Print Name of Participant_____

Signature of Participant _____ Thumb impression

Date: _____

If illiterate: A literate witness must sign (if possible, this person should be selected by the participant and should have no connection to the research team). Participants who are illiterate should include their thumb-print as well. I have witnessed the accurate reading of the consent form to the potential participant, and the individual has had the opportunity to ask questions. I confirm that the individual has given consent freely.
Name of witness_____ Signature of witness _____Date _____

Statement by the researcher/person taking consent: I have accurately read out the information sheet to the potential participant, and to the best of my ability made sure that the participant understands that the following will be done:10 ml of blood sample will be collected along with palmar prints, information required on clinical investigations, radiological investigations for study purpose will be taken. We will ask some questions about your health. I confirm that the participant was given an opportunity to ask questions about the study, and all the questions asked by the participant have been answered correctly and to the best of my ability. I confirm that the individual has not been coerced into giving consent, and the consent has been given freely and voluntarily.

A copy of this ICF has been provided to the participant.
Print Name of Researcher/person taking the consent_____
Signature of Researcher /person taking the consent_____
Date: _____

ರೋಗಿಯ ಮಾಹಿತಿಯುಕ್ತ ಸಮ್ಮತಿ:

ಈ ಮಾಹಿತಿಯುಕ್ತ ಸಮ್ಮತಿಯ ನಮೂನೆ ಫಾರ್ಮ್ OBG ವಿಭಾಗದಲ್ಲಿ ಹಾಜರಾಗುವ ಮಹಿಳೆಯರಿಗೆ ಮಾತ್ರ, ಮತ್ತು ಯಾರು ಪ್ರೀಕ್ಲಾಂಪ್ಸಿಯಾದ ರಲ್ಲಿ ಆಂಜಿಯೊಟೆನ್ಸಿನೊಜೆನ್ ಮತ್ತು ಘಟಕ V ಜೀನ್ ಬಹುರೂಪತೆಗಳು ಮೌಲ್ಯಮಾಪನ ಒಳಗೊಂಡ ಸಂಶೋಧನಾ ಯೋಜನೆಯಲ್ಲಿ ಭಾಗವಹಿಸಲು ಆಹ್ವಾನಿಸುತ್ತಿರುವಿರಿ. ಸಂಶೋಧನಾ ಯೋಜನೆಯ ಶೀರ್ಷಿಕೆ "ಪ್ರೀಕ್ಲಾಂಪ್ಸಿಯಾದ ರಲ್ಲಿ ಆಂಜಿಯೊಟೆನ್ಸಿನೊಜೆನ್ ಮತ್ತು ಘಟಕ **V** ಜೀನ್ ಬಹುರೂಪತೆಗಳು ಮೌಲ್ಯಮಾಪನ" ಆಗಿದೆ.

ಮುಖ್ಯ ಪರೀಕ್ಷಕರ ಹೆಸರು	:	ಕೃಷ್ಣವೇಣಿ . ಸಿ.
ಮಾರ್ಗದರ್ಶಿಯ ಹೆಸರು	:	ಡಾ. ಮೀತೆಶ್ ಶೆಟ್ಟಿ
ಸಂಸ್ಥೆಯ ಹೆಸರು	:	ಶ್ರೀ ದೇವರಾಜ ಅರಸ್ ಉನ್ನತ ಶಿಕ್ಷಣ ಅಕಾಡೆಮಿ ಮತ್ತು ಸಂಶೋಧನೆ

ಭಾಗ **I:** ಮಾಹಿತಿ ನಮೂನೆ:

ಪೀಠಿಕೆ:

ನಾನು ಕೃಷ್ಣವೇಣಿ . ಸಿ. ಒಬ್ಬ ಎಂ. ಫಿಲ್ ವಿದ್ಯಾರ್ಥಿಸರು ಪ್ರೀಕ್ಲಾಂಪ್ಸಿಯಾದ ಮೇಲೆ ಪ್ರೌಢಪ್ರಬಂಧದಲ್ಲಿ ಕೆಲಸ ಮಾಡುತ್ತಿದ್ದಾರೆ. ನಾನು ನಿಮಗೆ ಮಾಹಿತಿಯನ್ನು ನೀಡಲು ಮತ್ತು ಈ ಸಂಶೋಧನೆಯ ಭಾಗವೆಂದು ಆಮಂತ್ರಿಸಲು ಹೋಗುತ್ತಿದ್ದೇನೆ. ನೀವು ಸಂಶೋಧನೆಯಲ್ಲಿ ಭಾಗವಹಿಸಬಹುದು ಅಥವಾ ಬೇಡವೇ ಎಂಬುದನ್ನು ಇಂದು ನಿರ್ಧರಿಸುವ ಹಾಗೆ ಇಲ್ಲ. ನೀವು ನಿರ್ಧರಿಸುವ ಮುನ್ನ, ನೀವು ಯಾರಿಗಾದರೂ ಮಾತನಾಡಬಹುದು ನಿಮ್ಮ ಜೊತೆ ಸಂಶೋಧನೆಯ ಬಗ್ಗೆ ಹಿತಕರವಾದ ಭಾವನೆಯನ್ನು ವ್ಯ ಕ್ತ ಪಡಿಸು ತ್ತೇನೆ. ನಿಮಗೆ ಅರ್ಥವಾಗದ ಕೆಲವು ಪದಗಳು ಇರಬಹುದು . ನನಗೆ ಮಾಹಿತಿಯನ್ನು ನಿಲ್ಲಿಸಲು ನೀವು ನನಗೆ ಕೇಳಿದರೆ ನಾನು ಅದನ್ನು ವಿವರಿಸಲು ಸಮಯ ತೆಗೆದುಕೊಳ್ಳುತ್ತದೆ. ನಿಮಗೆ ಪ್ರಶ್ನೆಗಳಿದ್ದರೆ ನಂತರ, ನೀವು ನನಗೆ ಕೇಳಬಹುದು, ಅಧ್ಯಯನದ ವೈದ್ಯರನ್ನು ಅಥವಾ ಸಿಬ್ಬಂದಿಯನ್ನು.

ಸಂಶೋಧನೆಯ ಉದ್ದೇಶ: ನಾವು ಈ ಸಂಶೋಧನೆಯನ್ನು ಮಾಡುತ್ತಿರುವುದು ಪ್ರಿಕ್ಲಾಂಪ್ಸಿಯ ಅನುವಂಶೀಯ ಕಾರಣಗಳನ್ನು ಕಂಡುಕೊಳ್ಳಲ್ಲಿಕ್ಕೆ.

ಸಂಶೋಧನೆಯ ಹಸ್ತಕ್ಷೇಪದ ರೀತಿ: ಈ ಸಂಶೋಧನೆಯು 3 ml ರಕ್ತ ಸಂಗ್ರಹವನ್ನು ಒಳಗೊಂಡಿರುತ್ತವೆ.

ಪಾಲ್ಗೊಳ್ಳುವವರ ಆಯ್ಕೆ: ನಾವು ಪ್ರೀಕ್ಲಾಂಪ್ಸಿಯಾದ ರೋಗನಿರ್ಣಯ ಮಾಡುವ ಮಹಿಳೆಯರನ್ನುಮತ್ತು ಸಾಮಾನ್ಯ ಗರ್ಭಧಾರಣೆ ಮಹಿಳೆಯರನ್ನು ಆಹ್ವಾನಿಸುತ್ತಿರುವಿರಿ (ಪ್ರೀಕ್ಲಾಂಪ್ಸಿಯಾದ ಇಲ್ಲದೆ).

ಸ್ವಯಂಪ್ರೇರಿತ ಭಾಗವಹಿಸುವಿಕೆ: ಈ ಅಧ್ಯಯನದಲ್ಲಿ ನಿಮ್ಮ ಪಾಲ್ಗೊಳ್ಳುವಿಕೆಯು ಸಂಪೂರ್ಣವಾಗಿ ವೈಯಕ್ತಿಕವಾಗಿದೆ.

ಇದರಲ್ಲಿ ಭಾಗವಹಿಸಲು ಅಥವಾ ಇಲ್ಲದಿರುವುದು ಎಂಬುದು ನಿಮ್ಮ ಆಯ್ಕೆಯಾಗಿದೆ. ನಿಮಗೆ ಭಾಗವಹಿಸಲು ಅಥವಾ ಬೇಡವೇ ಎಂಬುದನ್ನು, ನಿಮಗೆ ಈ ಕ್ಲಿನಿಕ್ / ಆಸ್ಪತ್ರೆಯಲ್ಲಿ ಎಲ್ಲಾ ಸೇವೆಗಳು ಸ್ವೀಕರಿಸಲು ಮುಂದುವರಿಯುತ್ತದೆ ಮತ್ತು ಏನೂ ಬದಲಾಗುವುದಿಲ್ಲ.ನಿಮಗೆ, ಈ ಸಂಶೋಧನಾ ಯೋಜನೆಯಲ್ಲಿ ಭಾಗವಹಿಸಲು ಒಂದು ವೇಳೆ ಬೇಡ ಎಂಬುದಾದರೆ, ನಿಮಗೆ, ವಾಡಿಕೆಯಂತೆ ಪ್ರಿಕ್ಲಾಂಪ್ಸಿಯ ಈ ಕ್ಲಿನಿಕ್ / ಆಸ್ಪತ್ರೆಯಲ್ಲಿ ಪ್ರಿಕ್ಲಾಂಪ್ಸಿಯ ಎಲ್ಲಾ ಚಿಕಿತ್ಸೆಯನ್ನು ನೀಡಲಾಗುತ್ತದೆ, ಮತ್ತು ನಾವು ನಂತರ ಅದರ ಬಗ್ಗೆ ಹೆಚ್ಚು ನಿಮಗೆ ತಿಳಿಸುವರು. ನೀವು ನಂತರ ನಿಮ್ಮ ಮನಸ್ಸನ್ನು ಬದಲಾಯಿಸಲು, ನಿಮ್ಮ ಹಿಂದಿನ ಒಪ್ಪಿಗೆ ಸಹ ಮತ್ತು ಭಾಗವಹಿವುದನ್ನು ನಿಲ್ಲಿಸಬಹುದು.

ಪ್ರಶ್ನೆಗಳನ್ನು ಸ್ಪಷ್ಟಪಡಿಸಲು ತಿಳುವಳಿಕೆ : ನೀವು ಈ ಅಧ್ಯಯನದಲ್ಲಿ ಭಾಗವಹಿಸ ದಿದ್ದರೆ, ನಿಮ್ಮ ಆಯ್ಕೆಗಳು ಯಾವುವು? ನೀವು ಈ ಅಧ್ಯಯನದಲ್ಲಿ ಪಾಲ್ಗೊಳ್ಳುವಂತೆ ಇಲ್ಲ ಎಂದು ಗೊತ್ತು, ನೀವು ಒಂದು ವೇಳೆ ಬಯಸಿದಲ್ಲಿ? ನಿಮ್ಮ ಪ್ರಶ್ನೆಗಳು ಯಾವುವು?

ಕಾರ್ಯವಿಧಾನಗಳು ಮತ್ತು ಶಿಷ್ಟಾಚಾರಗಳು: ನಾವು ಒಂದು ಸಿರಿಂಜ್ ಹಾಗೂ ಸೂಜಿ ಬಳಸಿ (ಒಂದು ಸೀಸೆ ತೋರಿಸಲು) 3 ml ರಕ್ತವನ್ನು ನಿಮ್ಮ ತೋಳಿನಿಂದ ತೆಗೆದುಕೊಳ್ಳಬಹುದು. ಸಂಶೋಧನೆಯು ಕೊನೆಯಲ್ಲಿ, 6 ತಿಂಗಳಲ್ಲಿ, ಯಾವುದೇ ಉಳಿದ ರಕ್ತದ

ಮಾದರಿಯು ನಾಶವಾಗುತ್ತವೆ.ಪ್ರಕ್ರಿಯೆಯ ವಿವರಣೆ: ರಕ್ತ ಒಂದು ಸಣ್ಣ ಪ್ರಮಾಣವನ್ನು, ಸುಮಾರು ಒಂದು ಟೀಚಮಚಕ್ಕೆಸಮಾನವಾಗಿರುತ್ತದೆ, ಒಂದು ಸಿರಿಂಜ್ ನಿಮ್ಮ ತೋಳಿನಿಂದ ತೆಗೆದುಕೊಳ್ಳಲಾಗುವುದು. ಈ ರಕ್ತವನ್ನು ಡಿಎನ್ಎ ಬದಲಾವಣೆ ಇರುವಿಕೆಯನ್ನು ಪರೀಕ್ಷೆ ಮಾಡಲಾಗುತ್ತದೆ. ನಮ್ಮಲ್ಲಿ ನಿಮ್ಮ ಆರೋಗ್ಯದ ಬಗ್ಗೆ ಕೆಲವು ಪ್ರಶ್ನೆಗಳಿರುತ್ತವೆ.
ಕಾಲಾವಧಿ: ಸಂಶೋಧನೆ ಒಟ್ಟು 6 ತಿಂಗಳ ಕಾಲ ನಡೆಯುತ್ತದೆ.

ಅಪಾಯಗಳು: ನಿಮ್ಮ ಮೇಲೆ ಯಾವುದೇ ಔಷಧದ ಪರೀಕ್ಷೆ ಮಾಡಲಾಗುವುದಿಲ್ಲ. ರಕ್ತ ಮಾದರಿ ಶುದ್ಧೀಕರಿಸಿದ, ಒಂದೇ ಬಳಕೆಗೆ ಮತ್ತು ಬಳಸಿ ಬಿಸಾಡುವ ಸಿರಿಂಜ್ ಮೂಲಕ ಸಂಗ್ರಹಿಸಲಾಗುತ್ತದೆ.

ಲಾಭಗಳು: ಈ ಅಧ್ಯಯನವು ಕೋಲಾರ ಪ್ರದೇಶದಲ್ಲಿ ಪ್ರೀಕ್ಲಾಂಪ್ಸಿಯಾದ ಆನುವಂಶಿಕ ಆಧಾರವು ಆವಿಷ್ಕಾರ ಸಾಧ್ಯವಾಗಿಸುತ್ತದೆ. ಈ ಅಧ್ಯಯನದ ಫಲಿತಾಂಶಗಳು (ಯಶಸ್ವಿಯಾದರೆ) ಇದು ಸಂಭವಿಸುವುದಕ್ಕೂ ಮುನ್ನ ಪ್ರೀಕ್ಲಾಂಪ್ಸಿಯಾದ ರೋಗನಿರ್ಣಯವನ್ನು ಶಕ್ತಗೊಳಿಸಬಹುದು.

ವೆಚ್ಚವನ್ನು - ಮರಳಿಸುವಿಕೆ: ನೀವು ಈ ಸಂಶೋಧನೆಗೆ ಪಾಲ್ಗೊಳ್ಳಲು ಹಣ ಅಥವಾ ಉಡುಗೊರೆಗಳನ್ನು ನೀ ಡಲಾಗುವುದಿಲ್ಲ.

ಗೋಪ್ಯತೆ: ನಾವು ಸಹಭಾಗಿ ಗುರುತಿಸುವಿಕೆಯನ್ನು ಹಂಚಿಕೆ ಮಾಡಲಾಗುವುದಿಲ್ಲ. ನಾವು ನಿಮ್ಮಲ್ಲಿ ಸಂಗ್ರಹಿಸಿದ ಮಾಹಿತಿಯನ್ನು ಗೋಪ್ಯವಾಗಿಡಲಾಗುತ್ತದೆ ಮತ್ತು ಈ ಯೋಜನೆಯಲ್ಲಿ ಒಳಗೊಂಡಿರುವ ಸಂಶೋಧಕರು ಮಾತ್ರ ಪ್ರವೇಶವನ್ನು ಹೊಂದಿರುತ್ತಾರೆ.

ಫಲಿತಾಂಶಗಳ ಹಂಚಿಕೆ: ಈ ಅಧ್ಯಯನವು ಪಡೆದ ಫಲಿತಾಂಶಗಳನ್ನು ವೈಜ್ಞಾನಿಕ / ವೈದ್ಯಕೀಯ ಪತ್ರಿಕೆಗಳಲ್ಲಿ ಪ್ರಕಟಿಸಲಾಗುವುದು. ಫಲಿತಾಂಶಗಳನ್ನು ವೈಜ್ಞಾನಿಕ / ವೈದ್ಯಕೀಯ ಸಮಾವೇಶಗಳಲ್ಲಿ ಪ್ರಸ್ತುತಪಡಿಸಲಾಗುವುದು.

ನಿರಾಕರಿಸಬಹುದು ಅಥವಾ ಹಿಂಪಡೆ: ನೀವು ಈ ಸಂಶೋಧನೆಗೆ ಪಾಲ್ಗೊಳ್ಳುವಂತೆ ಇಲ್ಲ ನೀವು ಒಂದು ವೇಳೆ ಹಾಗೆ ಬಯಸಿದಲ್ಲಿ ಮತ್ತು ಭಾಗವಹಿಸಲು ನಿರಾಕರಿಸುವುದರೊಂದಿಗೆ ಯಾವುದೇ ರೀತಿಯಲ್ಲೂ ಈ ಆಸ್ಪತ್ರೆಯಲ್ಲಿ ನಿಮ್ಮ ಚಿಕಿತ್ಸೆಯ ಮೇಲೆ ಯಾವುದೇ ಪರಿಣಾಮ ಬೀರುವುದಿಲ್ಲ. ಈಗಲೂ ನೀವು ಈ ಕ್ಲಿನಿಕ್ ನಲ್ಲಿ ಇಲ್ಲದಿದ್ದರೆ ಎಲ್ಲಾ ಅನುಕೂಲಗಳು ಹೊಂದಿರುತ್ತದೆ . ನೀವು ಯಾವುದೇ ಸಮಯದಲ್ಲಿ ಪಾಲ್ಗೊಳ್ಳುವಾಗ ಸಂಶೋಧನೆಯನ್ನು ನಿಲ್ಲಿಸಬಹುದು ಇಲ್ಲಿ ನಿಮ್ಮ ಹಕ್ಕಿನ ಯಾವುದೇ ಒಬ್ಬ ರೋಗಿಯಾಗಿ ನಿಮ್ಮನ್ನು ಸೋಲದೇ ಬಯಸುತ್ತೇನೆ. ಈ ಕ್ಲಿನಿಕ್ ನಲ್ಲಿ ನಿಮ್ಮ ಚಿಕಿತ್ಸೆಗೆ ಯಾವುದೇ ರೀತಿಯಲ್ಲೂ ಪರಿಣಾಮ ಬೀರುವುದಿಲ್ಲ.

ಯಾರನ್ನು ಸಂಪರ್ಕಿಸಬಹುದಾಗಿದೆ: ಅಧ್ಯಯನವು ಪ್ರಾರಂಭಿಸಿದ ನಂತರ ನಿಮಗೆ ಯಾವುದೇ ಪ್ರಶ್ನೆಗಳನ್ನು ಹೊಂದಿದ್ದರೆ ನಮಗೆ ಈಗ ಅಥವಾ ನಂತರ ಕೇಳಬಹುದು. ನೀವು ಇಚ್ಛಿಸಿದರೆ ನಂತರ ಪ್ರಶ್ನೆಗಳನ್ನು ಕೇಳಬಹುದು, ಈ ಕೆಳಗಿನ ವ್ಯಕ್ತಿಯನ್ನು ಸಂಪರ್ಕಿಸಬಹುದಾಗಿದೆ:

ಶ್ರೀಮತಿ ಕೃಷ್ಣವೇಣಿ . ಸಿ.
ಅಂಗರಚನಾಶಾಸ್ತ್ರ ಇಲಾಖೆ
ಶ್ರೀ ದೇವರಾಜ ಅರಸ್ ವೈದ್ಯಕೀಯ ಕಾಲೇಜು, ಕೋಲಾರ.
ದೂರವಾಣಿ ಸಂಖ್ಯೆ: 9972692715
ಇಮೇಲ್: ಕೃಷ್ಣ. ಐ@ಹಾಟ್ಮೇಲ್. ಕಾಮ್

ಭಾಗ **II**: ಒಪ್ಪಿಗೆ ಪ್ರಮಾಣಪತ್ರ

ನಾನು ಮೇಲ್ಕಂಡ ಮಾಹಿತಿಯನ್ನು ಓದಲು, ಅಥವಾ ಇದು ನನಗೆ ಓದಲು ಮಾಡಲಾಗಿದೆ. ನಾನು ಅದರ ಬಗ್ಗೆ ಪ್ರಶ್ನೆಗಳನ್ನು ಕೇಳಲು ಅವಕಾಶ ಹೊಂದಿದ್ದವು ಮತ್ತು ನಾನು ಯಾವುದೇ ಪ್ರಶ್ನೆಗಳನ್ನು ಕೇಳಿದಾಗ ನ ನ ಗೆ ತೃಪ್ತಿಯಾಗಿ ಉತ್ತರಿಸಲಾಗಿದೆ. ನಾನು ಈ ಸಂಶೋಧನೆಯಲ್ಲಿ ಭಾಗವಹಿಸಲು ಸಮ್ಮತಿಯನ್ನು ಇಚ್ಚಿಸುತ್ತೇನೆ .

ಭಾಗವಹಿಸುವವರ ಹೆಸರು _____

ಅಭ್ಯರ್ಥಿಯ ಸಹಿ _____

ದಿನಾಂಕ_____

ದಿನ/ ತಿಂಗಳು/ ವರ್ಷ

ಅನಕ್ಷರಸ್ಥ ಇದ್ದರೆ

ಸಾಕ್ಷರ ಸ್ಥ ಸಾಕ್ಷಿ ಸಹಿ ಮಾಡಬೇಕು (ಸಾಧ್ಯವಾದರೆ ಈ ವ್ಯಕ್ತಿ ಭಾಗವಹಿಸಿದ ವ್ಯಕ್ತಿಯಿಂದ ಆಯ್ಕೆ ಮಾಡಬೇಕು ಮತ್ತು ಸಂಶೋಧನಾ ತಂಡ ಯಾವುದೇ ನಂಟಿರುವುದಿಲ್ಲ) ಅನಕ್ಷರಸ್ಥ ಭಾಗಿಗಳು ತಮ್ಮ ಹೆಬ್ಬೆರಳು ಮುದ್ರಣ ಒಳಗೊಂಡಿರಬೇಕು.

ನಾನು ಸಂಭಾವ್ಯ ಸ್ಪರ್ಧಿ ಒಪ್ಪಿಗೆ ರೂಪ ಕರಾರುವಾಕ್ಕಾದ ಓದುವ ಸಾಕ್ಷಿಯಾಗಿದ್ದರು ಮತ್ತು ವೈಯಕ್ತಿಕ ಪ್ರಶ್ನೆಗಳನ್ನು ಕೇಳಲು ಅವಕಾಶ ಹೊಂದಿದೆ. ನಾನು ಮಾಲಿಕ ಮುಕ್ತವಾಗಿ ಖಚಿತಪಡಿಸಲು ಒಪ್ಪಿಗೆ ನೀಡಿದೆ.

ಸಾಕ್ಷಿಯ ಹೆಸರು_____ ಮತ್ತು ಸಹಭಾಗಿ ಹೆಬ್ಬೆಟ್ಟಿನ ಮುದ್ರಣ

ಸಾಕ್ಷಿಯ ಸಹಿ_____

ದಿನಾಂಕ_____

ದಿನ/ ತಿಂಗಳು/ ವರ್ಷ

ಸಂಶೋಧಕ / ವ್ಯಕ್ತಿ ಸಮ್ಮತಿಯಿಂದ ತೆಗೆದುಕೊಳ್ಳುವ ಹೇಳಿಕೆ

ನಾನು ನಿಖರವಾಗಿ ಸಂಭಾವ್ಯ ಸಹಭಾಗಿಗೆ ಮಾಹಿತಿ ಹಾಳೆಯಲ್ಲಿ ಓದಲು, ಮತ್ತು ನನ್ನ ಸಾಮರ್ಥ್ಯವನ್ನು ಅತ್ಯುತ್ತಮ ಎಂದು ಖಚಿತಪಡಿಸಿಕೊಳ್ಳಲು ಸಹಭಾಗಿ ಗೆ ಅರ್ಥ ಮಾಡಲಾಗುತ್ತದೆ.

೩ ಮಿಲಿ ರಕ್ತ ಸಂಗ್ರಹಿಸಲಾಗುತ್ತದೆ

ಡಿಎನ್ಎ (RELP) ವಿಶ್ಲೇಷಣೆ ಮಾಡಲಾಗುವುದು.

ನಾನು ಸಹಭಾಗಿ ಅಧ್ಯಯನದ ಕುರಿತು ಪ್ರಶ್ನೆಗಳನ್ನು ಕೇಳಲು ಅವಕಾಶ ನೀಡಲಾಗುತ್ತದೆ, ಮತ್ತು ಭಾಗವಹಿಸಿದ ವ್ಯಕ್ತಿಯಿಂದ ಕೇಳಿದಾಗ ಎಲ್ಲಾ ಪ್ರಶ್ನೆಗಳನ್ನು ಸರಿಯಾಗಿ ಮತ್ತು ನನ್ನ ಸಾಮರ್ಥ್ಯದ ಅತ್ಯುತ್ತಮವಾಗಿ ಉತ್ತರಗಳನ್ನು ನೀಡಲಾಗಿದೆ. ನಾನು ವೈಯಕ್ತಿಕ ಸಮ್ಮತಿಯನ್ನು ಮುಕ್ತವಾಗಿ ಮತ್ತು ಸ್ವಯಂಪ್ರೇರಣೆಯಿಂದ ನೀಡಲಾಗಿದೆ.

ಈ ಐಸಿಎಫ್ ಪ್ರತಿಯನ್ನು ಸಹಭಾಗಿ ಗೆ ಒದಗಿಸಲಾಗಿದೆ.

ಮುದ್ರಣ -ಸಮ್ಮತಿಯನ್ನು ತೆಗೆದುಕೊಳ್ಳುವ ಸಂಶೋಧಕ / ವ್ಯಕ್ತಿಯ ಹೆಸರು_____

ಸಮ್ಮತಿಯನ್ನು ತೆಗೆದುಕೊಳ್ಳುವ ಸಂಶೋಧಕ / ವ್ಯಕ್ತಿಯ ಸಹಿ_____

ದಿನಾಂಕ_____

ದಿನ/ ತಿಂಗಳು/ ವರ್ಷ

10. SUMMARY

- The 3 serum biomarkers individually are the (sFlt1, PIGF) first south Indian study with a huge sample size of 300 subjects.
- sEng is the 2nd study in South Indian population.
- The maternal serum concentration of sFlt1 and sEng levels in normotensive pregnant women is low compared to PE women.
- The maternal serum concentration of PIGF levels in normotensive pregnant women is high compared to PE women.
- If we compare the sFlt1 and sEng levels in early and late gestational weeks, in late gestational weeks in controls and PE the levels were highly significant than early gestational weeks of PE and controls. So sFlt1, sEng and PIGF levels can be used as a diagnostic marker in the third trimester for PE in the Kolar population. Rather than an individual marker combination of markers were more significant.
- Serum F levels in PE is the first study and we conclude that F is an added risk factor for PE. The maternal serum F level of ≥1.8 mg/L can be considered as a diagnostic biomarker for predicting PE and the related pregnancy outcomes in Kolar like fluoride endemic areas.
- It can be a routine biochemical laboratory diagnostic marker under routine biochemical tests especially in F endemic areas.
- Combination of 3 objectives with Histopathological evidence is a first study. It indicates that there was a good correlation between serum F levels, and the 3serum biomarkers will definitely help us to pave a way for diagnostic marker in PE in Kolar population.

11. CONCLUSION

The thesis titled **"Serum Biomarkers and Fluoride Estimation with Histopathological Changes in Placentae of Pre-Eclampsia"** is a case-control study with 3 objectives: serum biomarkers, serum F levels and histopathology of placentae as evidence. The serum F levels, serum biomarkers shows significant in PE to Normotensive pregnant women, along with the evidence of histopathology were observed. So molecular level also present study showed significant changes. Due to high F, PE placentae when compared to normotensive placentae had showed statistically significant changes in DVH, hypervascularity, maturity of the villi, avascular villi, crowding of villi and so on. Firmly adhering to the inclusion and exclusion criteria, subjects were selected for the study, and blinding of samples were followed by coding and decoding to avoid bias. After the reports of the parameters were known, the codes were de-coded and samples were assigned to designated groups. The decoding process was done by another external observer selected exclusively for decoding purpose. The decoded data were arranged into different groups and thus bias was totally avoided.

This is the first study to perform with the combinations of serum biomarkers, histopathological changes and F. So F in PE itself is the first study. So this will pave a way in thinking that F like components will adverse the disease condition and more chances of increasing in the incidence of PE like disorders especially in F affected areas. So from this study we need to start a new way of thinking and it is difficult to say it may help for the pathophysiology of PE, but can predict the diagnostic marker for PE especially for Kolar population.As there was an increase incidence of PE. So the epigenetic effects have a lot of influence and can worsen the PE like conditions.