"HISTOPATHOLOGICAL STUDY OF VASCULAR CHANGES IN PLACENTA IN PRE-ECLAMPSIA USING HISTOCHEMISTRY"

By

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DOCTOR OF MEDICINE

IN PATHOLOGY

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LIST OF ABBREVIATIONS

- PE - Pre-eclampsia
- PAS - Periodic acid Schiff
- IUGR - Intrauterine growth retardation
- BMT - Basement membrane thickening
- FN - Fibrinoid necrosis
- PAS-Periodic acid Schiff
- MT-Masson's trichrome
- VS-Verhoeff's stain
- AT 1 - Angiotensinogen 1
- VEGF - Vascular endothelial growth factor
- NK Cell - Natural Killer cell
- DV - Decidual Vasculopathy
- PIH - Pregnancy induced hypertension

ABSTRACT

INTRODUCTION

Preeclampsia is a threat that affects globally, and more so in developing countries. It occurs in 5-8% of pregnancies worldwide, and is second most common cause of maternal and fetal death. The pathological changes in placenta of pre-eclampsia reflects the pathogenesis of the disease condition.

OBJECTIVES OF THE STUDY

To compare vascular changes in stem villi, intermediate villi and terminal villi in placenta of preeclampsia and placenta from normal pregnancy using special stain like Periodic acid Schiff(PAS), Masson trichome and Verhoeff's stain.

To compare vascular changes in spiral artery in placenta of preeclampsia with placenta from normal pregnancy.

MATERIALS AND METHODS:

A total of 120 placentas were included in the study. 60 cases were from preeclampsia patients of which 20 were from severe preeclampsia and 40 from mild pre-eclampsia and 60 were gestational age matched controls. Placental tissue were examined for gross and microscopic changes under the light microscopy using hematoxylin and eosin stained sections. Representative sections were also screened using special stains like periodic acid Schiff to look for basement membrane thickening, Masson's trichrome for medial hypertrophy and Verhoeff's stain for elastic fibre .SPSS 22 ,USA was used for descriptive and analytical data. Chi square was the test of significance. p value <0.05 was considered significant.

RESULTS

Most of our pre-eclampsia cases were in the age group of 20-25 years with significant low birth weight, low placental weight, less diameter and thickness of placenta as compared to control.. The stem villi showed increased stromal fibrosis, thrombosis and medial hypertrophy. The intermediate and terminal villi showed increased basement membrane thickening and fibrinoid necrosis in more than three percent of villi. Terminal villi also showed increased number of avascular villi and increased elastic content in the capillaries. All the parameters were statistically significant when compared between the two groups and these changes were related with the severity of pre-eclampsia.

CONCLUSION

Vascular changes in the villi of the preeclampsia placenta is the basis of the other gross and microscopic changes in the placenta. These changes may bring about differences in function of placenta. Vascular changes and products released may be the reason for the pathogenesis, clinical sequelae onset of disseminated intravascular coagulation, maternal inflammatory syndrome and poor outcome in pre-eclampsia which needed further study.

Keywords:

Pre-eclampsia, Vascular changes, Histochemistry

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INTRODUCTION

Preeclampsia is a threat which affects globally particularly developing countries. It affects 5-8% of pregnancies worldwide, and is second most common cause of maternal and fetal death.[1] The high incidence of pre-eclampsia in developing countries is because of malnutrition, hypoproteinemia and poor obstetric facilities. Overall 10-15% of maternal death directly arises as a consequence of preeclampsia and eclampsia.[2]

Preeclampsia is known to cause morbidity and mortality in the growing fetus such as fetal growth restriction, low birth weight, spontaneous or iatrogenic preterm delivery, respiratory distress syndrome leading to increased admissions to neonatal intensive unit.[3]

Numerous theories have been put forward to describe the pathogenesis of preeclampsia. Some of them are impaired remodeling of the spiral arteries which becomes tortuous, thick walled and narrow leading to reduced blood flow. Few other theories include genetic predisposition, immunological mediated theory, role of vasoactive agents and inflammatory changes and oxidative stress. [4]

On microscopy placenta in preeclampsia shows numerous changes such as increased number of villi, prominence of villous trophoblastic cells, irregular thickening of the trophoblastic basement membrane, abundance of syncytial knot, lack of vasculo-syncytial membranes and increased content of stromal collagen.[5] Studies done using special stains like Periodic acid Schiff and Van Gieson in preeclampsia have shown better appreciation of histopathological changes like increased syncytial knots, paucity of vascular syncytio membrane, basement membrane thickening fibrinoid necrosis and stromal fibrosis of villi.[6]

Although villous changes in placenta of pre-eclampsia are extensively studied not much studies have described the vascular changes in spiral arteries, changes in the vessels of stem villi, intermediate and terminal villi. Hence this study aims at study of vascular changes in pre-eclampsia.

AIMS AND OBJECTIVES

1.To compare vascular changes in stem villi, intermediate villi and terminal villi in placenta of preeclampsia and placenta from normal pregnancy using special stain like Periodic acid Schiff (PAS), Masson's trichrome and Verhoeff's stain

2.To compare vascular changes in spiral artery in placenta of pre-eclampsia with placenta from normal pregnancy.

REVIEW OF LITERATURE

HISTORICAL ASPECTS: [7]

In the Old Testament, placenta was considered as the external soul and sometimes was described as being tied up in the so-called "bundle of life" which probably included the umbilical cord. Placenta is derived from Greek word "PLAKOUS" meaning circular cake.

The earliest insight into this organ comes from drawings of the placenta from illustrators such as Andreas Vesalius. The designation placenta was introduced by Gabriele de Falloppi. Others, however believe that this designation, placenta, stemmed from Realdus Columbus in 1559.

The word chorion and chori meaning "separate" or "distinct" was thought to be first used by Aristotle. The word amnion was introduced by Galen which means 'Lamp' in Greek. Arantius (1564) gave the idea that there was continuity between the maternal and fetal vascular systems. Harvey (1651) opiniated that fetal arterial and venous circulation was linked with the placenta.8 Later, John Mayow (1643-1679) described placental circulation in detail. During the same time Malphigii (1660) established that capillary network was the anatomical basis for the placental regional circulation.

John Hunter and William Hunter (1750) described about the anastomoses between maternal and fetal vessels which was linked through intervillous spaces. William Hunter described decidua and distinguished decidua parietalis from decidua capsularis while John Hunter described the decidua basalis in detail.

Langhans (1882) demonstrated clearly, that villi were covered by two layers of cells. The inner layer of the cells of the villi, the cytotrophoblast that line the intra-villous space, are referred to as Langhans cells.

Hubrecht (1889) introduced the term trophoblast to distinguish the portion of blastocyst cells that do not contribute to the cellular portion of the embryo. Eventually the syncytial nature of the superficial layer of the chorionic villi was demonstrated which is now generally referred to as the syncytiotrophoblast. Wislochi and Dempreyin (1950) published the electron microscopic appearance of placenta.

DEVELOPMENT OF PLACENTA[5]:

Normal formation, implantation and development of placenta ensures adequate feto-maternal transfer of nutrients and waste products which in turn reflects a fetal development.

Placenta inspite of having a very short life span has a varied appearance during the process of development. Also the histological appearance varies from one area to another area.

After fertilization the blastocysts attaches itself to the endometrium at the implantation pole through the proliferation of trophoblastic cell mass that implants to the endometrial stroma.

The series of development that occurs post ovulation are as follows-

Day 7th -

Trophoblast forms a plaque that differentiates into inner layer cytotrophoblast and outer layers of syncytiotrophoblast. These goes on proliferating to form cell mass.

Day 10th to 13th

Series of intercommunicating clefts or lacunae appear in this cell mass by engulfment within syncytiotrophoblast by endometrial capillaries. These spaces are filled with maternal plasma. True maternal blood flow is established only later (12th week of gestation).These lacunae are incompletely separated by trabeculae columns of syncytiotrophoblasts.

14th day to 21th day

The trabeculae column of syncytiotrophoblast gets radially oriented and acquires a cellular core produced by proliferating cytotrophoblastic cells at the chorionic base. These radially oriented column serves as framework from which primary villi will develop later. Placenta at this stage is a labyrinthine, and trabeculae are called primary villous stems. Distal end of villous stem the cytotrophoblast grows continuously into the decidual attachment of basal plate and at the same time in the villous stem mesenchymal core starts appearing .The cytotrophoblasts not invaded by mesenchyme but still anchored to decidual (cytotrobhoblastic cell columns) grows laterally to form trophoblastic shell and splits the syncytium into peripheral syncytium on decidual side and definitive syncytium on fetal side.

Peripheral syncytium disintegrates to form nitabuch's layer. Cytotrobhoblastic shell helps in rapid circumferential growth of developing placenta with expansion of intervillous space into which sprouts from primary villous stem extent. On 21 st day these sprouts form the primary villi and placenta becomes a complete vascularized organ.

21st day to fourth month

The villi towards cavity degenerate-chorion leave. Thin rim of decidua covering this area disappears to allow chorion leave to come into apposition with deciduae of opposite wall of uterus. Villi towards basalis on the side of chorion form chorionic frondosum that forms placenta. Placental septa appear during 3rd month and protrudes from the basal

plate into the intervillous space and divide the maternal space into 15-20 lobes. By the end of fourth month placenta has a definite form and growth occurs only due to development of villous tree.

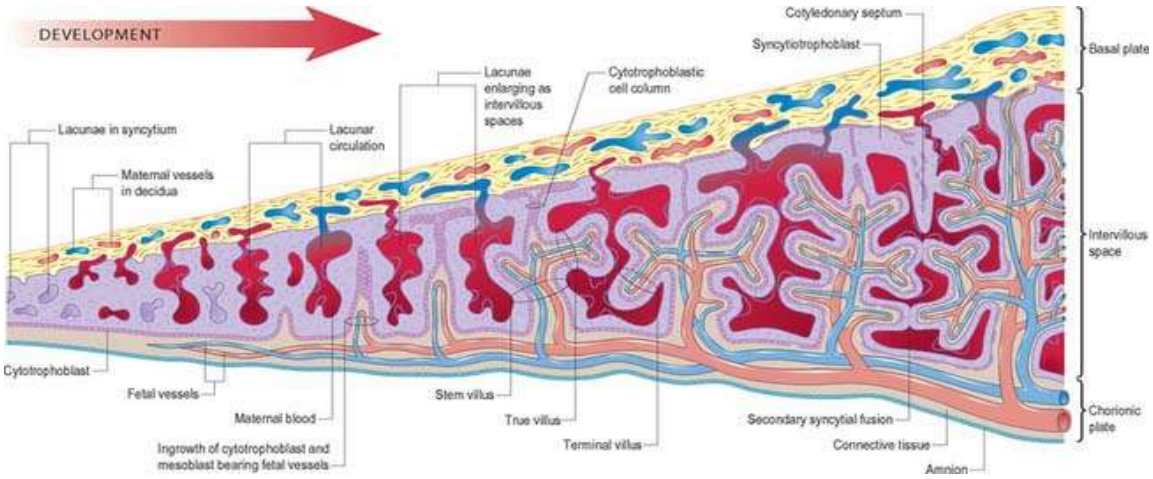


Fig1: Showing development of villi from left to right (http://basicmedical key.com)

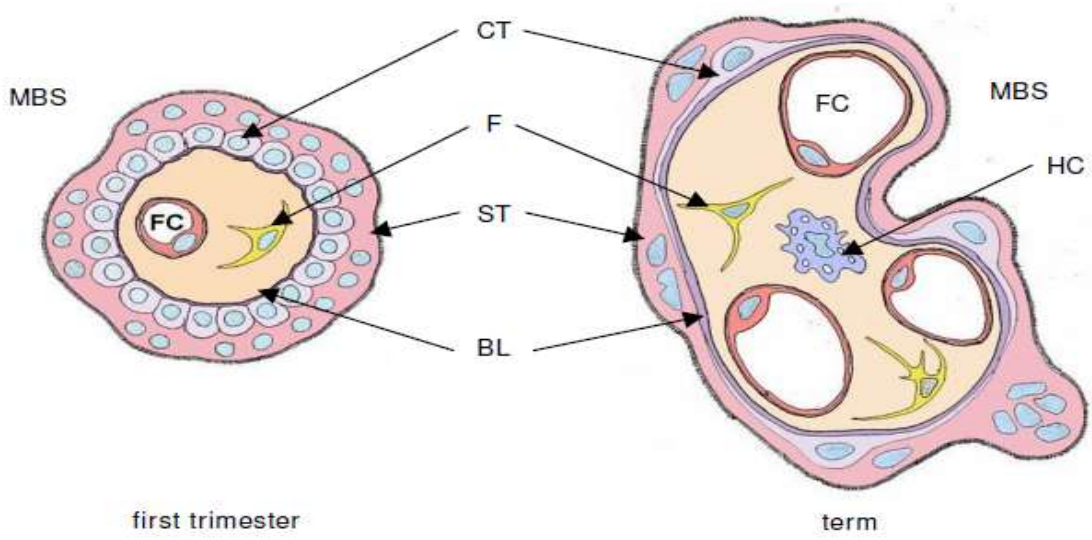


Fig 2 [8]: Evolution of chorionic villi. The chorionic villi, in direct contact with the maternal blood in the maternal blood space (MBS), consist of cytotrophoblastic cells (CT) and syncytiotrophoblast (ST) surrounding a core of mesenchymal cells including fetal capillaries (FC), fibroblasts (F) and Hofbauer cells (HC). BL: basal lamina.

NORMAL ANATOMY OF PLACENTA.[5]

Human placenta is discoid in shape. It is attached to the upper part of the uterine body either at the posterior or anterior wall. After delivery, placenta separates with the line of separation being through decidua spongiosum (intermediate spongy layer of the decidua basalis).

Normal placenta is fleshy, weighs around 500-700gm, and occupies 30% of uterine wall. It has a diameter of 15-20 cm with thickness ranging from 1.5-3.5 cm. Fetal surface is covered by amniotic membrane which is transparent. Normally umbilical cord is inserted in central and have three vessels (Two arteries and one vein). It is spongy to feel and has two surfaces namely maternal and fetal surface.

Fetal surface of placenta

It is covered by shiny and transparent amnion layer overlying the chorion. Umbilical cord is attached at or near its center. The radiating branches of the umbilical vessels beneath the amnion are visible.

Maternal surface of placenta

It is rough and spongy. Maternal blood gives it dull red color and remnants of the decidua basalis gives it shaggy appearance. Divided into 15-20 cotyledons by the septa. Margins of the placenta are formed by fused chorionic and the basal plate.

NORMAL HISTOLOGY OF THE PLACENTA.[5,9]

Placenta is limited by the amniotic membrane on the fetal side and by the basal plate on the maternal side. Between these two, lies the intervillous space which is filled with maternal blood and stem villi with their branches.

The histology of various parts of the placenta are as follows-

Amniotic membrane:

It is lined by a single layer of cuboidal epithelium loosely attached to adjacent chorionic plate and does not take part in placental formation.

Chorionic plate:

Forms the roof of the placenta and consists of syncytiotrophoblasts, cytotrophoblast, extraembryonic mesoderm with branches of umbilical vessels from outside inwards. Syncytiotrophoblast and cytotrophoblast are describe in detail later.

Basal Plate:

Forms the floor of the placenta and consists of compact and spongy layer of decidua basalis, layer of Nitabuch, cytotrophoblastic shell, Syncytiotrophoblast from outside to inward. Basal plate is perforated by the spiral arteries allowing entry of maternal blood into intervillous space.

Layer of Nitabuch:

It is a fibrinous layer formed at the junction of cytotrophoblastic shell with decidua due to fibrinoid degeneration of synctiotrophoblasts. It prevents excessive penetration of the decidua by the trophoblast. Nitabuch membrane is absent in placenta accreta and other morbidly adherent placentas

Intervillous space:

Numerous branching villi arising from the stem villi project into this space.It is lined internally on all sides by the syncytiotrophoblast and is filled with maternal blood

Chorionic villi

It is site where exchange of materno-fetal and feto-maternal blood takes place. The layers which separate maternal and fetal blood are called as vasculosyncytial or placental membrane. This layer is composed of - Syncytiotrophoblasts, an incomplete layer of cytotrophoblasts, trophoblastic basement membrane, connective tissue, endothelial basement membrane and fetal capillary endothelium.

Microscopic appearance of various cells lining a mature chorionic villus are as follows-

Syncytiotrophoblasts

Also called syncytium are multinucleated cells. In term placenta, the nucleus piled up to form syncytial knots. Helps in complex maternal fetal transfer by following mechanism-

a) Helps in catabolism and resynthesize of proteins and fat

b) Synthesis of hormones

c) Helps in facilitated transfer of glucose

d) Transfer of gases and water by diffusion

e) Active transport of amino acid and electrolytes

Villous cytotrophoblasts

It is second trophoblastic layer. These are cuboidal to polyhedral cells having well-defined cell border, vesicular nucleus with dispersed chromatin. Cytoplasm appears clear to slightly granular and basophilic. Occasional mitotic figure seen

Trophoblastic basement membrane

The villous trophoblast is separated from the underlying stroma by the trophoblastic basement membrane which at light microscopic level has a fibrillary structure and measures 20-50nm in thickness. Basement membrane is made up of collagen 1V, Laminin and heparin sulphate.

Villous stroma

Contains fixed connective tissue cells and connective tissue fibers consisting of pre-collagen and collagen, free connective tissue cells (hofbauer cells) and fetal vessels.

In early pregnancy, stromal cells are present in the form of undifferentiated stromal cells or mesenchymal cells. Later mesenchymal cell differentiates to form fibroblasts which further differentiate into myofibroblasts during later pregnancy.

Synctial knots

These are aggregates of syncytiotrophoblasts**.** Apoptotic changes is seen in few syncytial knots which are shedded into the maternal circulation**..**These apoptotic knots have densely packed nuclei showing chromatin condensation and occasional degenerative change**.** Mitotic figure is absent.

Hofbauer cells

These cells are round, ovoid or reniform, measure about 25 ums in diameter and have an eccentrically placed nucleus. Their cytoplasm is coarsely vacuolated during early pregnancy but as gestation progresses the vacuoles decrease in number and size and intracytoplasmic granules become more apparent. Hofbauer cells are simple macrophages are present in the villi at a very early stage of development and persists throughout pregnancy. As the gestation progresses the villous stroma become denser and these cells are masked, they are visible only when there is stromal oedema.

Their main function is immune and non-immune phagocytosis. These cells can trap maternal antibodies that crosses over placental tissues and are probably important source of cytokines, prostaglandins and thromboxane released from the placenta. Most recently it has been claimed that these cells express sporty proteins which modulates branching of villous tree and thus play a role in placental development.

VILLOUS DEVELOPMENT AND VILLOUS TYPES.[9]

The branching of the villous tree can be divided based on calibre, stoma vasculature, function, position within the villous tree and development

The different types of villi are

1. Mesenchymal villi
2. Immature intermediate villi
3. Mature intermediate villi
4. Stem villi
6. Terminal villi

Mesenchymal villi

It develop in beginning of fifth week and is composed of primitive stromal core with loosely arranges collagen. It has poorly developed fetal capillaries, highest mitotic index and few Hofbauer cells. It get transformed into immature intermediate villi

Immature intermediate villi

It develops around eighth week and compromise the majority of villi. At term only few clusters are present**.** These are bulbous in shape with a thick trophoblastic cover and have prominent cytotrophoblast**,** stroma is of reticular type and it also have fluid filled stromal channels.

They further develop to form stem villi.

Stem villi

It starts appearing from eighth week and at term forms 20-25% of placental volume. These are large trunk and branches of villous tree located mostly beneath cord insertion in the central subchorionic area of the placenta. Its main function is to support the structure

of villous tree. They have thick trophoblastic cover with identifiable cytotrobhoblasts on about 20% of the villous surface. They are mature villi mostly covered by fibrinoid necrosis

The stroma of stem villi contains bundles of collagen, occasional fibroblasts and mast cells and few macrophages .Stem villi contain arteries and veins along with arterioles, venules and capillaries.

Mature intermediate villi

They are long, slender. Its stroma contains loose bundles of connective tissue fibres Mature intermediate villi contains many capillaries, small terminal arterioles and collecting venules and forms 25% of the placental volume. They form a zigzag configuration due to branching of terminal villi.

Terminal villi

Terminal villi forms end branch of villous tree. They form grape like outgrowth of mature intermediate villi due to peripheral branching of intermediate villi. There are connective tissue fibers and few macrophages with trophoblastic in close contact with the capillaries. These are filled with numerous fetal capillaries that forms vasculosyncytial membrane with syncytiotrophoblast.

Table 1: Comparison between different types of villi [9]

Villous type	When present	When maximum	% of volume at term	Size(um)	Characteristics features
Mesenchymal villi	5 weeks term	0-8 weeks	<1%	120-250	Primitive stroma. Thick trophoblastic cover, few vessels
Immature intermediate villi	8 weeks term	14-20 weeks	5-10%	100-200	Reticular stroma with fluid filled stromal channels
Stem villi	12 weeks term		20-25%	150-300	Fibrotic stroma, myofibroblastic perivascular sheath, large vessels.
Mature intermediate villi	Third trimester		25%	80-150	Cellular stroma with <50% capillaries
Terminal villi	Term		40-50%	60	>50% capillaries

FUNCTION OF PLACENTA.[7]

The placenta is a crucial organ which plays critical roles in maintaining and protecting the developing foetus. It is directly responsible for modulating the maternal environment which is necessary for normal development of foetus. It an active endocrine organ which secretes hormones, growth factors and cytokines.

Placental function can be summarized as follows:

1. Nutritive Function: It helps in the exchange of nutrient between foetus and mother
2. Excretory function: It helps in the exchange of metabolic wastes like urea, uric acid and creatinine from foetus to mother circulation. It also helps in pH regulation and water balance which are later taken by kidney.
3. Respiratory function :It also helps in exchange of oxygen and carbon dioxide
4. Endocrine function: Placenta produces glycoprotein and steroid hormones, which help maintain homeostasis.
5. Barrier function: It protects the foetus against pathogens and maternal immune system.
6. Hematopoisis of the bone marrow (During early stage of pregnancy)
7. Placental transfer of heat: Fetal heat loss is dependent on umbilical blood flow through the placenta.
8. Immunologic function: Placenta plays a fundamental role in the immunological acceptance of the fetal allograft.
9. Numerous metabolic and secretory function of liver are performed by placenta

Histopathological finding in abnormal placenta.

Macroscopic findings-

1. Calcification:

Placental calcification commonly increases with gestational age. It is uncommon before 36 weeks of gestation. Grossly identifiable calcification is seen in 14-37% of placentae at term [10,11]. These appear as small, firm to hard, scattered, whitish plaques on the maternal surface and give a gritty feel while sectioning. Histologically it is seen as basophilic material on H &E and appears black after Von Kossa stain. The incidence of

histologically detectable calcification is higher in term placenta. A recent study has concluded that the main form of calcification in the placenta is of the metastatic type but dystrophic calcification of dead or degenerative villi can also occur.

2. Infarction:

Placental infarcts focal parenchymal lesions and appears and are macroscopically seen. It appears due to rapid loss of arterial blood supply. A recent infarct is triangular lesion with the base of the infarct pointing towards the basal plate and appears dark red in colour where an old infarct appears yellow or white in colour. Most placental infarcts are due to thrombotic occlusion of the maternal arteries. About 25% of normal placentas contain infarcts involving less than 5% of the placental parenchyma. Excess of placental infarction and retroplacental hematoma can be attributed to abnormalities of spiral arteries which predispose to thrombosis and vessel rupture. Excessive placental infarctions lead to greater probability of IUGR, fetal hypoxia and fetal death [12]

3. Retroplacental hematoma:

Retroplacental haematoma lies between and separates the basal plate of the placenta and the uterine wall. They are usually soft, red and can be easily separated from the maternal placental surface. They can be associated with abruption placentae, pre-eclampsia, preterm delivery and placentae with large areas of infarction.[6]

4. Perivillous and subchorionic fibrin deposit:

Plaques of perivillous fibrin deposition are seen mainly in the peripheral areas of the placenta and are related to adverse fetal outcome when the deposits occupy more than 30% of villous surface.[14,15]

Microscopic findings-

1. Excessive number of syncytial knots

Syncytial knots are considered as an accumulation of apoptotic nuclei that arises from surface of villi and protrudes into the intervillous space and is considered as degenerative process.[16] Some theories suggest that they occur due to hypoperfusion of villi secondary to obliteratively lesion of fetal stem arteries. Another study demonstrated using phase contrast microscopy that knots are representative of reactivation of syncytiotrophoblast. In another study it was shown that reduced intra placental oxygenation resulted in increased syncytial knotting. Normally they are uncommon before 32 weeks of gestation .At term 10-30 of the villi will have knots. Increased syncytial knots are seen in pre-eclampsia, gestational diabetes and IUGR. If syncytial knots are seen in more than 30% of the villi they are considered excessive and indicates ischemia .[17] Excessive knots are also associated with fetal stress. [18] There are many theories which explain the formation of these syncytial knots. One study done by examining the placentae under phase contrast microscopy tells that they represent reactivation of the syncytiotrophoblast .[19]

Other studies tell that they occur as a result of hypo perfusion of villi secondary to obliterative lesion of fetal stem arteries. Recent studies have demonstrated that reduced intra-placental oxygenation resulted in aggregated, web like arranged villous profiles with impressive syncytial knotting.[16] It is seen in condition like pre-eclampsia.[20]

2. Vasculo-syncytial membranes:

Vasculo-syncytial membrane is attenuated anuclear syncytiotrobhoblast which are stretched over and are in close apposition to a capillary. It was first described by Bremer in 1916. These membrane is uncommon before 32 weeks and rapidly increase between 32 weeks and term. At term 20% of villi show vasculosyncytial membrane. [21]

Vasculo-syncytial membrane rapidly increases in them between 32 weeks and term and is relatively uncommon before 32 weeks. Deficiency of vasculosyncytial membrane (<5%) of villi showing vasculo-syncytial membranes is seen in placenta from women having pre-eclampsia, diabetes mellitus and Rh incompatibility.[22]

It is an indicator of fetal villous circulation.

3. Fibrinoid necrosis of villi

Fibrinoid necrosis appears as homogeneous PAS positive deposits and contains few degenerate syncytial nuclei. On immuno-histochemical studies it is known to contain laminin, collagen IV, oncofetal protein and fibronectin without any fibrin material. Their normal function is to provide mechanical stability of placenta and shaping of intervillous space.

Normally 3% of the villi in mature placentas show fibrinoid necrosis. [16]. More than 3% of the villi having fibrinoid necrosis is seen in complicated pregnancies including pre-eclampsia,diabetis and materno-fetal rhesus incombatibility.[23,24,25]

It is hallmark of an immunological reaction within the trophoblastic tissue.

4. Stromal fibrosis:

There is a little collagen in the stroma of mature villi. Throughout pregnancy stromal collagen gradually increases and at term the stroma shows a delicate network of fibrous tissue. More 3 percent of the villi is seen in prolonged pregnancies, pre-eclampsia as well as in placenta from diabetic women.[26] It is considered as a morphological hallmark of reduced villous perfusion. [16]

5. Basement membrane thickening:

Exact thickness of the cytotrophoblastic basement membrane is not known because of interobserver variability in assessing the thickness. They can be demonstrated by using special stain by PAS and IHC for collagen 4 or laminin. Thick basement membrane is seen in placenta from women having pre-eclampsia, essential hypertension or IUGR, diabetes, materno-fetal rhesus incompatibility and from women who smoke.[27,28] It occurs due to chronic villous ischemia and is associated with impaired transport across chorionic villous membrane. Cytotrophoblastic hyperplasia appears to be an indicator of ischemia and ischemic severity.

6. Cytotrophoblastic cell proliferation

Villous cytotrobhoblastic cells proliferation are prominent in early stages of gestation and this proliferation disappears as pregnancy advances.[29] However, in placenta from diabetic women, maternofetal rhesus incombatibility, pre-eclampsia and idiopathic intrauterine growth retardation of the fetus the cytotrophoblastic proliferation will increase even if pregnancy advances.[30,31]

This increase in such cells occurs because of failure of cytotrophoblastic regression and a proliferation of cytotrobhoblastic cells.

7.Villous edema

Villous edema is usually not found in term pregnancy. If found in later pregnancy it can be because of diabetic women, pre-eclampsia as well as in placental infections like syphilis, toxoplasmosis, parvovirus and cytomegalovirus and also in placentas containing a large haemangiomas or metastasis from a fetal neuroblastoma, congenital nephrotic syndrome, fetal cardiac disorders, fetal anaemia, hydatidiform mole and maternal hyperthyroidism.[32,33,34]

8. Vessels in terminal villi

Normally terminal villi of the mature placenta contain two and six sinusoidally dilated capillary vessels, that occupy the majority of the cross-sectional area of the villi.

Three abnormalities of villous vasculature can be seen- Avascualarity, hypovacularity and hypevasularity.

Avascular villi – Placentas from were noted by Gruenwald in placentae from babies of low birth weight and stillborn infants.[35]

Avascular villi-It is classified in three group. Small foci are the finding of 3 or more foci of 2 to 4 terminal villi showing bland hyaline fibrosis and total loss of villous capillaries of the villous stroma. Intermediate foci are 5 to 10 villi, and large foci are more than 10 villi showing hyaline fibrosis .[36]

Hypo-vascular villi - seen in placentae from prolonged pregnancies .[36]

Hyper-vascular villi (cholangiosis), also called diagnosed when 10 villi, each with 10 or more vessels in 10 or more infarcted area is seen under 10X objective as described by Altshuler and he also found that cholangiosis was associated with perinatal death and congenital malformation.[37]

9. Obliterative endarteritis

This is characterised by an apparent swelling of the intimal cells of the fetal stem arteries with thickening of the subendothelial basement membrane. It is seen in pre-eclampsia, maternal-fetal rhesus incombatibity, diabetic women and premature onset of labour.[38] It is an indication of uteroplacental ischemia and/or fetal hypoxia and leads to reduced fetal perfusion of the villi, stromal fibrosis and excessive formation of syncytial knots.[4]

10. Spiral artery atherosis

Atherosis is seen in the intradecidual portion of the spiral arteries in the placental bed biopsy which is characterised by fibrinoid necrosis of the vessel wall with an accumulation of fat-containing macrophages and a mononuclear perivascular infiltrate. This features is seen in pregnancy complicated by pre-eclampsia,fetal intrauterine growth restriction,hydatidiform mole [41] and in the maternal thrombophilic conditions such as homocysteinaemia and antiphospholipid antibody syndrome.[42.] This occurs due to complete lack of trophoblastic invasion and is indicative of impaired development of the uteroplacental vaculature.[4]

11. Medial hypertrophy

Media Hypertrophy is hypertrophy of tunica media in the arteries of stem villi. It is usually seen in severe pre-eclampsia. It is secondary to development of hypertension and acts as a protective mechanism of the blood vessels against high pressure. A study done in severe pre-eclampsia with mean diastolic blood pressure 110mmHg showed hypertrophy of tunica media. This hypertrophy may be secondary to the development of hypertension and may be a protective mechanism of the vessels against high pressure. [41,42]

12. Elastic content in vessel wall

It is characterised by increased proliferation and elastic secretion leading to thick vessel wall seen in conditions that increases the local stress. It is a protective phenomenon adapted by the placental blood vessel tree against stressful factors. It has been proved in hypertensive disorders where increased pressure leads to increase in the elastic content of the vessels.

Hypertensive disorder of pregnancy

Pregnancy induced hypertension is defined as the hypertension that develops as a direct result of the gravid state. It includes:

1. Gestational hypertension
2. Preeclampsia
3. Eclampsia

GESTATIONAL HYPERTENSION:

Gestational hypertension is defined as development of hypertension with blood Pressure >140/90 mm of Hg for first time during pregnancy without proteinuria. Blood Pressure return to normal within 12 weeks post-partum. Final diagnosis is made only post-partum.

The patient may have other signs of pre-eclampsia, for example, epigastric discomfort or thrombocytopenia.

PRE-ECLAMPSIA

Pre-eclampsia is defined as blood pressure greater than 140/90 mm of Hg with proteinuria after 20 weeks of pregnancy.

American College of Obstetrics and Gynecologists classify preeclampsia as mild and severe.

Other classification by Lindheimer and co-workers classifies pre-eclampsia as severe and non-severe pre-eclampsia.

Table 2: Indicators of severity of gestational hypertensive disorders

Abnormality	Non-severe	Severe
Diastolic blood pressure	<110 mm Hg	>110 mm Hg
Systolic blood pressure	<160 mm Hg	>160 mm Hg
Proteinuria	<2+	>3+
Headache	Absent	Present
Visual disturbance	Absent	Present
Upper abdominal pain	Absent	Present
Oliguria	Absent	Present
Serum creatinine	Normal	Elevated
Thrombocytopenia	Absent	Present
Serum transaminase elevation	Minimal	Marked
Fetal growth restriction	Absent	Obvious

3.Eclampsia

Eclampsia is seizures in a woman with preeclampsia that cannot be attributed to other causes. It is classified according to the time of onset as antepartum, intrapartum and postpartum or intercurrent.

EPIDEMIOLOGY

Preeclampsia is a major public threat in pregnancy globally in developing countries. It occurs in 5-8% of pregnancies worldwide, and is second most common cause of maternal and fetal death. The incidence is high in developing countries because of malnutrition,

hypoproteinemia and poor obstetric facilities. Overall 10-15% of maternal death directly arises as a consequence of preeclampsia and eclampsia.

A systematic review by the World Health Organization have shown that hypertensive disorders is responsible for 16% of all maternal deaths in developed countries, 9% of maternal deaths in Africa and Asia, and 26% in Latin America and the Caribbean which has highest incidence.[43]

Pre-eclampsia is associated with morbidity and mortality both in the mother and the fetus. In mother, it can cause cardiac dysfunction or arrest, respiratory compromise, coagulopathy, and liver failure, renal failure, stroke.[44]

In fetus it is associated with fetal IUGR, small for gestational age, prematurity (neonatal death and long term neonatal morbidity). In developing countries infant mortality is associated with pre-eclampsia in one quarter of stillbirths and neonatal deaths which is three times higher when compared to developed countries due to the lack of neonatal intensive care facilities.[44]

In non-pregnant women of developing countries hypertension is a common problem which increases the incidence of pre-eclampsia as chronic hypertension is one of the main risk factor of pre-eclampsia.[45]

Pre-eclampsia contributes to approximately 12 to 25% of fetal growth restriction and small for gestational age infants as well as 15 to 20% of all preterm births and is associated with complications of prematurity leading to neonatal morbidity and mortality.[46]

RISK FACTORS FOR PRE-ECLAMPSIA

I) Pre-conceptional and/or chronic risk factors:

• Nullipara/ primipara/ teenage pregnancy-Nulliparity almost triples the risk of preeclampsia,hence it is a strong risk factor.[47]

• History of previous pre-eclampsia

• Age interval between pregnancies

• Family history-A family history of preeclampsia nearly triples the risk of preeclampsia.[47]

II) Presence of specific underlying disorders

• Pre-existing medical conditions like hypertension, obesity obesity, and vascular disorders (renal disease) are associated with preeclampsia.[48,49]

Compared to general population women with underlying chronic hypertension have a 10-25% risk of developing preeclampsia.[50,51,52]

The overall risk of developing preeclampsia with pre-gestational diabetes is approximately 21%.[53,54]

The overall risk of preeclampsia due to obesity increases by approximately 2- to 3-fold times.[55]

• Activated protein C resistance, protein S deficiency

• Autoimmune disease

-Preeclampsia is also known to occurs more frequently among pregnant women with associated autoimmune conditions such as systemic lupus erythematosus and antiphospholipid antibody syndrome.[47]

• Hyper homocysteinemia

• Sickle cell disease and trait

III) Exogenous factors

- Smoking (risk reduction)
- Stress, work related psychosocial strain

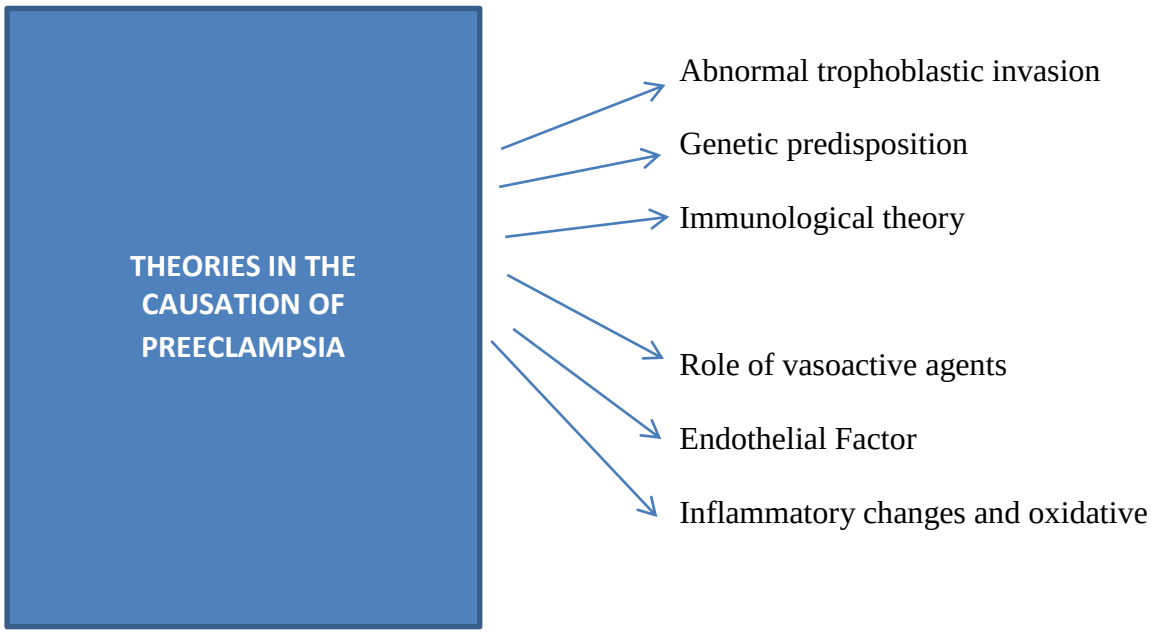
IV) Pregnancy associated risk factors

- Multiple pregnancy
- Structural congenital anomalies
- Hydrops fetalis
- Chromosomal anomalies (trisomy)

V) Placental factors

Excess placental volume (hydatidiform moles and multi-fetal gestations).[56,57]

THEORIES ABOUT ETIOPAHTHOGENESIS OF PRE-ECLAMPSIA



1.ABNORMAL TROPHOBLASTIC INVASION

Cytotrophoblast cells invade the uterine spiral arteries during early human pregnancy and completely remodel it into large capacitance vessels with low resistance replacing not only the endothelial layers of these vessels but also causes subsequent destruction of the highly muscular tunica media. [58] The uterine spiral arteries are lined exclusively by cytotrophoblast by the end of the second trimester of pregnancy.

Shallow placental cytotrophoblast invasion of uterine spiral arterioles in preeclampsia, leads to reduced placental perfusion and thus causing placental insufficiency.

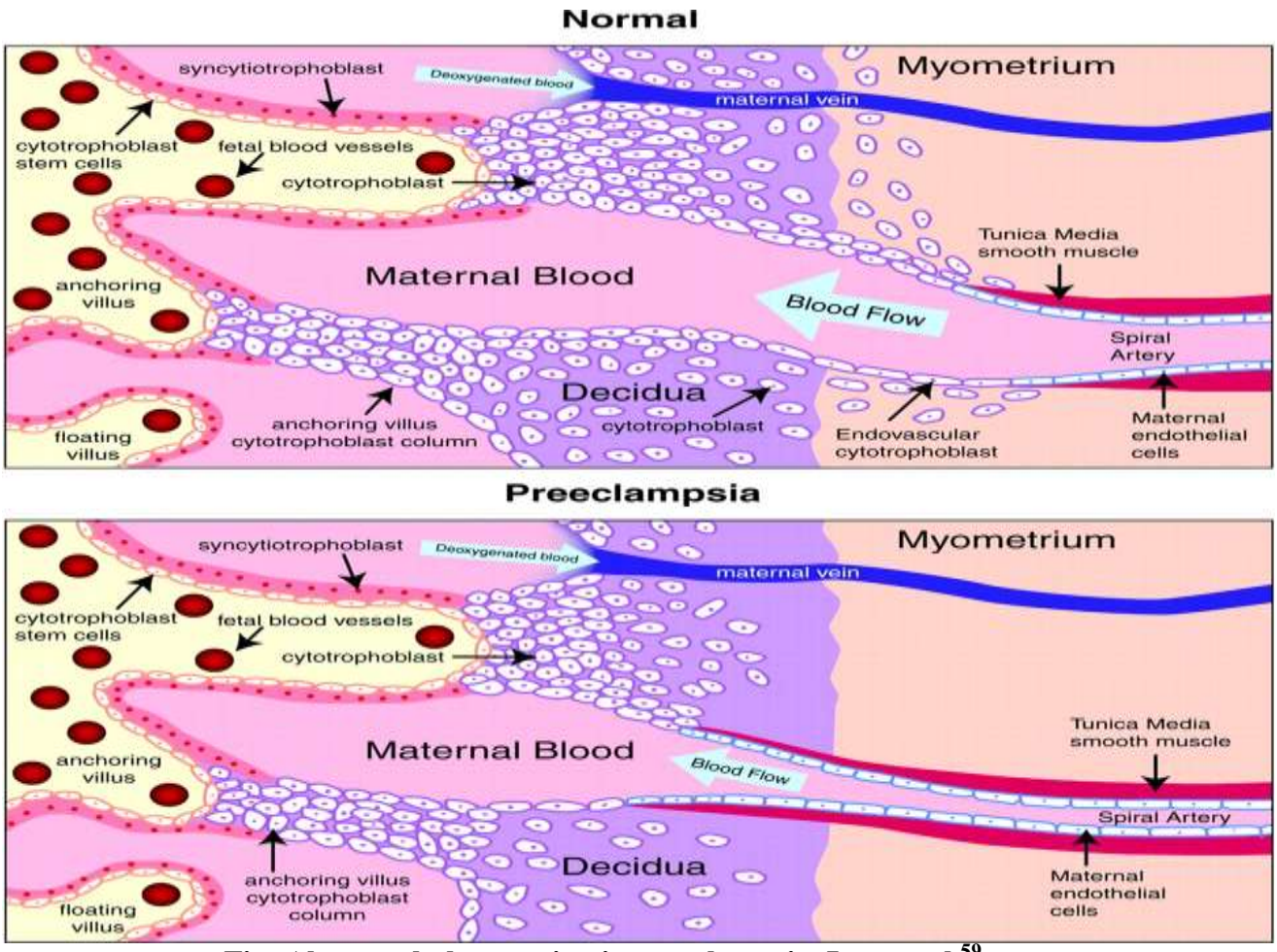


Fig: Abnormal placentation in preeclampsia. Lam et al.[59]

2.GENETIC PRE-DISPOSITION

A wide variety of genes in associations with preeclampsia have been identified. These genes probably interact in the hemostatic and cardiovascular systems.It also has role in the inflammatory response. Some of the genes which have been identified, and in candidate gene studies they have provided evidence of linkage to several genes includes angiotensinogen on 1-q42–43 and eNOS on 7q36; other main important loci are 2p12, 2p25,9p13, and 10q22.1.16 [60]

3.IMMUNOLOGICAL THEORIES

A study showed that sera from women with preeclampsia contain autoantibodies has stimulatory effect on cardiac myocytes, trophoblast cells, endothelial cells, mesangial cells, and vascular smooth muscle cells by reacting with the Angiotensinogen 1(AT1) receptor. These autoantibodies activate AT1 receptors on and are thought to have role in pathogenesis in preeclampsia.[61]

4.ROLE OF VASOACTIVE AGENTS

Normally, pregnant women have refractoriness to vasopressor substances namely. Angiotensin II, nor epinephrine and vasopressin. In preeclampsia this refractoriness is lost and there is increased vascular reactivity.

Vasoactive agents which bring about these changes are:

i. Prostaglandins: The exact mechanism how prostaglandins mediate vascular reactivity during pregnancy is not known however there is elevation of plasma and urine levels of thromboxane whereas there is reduced synthesis of prostaglandins, such as prostacyclin leading to vasoconstriction.[62]

ii. Endothelins: Endothelial-derived factor endothelin play a role in preeclampsia. It is the vasoconstrictor and is released due to endothelial damage .Increased production of endothelin may play a role in pathophysiology of preeclampsia.[63]

iii. Vascular Endothelial Growth Factor (VEGF): It is a glycoprotein which causes vasculogenesis in human placenta and plays a role in microvascular permeability. A study showed low level of VEGF levels in maternal serum in pre-eclampsia compared to normotensive pregnancy and non-pregnancy state which explains the pathophysiology of early-onset pre-eclampsia contributed by endothelial dysfunction.[63]

Circulating Angiogenic Factors in Preeclampsia: In a study in rodents a casual association of two endogenous antiangiogenic proteins namelye fms-like tyrosine kinase 1 (sFlt1) and soluble endoglin; have been suggested which circulates in the blood and have profound agonistic effect on VEGF and placental growth factor .[64]

5.ENDOTHELIAL FACTOR

Endothelial cell activation due to damage plays an important role in the pathogenesis of pre-eclampsia. The normal endothelium is lined by a single layer of endothelial cells lining the vessel lumen and is in contact with the blood. Any damage to endothelium activates the coagulation pathway and increases sensitivity to vasopressin agents.[64]

6.INFLAMMATORY CHANGES AND OXIDATIVE STRESS-

Preeclampsia is associated with oxidative stress. In pre-eclampsia, there is imbalance between prooxidants and antioxidants leading to cell or tissue damage.

Vascular endothelial damage which is thought to be due to free radical mediated lipid peroxidation plays an important role in the pathophysiological mechanism of preeclampsia. There are data which suggests that there is increase in lipid peroxidation products and decrease in antioxidant activity in preeclampsia compared with normal pregnancy.

Plasma level of reduced ascorbic acid a water-soluble antioxidant, are decreased in mild and severe preeclampsia. Levels of α tocoferol and β carotene which are lipid soluble antioxidants, having capacity to quench free oxygen radicals are decreased only in severe form of preeclampsia.[65]

Newer theory on pathogenesis of pre-eclampsia:

• **Role of Natural Killer (NK) cells:**

Aberrant NK cell activation may be the cause of preeclampsia, but their exact role is still uncertain. It has been hypothesized that NK cell play a role in the remolding of the spiral arteries in stripping off the muscular wall of the spiral arteries and displacing endothelial cells that line these vessels making them low resistance channels. The immunomarker of NK cell is CD56 [66]

• **Placental Apoptosis in Preeclampsia:**

A study showed that there was an increased cytotrophoblast apoptosis in preeclampsia .[67] Apoptotic cascade initiation phase takes place in cytotrophoblast while execution phase in syncytiotrophoblasts. Apoptotic nuclei accumulate as syncytial knots. Though these particles are released into maternal circulation. An increase in the circulation of these particles may be a reason for maternal endothelial disruption in preeclampsia. A recent

study supported the above hypothesis and detected apoptotic syncytiotrophoblastic microparticles (STMP) in the serum by Elisa method.[68]

Brief review of histopathological changes in placenta in pre-eclampsia patient

Following is a review of the histopathological changes of placentae in preeclampsia Pre-eclampsia, with important contributions. The common pathological features in placenta in preeclampsia which is specific to the disease is infaction, intervillous thrombosis, abruption placentae and decidual arteriopathy. Other changes are nonspecific.[69,70]

Studies using special stain like per-iodic acid Schiff and Von Gieson showed increased syncytial knots, paucity of vascular syncytio membrane, fibrinoid necrosis, basement membrane thickening and villous stromal fibrosis and avascular villi.[7,71] Other studies in addition showed areas of marked branching angiogenesis and intramural lipid deposition in the wall of uterine vessels.[72]

The stem villi of the placenta in preeclampsia has numerous arteriosclerotic blood vessel with endothelial degeneration with progressive fibrosis, villous perivasculitis and narrowing of lumen and there is decreased diameter and density of fetal blood vessels in transverse villi which was showed in study done on morphometrical analysis. [73] Other study on morphometry showed that terminal villi and surface area were compromised in early onset preeclampsia cases, late onset had no impact on peripheral villi or vascular feature.[74]

Total decidual vasculopathy which is specific finding in preeclampsia correlated with adverse maternal and fetal outcome, more relatively with perinatal mortality.[75]

METHODOLOGY

Type of study

Observational study

Duration of study

The duration of study was from February 2016 to October 2017(1 Year 8 months)

Source of data

All the placenta specimen which were sent from Department of Obstetrics and Gynecology of R.L Jalappa Hospital and Research Centre to the Department of Pathology, Sri Devaraj Urs Medical College. A detail of the procedure was explained to the patient in their own language and consent was taken.

Inclusion criteria

Placenta from all cases of preeclampsia

Exclusion criteria:

1. Chronic hypertension
2. Fetal congenital abnormality in newborn.
3. Twin pregnancy.
4. Hypothyroid patient.
5. Clinically detected other medical condition like Heart disease, Systemic lupus erythematous, Rh incompatibility.

Sample size

Sample size was estimated from study done by Jena M et al[7] by comparing all the histological features of placenta in both control group and PIH group using the following formula for difference in proportion:

- N= 2 (Zα/2 + Zβ /)2 P (1-P) / (P1 – P2)2
- Independent T test and Chi square test will be applied.
- At α=0.01, Power is 90%
- Zα/2 at 0.01=2.58, Zβ at 90%=1.28
- P1 =76%, P2 =100%, P= P1 + P2 /2
- Using this formula P=88%
- And N=55 in each group

By using 10% non-responsive rate 55+5 = 60 cases and 60 controls (120 subjects) were estimated.

Method of collection of data (including sampling procedure, if any):

The clinical history like age of mother, blood pressure, severity of pre-eclampsia(American college of Obstetrics and Gynecologists) and birth weight of neonate was recorded. Only the placenta from term pregnancy were collected. The placentae were washed in running tap water to remove any blood clot and weight, diameter and thickness, nature of membrane, insertion of umbilical cord and number of vessels in umbilical cord were noted following which it was cut in regular interval of one cm (Bread and slice technique) and gross lesions like calcification and infarction were noted. Infarction involving more than 5% of placental parenchyma was considered significant.

The whole specimen was left for fixation in 10% neutral buffered formalin for 24 hours. After fixation five tissue bits were taken from periphery, five from central region and one bit from umbilical cord. These tissue bits were put in labeled cassettes and were processed routine histopathological processing of our department using LEICA Tissue Processor

TISSUE PROCESSING

- Formalin ---------- 7 Hours
- Water ---------- 20 Minutes
- 60% Isopropyl alcohol---------- 1 Hour
- 70% Isopropyl alcohol ---------- 1 Hour
- 80% Isopropyl alcohol---------- 1 Hour
- 90% Isopropyl alcohol---------- 1 Hour
- 100% Isopropyl alcohol---------- 1 Hour
- 100% Isopropyl alcohol---------- 1 Hour
- Chloroform I ---------- 1 Hour
- Chloroform II ---------- 1 Hour
- Paraffin Wax I ---------- 1 Hour
- Paraffin Wax II ---------- 1 Hour
- Total ---------- 17 Hours 20Minutes

After processing the section were embedded and tissue section of 5 um was cut by the routine histopathological procedure of our department.

The slides were stained with hematoxylin and eosin using by following steps-

- The section was deparrafinize and hydrated through grading of alcohols to water
- It was kept in Harris hematoxylin for 4 mins
- Rinsed in running tap water for 5 mins
- Differentiated in 1 % acid alcohol for 5 secs
- Washed well in Tap water for 5 mins (Until Blueing)
- Dipped in Eosin for1 min
- Rinsed in Tap water for 5 mins
- Dehydrated in grading of alcohols in ascending order.

- Dipped in Xylene (Clearing)
- Mounted in DPX

All the hematoxylin and eosin slides were screened under the microscope for the presence of following histopathological changes. Stem villi were looked for any stromal fibrosis, medial hypertrophy and thrombosis. Intermediate villi were looked for fibrinoid necrosis. In term placenta ≤ 3 percent of villi can be fibrotic and if more than 3 percent of villi in a placenta are fibrotic it is considered as abnormal. Terminal villi were looked for fibrinoid necrosis and avascularity which was classified as large foci, intermediate foci and small foci of avascular villi. Small foci are the finding of 3 or more foci of 2 to 4 terminal villi showing bland hyaline fibrosis of the villous stroma and total loss of villous capillaries. Intermediate foci are 5 to 10 villi, and large foci are more than 10 villi showing hyaline fibrosis.Also spiral artery was searched for acute atherosis changes like thrombosis and foam cell infiltration.

Slides were selected for special staining like periodic acid Schiff (PAS), Masson's trichrome (MT) and Verhoeff's stain(VS) and steps of staining procedure was as follows-

PROCEDURE FOR PERIODIC ACID SCHIFF STAINING

(Control-Appendix)

- Deparaffinised section was hydrated through graded alcohols to water.
- Sections was brought to water and oxidised with 1% periodic acid for 10 min.
- Section was washed with distilled water
- It was covered with Schiff's reagent for 20 minutes and then washed in tap water for 10 minutes.
- The nuclei were stained with an Alum Hematoxylin for one minute.
- Then it was dehydrated in graded alcohols, dipped in xylene and mounted in DPX.

RESULT:

- PAS positive material stain magenta pink to red
- nuclei stain blue.

PROCEDURE FOR MASSON'S TRICHROME STAINING

(Control-Aorta)

- Deparaffinised section was hydrated through graded alcohols to water.
- Section was kept in bouin's solution overnight
- Section was washed in running water till yellow color disappeared.
- Weigert's Hematoxylin was added for 15-20 min and will be differentiated in acid alcohol
- It will be washed in water for 5 minutes.
- Beibrich scarlet –Acid fuschin was added and rinsed in water after 10 minutes.
- Mordant (PTAH) was added and kept for 10 minutes.
- 2.5% Methylene blue was added for 2 sec
- 2% acetic acid for 5 minutes
- Section was dehydrated, cleared in xylene and mounted finally.

RESULT:

Muscle, cytoplasm, keratin- red,

Collagen and cartilage-blue/green

Nuclei -black

PROCEDURE FOR VERHOFF'S STAIN (VS)

(Control-Aorta)

- Deparaffinised section was hydrated to distilled water.

- It was then stained in freshly prepared verhoeff's mixture in a closed container for 15 minutes and washed in water.
- It was then differentiated in 2% Ferric chloride solution, checking each section microscopically until the nuclei and fine elastic fibres were stained black with weakly stained background.
- It was then washed in water and then in 95% alcohol momentarily to remove iodine colouration and washed in water for 5 minutes.

RESULTS:

- Nuclei and elastic fibres-Black
- Collagen fibres-Red

The slides were screened for the following histological findings –

Different stain used to observe histopathological parameters

Parameters	H&E	PAS	MT	VS
Basement Membrane Thickening		√		
Fibrinoid necrosis	√	√		
Thrombosis	√			
Medial hypertrophy			√	
Stromal fibrosis	√	√	√	
Elastic content				√
Avascular villi	√			

Morphometry analysis for measuring villi size and medial hypertrophy was done using Primo star Ziess microscope, Axion cam ERc 5s Ziess Camera, Using software ZEN2.3, version 2.3.69.01000

In this study, microscopic appearance of all the three villi were identified based on the size the stem villi were largest in diameter and has arteries and veins, intermediate was second largest and consisted of capillaries and gave branching to terminal villi while terminal villi were smallest in diameter with 6-10 capillaries without any branching. Morphometry was done in any case of confusion between size of terminal and intermediate villi and villi with the diameter less than 60 um was considered as terminal villi.

Areas of fibrinoid necrosis were excluded as the morphology of villi was hampered.

The medial hypertrophy was measured in micrometer in Masson's trichrome stained slides using morphometry. The stem villi vessels having largest diameter was measured by morphometry.

Statistics analysis

The data was entered into Microsoft excel data sheet. Analysis was done using SPSS 22(Statistical package for social sciences version 22), USA. Chi square test was the test of significance used to find the association between the various gross and histological parameters in placenta from pre-eclampsia and that of control group. p- value <0.05 was considered statistically significant while p-value of <0.001 was considered highly significant. The parameters where chi-square test was not applicable, Fisher exact test was used. Bar diagram was plotted to show the association for each parameter.

For the sake of comparison of our studies with other studies p value was calculated separately for mild pre-eclampsia and severe pre-eclampsia group.

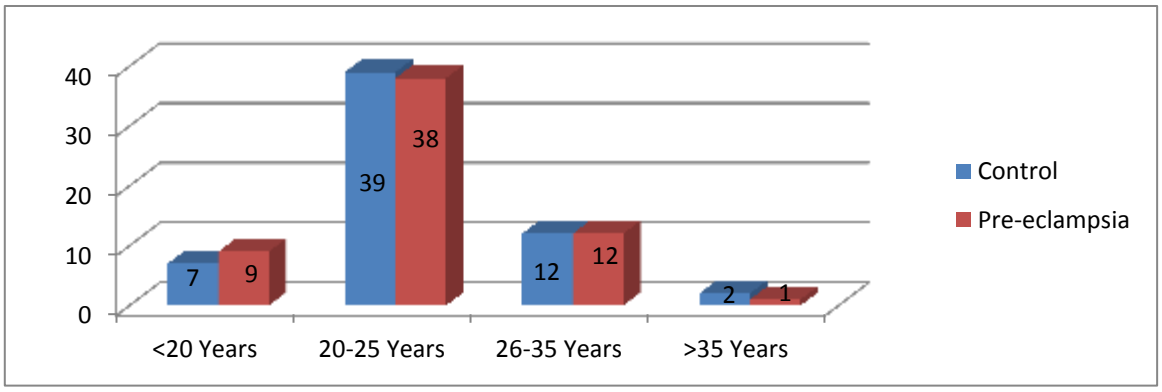
RESULTS

A total of 120 placentae were studied out of which 60 (50%) placentae were from normal term pregnancy which formed the control group and 60 (50%) placentae were from pregnancy with pre-eclampsia (BP > 140/80). Of these 40 cases were of mild pre-eclampsia, 20 cases of severe pre-eclampsia. All the mothers in the control group and the pre-eclampsia group satisfied the selection criteria.

Table 3: Age distribution among cases and controls

Age Group	Control (n=60)	Pre-eclampsia (n=60)	Pre-eclampsia	
			Mild PE(n=40)	Severe PE(n=20)
<20 Years	07 (11.67%)	9 (15%)	6 (15%)	3(15%)
20-25 Years	39 (65%)	38 (63.33%)	25 (62.5%)	13(65%)
26-35 Years	12 (20%)	12 (20%)	8(20%)	4(20%)
>35 Years	02 (3.33%)	1 (1.67)	1(1.67%)	0

Chart 1: Age distribution among cases and control



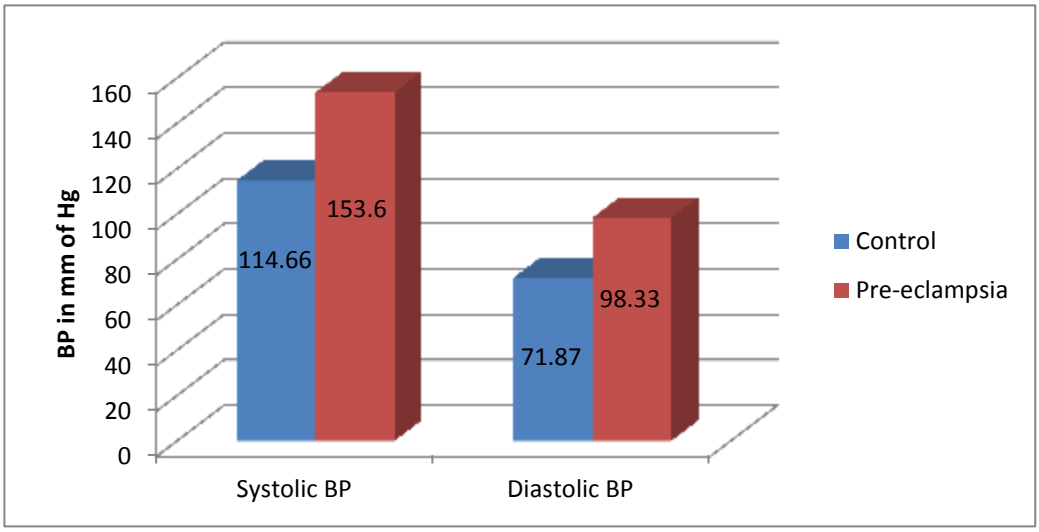
Majority of cases and controls belonged to the age group of 20 to 25 years .The mean age of the mothers was 24.08 ±3.90 years in control group and 24.17±3.6 in pre-eclampsia.

In mild pre-eclampsia the mean age was 24.38+3.78 years and 23.75+3.28 years in severe preeclampsia.

Table 4: Mean Systolic and diastolic Blood Pressure in each group

Blood pressure (mm of Hg)	Control (n=60)		Pre-eclampsia (n=60)		Pre-eclampsia			
					Mild PE (n=60)		Severe PE (n=20)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Systolic	114.66	5.48	153.6	9.17	148.65	5.91	163.5	5.87
Diastolic	71.87	6.74	98.33	8.98	92.5	4.15	110	0.00

Chart 2: Mean systolic and diastolic Blood Pressure in each group



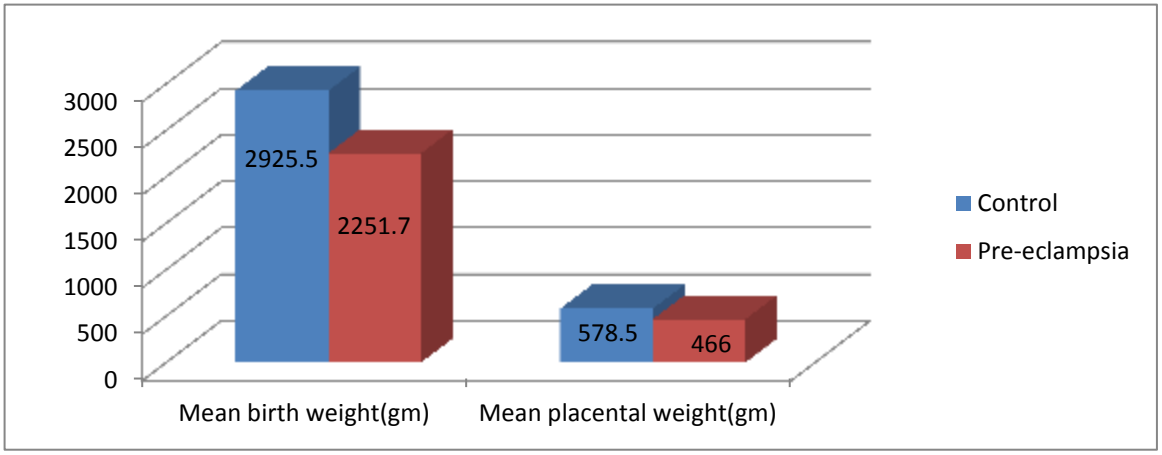
Mean systolic blood pressure in control group was 114.63±5.48 mm of Hg and in pre-eclampsia was 153.6±9.17. In mild pre-eclampsia mean systolic BP was 148.65 ± 5.91 mm of Hg and in severe pre-eclampsia it was 163.50±5.87 mm of Hg.

The mean diastolic blood pressure in control group was 71.87±6.74 mm of Hg and in pre-eclampsia was 98.33±8.98. In mild pre-eclampsia group mean diastolic pressure was 92.50± 4.15 mm of Hg, in severe pre-eclampsia group was 110.0±0.00 mm of Hg.

Table 5: Mean of Birth weight and Placenta Weight

Parameters	Control(n=60)		Pre-eclampsia (n=60)		Mild PE (n=40)		Severe PE (n=20)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Birth weight (gm)	2925.5	0.49	2251.7	0.69	2255.8	0.68	2243.5	0.72
Placenta weight (gm)	578.500	95.36	466.0	47.34	472.50	48.50	453.00	43.18

Chart 3:Mean birth weight and placenta weight



The mean birth weight was 2925.5±0.49 gm in control and 2251.7±0.69 gm in pre-eclampsia. The result was statistically significant between the two group (p <0.001).

In mild pre-eclampsia the mean birth weight was 2255.8±0.68 gm and 2243.5±0.72 gm in severe pre-eclampsia. The result was statistically significant between three groups. All the parameters were lowest in Severe Pre-eclampsia< Non-severe pre-eclampsia < Control.

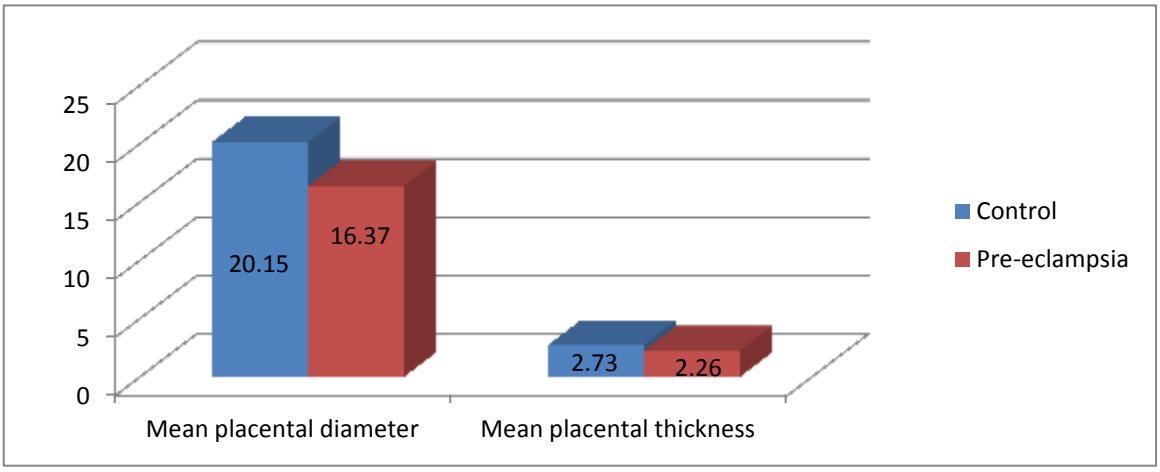
The mean placenta weight was 578.50±95.36 gm in control and 466.0±47.34 in pre-eclampsia. The result was statistically significant between the two groups (p <0.001).

In mild pre-eclampsia the mean placenta weight was 472.50±48.50 gm and 453.00+43.18 gm in severe pre-eclampsia. All the parameters were lowest in Severe Pre-eclampsia< Non-severe pre-eclampsia < Control.

Table 6: Mean placental Diameter and thickness

Parameters	Control(n=60)		Pre-eclampsia (n=60)		Pre-eclampsia			
					Mild PE (n=40)		Severe PE (n=20)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Placental Diameter (cm)	20.15	2.36	16.37	3.81	17.81	3.90	13.48	0.72
Placental Thickness(cm)	2.73	0.34	2.26	0.38	2.31	0.36	2.15	0.40

Chart 4: Mean placental Diameter and thickness



Mean diameter of placentae in control was 20.15±2.36 cm and 16.37±3.81 and in pre-eclampsia. The result was statistically significant between the two groups. ($p<0.001$)

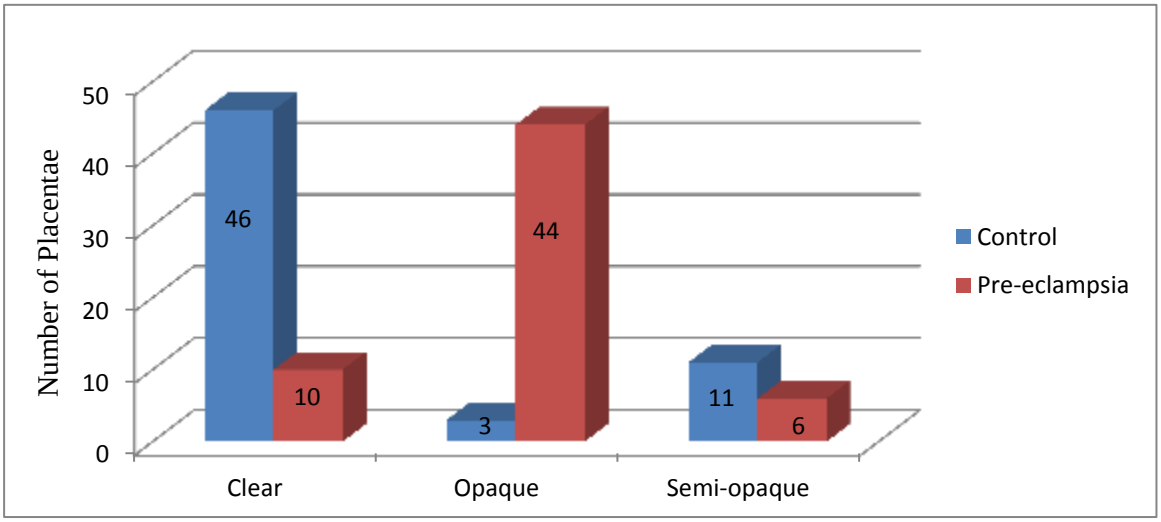
Mild pre-eclampsia group had mean diameter of 17.81±3.90 cm and 13.48±0.72 cm in severe pre-eclampsia. All the parameters were lowest in Severe Pre-eclampsia< mild pre-eclampsia < Control

The mean thickness of placentae of control group was 2.73±0.34 cm and 2.26±0.38 cm in pre-eclampsia. The result was statistically significant between the two groups ($p<0.001$). In mild pre-eclampsia group the mean thickness of placenta was 2.31±0.36 cm and that of severe pre-eclampsia group was 2.15±0.40 cm. All the parameters were lowest in Severe Pre-eclampsia< mild pre-eclampsia < Control

Table 7: Distribution of appearance of membrane of placenta

Membrane of placenta	Control(n=60)	Pre-eclampsia (n=60)	Pre-eclampsia		P-Value
			Mild PE (n=40)	Severe PE (n=20)	
Clear	46(76.67%)	10(16.67%)	7(17.5%)	3(15%)	<0.001
Opaque	3(5%)	44(73.33%)	29(72.5%)	15(75%)	
Semi Opaque	11(18.33%)	6(10%)	4(10%)	2(10%)	

Chart 5 :Distribution of appearance of membrane of placenta in percentage

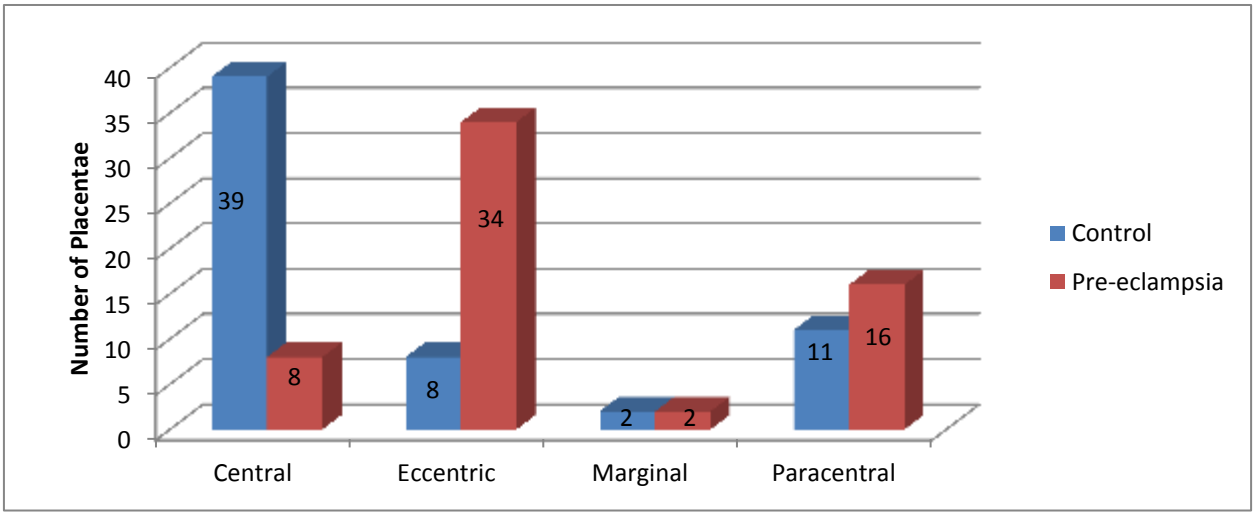


Most common type of placental membrane in control group was clear membrane while in both severe and non-severe pre-eclampsia it was opaque membrane . The result was statistically significant.(p-Value<0.001)

Table 8: Insertion of umbilical cord

Insertion of cord			Pre-eclampsia		p- value
	Control	Preeclampsia	Mild PE	Severe PE	
Central	39(65%)	8(13.33%)	6(15%)	2(10%)	<0.001
Eccentric	8(13.33%)	34(56.67%)	25(62.5%)	9(45%)	
Marginal	2(3.33%)	2(3.33%)	0(0%)	2(10%)	
Paracentral	11(18.3%)	16(26.67%)	9(22.5%)	7(35%)	

Chart 6: Distribution of insertion of cord in percentage

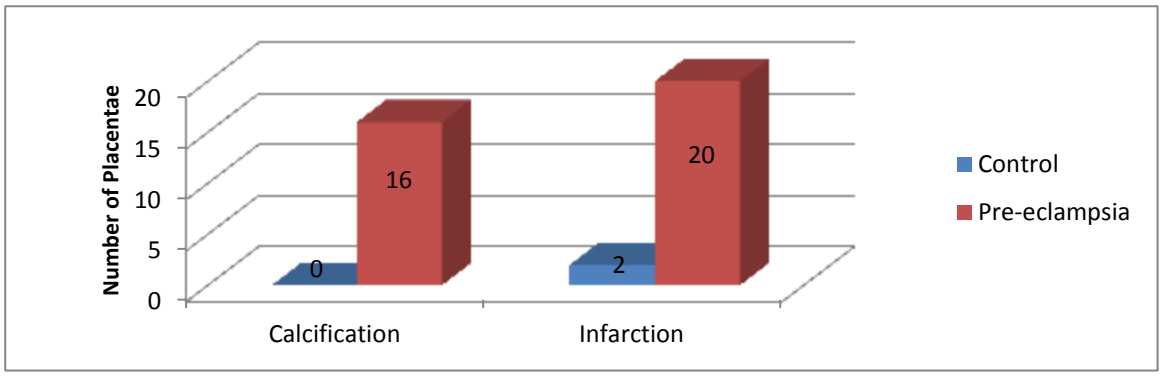


Umbilical cord was centrally inserted in majority of the control placenta (65%) whereas eccentric insertion was common in pre-eclampsia. The result was statistically significant in between two groups. (p value < 0.001)

Table 9: Distribution of placenta having Calcification and infarction

	Control	Pre-eclampsia	Pre-eclampsia		p-Value
			Mild PE	Severe PE	
Calcification	0 (0%)	16 (26.67%)	4 (10%)	12 (60%)	< 0.001
Infarction	2 (3.33%)	20 (33.33%)	5 (12.5%)	15 (75%)	< 0.001

Chart 7: Distribution of calcification and infarction of placenta



Calcification was seen in 16/60 (26.67%) of cases of pre-eclampsia of which 12/20 (60%) of placentae in severe pre-eclampsia and 4/40 (10%) of mild pre-eclampsia. None of the control group showed calcification.

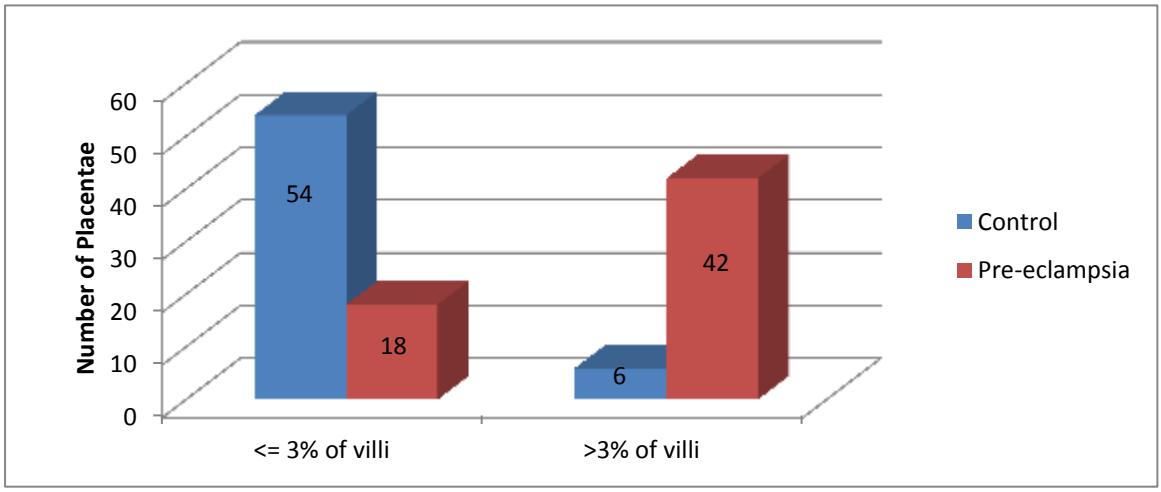
Infarction involving more than 5% of parenchyma was seen in 20/60 (33.33%) of placenta in pre-eclampsia and 2/60 (3.33%) in control groups .

15/20 (75%) of placentae of severe pre-eclampsia group and 5/40 (12.5%) mild pre-eclampsia cases. The result was statistically significant (p value<0.001).

Table 10: Incidence of stromal fibrosis in stem villi in each group

Percentage of stem villi stromal fibrosis	Control (n=60)	Pre-eclampsia (n=60)	Pre-eclampsia		p-value
			Mild PE(n=40)	Severe PE (n=20)	
≤ 3% of villi	54 (90%)	18 (30%)	17 (42.5%)	1 (5%)	<0.001
>3% of villi	6 (10%)	42 (70%)	23 (57.5%)	19 (95%)	

Chart 8: Incidence of stromal fibrosis in each group



Increase in villous stromal fibrosis, involving more than 3 % of villi was seen in 6 /60 (10%) placenta in control group and 42/60 (70%) in pre-eclampsia group .The result between the two group was statistically significant (p<0.001).

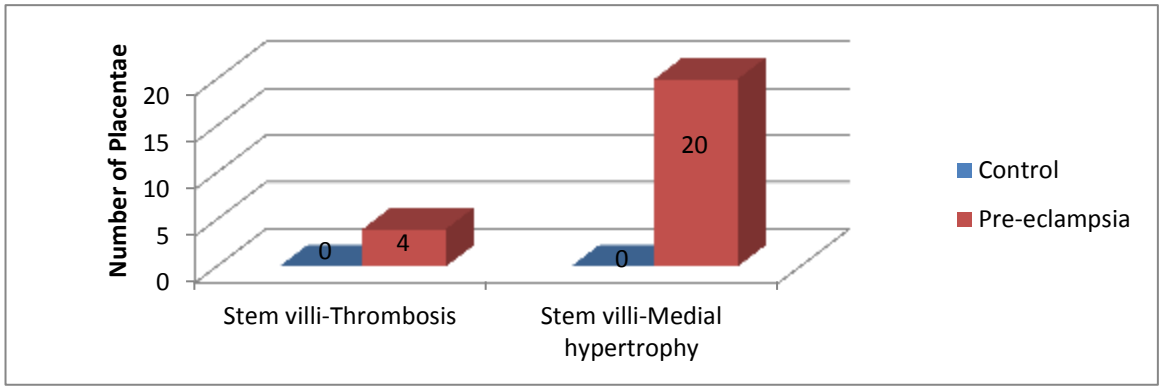
23/40 (57.5%) placentae of the mild pre-eclampsia group and 19 /20 (95%) placentae of severe pre-eclampsia group showed increased stromal fibrosis involving > 3% of villi.

The difference of proportion of placentae showing increase in stromal fibrosis in >3 percent of the villi between the three groups was statistically significant (p<0.001). This shows that increase in villous stromal fibrosis is commonly seen in severe pre-eclampsia >mild pre-eclampsia > Control

Table 11: Incidence of thrombosis and medial hypertrophy in Stem villi

Percentage of stem villi stromal fibrosis	Control	Pre-eclampsia	Pre-eclampsia	
			Mild PE	Severe PE
Stem villi- Thrombosis	0	4 (6.67%)	0	4 (20%)
Stem villi -Media hypertrophy	0	20 (33.33%)	0	20 (100%)
Total	60	60	40	20

Chart 9: Incidence of thrombosis and media hypertrophy of stem villi.



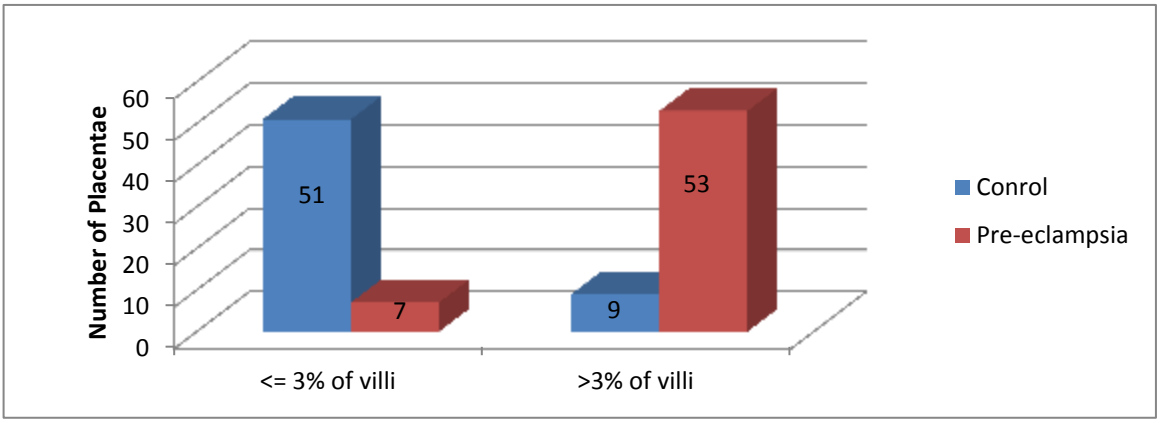
Thrombosis of stem villi is seen in 4/20 (20%) in pre-eclampsia while none in the control group. All the four cases belonged to severe pre-eclampsia.

The mean diameter of medial thickness was 19.88±1.48 um in pre-eclampsia and 6.43±0.63 um in control. In severe pre-eclampsia the mean diameter of media was 21.27±1.51 and 19.18±0.85 in mild pre-eclampsia. There was three fold times increase in medial thickness in pre-eclampsia as compared to control

Table 12: Incidence of basement membrane thickening of intermediate villi

Intermediate villi - Basement membrane thickening	Control (n=60)	Pre-eclampsia (n=60)	Pre-eclampsia		p-Value
			Mild PE (n=40)	Severe PE (n=20)	
≤ 3% of villi	51 (85%)	7 (11.67%)	6 (15%)	1 (5%)	<0.001
>3% of villi	9 (15%)	53 (88.33%)	34 (85%)	19 (95%)	

Chart 10 : Incidence of basement membrane thickening of intermediate villi



Basement membrane thickening in >3 percent of the villi was seen in 9 /60 (15%) placentae of the control group and 53/60 (88.33%) of placenta in pre-eclampsia. The result was statistically significant between the two groups (p<0.001).

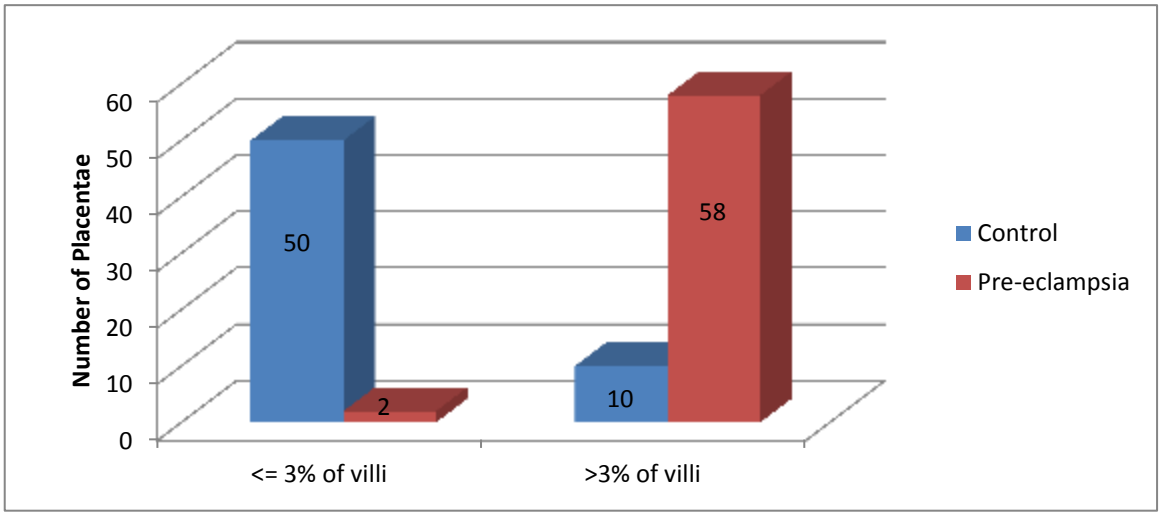
While on sub grouping 34 /40 (85%) placentae of mild pre-eclampsia and 19 /20(95%) of severe pre-eclampsia group showed this change.

The difference of proportion of placentae showing basement membrane thickening in >3 percent of the villi between the three groups was statistically significant (p<0.001). This shows that basement membrane thickening is more commonly seen in placentae of severe pre-eclampsia>mild pre-eclampsia > Control

Table 13: Incidence of fibrinoid necrosis in intermediate villi

Intermediate villi –Fibrinoid necrosis	Control (n=60)	Pre-eclampsia (n=60)	Pre-eclampsia		p- Value
			Mild PE (n=40)	Severe PE(n=20)	
≤ 3% of villi	50 (83.33%)	2 (3.33%)	1 (2.5%)	1 (5%)	< 0.001
>3% of villi	10 (16.67%)	58 (96.67%)	39 (97.5%)	19 (95%)	

Chart 11: Incidence of Fibrinoid necrosis in intermediate villi



Fibrinoid necrosis was seen in >3 percent of the villi in 10 /60 (16.67 %) placentae of the control group and 58 (96.67%) of pre-eclampsia group. The result was significant between the two groups (p<0.001).

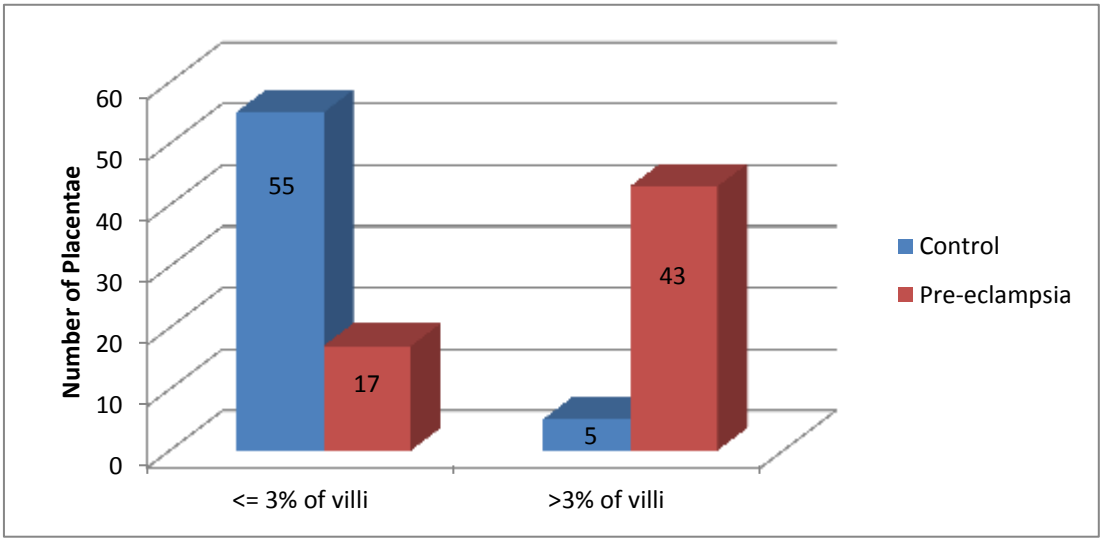
On sub grouping 39/40 (97.4%) placentae of mild pre-eclampsia and 19/20 (95%) placenta of severe pre-eclampsia showed this change.

The difference of proportion of placentae showing fibrinoid necrosis in >3 percent of the villi between the three groups was statistically very significant (p<0.001). This shows that fibrinoid necrosis is commonly seen in placentae of severe pre-eclampsia > mild pre-eclampsia > Control

Table 14: Incidence of basement membrane thickening of terminal villi

Terminal villi basement membrane thickening	Control(n=60)	Pre-eclampsia (n=60)	Pre-eclampsia		p-Value
			Mild PE (n=40)	Severe PE (n=20)	
≤ 3%	55 (91.67%)	17 (28.33%)	17 (42.5%)	0 (0%)	< 0.001
>3%	5 (8.33%)	43 (71.67%)	23 (57.5%)	20 (100%)	

Chart 12: Incidence of basement membrane thickening of terminal villi



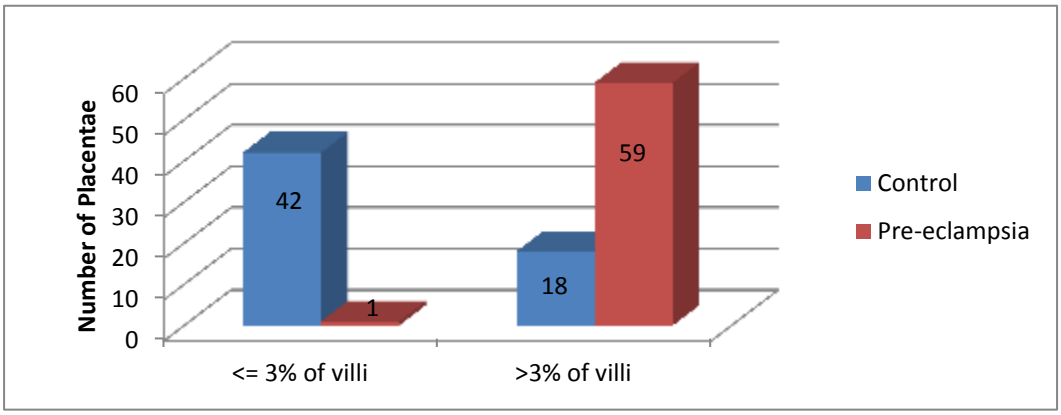
Basement membrane thickening in >3 percent of the villi was seen in 5/60 (8.3%) placentae of the control group and 43 (71.67%) of placenta in pre-eclampsia group. On sub grouping 23 /40 (57%) placentae of mild-preeclampsia and 20/20 (100%) of severe pre-eclampsia showed this change.

The difference of proportion of placentae showing basement membrane thickening in >3 percent of the villi between the three groups was statistically highly significant (p<0.001). This shows that basement membrane thickening is more commonly seen in severe pre-eclampsia>mild pre-eclampsia > Control

Table 15: Incidence of fibrinoid necrosis of terminal villi

Terminal villi – fibrinoid necrosis	Control (n=60)	Pre-eclampsia (n=60)	Pre-eclampsia		p-value
			Mild PE (n=40)	Severe PE (n=20)	
≤ 3% of villi	42 (70%)	1 (1.67%)	1 (2.5%)	0 (0%)	< 0.001
>3% of villi	18 (30%)	59 (98.33%)	39 (97.5%)	20 (100%)	

Chart 13: Incidence of fibrinoid necrosis of terminal villi



Fibrinoid necrosis was seen in >3% of the villi in 18/60 (30%) placentae of the control group and 59(98.33%) of placenta in pre-eclampsia group. The result was statistically significant between the two groups.

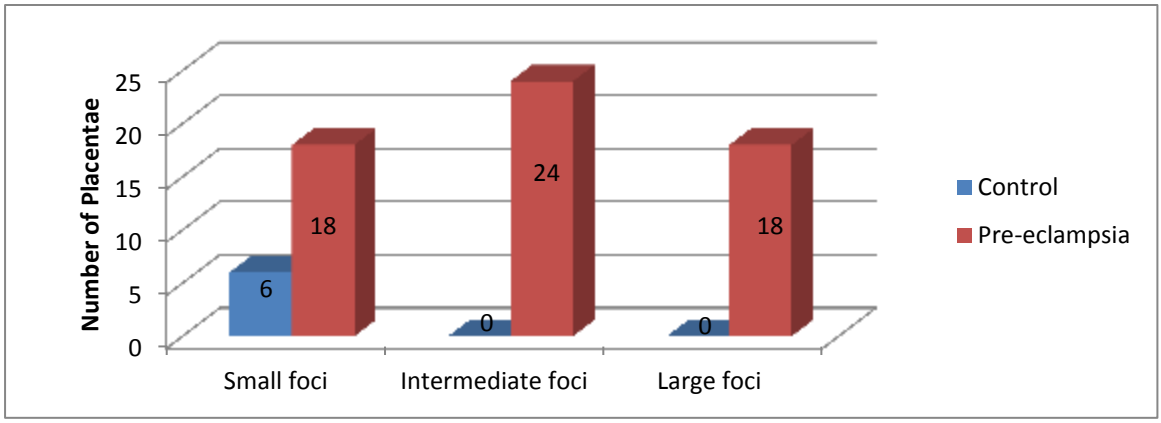
On sub grouping 39 /40 (97.5%) placentae of mild pre-eclampsia group and 20 /20(100%) of placenta of severe pre-eclampsia group showed this change.

The difference of proportion of placentae showing fibrinoid necrosis in >3 % of the villi between the three groups was statistically significant (p<0.001). This shows that fibrinoid necrosis is commonly seen in severe pre-eclampsia>mild pre-eclampsia > Control

Table 16: Showing incidence of avascular villi

Avascular villi	Control (n=60)	Pre-ecalmpsia (n=60)	Pre-eclampsia		p-Value
			Mild PE (n=40)	Severe PE (n=20)	
Small Foci	6 (10%)	18 (30%)	18 (45%)	0 (0%)	< 0.001
Intermediate Foci	0 (0%)	24 (40%)	22 (55%)	2 (10%)	
Large Foci	0 (0%)	18 (30%)	0 (0%)	18 (90%)	
Absent	54 (90%)	0 (0%)	0 (0%)	0 (0%)	

Chart 14 : Incidence of avascular villi in placenta

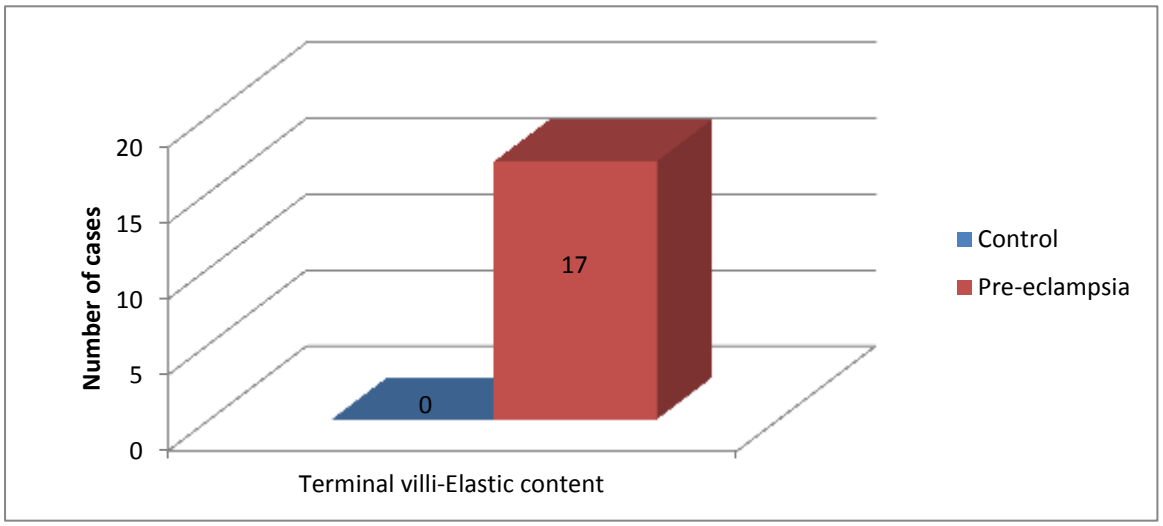


Avascular villi in control group having large foci was present in 18/60 (30%) placenta in pre-eclampsia while it was absent in control group. All the cases having large foci of avascular villi belonged to severe pre-eclampsia.

Table 17: Showing incidence of elastic content in terminal villi

Elastic content –Terminal villi	Control(n=60)	Pre-eclampsia (n=60)	Pre-eclampsia	
			Mild PE (n=40)	Severe PE (n=20)
Present	0	17 (28.33%)	0	17 (85%)

Chart 15: Incidence of elastic content of terminal villi

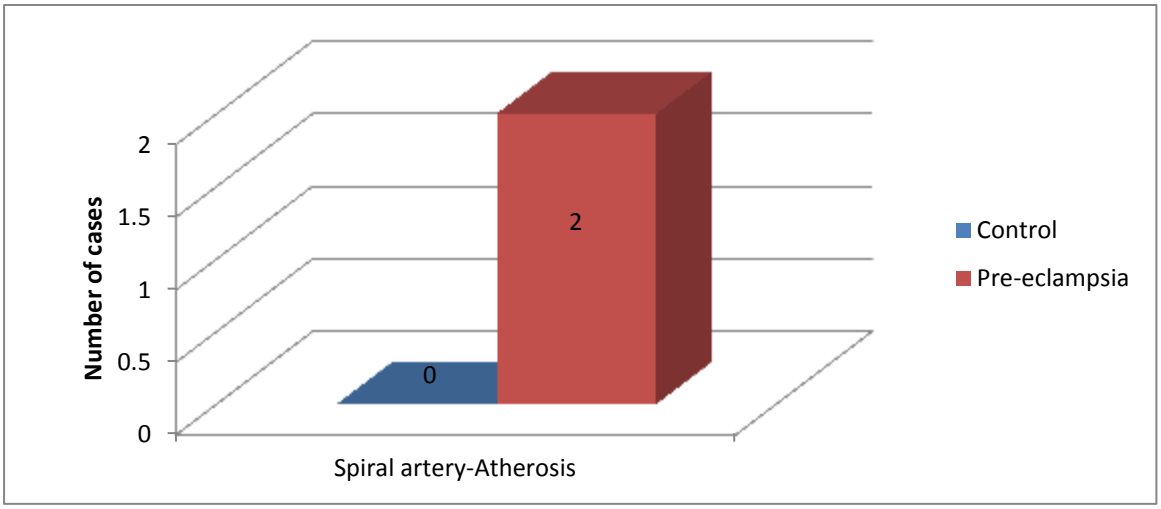


Increased elastic content in terminal villi was seen in 17/60 (28.33%) case of pre-eclampsia. All these cases were of severe pre-eclampsia group while none of the placenta in mild pre-eclampsia and control group showed increased elastic content.

Table 18 : Incidence of atherosis of spiral artery

Spiral artery atherosis	Control (n=60)	Pre-eclampsia	Pre-eclampsia	
			Mild PE (n=40)	Severe PE (n=20)
Present	0	2 (3.33%)	0	2 (10%)

Chart 16: Incidence of atherosis of spiral artery



Two cases of spiral artery in pre-eclampsia shows atherosis. Both the cases belonged to severe pre-eclampsia group while none of the cases of mild pre-eclampsia and control group showed this finding.

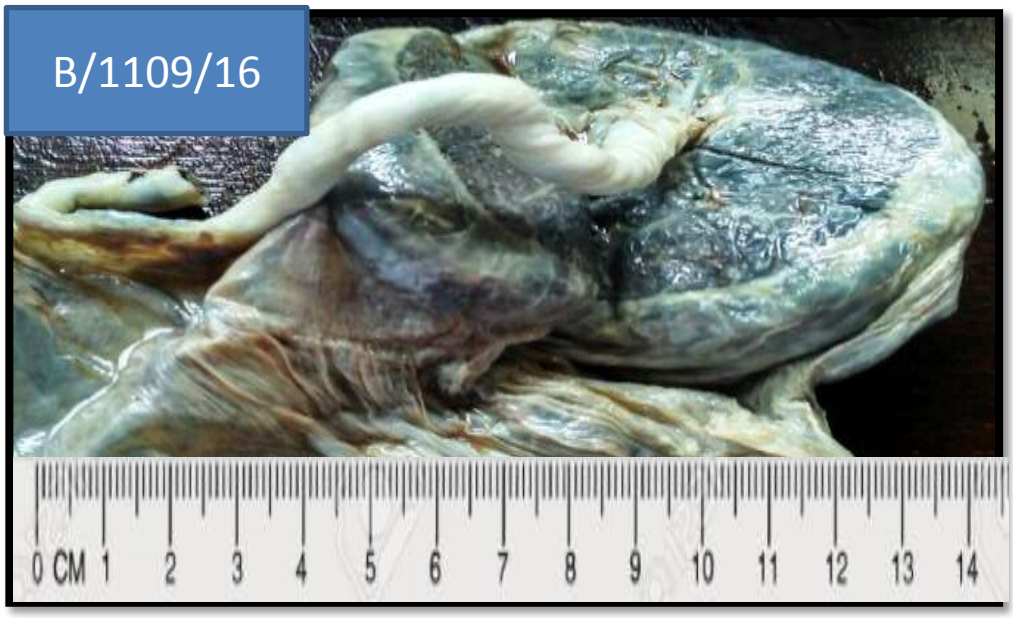


Fig 4: Central position of cord in normal pregnancy

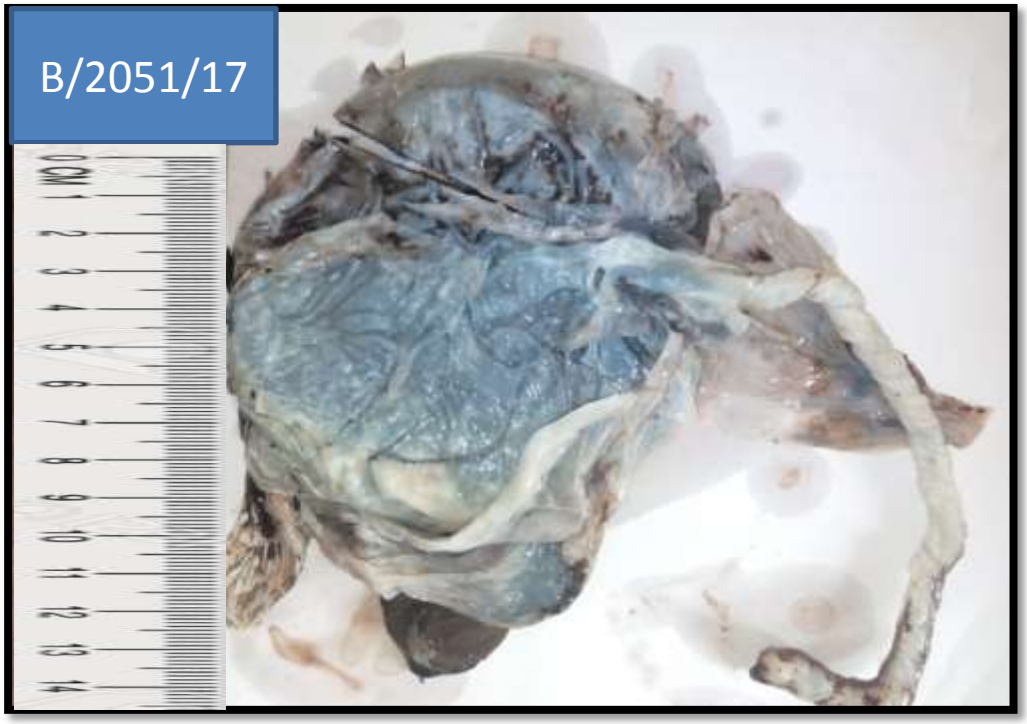


Fig 5: Eccentrically placed cord in pre-eclampsia

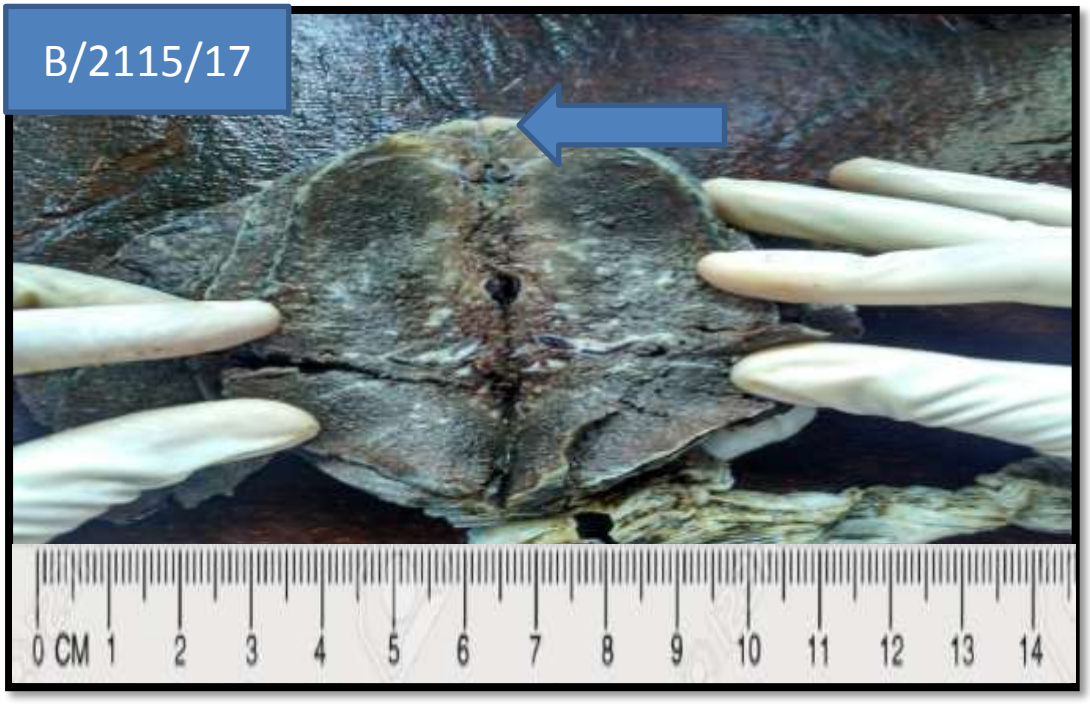


Fig 6: Area of calcification in pre-eclampsia

Fig 7: Areas of infarction in pre-eclampsia

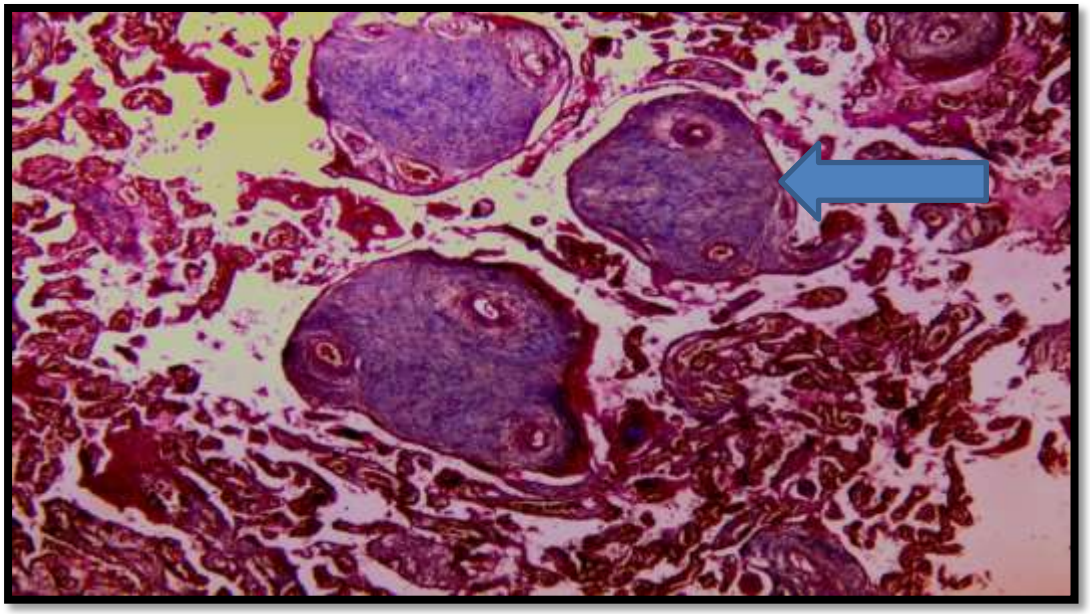


Fig 8: Stromal fibrosis of stem villi in pre-eclampsia. (Masson's Trichrome,10X)

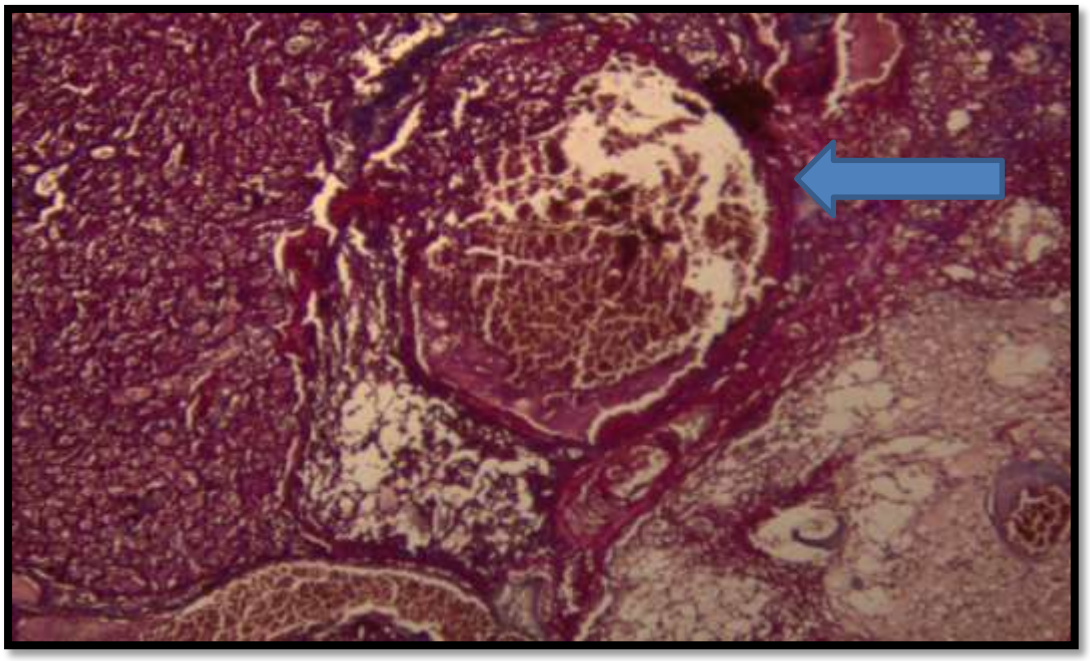


Fig 9: Thrombosis in stem villi vessel in pre-eclampsia. (Masson's Trichrome,40X)

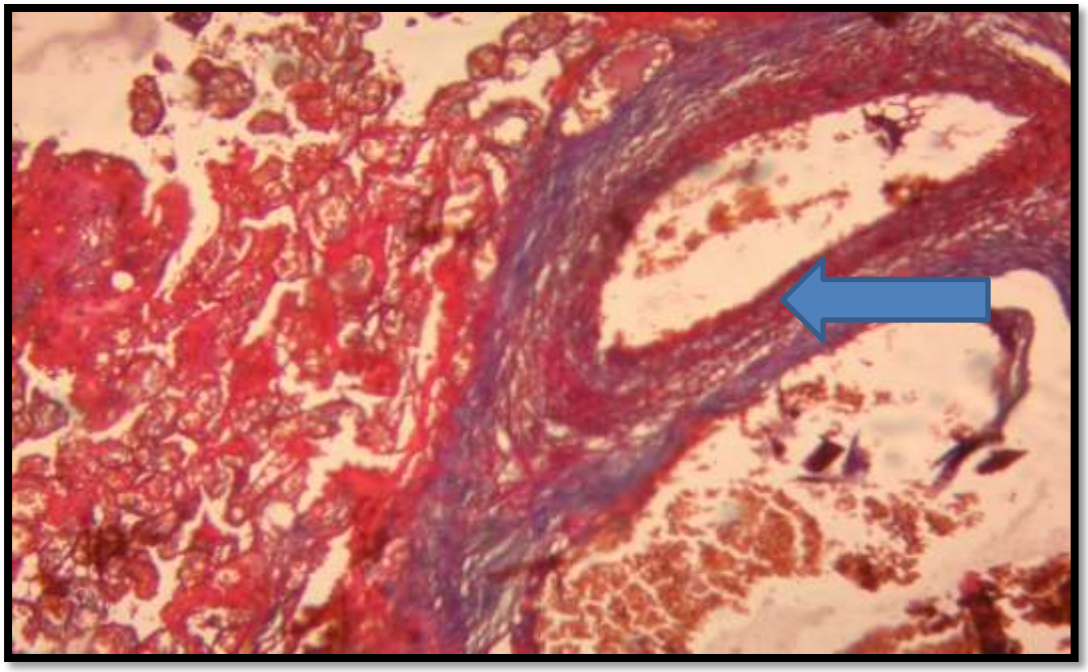


Fig 10: Medial hypertrophy in stem villi artery in pre - eclampsia.

(Masson'sTrichrome,10X)

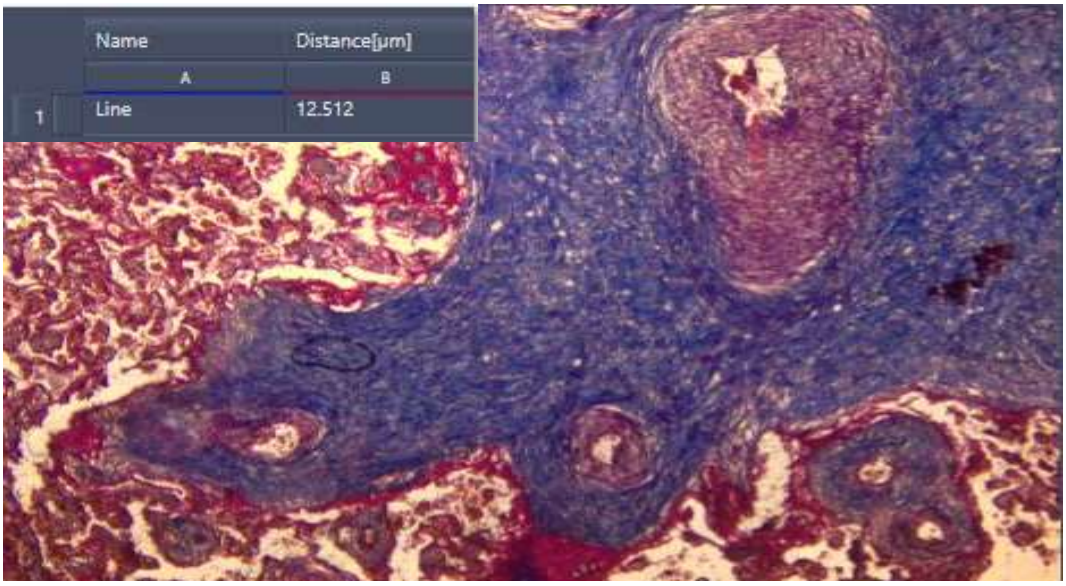


Fig 11: Measurement of medial hypertrophy in stem villi artery in pre-eclampsia by morphometry. (Masson's trichrome 10X)

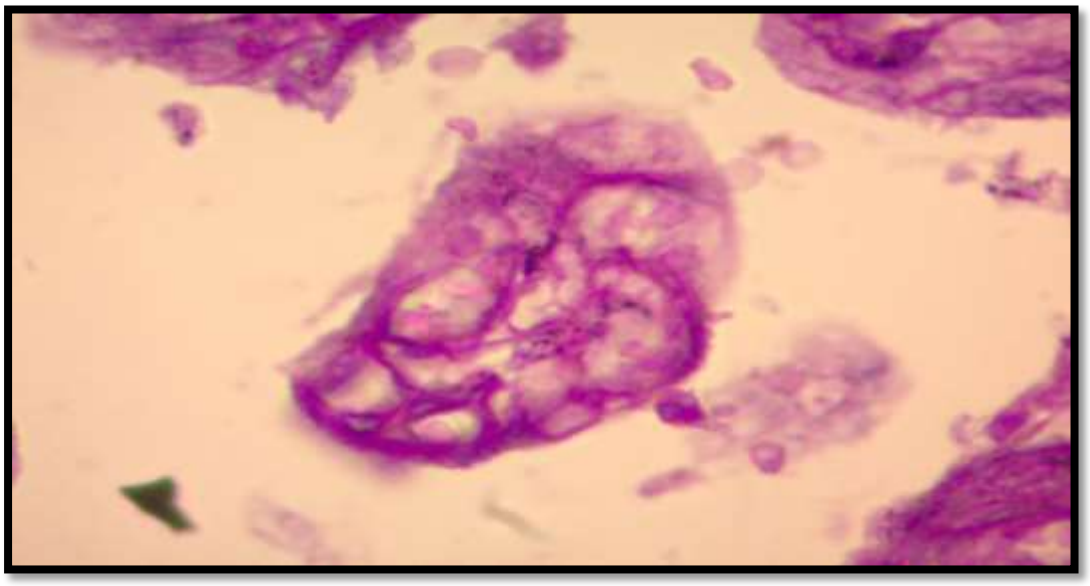


Fig 12: Normal basement membrane thickness in control. (PAS,100X)

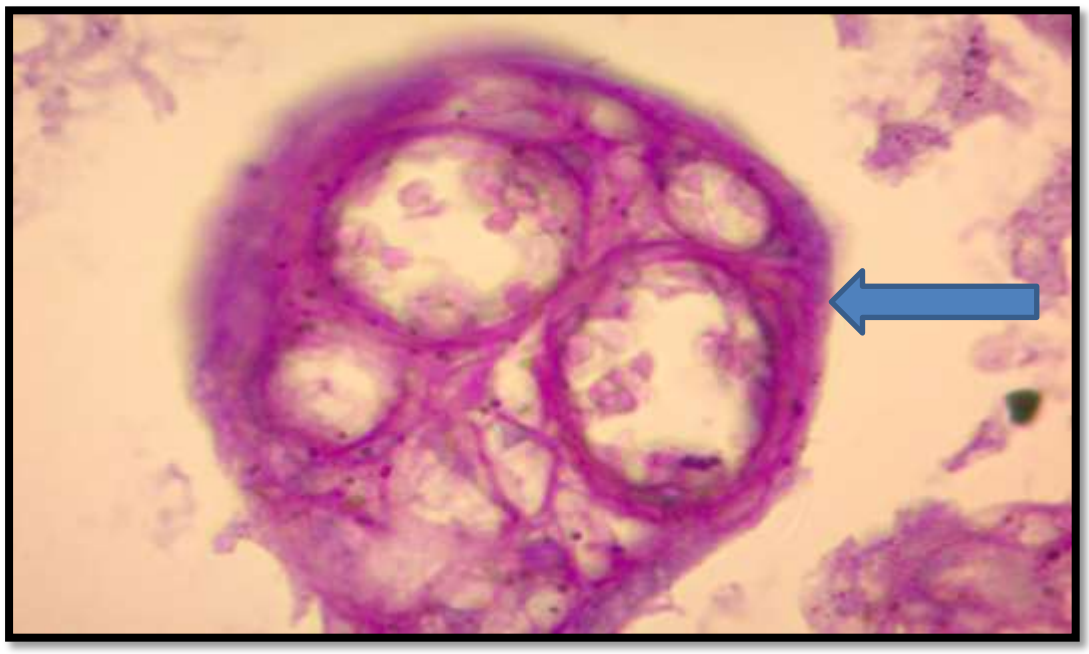


Fig 13: Cytotrophoblastic basement membrane thickening in pre-eclampsia.

(PAS,100X)

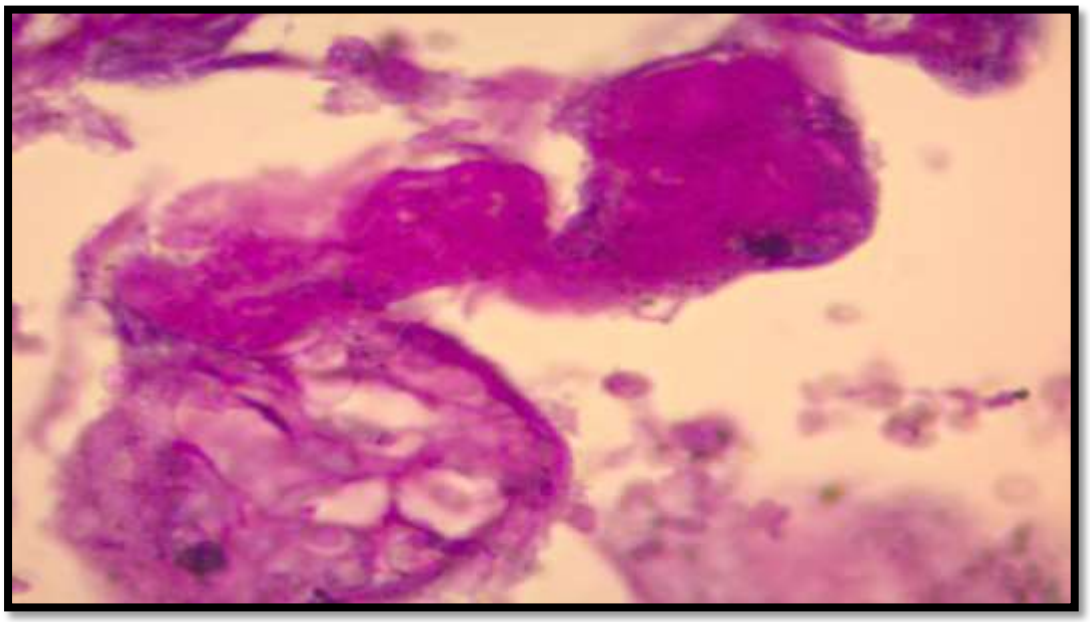


Fig 14: Fibrinoid necrosis of intermediate villi in pre-eclampsia. (PAS,100X)

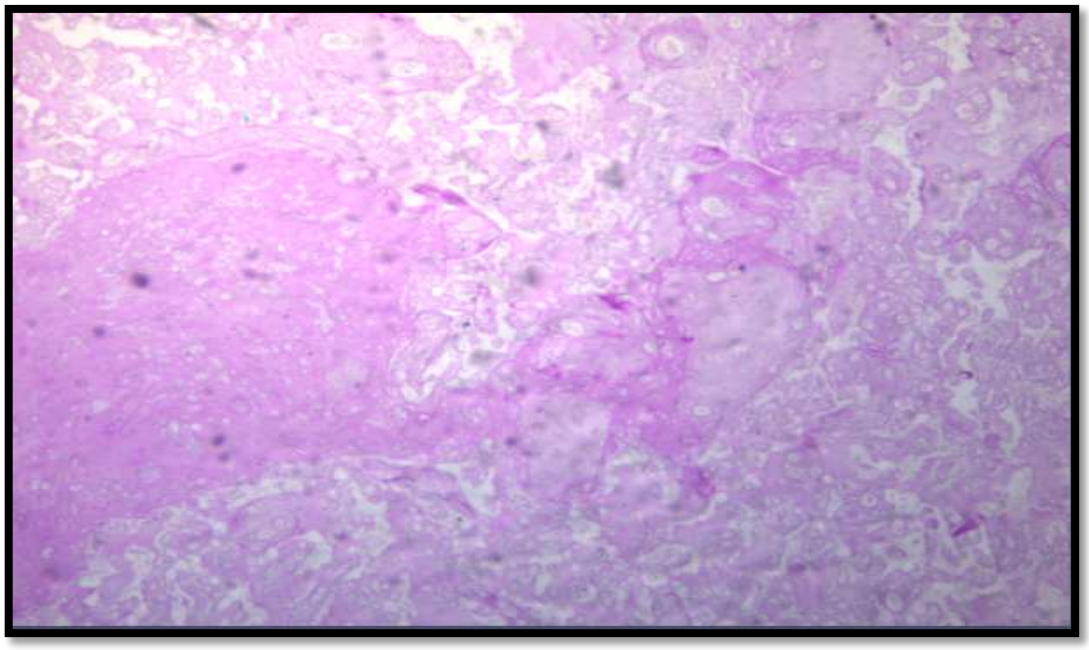


Fig 15: Avascular villi in preeclampsia. (PAS,10X)

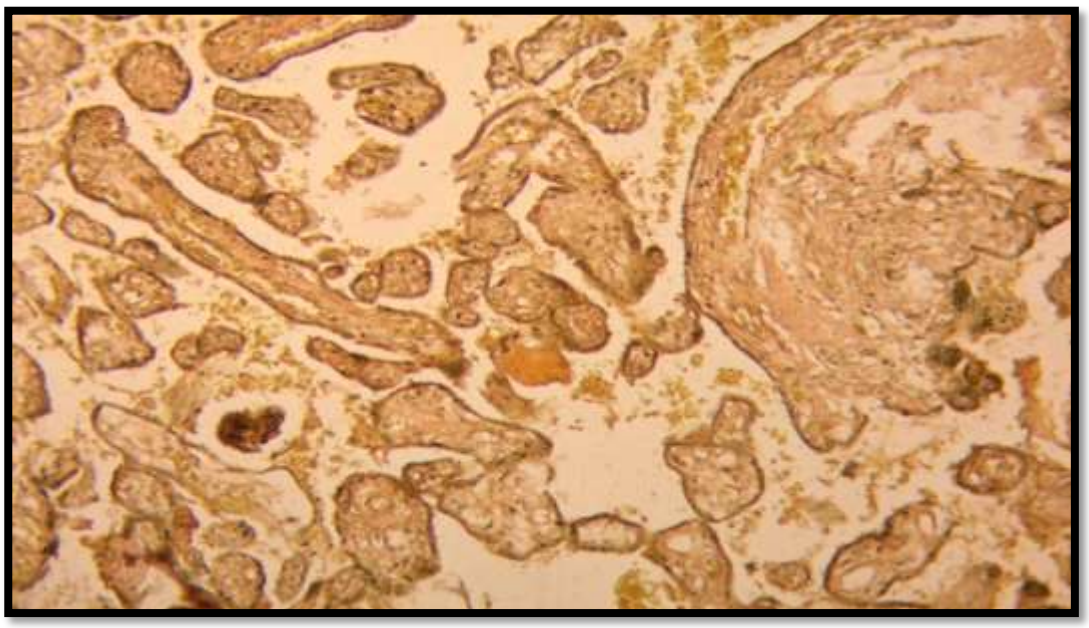


Fig 16: Normal elastic content in terminal villi capillaries in control group.

(Verhoeff's stain ,10X)

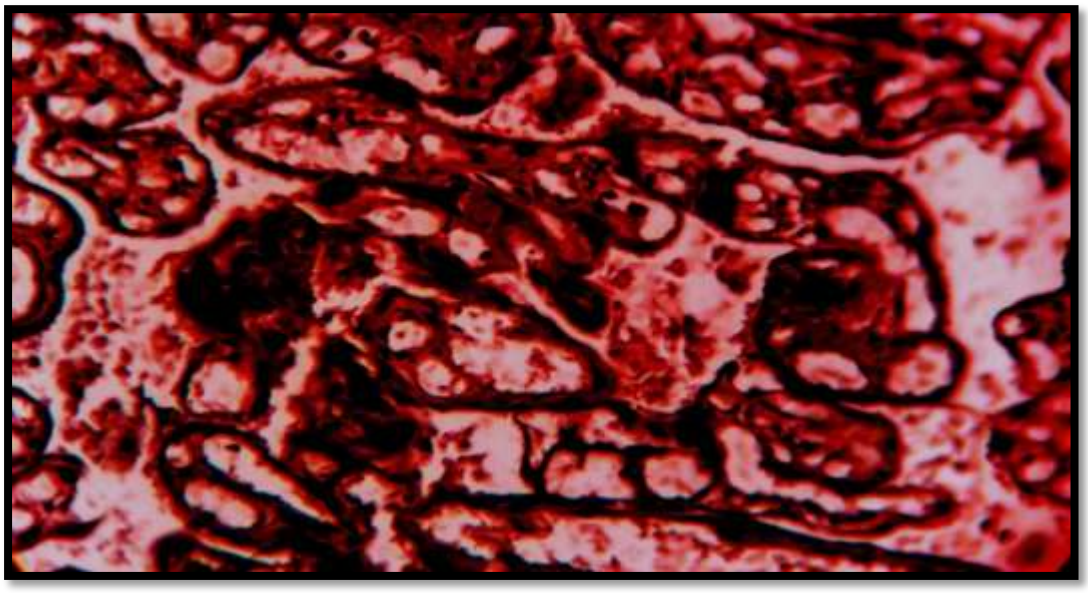


Fig 17: Increased elastic content in terminal villi in pre-eclampsia.

(Verhoeff's stain, 40X)

DISCUSSION

Placenta is a complex organ that connects mother and fetus. Placental function is highly influenced by its anatomical structure. Morphology and cellular architecture of placenta is important for adequate oxygen delivery to the fetus from the mother. Successful placental development is therefore essential for fetal growth and well-being after 20 weeks of gestation and is required for adequate maternal blood supply to the placenta. This has been being demonstrated by normal uterine artery Doppler .[76]

Placenta is connected to both fetus and mother. Thus, any disease process affecting the mother or the fetus also has a great impact on placenta. Placental architecture is modified in disease like pre-eclampsia affecting the mother as a result it affects the fetus too.

Hence, this study was taken up to look into the vascular changes that occurs at the level of terminal villi, intermediate villi and stem villi as these changes have profound effect both on the fetus as well as the mother. A total of 120 placentas were studied, this included 60 from mothers with normal pregnancies, considered as control group and 60 from mothers with pre-eclampsia group, of this 40 placenta were from mother with mild pre-eclampsia and 20 from mothers with severe pre-eclampsia. The gross and microscopic lesions were analysed, quantified and compared between the two groups.

Most of our cases and control belong to the age group of 20-25 years. Study done by Ranga MK et al found pregnancy induced hypertension (PIH) more common in age group of 25-29 years. With mean age of PIH mothers being 25.6 ± 3.8 years. [77]

There is association of pre-eclampsia with extremes of childbearing age group.[78] Multiple studies have shown a higher incidence of pre-eclampsia association with women of older age

group independent of the parity. However, many of these studies have not taken into account the related medical illness of the mother before conception.[78,79]

In the present study, the birth weight of babies born to pre-eclamptic mother was lower (2251.7 gm) than the control group (2925.5 gm) and there was also significant correlation with the severity of pre-eclampsia. The comparison of present study with study by Salam et al [80] is described in a table no 19. Our findings were also comparable with findings of Shankar et al [73], Udainia et al [81] and Majumdar et al. [82]

The pathological changes that occurs in pre-eclampsia leads to limited blood flow to the placenta predisposing to IUGR and lower birth weight. The same has been proofed by the Doppler studies which has shown limited blood flow to placenta.[76]

The weight of the placenta gives us a clue of undergoing pathological process within the placenta. The weight changes depend not only on the disease process but also on the severity of the disease. In the present study, the mean weight of the placenta in pre-eclampsia was less (466.0 gm) compared to controls (578.5 gm) and there was also significant positive correlation with the severity of pre-eclampsia. This is in correlation with other studies shown in the table 19 and other studies done by Salam et al [80], Udainia et al [81] , Majumdar et al [82] and Sankar et al [73] .

Fox H [23] in 1968 reported that placentae tend to be smaller in preeclampsia than those in uncomplicated pregnancies. Reduced placental weight results due to reduced blood flow to the placenta in pathological condition when the compensatory mechanism fails.

Table 19: Comparison of mean birth weight and mean placental weight.

	Mean Birth weight (gm)		Mean placenta weight (gm)	
	Control	**Pre-eclampsia**	**Control**	**Pre-eclampsia**
Present study	2925.5	2251.7	578.5	466.00
Salam et al [80]	3460	2920	578.4	477.4
Udainia et al [81]	2640	2280	495	405.67
Majumdar et al [82]	2800	2040	485.85	399.10
Sankar K et al [73]	2635.71	2037.39	470.64	401.23

The mean diameter and thickness of the placentae of pre-eclampsia group was significantly less than the control group. This was comparable to the findings of Sankar KD et al [73], Kishwara et al [83] and Ranga MK et al[77]. The cause of this reduced diameter in pre-eclampsia was thought to be due to pathological process interfering with the normal placental growth[73]. The comparison of mean placental diameter and mean placental thickness of present study with other studies are shown in table no 20

Table 20: Comparison of mean placental diameter and thickness with other studies.

	Mean placental diameter(cm)		Mean placental thickness(cm)	
	Control	**Pre-eclampsia**	**Control**	**Pre-eclampsia**
Present study	20.15	16.37	2.73	2.26
Sankar KD et al [73]	17.21	15.91	1.82	1.48
Kishwara et al [83]	18.80	16.08	1.59	1.51
Ranga MK et al [77]	19.1	14.1	2.4	1.9

Opaque membrane was found more commonly in pre-eclampsia. While control group showed predominantly clear membrane. The possible theory is that the factors released during the development of preeclampsia may get deposited on the placental membrane leading to opacity of the placenta.

Eccentric insertion of the cord results in an altered distribution of fetal blood in the placenta leading to weaker chorionic vascular distribution and unequal distribution of blood flow to the placenta which also leads to reduced birth weight for a given placental weight. The incidence of eccentric insertion of the umbilical cord in the pre-eclampsia group was more than that of the control group. This is in concurrence with the findings of Dekan S et al [85] and Vinnars MT et al .[86]

Kurdukar et al [87] have reported that thrombotic occlusion of maternal uteroplacental vessel is responsible for infarction. Infarction involving more than 5% of placental parenchyma has clinical significance as it causes placental ischemia by reducing the amount of placental tissue available for nutrition of the fetus. He found out that the foci of infarction increased as the severity of pre-eclampsia increased.

Jain et al [88] have shown that extensive infarcts are associated with higher incidence of fetal hypoxia and intrauterine death. In the present study, infarction involving more than 5% of placental parenchyma was more in pre-eclampsia than the control group and we also found positive correlation with the severity of pre-eclampsia. This comparison of our findings and findings of Vinnars MT et al [86], Narasimha et al [84], Ranga et al [77] are described in table no 21

Table 21: Comparison of Infarction percentage with other studies

	Infarction		
	Control	**Mild PE**	**Severe PE**
Present study	3.33%	12.5%	75%
Vinnars MT et al [86]	5.1%	17.1%	39.7%
Narasimha et al [84]	4%	10%	21%
Ranga MK et al [77]	6.7%	36.7%	

Calcification was seen in pre-eclampsia placentae in the present study. Calcification is regarded as an evidence of placental senescence or degeneration as it is increased at term. It is not associated with adverse fetal outcome. No statistical significance was observed between the two groups by Jain et al [88] but increased incidence was observed in PIH placentae by Kurdukar et al [87] and Manjunatha et al [89]. In present study, we found 60% of placenta in severe pre-eclampsia showing calcification ,10% of non-severe pre-eclampsia while none of the control group showed calcification and the result was statistically significant.

MICROSCOPIC LESIONS:

In this study, microscopic appearance of all the three villi were identified based on the size**.** Morphometry was done in all case to differentiate between the size of terminal and intermediate villi and villi with the diameter less than 60 um was considered as terminal villi. Morphometry was also applied to determine medial hypertrophy in stem villi vessel.

Stem Villi

The microscopic lesions in the stem villi were assessed and quantified as percentage of placenta in control, mild pre-eclampsia and severe pre-eclampsia group showing stromal fibrosis in more than 3% of the stem villi, thrombosis and media hypertrophy in the vessels of the stem villi. These were compared with two other studies.

Increase in villous stromal fibrosis (> 3% of villi) in the PIH group has been reported in all the previous studies done by Jena M et al [6] , Shankar et al [73] and in the present study. Increase in the stromal fibrosis in the stem villi was attributed to the obliterative endarteritis which occurred as a consequence of pre-eclampsia.

Thrombosis of the stem villi was seen in 4/20 (20%) cases of severe preeclampsia in the present study while none of the non-severe pre-eclampsia and control group showed this finding. Our study was comparable with the studies done by Narasimha A et al [84] and Sankar e al [73]. Thrombosis of stem villi leads to reduces the lumen of blood vessel of stem villi which in turn fails to establish a network into the terminal villi resulting in avascular villi due to absence of capillaries. This causes reduced perfusion of placenta causing oxidative stress a known mechanism in pathophysiology of pre-eclampsia.

This study also showed an increase in medial thickness of the stem villi in severe pre-eclampsia group as compared to mild pre-eclampsia and control group. This finding is similar with the study done by Stenmark et al. [90] Increased pressure in pre-eclampsia causes proliferation and elastin secretion of the smooth muscle cells leading to thickening of vessel wall. This hypertrophy may be a protective mechanism adapted by the placental blood vessel tree against local stress. Similar findings was done by Las Heras and Haust [41,42] using electron microscopy which showed increase in proliferation of smooth muscle cells in the

media of stem villi arteries in toxemic placentae. Similar finding was seen by Baran PO et al using verhoeff's stain.[91]

In our study, we used Masson's trichrome stained slides for assessing the medial hypertrophy. Medial hypertrophy was found in all the twenty cases of severe pre-eclampsia while none of the mild pre-eclampsia and control group showed this finding. The mean medial thickness in pre-eclampsia was 21.2 um in severe preeclampsia and 19.11 um in non-severe preeclamsia and 6.43 um in control group. This measurement of media hypertrophy was not described in literature and exponential 30% increase of mean diameter bêtween pre-eclampsia and control group has been described .[92]

Intermediate villi

The microscopic lesions in intermediate villi were assessed and quantified as percentage of villi showing basement membrane thickening and fibrinoid necrosis. A value of >3% villi showing these changes was considered significant.

In the present study, significant fibrinoid necrosis was seen in intermediate villi. Fibrinoid necrosis appears as eosinophilic glossy substance which initially starts in the capillaries of intermediate villi and later involve the entire villi. The findings of present study, was in conformity with earlier studies which also showed increase in fibrinoid necrosis.

Study done in women having severe pre-eclampsia by Estellés Amparo et al [93] showed increased abnormal expression of Type 1 Plasminogen Activator Inhibitor and Tissue Factor. The study also showed significant increase in plasminogen activator inhibitor (PAI-1) levels in both plasma and placenta from mothers with pre-eclampsia compared to normal pregnant women which result in higher fibrin deposition in the capillaries of villi leading to fibrinoid necrosis.

Basement membrane thickening identified by PAS stain was seen in 9 /60 (15%) placentae of the control group, 34 /40 (85%) placentae of mild pre-eclampsia and 19 /20(95%) of the placenta of severe pre-eclampsia group.

The comparison of our finding with other studies are shown in table no Fauk et al [94] suggested that basement membrane thickening is secondary to placental ischemia and that the cytotrophoblast secretes basement membrane material. Hence, cytotrophoblastic proliferation is seen as basement membrane thickening in placental ischemia of PIH group.

Terminal villi

The microscopic lesions in intermediate villi were assessed and quantified as percentage of villi showing basement membrane thickening and fibrinoid necrosis. A value of >3% villi showing these changes was considered significant. In the present study, the terminal villi showed significant fibrinoid necrosis and basement membrane thickening. The comparison of present study with other studies are shown in table no 22.

Table 22: Comparison of Basement membrane thickening and fibrinoid necrosis with other studies

Parameters	**Present study**		**Jena M et al [7]**		**Navbir P et al [95]**		**Narasimha etal [84]**	
	Control (n=60)	PE (n=60)	Control (n=50)	PE (n=50)	Control (n=30)	PE (n=30)	Control (n=37)	PE (n=63)
BMT	8%	71%	2%	76%	0%	70%	0%	49.25%
Fibrinoid necrosis	30%	98.3%	76%	100%	73.33%	90%	29.72%	97.82%

Avascular villi reflect failure of vascular organisation. In the present study, also we found increased avascular villi in pre-eclampsia (30%) as compared to control which showed only small foci (10%). This finding correlated with the severity of pre-eclampsia and cases from severe pre-eclampsia showed large foci of avascular villi (90%), while mild pre-eclampsia showed intermediate foci in majority (55%) of cases. It was found to increase in pre-eclampsia in the study done by Sankar KD et al [73] and Kaur P et al [96].

Elastic stain was looked for in the blood vessel wall of terminal villi by using verhoeff's stain. This elastic villus was increase in all cases of severe preeclampsia as compared to on severe preeclampsia and control group. This finding was compared with studies done by Baran PO et al using Verhoeff's stain and Stenmark et al which also showed significant increase of elastic fibre in terminal villi which was dark in color.[90,91]

We already know that elastic tissue fibers increase in systemic hypertension therefore increase in elastic tissue fibers in placental terminal villi during pre-eclampsia may be induced by the hypertension which is a part of protective mechanism.

Spiral artery atherosis was found only in two cases of severe preeclampsia. They appeared as fibrinoid necrosis of arterial wall with subendothelial lipid filled foamy macrophages and perivascular cuff of lymphocytes.[4]

The cause and consequences of the histological features of acute atherosis are not fully understood.[97,98]. Studies have shown that there might be an immunologic component in the development of acute atherosis and that acute atherosis might represent a marker of autoimmune disease. [99,100] Acute atherosis of spiral artery narrows the lumen of the spiral artery causing reduced perfusion of the placenta in pre-eclampsia with the net result of maternal hypertension and proteinuria.[101]

Several issues hamper detection of spiral arterioles as they are found more in the deeper decidual or myometrial segments of the spiral arteries as compared to superficial segments. In present study delivered placenta was only tissue available for pathological examination and they have only a thin layer of decidua left. Placental bed biopsies are preferred mode of examination for studying spiral arteries.[102]

Study done by Jan V et al [103] found on vascular changes in spiral artery bed biopsies in pre-eclampsia showed that placental bed disorders such as acute atherosis was related to increased long-term risk of cardiovascular disease. Therefore, placental examination is crucial for retrospective investigation of pregnancy complications and outcomes.

Acute atherosis in spiral arteries of preeclampsia patient has been reported to occur in 5% to 40% of patients with preeclampsia [104]. The variable frequency of acute atherosis may be explained due to variation in the number of tissue sections taken including size and sample, location of tissue sections, variation in tissue staining methods that is haematoxylin and eosin only or with addition immunohistochemistry done on section to identify foam cells and differences in the pathologist's diagnostic skill. Our study due to limitation of sampling of tissue could not identify the lesion adequately.

CONCLUSION

Vascular changes in the villi of the pre-eclampsia placenta is the basis of the other gross and microscopic changes in the placenta. These changes may bring about differences in function of placenta.

Microscopic examination of stem villi in placenta from pre-eclampsia patient showed increased stromal fibrosis, medial hypertrophy and thrombosis in stem villi, increased basement membrane thickening and fibrinoid necrosis in intermediate villi as well as terminal villi. In addition, terminal villi also showed increased number of avascular villi and increased elastic content as compared to control. These changes were maximum in severe pre-eclampsia > mild pre-eclampsia >Control.

Quantitative determination of placental changes is essential in study of placenta as normal pregnancies can also show similar placental changes due to ageing.

Vascular changes and products released may be the reasons for the onset of disseminated intravascular coagulation, maternal inflammatory syndrome and poor fetal outcome in pre-eclampsia**.** More studies in this regard should be done**.**

SUMMARY

1. A total of one hundred and twenty placentae were studied. Sixty placentae were from pre-eclampsia patients and other sixty were gestational age matched control. Pre-eclampsia group was further divided into mild pre-eclampsia (forty placentae) and severe pre-eclampsia (twenty placentae).

2. Most of our preeclampsia cases were in the age group of 20-25 years with significant low birth weight babies, low placental weight, less diameter and less thickness as compared to controls and was related to the severity of pre-eclampsia.

3. The gross findings of extensive infarction and calcification was increased in pre-eclapsia and as compared to control and this comparison was statistically significant ($p<0.001$)
4. Significant microscopic changes such as increased villous stromal fibrosis, medial hypertrophy and presence of thrombosis in stem villi was more in preeclampsia as compared to control ($p<0.001$)
5. The findings of basement membrane thickening and fibrinoid necrosis in intermediate villi was significantly higher in pre-eclampsia as compared to control ($p<0.001$).
6. The findings of basement membrane thickening, fibrinoid necrosis, avascular villi and elastic content in terminal villi was significantly higher in cases of preeclampsia as compared to control ($p<0.001$).
7. In our study, spiral artery changes were found only in two cases. Hence placental bed biopsies are recommended for studying pathological changes in spiral arteries.

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ANNEXURES

PROFORMA

Title: "Histopathological study of vascular changes in preeclampsia using histochemistry''**.**

NAME:

AGE:

HOSPITAL NO:

BIOPSY NO:

CLINICAL DIAGNOSIS:

CLINICAL FINDING: a) Mother- → 1. Blood pressure
→ 2. Protein

b) Baby → 1. Weight
→ 2. Apgar score

GROSS:

1.Weight-

2.Diameter

3.Thickness

4.Visible calcification

5.Infaction

6.Umbilical cord vessels and knots

MICROSCOPY:

I)Changes in stem villi-

1.Diameter

2.Stromal fibrosis

3.Medial hypertrophy

4.Thrombosis

II)Changes in intermediate villi

1.Diameter

2.Basement membrane thickening

3.Fibrinoid necrosis.

III)Changes in terminal villi

1.Diameter

2.Avascular villi

3.Basement membrane thickening

4.Fibrinoid necrosis

5.Elastic content

I)Spiral artery lesions in the decidua-

Atherosis (Foam cell infiltration, fibrinoid necrosis,lymphocyte)

INFORMED CONSENT FORM

"**Histopathological study of Vascular changes in Placenta in Preeclampsia using Histochemistry**

I, _____the undersigned, agree to participate in this study and authorize the collection and disclosure of my personal information as outlined in this consent form.

I understand the purpose of this study, the risks and benefits of the procedure (contributing placenta after delivery) and the confidential nature of the information that will be collected and disclosed during the study. The information collected will be used only for research.

I have had the opportunity to ask questions regarding the various aspects of this study and my questions have been answered to my satisfaction.

I understand that I remain free to withdraw from this study at any time and this will not change my future care and treatment.

Participation in this study does not involve any extra cost to me.

Subject's name and signature /thumb impression Date:

Name and signature of witness

Date:

Name and signature of person obtaining consent

Date:

ರೋಗಿಯ ಮಾಹಿತಿ ಪತ್ರ

ಅಧ್ಯಯನದ ಶೀರ್ಷಿಕೆ: "Histochemistry ಬಳಸಿಕೊಂಡು ಪ್ರೀಕ್ಲಾಂಪ್ಸಿಯಾದ ಜರಾಯು ರಲ್ಲಿ ನಾಳೀಯ ಬದಲಾವಣೆಗಳನ್ನು Histopathological ಅಧ್ಯಯನ - ಒಂದು ಕೇಸ್ ನಿಯಂತ್ರಣ ಅಧ್ಯಯನದ" **ಅಧ್ಯಯನದ ಸ್ಥಳ**-.R.L. Jalappa ಆಸ್ಪತ್ರೆ ಮತ್ತು ರಿಸರ್ಚ್, Tamaka, ಕೋಲಾರ ಜೋಡಿಸಲಾದ ಶ್ರೀ ದೇವರಾಜ ಅರಸು ವೈದ್ಯಕೀಯ ಕಾಲೇಜು

ಮುಖ್ಯ ಗುರಿ ಪ್ರೀಕ್ಲಾಂಪ್ಸಿಯಾದ ರಲ್ಲಿ ಜರಾಯುವಿನ histopathological ಬದಲಾವಣೆಗಳನ್ನು ಅಧ್ಯಯನ ಮತ್ತು ಸಾಮಾನ್ಯ ಮಹಿಳೆಯರಲ್ಲಿ ಪ್ಲಾಸೆಂಟಾ ಅದನ್ನು ಹೋಲಿಕೆ ಮಾಡುವುದು.

ನೀವು ಪ್ರೌಢಪ್ರಬಂಧದಲ್ಲಿ ಅಂಗವಾಗಿ ರೋಗಶಾಸ್ತ್ರದ ವಿಭಾಗವು ನಡೆಸಿದ ಅಧ್ಯಯನದಲ್ಲಿ ಭಾಗವಹಿಸಲು ಕೋರಲಾಗಿದೆ. ಈ ಅಧ್ಯಯನವು ಪ್ರೀಕ್ಲಾಂಪ್ಸಿಯಾದ ರೋಗಿಯ ಜರಾಯು ಮೇಲೆ ಮಾಡಲಾಗುತ್ತದೆ. ಪ್ರೀಕ್ಲಾಂಪ್ಸಿಯಾದ ರೋಗಿಯ ಜರಾಯು ಸಾಮಾನ್ಯ ಅಥವಾ ಸಿಸೇರಿಯನ್ ನಂತರ ಸಂಗ್ರಹಿಸಲಾಗುವುದು ಮತ್ತು histopathological ಹೋಲಿಕೆ ಸಾಮಾನ್ಯ ಮಹಿಳೆಯರಲ್ಲಿ ಪ್ಲಾಸೆಂಟಾ ವಿರುದ್ಧ ಮಾಡಲಾಗುತ್ತದೆ.

ಈ ಅಧ್ಯಯನವು ನೈತಿಕ ಸಮಿತಿ ಒಪ್ಪಿಗೆ ನಡೆಯಲಿದೆ. ಸಂಗ್ರಹಿಸಿದ ಮಾಹಿತಿಯನ್ನು ಮಾತ್ರ ಪ್ರೌಢಪ್ರಬಂಧದಲ್ಲಿ ಮತ್ತು ಪ್ರಕಟಣೆಗೆ ಬಳಸಲಾಗುತ್ತದೆ. ಭಾಗವಹಿಸಲು ಒಪ್ಪಿಕೊಳ್ಳಲು ಯಾವುದೇ ಕಡ್ಡಾಯ ಇಲ್ಲ. ಸ್ವಯಂಪ್ರೇರಣೆಯಿಂದ ಅಧ್ಯಯನದಲ್ಲಿ ಭಾಗವಹಿಸಲು ಹೆಬ್ಬೆಟ್ಟಿನ ಗುರುತು ನೀಡಲು ಕೋರಲಾಗಿದೆ.

ಸಂಗ್ರಹಿಸಿದ ಎಲ್ಲಾ ಮಾಹಿತಿಯನ್ನು ಗೌಪ್ಯವಾಗಿ ಇಡಲಾಗುತ್ತದೆ ಮತ್ತು ಯಾವುದೇ ಹೊರಗಿನವ ಬಹಿರಂಗ ಮಾಡಲಾಗುವುದಿಲ್ಲ. ನಿಮ್ಮ ಗುರುತನ್ನು ತೋರಿಸಲಾಗುವುದಿಲ್ಲ. ನೀವು ಮೂಲ ತನಿಖೆ ನೀಡಬೇಕಾದ ಮತ್ತು ಈ ಸಂಶೋಧನೆ ಭಾಗವಹಿಸಲು ಯಾವುದೇ ಪರಿಶೀಲನಾ ಲಾಭವನ್ನು ಸ್ವೀಕರಿಸುವುದಿಲ್ಲ.

ಈ ಮಾಹಿತಿ ಸಮ್ಮತಿಯ ದಸ್ತಾವೇಜು ನೀವು ಅಧ್ಯಯನದ ಸಾಮಾನ್ಯ ಹಿನ್ನೆಲೆ ನೀಡಲು ಉದ್ದೇಶಿಸಲಾಗಿದೆ. ಕೆಳಗಿನ ಮಾಹಿತಿಯನ್ನು ಓದಲು ಮತ್ತು ನಿಮ್ಮ ಕುಟುಂಬ ಸದಸ್ಯರು ದಯವಿಟ್ಟು ಎಚ್ಚರಿಕೆಯಿಂದ ಚರ್ಚಿಸಬೇಕು. ಅಧ್ಯಯನದಲ್ಲಿ ನಿಮಗೆ ಯಾವುದೇ ಸಮಯದಲ್ಲಿ ಅಧ್ಯಯನ ಸಂಬಂಧಿಸಿದ ನಿಮ್ಮ ಪ್ರಶ್ನೆಗಳನ್ನು ಕೇಳಬಹುದು. ನೀವು ಅಧ್ಯಯನದಲ್ಲಿ ಭಾಗವಹಿಸಲು ಒಪ್ಪಿದರೆ ನೀವು ಅಧ್ಯಯನ ಮತ್ತು ಇಡೀ ಪ್ರಕ್ರಿಯೆಯನ್ನು ಭಾಗವಹಿಸಲು ಬಯಸುವ ರಸೀದಿಯನ್ನು ಪಡೆಯುವ ಒಂದು ಒಪ್ಪಿಗೆ ತಿಳಿಸಲು ನಮೂನೆಗೆ ಸಹಿ ಕೇಳಲಾಗುತ್ತದೆ. ನೀವು ವಿವರಣೆ ಇಲ್ಲದೆ ಅಧ್ಯಯನದಲ್ಲಿ ಯಾವುದೇ ಸಮಯದಲ್ಲಿ ಭಾಗವಹಿಸಲು ನಿಮ್ಮ ಸಮ್ಮತಿಯನ್ನು ಹಿಂಪಡೆಯಲು ಉಚಿತ ಮತ್ತು ಈ ನಿಮ್ಮ ಭವಿಷ್ಯದ ಕಾಳಜಿ ಬದಲಾಗುವುದಿಲ್ಲ.

ಯಾವುದೇ ಸ್ಪಷ್ಟೀಕರಣ ನೀವು ಸಂಶೋಧಕ ಸಂಪರ್ಕಿಸಲು ಉಚಿತ.

ಪ್ರಾಥಮಿಕ ಪರೀಕ್ಷಕ: ಡಾ ಪಾಂಡೆ ಸ್ವಾತಿ

ಸಂಪರ್ಕಿಸಿ: 9986419588

ಈಮೇಲ್ ಅಡ್ರೆಸ್: swatipandey30@gmail.com

PATIENT INFORMATIOM SHEET

STUDY TITLE: "**Histopathological study of Vascular changes in Placenta in Preeclampsia using Histochemistry."**

PLACE OF STUDY: Sri Devaraj Urs Medical College attached to R.L Jalappa Hospital and Research, Tamaka, Kolar.

The main aim is to study the histopathological changes of placenta in preeclampsia and compare it with placenta of normal women.

You are requested to participate in a study conducted by the department of pathology as a part of dissertation. This study will be done on placenta of preeclampsia patient. The placenta of preeclampsia patient will be collected after normal or caesarean section and a histopathological comparison will be done against placenta of normal women.

This study will be approved by the institutional ethical committee. The information collected will be used only for dissertation and publication. There is no compulsion to agree to participate. You are requested to sign/provide thumb impression only if you voluntarily agree to participate in the study.

All information collected from you will be kept confidential and will not be disclosed to any outsider. Your identity will not be revealed. You will have to pay for the basic investigation and you will not receive any monetary benefits to participate in this research.

This inform consent document is intended to give you a general background of study. Please read the following information carefully and discuss with your family members. You can ask your queries related to study at any time during the study. If you are willing to participate in the study you will be asked to sign an inform consent form by which you are acknowledging that you wish to participate in the study and entire procedure will be explained to you by the

study doctor. You are free to withdraw your consent to participate in the study any time without explanation and this will not change your future care.

For any clarification, you are free to contact the investigator.

PRINCIPAL INVESTIGATOR: Dr. Swati Pandey

Contact no: 9148832496

Email ID: swatipandey30@gmail.com

KEY TO MASTER CHART

BP-Sys-Blood Pressure-Systolic

BP-Dia-Blood Pressure-Diastolic

Pro-Protein

B.Wt-Birth weight

PT.Wt-Placenta weight

D-Diameter

Thic-Thickness

Mem-Appearance of membrane

O-Opaque

SO-Semi-opaque

C-Central

Ins-Insertion of umbilical cord

C-Clear

E-Eccentric

PC-Paracentral

M-Marginal

Cal-Calcification

Inf-Infarction

CV-Cord vessel number

SV-SF-Stem villi-Stromal fibrosis

SV-MT-Stem villi-Medial hypertrophy

SV-T-Stem villi-Thrombosis

IV-BMT-Intermediate villi-Basement membrane thickening

IV-FN-Intermediate villi-Fibrinoid necrosis

TV-BMT-Transverse villi-Basement membrane thickening

TV-AV-Transverse villi-Avascular villi

TV-FN-Transverse villi –Fibrinoid necrosis

TV-EC-Transverse villi-Elastic content

SA-A-Spiral artery-Atherosis

Dig-Diagnosis

1-Nonsevere pre-eclampsia

2-Severe pre-eclampsia

3.Control

	Name	Age	Hos no	Biopsy no	BP-Sy	BP-Dia	Pro	B-Wt	Pt-Wt	D	Thic	Mem	Ins	Cal	Inf	CV	SV-SF	SV-MT	SV-T	IV-BMT	IV-FN	TV-BMT	TV-AV	TV-FN	TV-EC	SA-A	Dia
1	Aruna	24	240528	B/66/16	150	90	1+	3.15	500	14	2	O	C	P	A	3	>3%	20.2	A	>3%	<3%	<3%	SF	>3%	A	A	1
2	Asha	21	245004	B/199/16	144	90	1+	3.08	510	15	2.2	O	E	A	A	3	<3%	18.5	A	<3%	>3%	<3%	SF	>3%	A	A	1
3	Yashmin	27	244721	B/201/16	160	110	3+	2.47	430	13	2.5	O	E	A	P	3	>3%	25.2	P	>3%	>3%	>3%	LF	>3%	P	A	2
4	Nagaveni	25	212759	B/265/16	150	90	1+	2.3	520	15	2.1	SO	E	A	A	3	<3%	18.6	A	>3%	>3%	>3%	SF	>3%	A	A	1
5	Shardamma	31	229765	B/266/16	150	90	1+	2.2	530	22	2.4	O	E	A	A	3	<3%	20.6	A	>3%	>3%	>3%	IF	>3%	A	A	1
6	Naseema taj	24	208546	B/287/16	150	90	1+	2.1	470	16	2.4	SO	E	A	P	3	<3%	19.5	A	>3%	>3%	>3%	SF	>3%	A	A	1
7	Rutima	20	155543	B/288/16	150	90	1+	2	400	21	2.2	SO	E	A	P	3	<3%	18	A	>3%	>3%	<3%	SF	>3%	A	A	1
8	Varalakshmi	28	250479	B/307/16	160	90	1+	2	420	20	2.5	O	E	A	P	3	<3%	19.8	A	>3%	>3%	>3%	IF	>3%	A	A	1
9	Vedashree	21	242255	B/390/16	160	110	3+	2.93	480	14	2.1	O	PC	A	P	3	>3%	23.5	P	>3%	>3%	>3%	LF	>3%	P	A	2
10	Salamma	26	243164	B/392/16	150	92	1+	2.53	510	13	3.2	C	E	A	A	3	>3%	19.6	A	>3%	>3%	<3%	SF	>3%	A	A	1
11	Parvathi	23	247554	B/393/16	160	110	2+	3.3	470	15	1.5	O	PC	P	A	3	>3%	20.2	P	>3%	>3%	>3%	LF	>3%	P	A	2
12	Arthi	23	254707	B/394/16	160	100	1+	2.1	430	18	2.5	O	PC	A	A	3	>3%	18.2	A	>3%	>3%	>3%	SF	>3%	A	A	1
13	Asha	25	182242	B/395/16	150	100	1+	2.2	450	16	2.4	O	PC	A	A	3	>3%	18.8	A	>3%	>3%	<3%	IF	>3%	A	A	1
14	Saritha	25	255928	B/436/16	160	110	2+	2.3	480	14	2.5	O	PC	A	P	3	>3%	21.2	P	>3%	>3%	>3%	LF	>3%	A	A	2
15	Veena	25	254618	B/437/16	150	90	1+	2.2	400	15	2.5	O	PC	A	P	3	<3%	18.2	A	>3%	>3%	>3%	SF	>3%	A	A	1
16	Pallavi	25	255077	B/493/16	150	90	1+	1.72	470	20	2.5	O	E	A	A	3	<3%	20.6	A	>3%	>3%	<3%	SF	<3%	A	A	1
17	Shilpa	24	255073	B/494/16	160	110	3+	2.06	500	14	2.4	SO	PC	A	A	3	>3%	20.4	A	>3%	>3%	>3%	LF	>3%	P	A	2
18	Triveni	23	255988	B/496/16	170	110	3+	2.02	520	13	1.5	O	C	P	A	3	<3%	22.5	A	<3%	>3%	>3%	LF	>3%	P	A	2
19	Netravathi	28	251769	B/626/16	160	110	3+	2.3	510	14	2.2	O	E	A	A	3	>3%	20.2	A	>3%	>3%	>3%	LF	>3%	P	A	2
20	Sheela	19	263634	B/734/16	180	110	3+	2.23	480	14	1.8	C	M	P	P	3	>3%	20.4	A	>3%	>3%	>3%	LF	>3%	P	A	2
21	Lakshmi	25	265500	B/735/16	170	110	3+	3	400	13	2	O	E	A	P	3	>3%	23	A	>3%	>3%	>3%	LF	>3%	P	A	2
22	Bhavya	20	265900	B/736/16	160	110	3+	1.84	500	14	2.1	O	M	P	P	3	>3%	20.1	A	>3%	<3%	>3%	LF	>3%	P	P	2
23	Madhumati	23	265199	B/737/16	160	110	1+	3	520	14	2.2	O	PC	A	A	3	>3%	20.2	A	>3%	>3%	>3%	LF	>3%	P	A	2
24	Shashitha	18	273241	B/915/16	150	100	1+	2.1	410	14	2.5	O	PC	A	A		<3%	19.5	A	>3%	>3%	<3%	SF	>3%	A	A	1
25	Shobha	20	273147	B/916/16	150	90	1+	2.5	430	15	2.5	O	PC	A	A		>3%	18	A	>3%	>3%	>3%	SF	>3%	A	A	1
26	Kalpana	32	368171	B/3159/16	160	110	3+	3.04	460	14	2.5	SO	PC	P	P	3	>3%	20.8	A	>3%	>3%	>3%	LF	>3%	P	A	2
27	Shalini	20	372932	B/3319/16	170	110	3+	2.1	410	13	3	C	E	P	P	3	>3%	20.3	A	>3%	>3%	>3%	LF	>3%	P	A	2
28	Ramya	21	380848	B/1326/17	150	90	1+	2	430	14	1.5	O	C	A	A	3	<3%	19.8	A	<3%	>3%	<3%	IF	>3%	A	A	1
29	Rekha	36	382243	B/1351/17	158	90	1+	2.1	400	14	2.4	O	PC	A	A	3	>3%	18.6	A	>3%	>3%	>3%	IF	>3%	A	A	1

30	Faseeha	20	375880	B/1352/17	160	90	1+	2.92	500	14	2.4	SO	E	A	A	3	>3%	18.5	A	>3%	>3%	<3%	IF	>3%	A	A	1
31	Ramya	21	384432	B/1693/17	150	92	1+	2.46	510	22	2.1	C	PC	A	A	3	>3%	20.2	A	>3%	>3%	>3%	IF	>3%	A	A	1
32	Shobha	23	305221	B/1694/17	140	90	1+	2.6	520	14	1.5	O	E	P	A	3	>3%	19.6	A	>3%	>3%	<3%	IF	>3%	A	A	1
33	Anitha	25	384687	B/1695/17	140	92	1+	1.63	400	13	2	O	E	A	A	3	>3%	18.8	A	>3%	>3%	>3%	IF	>3%	A	A	1
34	Shivrani	20	385623	B/1696/17	144	90	1+	3.78	410	13	3	O	PC	A	A	3	>3%	18.5	A	>3%	>3%	>3%	IF	>3%	A	A	1
35	Uma	22	463448	B/1816/17	160	110	3+	1.47	410	12	2	O	C	P	P	3	>3%	20.72	A	>3%	>3%	>3%	IF	>3%	P	A	2
36	Mamatha	25	466215	B/1889/17	160	110	3+	2	400	13	2	C	E	P	P	3	>3%	20.6	A	>3%	>3%	>3%	LF	>3%	P	A	2
37	Rajeshwari	23	469555	B/1890/17	170	110	3+	2.2	410	14	1.6	O	PC	P	P	3	>3%	22.4	A	>3%	>3%	>3%	LF	>3%	P	A	2
38	Pushpa	23	389052	B/2052/17	150	90	1+	3.3	520	14	2	O	E	A	A	3	<3%	20.2	A	<3%	>3%	>3%	IF	>3%	A	A	1
39	Rohini	24	382954	B/2014/17	150	100	1+	3.84	490	20	2.5	C	E	A	A	3	>3%	19.5	A	>3%	>3%	>3%	IF	>3%	A	A	1
40	Asma taj	24	390831	B/2015/17	140	100	1+	1.07	480	18	2.7	O	C	P	A	3	>3%	20.2	A	>3%	>3%	<3%	IF	>3%	A	A	1
41	Swetha	22	390423	B/2029/17	160	110	3+	1	420	14	2.8	O	E	P	P	3	>3%	20	A	>3%	>3%	>3%	LF	>3%	P	A	2
42	Uma	30	391369	B/2030/17	140	92	1+	1	510	21	1.5	C	E	A	A	3	>3%	18.6	A	>3%	>3%	>3%	IF	>3%	A	A	1
43	Anitha	27	391457	B/2034/17	140	90	1+	2.54	540	20	3	O	C	A	A	3	>3%	18.5	A	<3%	>3%	<3%	SF	>3%	A	A	1
44	Heerabai	24	392510	B/2061/17	140	90	1+	2	430	20	2.4	O	E	P	A	3	>3%	19.5	A	>3%	>3%	>3%	IF	>3%	A	A	1
45	Sudharani	24	392551	B/2062/17	150	90	1+	2.4	420	22	2.3	C	PC	A	A	3	>3%	20.6	A	>3%	>3%	<3%	IF	>3%	A	A	1
46	Anjali	25	394089	B/2101/17	150	100	1+	2.07	500	22	2.1	O	E	A	A	3	>3%	19.8	A	>3%	>3%	>3%	SF	>3%	A	A	1
47	Rekha	21	394170	B/2102/17	160	110	3+	1.2	420	13	2	O	E	P	P	3	>3%	23.4	A	>3%	>3%	>3%	IF	>3%	A	A	2
48	Lakshmidevi	23	394069	B/2115/17	140	90	1+	1.48	400	18	2.1	O	E	P	A	3	>3%	18	A	<3%	>3%	<3%	SF	>3%	A	A	1
49	Kavita	23	390425	B/2116/17	170	110	3+	1.01	410	13	2.2	O	E	A	P	3	>3%	20	A	>3%	>3%	>3%	LF	>3%	A	A	2
50	Zarima firdos	28	367903	B/2035/17	150	90	1+	3.3	500	25	2.5	C	C	A	A	3	>3%	19.6	A	<3%	>3%	>3%	IF	>3%	A	A	1
51	Jasmiya taj	20	388337	B/2051/17	150	90	1+	2.35	510	24	2.4	O	E	A	A	3	>3%	18.2	A	>3%	>3%	<3%	IF	>3%	A	A	1
52	Nagin taj	29	394556	B/2126/17	160	110	3+	3.4	430	14	2	O	E	A	P	3	>3%	20.2	A	>3%	>3%	>3%	LF	>3%	P	P	2
53	Sowjanya.M	26	311169	B/2127/17	150	100	1+	2.48	500	22	2.3	C	C	A	A	3	>3%	18.8	A	>3%	>3%	>3%	SF	>3%	A	A	1
54	Aruna	21	386674	B/2139/17	140	92	1+	1.75	510	24	2.5	O	E	A	A	3	>3%	20.6	A	>3%	>3%	<3%	SF	>3%	A	A	1
55	Bhargavi	24	386863	B/2140/17	140	100	1+	1.63	490	22	2.5	O	E	A	A	3	<3%	19.8	A	>3%	>3%	>3%	IF	>3%	A	A	1
56	Annaporna	23	391487	B/2148/17	150	90	1+	1.4	540	22	2.4	O	E	A	A	3	<3%	18.6	A	>3%	>3%	>3%	SF	>3%	A	A	1
57	Chaitra	24	385529	B/2149/17	160	90	1+	1.01	550	14	2	O	E	A	P	3	<3%	18	A	>3%	>3%	<3%	IF	>3%	A	A	1
58	Pushpamma	35	393185	B/2161/17	150	90	1+	1.87	490	22	2.4	O	E	A	A	3	<3%	19.6	A	>3%	>3%	>3%	SF	>3%	A	A	1
59	Priyanka	25	360192	B/2162/17	150	100	1+	3.06	500	14	2	O	E	A	A	3	<3%	18.8	A	>3%	>3%	>3%	IF	>3%	A	A	1
60	Bgagyamma	24	249575	B/2255/17	150	90	1+	2.01	400	13	2	O	E	A	A	3	<3%	18.2	A	>3%	>3%	>3%	IF	>3%	A	A	1
61	Renuka	20	189712	B/2343/15	110	70	Ab	2.8	620	21	2.7	C	C	A	A	3	>3%	6.6	A	<3%	<3%	<3%	A	<3%	A	A	3
62	Deepa	20	187500	B/2344/15	120	80	Ab	2.7	720	20	2.5	C	C	A	A	3	<3%	5.22	A	<3%	<3%	<3%	A	<3%	A	A	3
63	Rekha	26	190200	B/2358/15	120	70	Ab	2.6	710	20	2.5	C	E	A	A	3	<3%	6.08	A	<3%	<3%	<3%	A	<3%	A	A	3

64	Basheera	24	192391	B/2403/15	120	70	Ab	2.6	540	18	3.2	C	PC	A	A	3	<3%	6.32	A	<3%	<3%	<%3	A	<3%	A	A	3
65	Chaitra	23	129240	B/2422/15	120	70	Ab	3.1	520	20	3.3	C	PC	A	A	3	<3%	6.13	A	<3%	<3%	<3%	A	<3%	A	A	3
66	Munilakshma	21	194317	B/2452/15	120	80	Ab	2.8	470	21	3.2	C	PC	A	A	3	<3%	6.22	A	<3%	<3%	<3%	SF	<3%	A	A	3
67	Roopa	20	194270	B/2453/15	120	80	Ab	3.2	510	22	3.2	C	PC	A	A	3	<3%	7.41	A	<3%	<3%	<3%	A	<3%	A	A	3
68	Radha	36	182051	B/2455/15	120	88	Ab	3	560	18	3.5	C	C	A	A	3	<3%	7.5	A	<3%	<3%	<3%	A	<3%	A	A	3
69	Nagaveni	22	198723	B/2532/15	110	70	Ab	3.4	480	22	3.2	C	C	A	A	3	<3%	6.57	A	<3%	<3%	<3%	A	<3%	A	A	3
70	Roopa	21	136020	B/2537/15	120	70	Ab	2.8	520	22	3	C	C	A	A	3	<3%	6.2	A	<3%	<3%	<3%	A	<3%	A	A	3
71	Nagarathna	24	199154	B/2545/15	120	80	Ab	2.6	540	20	3.3	C	PC	A	A	3	<3%	6.22	A	<3%	<3%	<3%	A	<3%	A	A	3
72	Ashwini	20	199964	B/2559/15	110	70	Ab	2.5	500	18	2.8	C	PC	A	A	3	<3%	6.32	A	<3%	<3%	<3%	A	<3%	A	A	3
73	Harshiya	37	202058	B/2616/15	110	70	Ab	2.6	520	20	3	C	C	A	A	3	<3%	7.41	A	<3%	<3%	<3%	SF	<3%	A	A	3
74	Anusha	22	240528	B/67/16	110	60	Ab	3	550	14	2.3	C	C	A	A	3	>3%	6.57	A	>3%	>3%	<3%	A	>3%	A	A	3
75	Pratima	23	162627	B/198/16	110	70	Ab	2.96	500	23	2.6	C	C	A	A	3	>3%	5.22	A	<3%	>3%	<3%	A	>3%	A	A	3
76	Sakamma	21	241092	B/200/16	112	70	Ab	3.06	520	20	3.2	SO	C	A	A	3	<3%	6.13	A	<3%	<3%	<3%	A	<3%	A	A	3
77	Suma	30	243005	B/391/16	100	60	Ab	3	600	22	2.6	C	C	A	A	3	>3%	6.08	A	<3%	<3%	<3%	A	<3%	A	A	3
78	Parvathi	23	247554	B/393/16	110	70	Ab	3.3	550	21	2.6	O	C	A	P	3	>3%	7.5	A	<3%	>3%	<3%	A	>3%	A	A	3
79	Keertana	23	245774	B/495/16	120	80	Ab	2.82	300	20	2.4	C	C	A	A	3	<3%	6.2	A	<3%	<3%	<3%	A	<3%	A	A	3
80	Meenakshi	20	255077	B/624/16	110	60	Ab	2.62	550	13	2.5	SO	C	A	A	3	<3%	6.6	A	<3%	<3%	<3%	A	>3%	A	A	3
81	Shashikala	24	255073	B/625/16	110	70	Ab	2.25	320	21	3.1	C	PC	A	A	3	<3%	5.22	A	<3%	<3%	<3%	SF	<3%	A	A	3
82	Rajeshwari	21	179697	B/627/16	120	70	Ab	3.18	500	22	2.6	C	C	A	A	3	<3%	6.32	A	>3%	>3%	>3%	A	>3%	A	A	3
83	Prema	23	255988	B/1107/16	120	80	Ab	2.8	580	23	2.8	C	C	A	P	3	<3%	6.6	A	<3%	<3%	<3%	A	<3%	A	A	3
84	Radhamma	24	242673	B/1108/16	110	70	Ab	3.02	630	22	2.2	C	M	A	A	3	>3%	6.22	A	<3%	<3%	<3%	A	>3%	A	A	3
85	Damethi	18	243083	B/1109/16	120	80	Ab	2	680	14	2.5	C	C	A	A	3	<3%	6.13	A	<3%	<3%	<3%	A	<3%	A	A	3
86	Nagaveni	22	245001	B/2523/16	110	70	Ab	3	500	21	3.2	SO	E	A	A	3	<3%	6.08	A	>3%	<3%	>3%	A	>3%	A	A	3
87	Aruna	28	243945	B/2524/16	110	60	Ab	2.5	520	20	2.6	C	C	A	A	3	<3%	7.41	A	<3%	<3%	<3%	A	<3%	A	A	3
88	Dhakshyani	30	243931	B/2525/16	120	70	Ab	2.6	510	18	2.3	C	PC	A	A	3	<3%	6.2	A	<3%	>3%	<3%	A	>3%	A	A	3
89	Lakshmiya	21	224392	B/2526/16	120	80	Ab	2.85	480	20	2.8	O	C	A	A	3	<3%	6.57	A	<3%	<3%	<3%	SF	<3%	A	A	3
90	Geeta	23	200642	B/2590/16	110	70	Ab	3.1	510	14	3.1	C	C	A	A	3	<3%	7.5	A	<3%	<3%	<3%	A	>3%	A	A	3
91	Sunitha	27	243067	B/2591/16	120	80	Ab	2.56	520	22	2.6	SO	C	A	A	3	<3%	7.41	A	>3%	<3%	>3%	A	>3%	A	A	3
92	Aruna	25	67231	B/2592/16	110	70	Ab	2.85	550	23	2.2	C	C	A	A	3	<3%	6.22	A	<3%	<3%	<3%	A	<3%	A	A	3
93	Asma taj	22	243913	B/2593/16	110	60	Ab	3.66	570	21	3.1	SO	PC	A	A	3	<3%	6.13	A	<3%	<3%	<3%	A	<3%	A	A	3
94	Bhavani	26	244703	B/629/17	120	80	Ab	2.5	560	14	2.5	C	M	A	A	3	<3%	6.32	A	>3%	>3%	<3%	A	>3%	A	A	3
95	Rani	25	243779	B/630/17	110	70	Ab	3	580	20	2.5	C	C	A	A	3	<3%	6.08	A	<3%	<3%	<3%	A	<3%	A	A	3
96	Gayathri	31	244685	B/631/17	126	80	Ab	3.23	590	21	2.8	C	C	A	A	3	<3%	5.22	A	<3%	<3%	<3%	A	<3%	A	A	3
97	Rajani	20	244620	B/774/17	110	66	Ab	2.29	540	22	3.1	SO	E	A	A	3	<3%	6.6	A	<3%	<3%	<3%	A	>3%	A	A	3

98	Pavithra	21	244316	B/775/17	110	70	Ab	2.6	680	20	2	C	C	A	A	3	<3%	6.57	A	<3%	<3%	<3%	A	<3%	A	A	3
99	Jamuna	24	244994	B/776/17	120	70	Ab	2.4	600	19	2.6	C	C	A	A	3	<3%	6.2	A	<3%	<3%	<3%	A	<3%	A	A	3
100	Suvama	23	244307	B/777/17	120	80	Ab	2.8	680	23	2.4	C	C	A	A	3	<3%	7.5	A	>3%	>3%	>3%	A	>3%	A	A	3
101	Soumya	26	360264	B/1057/17	110	80	Ab	2.9	720	20	2.5	C	E	A	A	3	<3%	7.5	A	<3%	<3%	<3%	A	<3%	A	A	3
102	Girija	24	389747	B/1058/17	110	70	Ab	3.26	710	21	2.6	SO	C	A	A	3	<3%	7.41	A	<3%	<3%	<3%	A	<3%	A	A	3
103	Varalakshmi	21	389940	B/1071/17	120	60	Ab	2.58	710	22	2.2	C	PC	A	A	3	<3%	6.22	A	<3%	<3%	<3%	A	>3%	A	A	3
104	Chaitra	25	386574	B/1072/17	110	70	Ab	2.12	600	21	3.1	C	C	A	A	3	<3%	6.13	A	<3%	<3%	<3%	A	<3%	A	A	3
105	Pavithra	30	254782	B/1175/17	110	70	Ab	2.8	590	20	2.6	C	E	A	A	3	<3%	6.22	A	<3%	<3%	<3%	A	<3%	A	A	3
106	Lalitha	30	316902	B/1176/17	110	66	Ab	4.29	550	21	2.5	SO	PC	A	A	3	<3%	6.18	A	>3%	>3%	>3%	A	>3%	A	A	3
107	Kalpana	21	390446	B/1177/17	120	80	Ab	4.31	580	22	2.7	O	C	A	A	3	<3%	5.32	A	<3%	<3%	<3%	A	<3%	A	A	3
108	Shilpa	21	390727	B/1178/17	110	70	Ab	2.4	720	23	2.5	C	C	A	A	3	<3%	6.5	A	<3%	<3%	<3%	A	<3%	A	A	3
109	Ramya	23	328112	B/1310/17	110	80	Ab	3.5	500	20	3.1	C	C	A	A	3	<3%	6.57	A	>3%	>3%	<3%	A	>3%	A	A	3
110	Ruksar	23	464647	B/1820/17	120	70	Ab	2.32	510	21	2.5	C	C	A	A	3	<3%	6.2	A	<3%	<3%	<3%	A	<3%	A	A	3
111	Mubarak	31	464696	B/1821/17	120	80	Ab	2.6	710	20	2.6	SO	C	A	A	3	<3%	6.22	A	<3%	<3%	<3%	A	<3%	A	A	3
112	Savitha	22	455577	B/822/17	120	80	Ab	2.8	710	21	2.6	C	C	A	A	3	<3%	6.13	A	>3%	>3%	<3%	SF	>3%	A	A	3
113	Bhagyamma	23	463809	B/1817/17	120	70	Ab	3	520	23	2.5	C	E	A	A	3	<3%	6.32	A	<3%	<3%	<3%	A	<3%	A	A	3
114	Jyothika	31	375961	B/1818/17	120	80	Ab	3.2	530	20	2.6	SO	C	A	A	3	<3%	6.08	A	<3%	<3%	<3%	A	>3%	A	A	3
115	Ramika	25	379225	B/1819/17	120	66	Ab	4	710	18	3.1	C	C	A	A	3	<3%	7.41	A	<3%	<3%	<3%	A	<3%	A	A	3
116	Lakshmi	22	465692	B/1912/17	110	70	Ab	4.2	720	18	2.7	C	C	A	A	3	<3%	7.5	A	<3%	<3%	<3%	A	<3%	A	A	3
117	Ramya	23	470433	B/1924/17	110	66	Ab	3.5	720	20	3.1	C	E	A	A	3	<3%	6.57	A	<3%	<3%	<3%	A	<3%	A	A	3
118	Pavithra	24	469614	B/1925/17	110	70	Ab	3.6	710	21	2.5	C	C	A	A	3	<3%	6.2	A	<3%	<3%	<3%	A	<3%	A	A	3
119	Saraswathi	24	465197	B/1926/17	110	60	Ab	3	710	20	2.7	SO	C	A	A	3	<3%	5.22	A	<3%	<3%	<3%	SF	<3%	A	A	3
120	Manisha	22	467813	B/1997/17	110	70	Ab	2.5	600	22	2.5	C	E	A	A	3	<3%	6.6	A	<3%	<3%	<3%	A	<3%	A	A	3