1. INTRODUCTION

The intrauterine existence of fetus solely depends on one organ “The Placenta”. Placenta is a vital organ playing central role in pregnancy. It maintains pregnancy and promotes normal fetal development and serves as a major organ for transfer of essential elements between mother and fetus.\textsuperscript{1} It has a significant role in health of the fetus because it is the sole source of nutrients for the fetus and first line of defense from the external world. Placental function is highly influenced by its anatomical structure.\textsuperscript{2} One of the key roles of placenta is the transport of maternal nutrients to the fetus. Many placental diseases result in fetal growth restriction or even fetal death.\textsuperscript{3} Placental efficiency is improved by increase in placental nutrient transfer capacity and thus permits increase in the number of grams fetal weight. Placental insufficiency is major cause of impaired fetal growth.\textsuperscript{1} Therefore placenta has been implicated with aberrant fetal growth which is associated with pregnancy pathologies. Thus histology and morphology of placenta is considered essential.

There are many well established causes of intrauterine growth restriction (IUGR), and preeclampsia being one of them. Preeclampsia is a systemic disorder defined as development of hypertension and proteinuria after 20 weeks of gestation in previously normotensive woman. Preeclampsia affects 5 to 7 percent of women worldwide and is a major cause for maternal and neonatal morbidity and mortality.\textsuperscript{4} It is one of the disorders of pregnancy which is accompanied by pathological changes in placenta and is associated with high perinatal morbidity and mortality.\textsuperscript{5} Preeclampsia contributes to complications like preterm birth, perinatal death, IUGR and is directly associated with 10 to 15 % of maternal deaths. The incidence being 3 to 7 % in nulliparas and 1 to 3 % in multiparas. Pathophysiology of this multisystem disorder characterized by abnormal vascular response to placentation still remains unclear.\textsuperscript{6}

Preeclampsia is a major unsolved problem in feto-maternal medicine and is a primary cause of placental insufficiency.\textsuperscript{7} Despite decades of research on this condition there is no significant improvement to predict preeclampsia prior to the onset of symptoms.\textsuperscript{8} Hypertension in pregnancy is found to be associated with various histological changes in placenta. These changes eventually lead to poor fetal outcome.\textsuperscript{9} Abnormal
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cytotrophoblast invasion leads to placental ischemia and endothelial dysfunction which characterizes preeclampsia.⁸

Etiology of preeclampsia remains unknown, currently accepted hypothesis are placental ischemia hypothesis, genetic hypothesis, immune maladaptation hypothesis and hypothesis of imbalance between free oxygen radicals and scavengers in favor of oxidants.¹⁰ A long standing hypothesis suggests that preeclampsia develops as a consequence of immune maladaptation between mother and fetus leading to impaired tissue and arterial invasion by trophoblastic cells followed by worsened placental perfusion. This results in chronic hypoxia in intervillous space and is expected to trigger placental necrosis.¹¹

Pathogenesis of preeclampsia is believed to be multifactorial although it remains a subject of extensive research. It is accepted that the presence of placenta rather than fetus is responsible for development of preeclampsia, thus placenta plays a vital role in development of preeclampsia but the severity and progress is significantly affected by maternal response to factors and proteins derived from placenta.⁴ Although the cause of preeclampsia is unknown evidence strongly implicates placenta and anatomical examination shows that the part of placenta that is most affected by this syndrome is fetal maternal interface.¹²

Preeclampsia is a disorder of vascular endothelial malfunction and vasospasm.¹³ Placenta is the key organ in pathogenesis of preeclampsia and its removal abolishes the disease. Pathological examination of placentae of preeclamptic women show abnormalities like infarcts, atherosis, thrombosis and chronic inflammation.¹⁴ A fetus is not a requisite for preeclampsia although chorionic villi are essential. Preeclampsia is characterized by abnormalities like vascular endothelial damage subsequently leading to vasospasm.⁹ Previously demonstrated histochemical observations indicate changes in intensities and distribution of important placental enzymes. Trophoblastic dysfunction may lead to placental insufficiency in preeclampsia. Disappearance of the disease immediately after delivery indicates that preeclampsia is a “placental disease”.⁷

This study reviews the importance of morphological and histochemical changes of placentae associated with preeclampsia.
2. AIM AND OBJECTIVES

AIM:

Histology and histochemistry of placenta in pregnancies complicated by preeclampsia.

OBJECTIVES:

1. To study morphology of normotensive and preeclamptic placentae.
2. To study histology of normotensive and preeclamptic placentae.
3. To study placental alkaline phosphatase activity in normotensive and preeclamptic placentae.
4. To analyse the histochemical distribution of glycogen in normotensive and preeclamptic placentae.
5. To analyse the histochemical distribution of glycosaminoglycans in normotensive and preeclamptic placentae.
6. To evaluate histochemical distribution of lipids in normotensive and preeclamptic placentae
7. To compare the morphological, histological and histochemical findings of preeclamptic placentae with normotensive placentae.
8. To compare the present findings with previous workers.
3. PLACENTA

3.1 PLACENTA:

Placenta is a feto-maternal organ which is a primary site of nutrient and gas exchange between the mother and fetus.\textsuperscript{15}

Figure 1

After fertilization and implantation is accomplished syncytiotrophoblast secretes a hormone called human chorionic gonadotrophin. This hormone prolongs the life of corpus luteum which continues to secrete oestrogen and progesterone during approximately the first two months of pregnancy. There after these and other hormones are province of definitive placenta. Menstruation does not occur and endometrium is now known as decidua of pregnancy. Decidua thickens further to form a suitable nidus for the conceptus.

As the blastocyst implants the syncytiotrophoblast digests and invades the endometrium including glands and walls of maternal blood vessels. Syncytiotrophoblast rapidly thickens towards embryonic pole and gradually thinner over the rest of the wall. After about 9-11 days of pregnancy lacunar spaces and microvillus lined clefts develop in syncytiotrophoblast. As the conceptus grows these lacunae enlarge to form an initial intervillous space. Microvillous trophoblastic walls are converted into an irregular
labyrinth which is further invaded first with cytotrophoblast and then with mesenchyme to form a radial array of secondary placental villi. Villous strands extend from the syncytial layer of chorion across the intervillous space. A layer of cytotrophoblast lined by vascularized fetal mesenchyme is present on their embryonic aspect. The villous strands extend to the layer of peripheral trophoblast which is opposed directly by excavated maternal tissues. Extravasated maternal blood continues to enter the intervillous space through spaces in layer of peripheral trophoblast.

As the intrasyncytial lacunae are developing a column of proliferating cytotrophoblast extends from chorionic plate through the syncytium to make direct contact with the maternal stroma. Cytotrophoblast proliferation further occurs laterally so that the neighboring outgrowths meet to form a spherical cytotrophoblastic shell around the conceptus. Capillaries from within the mesenchymal core now establish connections with the radicles of umbilical vessels in the general mesenchyme of chorion. Each villus now consists of vascularized mesenchymal core covered by a layer of cytotrophoblast which is again en sheathed by a layer of syncytium. These are now called the tertiary villi. Near the maternal interface these villi do not contain mesenchymal core but solid cytotrophoblastic cell columns which are continuous peripherally with the cytotrophoblastic shell. The developing placenta thus consists of tertiary chorionic villi connected to the maternal stroma by cytotrophoblastic columns called as anchoring villi.

Expansion of whole conceptus is accompanied by radial growth of villi and integrated tangential growth of trophoblastic shell and branching villous tree continuing till term. Eventually each stem villus forms a complex consisting of single trunk attached to the chorion at its base, from which arise the second and third order branches.

Each terminal villus commences as a syncytial outgrowth which is invaded by cytotrophoblastic cells which then develops a core of fetal mesenchyme and is finally vascularized by fetal capillaries. Terminal villi are specialized for exchange between the fetal and maternal circulations. Terminal villi continue to form and branch, projecting in all directions in intervillous space within the confines of definitive placenta throughout gestation.15
3.1.1 SHAPE AND SIZE OF PLACENTA:

A mature placenta is a flattened discoid mass which is circular or oval in outline and measures about 15 to 20 cms in diameter, 2 to 3 cms in thickness and weighs about 500 to 600 grams at term.\textsuperscript{16}

An expelled placenta consists of two surfaces:

- Fetal surface
- Maternal surface

**Fetal surface**: Macroscopically fetal surface or inner surface is covered with amnion and appears smooth, shiny and transparent. It is closely applied to the subjacent chorion which is mottled in appearance. The umbilical cord is attached near the center of this surface and branches of umbilical vessels radiate out under the amnion from this point. The veins are deeper and larger than the arteries. Fetal part of placenta is formed by villous chorion, chorionic villi arise from it and project into the intervillous space containing maternal blood.

**Maternal surface**: Maternal surface is granular in appearance and is divided into 15 to 30 lobes by a series of grooves. These lobes are termed as cotyledons. These grooves correspond to bases of incomplete placental septae which become increasingly prominent after third month. These placental septae extend from maternal aspect of intervillous space towards the chorionic plate, but they do not quite reach the chorionic plate. These septae are complex structures and comprise of components of cytotrophoblastic shell, residual syncytium, maternally derived material including decidual cells, occasional blood vessels, gland remnants, collagenous and fibrinoid extracellular matrix. The maternal part is formed by decidua basalis.\textsuperscript{15}

3.1.2 PLACENTAL TISSUES ARE ARRANGED AS:

A. Chorionic plate
B. Basal plate
C. Intervillous space
A. Chorionic plate: On the fetal aspect it is covered by amniotic epithelium. Stromal side of which has connective tissue layer carrying main branches of umbilical vessels. Adjacent to this is diminishing layer of cytotrophoblast and inner syncytial wall of intervillous space. Fusion between mesenchyme covered surfaces of amnion and chorion forms the connective tissue layer. This connective tissue is more fibrous and less cellular than the Wharton’s jelly of umbilical cord, except near the large vessels. The large vessels radiate and branch from the cord attachment until they reach the bases of the trunks of the villous stems. These branches of large vessels then enter and arborize within the intermediate and terminal villi.

B. Basal plate: The basal plate is thinned and progressively modified throughout second half of pregnancy. There is relative diminution of decidual elements and increased deposition of fibrinoid.

From fetal to maternal aspect it consists of:

- **Outer wall of intervillous space:** This comprises of syncytium, cytotrophoblast and fibrinoid matrix.
- **Rohr’s stria of fibrinoid:** This stria is irregularly interconnected. Strands pass from Nitabuch’s stria into the adjacent decidua.
- **Remains of cytotrophoblastic shell.**
- **Nitabuch’s stria of fibrinoid:** Nitabuch’s stria and basal decidua contain cytotrophoblast and multinucleate trophoblast giant cells that originate from mononuclear cytotrophoblast population which infiltrates the basal decidua during the first 18 weeks of pregnancy. The penetration of these cells is as far as the inner one third of the myometrium. They are not found in parietal decidua nor in the adjacent myometrium. Thus these placental bed giant cells appear to be a differentiative end stage in the extra villous trophoblastic lineage.
- **Maternal decidua:** It contains large and small decidual cells which are scattered in connective tissue framework and basal remnants of endometrial glands.

C. Intervillous space: Intervillous space is derived from lacunae that develop in syncytiotrophoblast and eventually coalesce. The maternal blood approaches the intervillous space through various layers of basal plate. Maternal blood enters this space from spiral endometrial arteries which open through the gaps in cytotrophoblastic shell
and discharge blood in the intervillous space. At term the walls of most spiral arteries consists of fibrinoid matrix within which cytotrophoblast is embedded. This allows expansion of arterial diameter to give an increased blood flow which is privileged in being independent of vasoconstrictors. This large space is drained by endometrial veins. The veins which drain the blood away from the space pierce the basal plate and join the tributaries of uterine veins. Numerous branch chorionic villi that arise from stem villi are continuously bathed with maternal blood that circulates through the intervillous space.\textsuperscript{15}

3.2 STRUCTURE OF PLACENTA:

Microscopically cross section of full term placenta shows cut sections of several chorionic villi.

Chorionic villi are essential structures involved in exchange between mother and fetus. Each stem villus has its base at the chorionic plate which progressively branch into intermediate and terminal villi.

Each villus has a core of connective tissue containing collagen type I, type III, type V, type VI and fibronectin. Type I collagen is often found as bundles while type III collagen fibers are thinner forming a meshwork. Collagen V and VI are found as fibers which are closely associated with type I and III. Collagen type IV and basal lamina associated molecules laminin are found in stroma in association with fetal vessels as well as in basal lamina of trophoblast. Cyto and syncytiotrophoblast overlie this matrix and are bathed by maternal blood in the intervillous space. Cohesion between the cells of cytotrophoblast is provided by numerous desmosomes. Desmosomes also provide cohesion between cyto and syncytiotrophoblast between their opposed plasma membranes.

Cytotrophoblast forms a continuous layer on the basal lamina in earlier stages but it gradually expends itself to form syncytiotrophoblast after the fourth month. As the cytotrophoblast decreases the syncytiotrophoblast becomes progressively thinner and becomes adjacent to the basal lamina over an increasingly large area. A few singly disposed cytotrophoblastic cells persist until term.

The cells of villous cytotrophoblast also known as langerhans cells are pale staining with only slight basophilia. Ultra structurally they show very few organelles and electron translucent cytoplasm. Cell organelles like a few clusters of ribosomes, narrow cisternae
of rough endoplasmic reticulum, Golgi apparatus and large mitochondria are seen in the cytoplasm. Also intermediate filaments particularly associated with desmosomes are seen. Between the desmosomes the membranes of adjacent cells show an intercellular gap of 20 nm which sometimes widens to accommodate microvillous cell projections from the cell surfaces.

The syncytial cytoplasm is more strongly basophilic. It is complex and more electron dense than that of Langerhans cells. Where the plasma membrane adjoins the basal lamina it shows complex infoldings into the cytoplasm. Surface bordering the intervillous space shows numerous long microvilli. Cytoplasm contains a free wealth of ribosomes, cisternae of granular endoplasmic reticulum, scattered Golgi complex, mitochondria, cytoskeleton of microfilaments, profusion of vesicles and vacuoles and numerous lysosomes and phagosomes. It is an intensely active layer across which most transplacental transport occurs. It is also responsible for the secretion of range of placental proteins into the maternal circulation which include chorionic gonadotrophin, chorionic somatomammotrophin and others.

Glycogen is present in both layers of trophoblast at all stages. Lipid droplets are also present in both layers, principally within the cytoplasm and basal lamina. These droplets diminish in number with advancing age and may represent fat in transit from mother to fetus. Membrane bound granular bodies occur particularly in cytoplasm of syncytiotrophoblast. Some of these are probably secretion granules. Lysosomes and phagosomes are concerned with degradation of materials engulfed from intervillous space.

In the immature placenta syncytial sprouts are found which represent first stages of development of new terminal villi. These later get invaded by cytotrophoblast and villous mesenchyme. Syncytial sprouts are also seen in the term placenta but the enclosed nuclei here are largely degenerative. Syncytial knots represent similar aggregates of degenerative nuclei. This represents a sequestration phenomenon which involves removal of senescent nuclear material from adjacent metabolically active areas of syncytium. These sprouts may detach and form maternal syncytial emboli. Daily a passage of some 100,000 such sprouts into the maternal circulation has been computed.
Fibrinoid deposits are found on villous surface in areas lacking syncytiotrophoblast which appears to be a repair mechanism in which fibrinoid forms a wound surface that is subsequently re-epithelialized by trophoblast. Tenascin is an extracellular matrix glycoprotein that is localized in the stroma adjacent to these sites.

Large reticulum cells, fibroblasts and large phagocytic Hofbauer cells are present in villous core. There is increase in mesenchymal collagen from network to fine fibers in early mesenchymal villi to densely fibrous stroma of stem villi of second and third trimester. Stromal channels found in immature intermediate villi is infilled by collagen after about 14th week to give the fibrous stroma characteristic of the stem villus.

The fetal vessels include arterioles and capillaries. Their endothelium contains fine cytoplasmic filaments. Pericytes may be found in close association with capillary endothelial cells. The vessels are surrounded externally by periendothelial basal lamina membrane. From second trimester onwards and later the terminal villi show dilated thin walled capillaries immediately adjacent to villous trophoblast. The two basal laminae are apparently fused to produce a vasculosyncytial interface.

3.3 PLACENTAL BARRIER

Blood in the fetal vessels is separated from maternal blood in the intervillous space by a placental barrier. Placental barrier is interposed between blood streams, but it is a selectively permeable barrier which allows water, oxygen and other nutritive substances and hormones to pass from mother to fetus and some products of excretion from fetus to mother.

3.3.1 LAYERS THAT CONSTITUTE THE PLACENTAL BARRIER ARE:

Six components separate the fetal circulation from maternal circulation throughout the first half of gestation. These components comprise the placental barrier, they are as follows:

Both chorionic villi and chorionic plate are entirely covered by:

- Syncytiotrophoblast: It is characterized by numerous small and dark staining nuclei.
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- Cytotrophoblast: Cytotrophoblast cells are present underlying the syncytiotrophoblast.
- Underlying trophoblastic basement membrane.
- Fetal loose connective tissue that constitutes the core of each villus.
- Basement membrane of fetal capillaries.
- Endothelium of fetal capillaries.

The thickness of placental barrier reduces progressively during gestation. After fourth month the villous syncytium becomes thinner and comes in direct apposition to subepithelial basal lamina over an increasing area. At term the cytotrophoblast layer is reduced to small fragments, thus placental barrier comprises of five layers instead of six. The fetal capillaries become dilated and approach the surface of terminal villi.

3.4 UTEROPLACENTAL CIRCULATION:

3.4.1 FETAL CIRCULATION: A large surface area is provided by branch chorionic villi of placenta. Exchange of materials between mother and fetus takes places through these various branch villi which arise from stem villi across very thin placental membrane. Poorly oxygenated blood leaves the fetus and passes through umbilical arteries to the placenta. These arteries further divide into various chorionic arteries before entering the chorionic villi. The blood vessels form an extensive arteriocapillary venous system within the chorionic villi which brings the fetal blood extremely close to the maternal blood. This system provides a large surface area for exchange of products between fetal and maternal blood. There is no intermingling of fetal and maternal blood cause of the placental barrier that lies between them. Thin walled veins converge to form the umbilical vein which carries oxygen rich blood to the fetus.

3.4.2 MATERNAL CIRCULATION: Maternal blood enters the intervillous space through 80 to 100 spiral endometrial arteries in decidua basalis. The blood flow from the spiral arteries is considerable at a higher pressure than intervillous space and is propelled in a jet like manner and spurs towards the chorionic plate which forms the roof of intervillous space. The blood flows slowly over the branch villi and allows exchange of products with the fetal blood. The blood returns through the endometrial veins to the maternal circulation.
3.5 FUNCTIONS OF PLACENTA:

The main functions of placenta is transfer of oxygen and nutrients.\textsuperscript{18,19}

3.5.1 PLACENTAL TRANSFER: Great surface area of placental membrane facilitates transport of substances in both the directions between placental and maternal blood.

Factors affecting placental transfer:

- Low molecular weight substances transfer more easily than those of high molecular weight. Water soluble substances up to weight 100 and lipid soluble substances up to weight of 600 or more can cross readily. However diffusion is limited when molecular weight is more than 1000. Ionized substances cross in a very small amount irrespective of molecular weight.

3.5.2 RESPIRATORY FUNCTION:

Oxygen and Carbon dioxide: Gases like oxygen, carbon dioxide and carbon monoxide cross the placenta by the process of simple diffusion. The rate of oxygen supply to fetus is 5ml/kg/min. The placental membrane is highly permeable to carbon dioxide. These gases diffuse freely across placenta.

3.5.3 NUTRITIVE FUNCTIONS OF PLACENTA:

Water: Water freely crosses the placenta. At term it reaches to 3.5 L/h.

Glucose: Transfer of glucose is by the process of facilitated diffusion. Glucose molecule combines with a carrier protein to form a lipid soluble complex. Because of increased consumption fetal glucose levels are lower than the mother.

Amino acids: Neutral straight chain amino acids, acidic and non-essential amino acids are synthesized in the placenta and not absorbed from the maternal serum. Basic amino acids like lysine and histidine are transferred by specific transport mechanism as these amino acids have higher concentration in the maternal blood.

Lipids: Lipids cross the placenta freely by the process of simple diffusion. In early pregnancy fatty acids, triglycerides and cholesterols are directly transported from the mother to the fetus. Later they are synthesized by the fetus. Levels of arachidonic acid are
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high as they are synthesized from cholesterol in the placenta. Fetus and placenta also can synthesize fatty acids from glucose.

**Water soluble vitamins**: Fetal concentration of these vitamins is higher than that of the mother. Water soluble vitamins are absorbed by active mechanisms. Although the placenta is impervious to vitamin A fetus can synthesize vitamin A from carotene which easily crosses the placenta. The level of fat soluble vitamins is lower in fetal serum as they are transferred slowly. Iron, calcium and phosphorous cross the placenta by the process of active transport. Parathormone and calcitonin do not cross the placenta.

**Electrolytes**: Sodium and Potassium readily cross the placenta by the process of simple diffusion. The requirement of water is more by the fetus and the transfer is dependent upon osmotic and hydrostatic pressures. Hormones such as vasopressin, oxytocin and prolactin are also thought to have a role in control of water exchange.

**Drugs**: Transfer of drugs through placenta is determined by their molecular weight and affinity for lipids and ionisation. Infectious agents like rubella virus, cytomegalovirus, coxsackie and poliomyelitis can cross the placental membrane and cause fetal infection.

**Steroids**: Steroids also cross the placenta readily.

**3.5.4 EXCRETORY FUNCTION**:

Products like uric acid and urea are excreted into the maternal blood by the process of simple diffusion.

**3.5.5 IMMUNOLOGICAL FUNCTION**:

Maternal immunoglobins provide protection to the fetus against infectious agents like measles, diphtheria etc. The fetus is provided with passive immunity by these antibodies against infectious diseases for some time after birth until the neonates own immune system starts functioning.
3.5.6 ENDOCRINE FUNCTION OF PLACENTA:

**Protein hormones:**

**Human Chorionic Gonadotrophins (hCG):**

hCG is also known as pregnancy hormone. It is a glycoprotein hormone with molecular weight of 38,400 daltons and it is a glycoprotein with highest carbohydrate residue. hCG is mainly synthesized by syncytiotrophoblast of placenta after synthesis from corpus luteum ceases. hCG is also produced by fetal kidney and malignant trophoblast. In a normal pregnancy shortly after implantation hCG can be detected in urine and plasma of a pregnant woman.

Functions of hCG:

- **Rescue of corpus luteum:** hCG continues the production of progesterone and maintains the pregnancy until third month.
- **hCG stimulation of fetal testis:** The peak of secretion of testosterone by fetal testis corresponds to the same time when maternal serum has maximum levels of hCG. There is only small amount of LH secretion by pituitary so hCG acts as LH during this period and promotes male sexual differentiation by helping in production of testosterone.
- **hCG stimulation of maternal thyroid.**
- **hCG promotes relaxin secretion by corpus luteum and it may also promote uterine vascular dilation and myometrial smooth muscle relaxation.**

**Human Placental Lactogen (hPL):**

hPL has a prolactin like activity and it is found to be concentrated in the syncytiotrophoblast and cytotrophoblast by around second or third week after fertilization. hPL is structurally and functionally more similar to pituitary growth hormone and prolactin, but its biological activity is less. The levels of hPL in the maternal blood show gradual rise with a plateau after 35-36 weeks. It’s proportional to placental mass.
Functions of hPL:

- Lipolysis and increase in the levels of free circulating free fatty acids. This helps in production of energy for both fetal and maternal metabolism.
- Angiogenesis that is production of fetal vasculature.
- Anti-insulin action.

Other Placental Hormones:

Chorionic adrenocorticotrophins:

This is a protein which is isolated from placental tissue. It is similar to ACTH. The concentration of ACTH increases as the pregnancy advances. ACTH does not cross the placenta. The placenta might produce ACTH which is then secreted into the mother and fetus.

Relaxin:

Relaxin is a peptide hormone. It is structurally similar to insulin. There are two relaxin genes H1 and H2. H2 genes are transcribed in corpus luteum of the ovary while H1 genes are expressed on placenta, decidua and fetal membranes. Relaxin acts on myometrial smooth muscles to stimulate adenyl cyclase thus promote uterine relaxation.

Oestrogen:

Placenta produces large amount of oestrogen and this production and synthesis of hormone is dependent on the steroidal precursors in the blood. The placental syncytiotrophoblast synthesizes oestriol from its fetal precursor dehydroepiandrosterone which is a product of adrenal glands of fetus. It is then hydroxylated in the fetal liver.

Maternal conditions that affect placental oestrogen synthesis:

- Glucocorticosteroid treatment: High dose of corticosteroid administration to pregnant woman causes a reduction in placental oestrogen formation as it inhibits ACTH secretion.
- Maternal hypertensive disorders: In maternal hypertensive disorders and diabetes fetal adrenal synthesis of dihydro-epiandrosterone is impaired because of decrease
in the uteroplacental blood flow and not because of reduction in the placental functions.

- Maternal renal disease: Pregnant woman with pyelonephritis show low levels of oestrogen in urine. This is probably because of low renal clearance.

**Progesterone:**

Progesterone is secreted by corpus luteum up to 6-7 weeks of gestation. Thereafter, it is synthesized by placenta. Maternal cholesterol is converted to pregnenolone in the mitochondria which is then converted to progesterone in the endoplasmic reticulum.

**Chorionic thyrotropin:** The placenta produces chorionic thyrotropin but its role in pregnancy is not yet clear.

**Parathyroid hormone related protein:** This hormone may serve as the parathyroid of the fetus.

**Human growth hormone variant:** This growth hormone variant gene is expressed in placenta. It is present in maternal plasma by 21 to 26 weeks and gradually increases in concentration by term.

**Hypothalamic like releasing hormones:** Human placenta produces hormones analogous to GnRH, TRH, CRH and somatostatin produced by the hypothalamus.
4. PREECLAMPSIA

4.1 PREECLAMPSIA

Preeclampsia is a hypertensive disorder of pregnancy. It is a pregnancy specific syndrome that can virtually affect every organ system. It is described as a condition with occurrence of hypertension, proteinuria and edema after 20 weeks of gestation in previously normotensive woman. It can be mild or severe.\textsuperscript{19}

4.1.1 CLASSIFICATION OF HYPERTENSION IN PREGNANCY: \textsuperscript{19}

**Gestational hypertension**: It is also called transitional hypertension of pregnancy. This is characterized by elevation of blood pressure alone without proteinuria for the first time during pregnancy. High blood pressure reverts back to normal within twelve weeks of pregnancy.

**Preeclampsia**: It is a multisystem disorder specific to pregnancy defined as gestational hypertension after 20 weeks of gestation associated with proteinuria. It can be mild or severe.

**Eclampsia**: It is defined as onset of convulsions in pregnant woman who is having preeclampsia. Onset of convulsions is after 20 weeks of gestation or 7 days postpartum.

**Preeclampsia or Eclampsia superimposed on chronic hypertension**: Preeclampsia or eclampsia may develop on preexisting chronic hypertension. The criteria include onset of proteinuria, hyperuricemia, thrombocytopenia and convulsions in case of eclampsia.

**Chronic hypertension**: This persists beyond 84 days of pregnancy. It is hypertension detected before 20 weeks of gestation.

4.1.2 DIAGNOSTIC CRITERIA FOR PREECLAMPSIA: \textsuperscript{9,19}

**Hypertension**: That occurs after 20 weeks of gestation in a woman with previously normal blood pressure.

- Systolic BP $\geq 140$ mmHg.
- Diastolic BP $\geq 90$ mmHg.
**Proteinuria** : Urinary excretion of > 300 mg protein in a 24 hours specimen.

**Oedema** : Generalized oedema or weight gain of at least 5 pounds in one week.

Any two of the above confirms preeclampsia.

### 4.1.3 PREECLAMPSIA CAN BE CLASSIFIED INTO MILD OR SEVERE:

**Mild-moderate**

BP is 140 to 159 mmHg systolic and/or 90 to 109 mmHg diastolic that occurs after 20 weeks of gestation (on 2 occasions at least 6 hours apart) and proteinuria is 300 mg/24 hours.

**Severe**

BP is ≥160 mmHg systolic and/or ≥110 mmHg diastolic (on 2 occasions at least 6 hours apart, while the patient is on bed rest) and proteinuria is 300 mg/24 hours.

### 4.1.4 RISK FACTORS: ¹⁹

- Nulliparous women
- Multiple pregnancy
- History of hypertension during previous pregnancy
- Hydatiform mole
- Maternal age more than 35 years
- Diabetes mellitus
- Obesity
- Low socioeconomic status
- Genetic predisposition
- Family history of preeclampsia

### 4.1.5 ETIOLOGY: ⁹

Important factors considered to cause preeclampsia are:

- **Immunological Factors** : A theory cited to account preeclampsia syndrome is dysregulation of maternal immune tolerance to paternally derived placental and
fetal antigens. There is also data that suggests that the risk of preeclampsia is enhanced in circumstances in which formation of blocking antibodies to placental antigenic sites might be impaired. Immune maladaptation has a major role in pathophysiology of preeclampsia.

- Maternal maladaptation to inflammatory and cardiovascular changes of normal pregnancy.
- **Genetic factors**: The hereditary predisposition in preeclampsia is likely to be the result of hundreds of genes, both maternal and paternal that control enzymatic and metabolic functions throughout every organ system.

### 4.1.6 PATHOGENESIS:

- **Abnormal trophoblastic invasion**: In normal pregnancy many cytotrophoblasts remain as single cells that detach from the basement membrane and form cell columns. Cytotrophoblasts from the distal ends of these columns invade the uterus and its arterioles. Thus endovascular invasion is a process in which these cells replace the endothelial and muscular linings of uterine arterioles and initiates maternal blood flow to the placenta and greatly enlarges the vessel diameter. Cells participating in endovascular invasion have two types of interaction with maternal arterioles.
  1) Large aggregates of cells are found in vessel lumen adjacent to the apical surface of endothelium or replace it in such a way that they appear directly attached to the vessel wall. Cytotrophoblasts colonize the smooth muscle layer of the vessel and lie within the vessel wall subjacent to the endothelium.
  2) Uterine spiral arterioles are invaded by endovascular trophoblasts and undergo extensive remodelling. Muscular linings and vascular endothelium is replaced by trophoblastic cells to enlarge the diameter of the vessel. The veins are superficially invaded. By late second trimester endothelial cells are no longer visible on spiral arterioles and they are lined exclusively by cytotrophoblasts.

However in preeclampsia there is incomplete trophoblastic invasion thus myometrial vessels are not lined with endovascular trophoblasts. Thus the deeper myometrial arterioles do not lose their endothelial lining and musculoelastic tissue and their mean external diameter is only half that of vessels in normal placentae. There is shallow
cytotrophoblastic invasion of the uterus and endovascular invasion does not proceed beyond terminal portions of spiral arterioles. Even if cytotrophoblasts gain access to the vessel wall they spread out in the vessel and fail to aggregate. They tend to remain as single cells and poorly anchor to vessel wall. This trophoblastic invasion correlates with severity of hypertensive disorder. Early preeclamptic arterial changes include endothelial damage insudation of plasma constituents into the vessel wall, myointimal cell proliferation and necrosis. Accumulation of lipid in myointimal cells and macrophages cause atherosis. Vessels affected by atherosis develop aneurysmal dilatation. Abnormally narrow lumen of spiral arterioles impairs placental blood flow. Diminished perfusion and hypoxia eventually leads to release of placental debris and incites inflammatory response.\textsuperscript{9,12}

**Figure 2**

Fig 2: Normal placental implantation shows proliferation of extravillous trophoblasts from an anchoring villus. These trophoblasts invade the decidua and extend into the walls of spiral arterioles to replace the endothelium and muscular wall to create low-resistance vessel. In preeclampsia there is incomplete invasion of spiral arteriolar wall by extravillous trophoblasts. This results in small caliber vessel and high resistance to flow.

- **Endothelial cell activation**: Endothelial cell activation is defined as altered state of endothelial cell differentiation. Various factors such as hypoxia, anti-endothelial cell antibodies and cytokines, reactive oxygen products, physical shear forces may be responsible for endothelial cell dysfunction in preeclampsia.
Endothelial activation or damage leads to secretion of variety of endothelial cell products which can provoke a vicious cycle of vasospasm, disruption of vascular integrity and micro thrombosis that persist until inciting factor is eliminated. Activation and dysfunction of vascular endothelium is provoked by secretion of unknown factors of placental origin in maternal circulation. Widespread endothelial cell changes are seen in preeclampsia. Significantly elevated levels of circulating endothelial cells are found in peripheral blood of preeclamptic women. Damaged endothelial cells produce less nitric oxide and secrete substances that promote coagulation.9

- **Atherosis:** Lesions in the spiral arteries at placental sites called acute atherosis and endothelial cell injury might be a mechanism responsible for diffuse vascular disease in patients with preeclampsia. Histologic changes in placental beds in preeclamptic women showed resemblance to vascular pathology associated with allograft rejection. The extent and severity of vascular lesions seem to be parallel to clinical severity of this syndrome.12

- **Vasospasm:** Increased resistance due to vascular constriction subsequently causes hypertension. Endothelial cell damage causes interstitial leakage through which blood constituents are deposited subendothelially. Mal distribution causes diminished blood flow, ischemia and leads to necrosis and other end organ disturbances.9

- **Nitric oxide:** Nitric oxide is a potent vasodilator which is synthesized form L arginine by endothelial cells. Inhibition of nitric oxide synthesis increases mean arterial pressure, decreases heart rate and reverses pregnancy induced refractoriness to vasopressors. Nitric oxide is likely the compound that maintains normal low pressure vasodilated state which is characteristic of fetoplacental perfusion. It is also produced by fetal endothelium. It appears that preeclampsia is associated with decreased endothelial nitric oxide synthase expression, thus increased nitric oxide inactivation.9

- **Prostaglandins:** Prostaglandins are among the primary vasoactive products of endothelial cells. These bioactive lipids play an important role in physiologic and pathophysiologic modulation of vascular tone as they have profound regulatory effects on vascular smooth muscle cells. The major product produced by endothelial cells is prostacyclin. Prostacyclin is a potent vasodilator and inhibitor
of platelet aggregation. As compared to normal pregnancy endothelial prostacyclin production is decreased in preeclampsia which ultimately leads to vasoconstriction.\textsuperscript{12}

- **Increased pressor response**: Pregnant women normally develop refractoriness to infused vasopressors. Studies showed that normotensive nulliparas remained refractory to infused angiotensin II while those who subsequently became hypertensive lost their refractoriness several weeks before the onset of hypertension.\textsuperscript{9}

- **Endothelins**: The endothelins are a family of 21 amino acid peptides. They are the most potent vasoconstrictors. Endothelin -1 is a vasoactive peptide. It exerts its biological actions on vascular smooth muscle cells. Vascular endothelin is secreted in insufficient amounts under normal conditions. Elevated Endothelin -1 concentration is found in preeclampsia which is a potent vasoconstrictor of human uterine and renal vascular beds, both of which are affected in this syndrome.\textsuperscript{12}
COMPLICATIONS

MATERNAL COMPLICATIONS:

- **Eclampsia**: The major complication of preeclampsia is eclampsia. Onset of convulsions in a woman with preeclampsia is termed as eclampsia. The seizures are generalized and may appear before, during or after labor. The incidence of eclampsia is reported to be 1 in 2000 deliveries in developed countries.\(^9\)
- **HELLP syndrome**: Hemolysis, Elevated liver enzymes and Low platelet count comprise of HELLP syndrome which is one of the serious complications of preeclampsia. Among women with severe preeclampsia 10% manifest with all three abnormalities which eventually leads to adverse outcomes like maternal deaths. HELLP syndrome accounts for most maternal deaths associated with hypertension.\(^20\)
- **Oliguria and Renal failure**: Increased glomerular permeability and damage which eventually causes proteinuria is an integral part of diagnosis of preeclampsia. Oliguria may occur secondary to hemoconcentration and decreased renal perfusion. Persistent oliguria may indicate acute tubular necrosis and acute renal failure in preeclampsia.\(^21\)
- **Cerebrovascular accident**: Acute and severe hypertension leads to cerebrovascular overregulation and vasospasm.\(^9\) Neurological complications like cerebral oedema, cerebral haemorrhage and seizures are associated with preeclampsia. Other central nervous system manifestations include headache, blurred vision, hyperreflexia.\(^21\)
- **Preterm labour and Postpartum hemorrhage.**\(^9\)
- **Shock**: Intravascular volume is already reduced in preeclampsia. Thus even slight loss of blood can lead to shock.\(^9,19\)
- **Sepsis**: Sepsis is due to increased incidence of induction, low resistance and operative interference.\(^19\)
- **Death**: Increased risk for maternal and fetal morbidity and mortality is associated with preeclampsia.\(^22\)
FETAL COMPLICATIONS

- **Growth restriction**: One of the major fetal complications in preeclampsia is growth retardation due to uteroplacental vascular insufficiency. Different degrees of fetal injury is associated with severe preeclampsia. The main impact on the fetus is undernourishment due to utero-placental vascular insufficiency which leads to growth retardation. Studies have shown that babies who suffered intrauterine growth retardation are more likely to develop hypertension, coronary artery disease and diabetes in adult life. Weight of the fetus is highly compromised. Fetal growth restriction is due to impaired gaseous exchange and unavailability of nutrients.\(^\text{23,24}\)

- **Intrauterine asphyxia**: Utero-placental hypoxia is related to abnormal placentation which is seen in preeclampsia. Depending on the severity of the preeclampsia it may lead to intrauterine hypoxia in the fetus.\(^\text{25}\)

- **Intrauterine death**: In severe preeclampsia fetal health and fetal weight is highly compromised leading to various degrees of fetal damage such as to cause fetal death. Intrauterine fetal death may also be due to accidental haemorrhage which is another complication of preeclampsia.\(^\text{19}\) Major complications seen in preeclampsia are intrauterine death, low birth weight and intrauterine growth restriction, thus preeclampsia has great implication on adverse neonatal outcome.\(^\text{26}\)

- **Prematurity**: Spontaneous onset of labour due to accidental haemorrhage or induction of labour are the causes for prematurity.\(^\text{19}\)

- **Oligohydramnios**: Renal agenesis, uteroplacental insufficiency and rupture of amnion results to chronic leakage of the amniotic fluid and may cause oligohydramnios. This may further lead to deformities like flattened facies, dislocated hips and positional abnormalities of hands and feet.\(^\text{24}\)

- **Placental infarction**: A localized area of ischemic tissue necrosis that is due to obstruction of the villous blood supply is termed as placental infarction. A large proportion of the placentae had histological signs of ischemia in pregnancies with mild or severe preeclampsia.\(^\text{27}\)
5. REVIEW OF LITERATURE

Further observations on distribution of phosphatases in mammalian placentae was a study conducted by Dempsey et al in the year 1947. They observed increased amount of alkaline phosphatase activity in human placentae at term. Preeclamptic placentae showed premature increase in alkaline phosphatase activity.\(^\text{28}\)

The metabolism of human placenta in vitro was a study conducted by Claude Villi in the year 1953. They observed that there was marked decrease in glycogen content of normotensive placenta at term. Placental glycogen begins to decrease at ten to twelve weeks of gestation and decreases steadily throughout gestation.\(^\text{29}\)

In the year 1963 Jeacock et al studied the activity of alkaline and acid phosphatase in the human placenta. They observed increased activity of placental alkaline phosphatase in preeclamptic placentae as compared to normotensive placentae.\(^\text{30}\)

Variations in enzymatic histochemistry of the placenta was a study conducted by Curzen P in the year 1964. They reported increased activity of placental alkaline phosphatase in preeclamptic placentae as compared to normotensive placentae.\(^\text{31}\)

Placental pathology in eclampsia and preeclampsia was a study conducted by Moqueo et al in the year 1964. Their results showed histological changes like extensive area of fibrosis, crowded villi, marked congestion of all vessels, disappearance of large areas of trophoblast, thinning of syncytium and narrowing of decidual arteriolar lumen with marked atheromatosis in preeclamptic placentae.\(^\text{32}\)

Comparative histochemical distribution of glycogen and alkaline phosphatase in the placenta was a study conducted by George Christie in the year 1967. They observed small concentration of glycogen in the stroma of normotensive human placenta. Rabbit placenta showed reduced amount of glycogen at term. Cat, dog and ferret placentae showed positive glycogen activity around blood vessels at term. Human placenta showed patchy distribution of alkaline phosphatase activity at term while rabbit placenta showed negative placental alkaline phosphatase activity at term.\(^\text{33}\)

Curzen et al in their study on enzyme assays in the management of pregnancy in the year 1970 reported that there is increase in alkaline phosphatase enzyme in normal pregnancy
and it can increase further or decrease in abnormal pregnancies. According to them abnormally high serum heat stable alkaline phosphatase levels represent placental damage and abnormally low levels indicate poor placental development.\textsuperscript{34}

Dempsey et al conducted a study on regional specializations in the syncytial trophoblast of early human placentae in the year 1971. They observed that trophoblast exhibits a well-marked layering of structure during first trimester. The apical surface of syncytium characteristically showed microvilli between which there were canals leading to small and large coated vesicles. They called this as the zone of absorption. Beneath this they observed a region predominantly occupied by cisternae lined by rough endoplasmic reticulum. This was termed as zone of secretion. Still deeper was a region containing organelles similar to those of cytotrophoblastic cells of Langhans. This was termed as zone of accrual. Under these layers were the cytotrophoblast cells of Langhans resting on thick basal lamina.

It was observed that the basal zone of syncytium resembled the cytotrophoblast in three features. I: Presence of glycogen was seen as individual granules or clumps in both the regions. II: A characteristic relationship was seen in both sites between dilated cisternae and mitochondria. III: Cytotrophoblast cells were attached by numerous desmosomes to the syncytium. Mitochondria of syncytium are small in the middle and outer zones and slightly more smaller in basal zone. Those in the Langhans cells are larger and more numerous than that of syncytium. Certain regions showed syncytial masses containing several nuclei but were lacking both Langhans cells and connective tissue cores. These masses were attached to terminal villi and were termed as syncytiial sprouts. There were few structures protruding into the intervillous space forming an extended base of microvilli. These were termed as syncytial protrusions. Occasionally well-defined clots were also observed in the intervillous space.\textsuperscript{35}

Studies conducted by Brosens et al in the year 1972 showed that in preeclampsia acute atherosis developed in muscular arteries of placental bed including spiral arteries. The whole thickness of vessel wall was affected cause of fibrinoid necrosis, there was accumulation of lipophages in the wall and mononuclear infiltrate around the damaged vessel. Atherosclerosis was seen in spiral arteries in preexisting hypertension and later developed acute atherosis when preeclampsia supervenes hypertension.\textsuperscript{36}
A study on quantity and distribution of placental glycogen was conducted by Robb et al in the year 1976. They reported that in first trimester the glycogen levels were high, but from about 12 weeks to term the levels were within a narrow range. There was no appreciable deviation in the glycogen levels of normal and a range of other clinical conditions.\(^\text{37}\)

Histopathology of placental insufficiency was a study conducted by Fox et al in the year 1976. They observed necrotic changes in villi, perivascular fibrin deposition, avascular villi and increased cytotrophoblast proliferation in preeclamptic placentae.\(^\text{38}\)

Uteroplacental arterial changes related to interstitial trophoblast migration in early human pregnancy was a study conducted by Pijnenborg et al in the year 1983. They found significant morphological alterations in spiral arteries such as swelling of endothelium, oedema and disruption of architecture of vessel wall and hypertrophy of individual smooth muscle cells. They concluded that these findings indicated that interstitial cytotrophoblasts may have a role to play in the preparation of myometrial segments of uteroplacental arteries for the second wave of endovascular trophoblast migration.\(^\text{39}\)

Francis et al in the year 1984 conducted a study in which they measured the total alkaline phosphatase in maternal plasma and placental extracts in normal and preeclamptic pregnancies. They concluded that total alkaline phosphatase concentration in placental tissue was higher in normotensive pregnancies as compared to preeclamptic pregnancies. However total plasma alkaline phosphatase was elevated during the last trimester in both normal as well as preeclamptic pregnancies.\(^\text{40}\)

Isemura M et al in the year 1985 investigated human placentae for distribution of glycosaminoglycans, collagens and fibronectin in which they showed presence of fibronectin and type IV collagen around fetal blood vessels and in stroma of placental villi. Glycosaminoglycans were indicated by alcian blue staining.\(^\text{41}\)

Placental morphology and clinical correlations in pregnancies complicated by hypertension is a study conducted by Bartl et al in the year 1985. Their result showed increased number of trophoblastic sprouts, trophoblastic hyperplasia, fibrinoid degeneration and necrosis in hypertensive pregnancies which increased even more with severity. These changes were rare in term placentae of healthy women. They concluded
that these changes may lead to retardation of placenta and immature placental perfusion which seems to be responsible for decreased fetal birth weight in case of hypertensive pregnancies.42

Studies by Khong et al in the year 1986 document partial or complete lack of physiological changes that is, only a part of the vessel wall is affected and physiological changes are seen only in decidua in pregnancies complicated by preeclampsia.43

Bloxam et al in their study on placental glycolysis and energy metabolism in preeclampsia in the year 1987 documented that there is significant metabolic abnormality in placentae of mothers with severe preeclampsia. Glycogen and glucose concentrations were high in preecalmptic placentae which supports the evidence of inhibited glycolysis.44

Histomorphometry of the human placenta in preeclampsia, associated with severe intrauterine growth retardation was a study conducted by Teasdale et al 1987. They observed that preeclamptic placentae were comparatively smaller than control placentae. There was reduction in transverse diameter of preeclamptic placentae.45

MS Thakur et al conducted a study in in the year 1988 in which they used sudan black stain to microscopically observe and monitor lipid production by microbes.46

Preeclampsia an endothelial cell disorder was a study by Roberts et al in the year 1989. They proposed that poorly perfused placental tissue release certain factors into the systemic circulation that leads to endothelial cell injury which in turn set a motion of dysfunctional cascade of vasoconstriction, coagulation and intravascular fluid redistribution that results in clinical syndrome of preeclampsia.47

In the year 1990 sudan black stain was used for staining lipids by Subramaniam et al in their study on evaluation of intracellular lipids by standardized staining with sudan black B fraction.48

In the year 1992 Suster S et al conducted studies using alcian blue to stain mucosubstances and found hydrophic degeneration of villi which indicates intravillous accumulation of sulphated mucosubstances. Thus connective tissue of placenta shows non specific stromal reaction to variety of noxious stimuli.49
Studies conducted by Arkwrith et al in the year 1993 showed presence of more glycogen in villi of preeclamptic placentae as compared to control placentae. Glycogen phosphorylase and glycogen synthase activity was much higher in cases with preeclampsia. Glycogen phosphorylase activity was higher in preeclamptic placentae but to smaller extent as compared to control placentae.\(^{50}\)

A study of placental bed spiral arteries and trophoblastic invasion in normal and preeclamptic placentae was conducted by Meekins et al in the year 1994. They reported that in preeclamptic placentae trophoblastic invasion was seen more in decidual than myometrial segments. Variation in morphological features was seen not only in different spiral arteries but also in different segment of same spiral artery. Endovascular trophoblastic invasion was complete, partial or isolated. Hyperplasia in the myometrial arteries and acute atherosis in decidual arteries was commonly seen in preeclampsia. Vascular changes seen in normal pregnancy were physiological.\(^{51}\)

Trophoblast and placental villous core production of lipid peroxides, thromboxane and prostacyclin in preeclampsia was a study conducted by Walsh et al in the year 1995. They concluded that increased production of lipid peroxides by placenta originates from both trophoblast and villous core compartments. Placenta secretes lipid peroxide and hence it could be a source of increased lipid peroxides in maternal circulation of women with preeclampsia. Increased placental vasoconstriction could be as a result of increased ratio of thromboxane to prostacyclin in the villous core.\(^{52}\)

In the year 1995 Salafia et al in their study on placental pathologic features of preterm preeclampsia found that chronic uteroplacental vasculitis, avascular villi, chronic villitis and hemorrhagic endovasculitis was more frequent in preeclampsia. They concluded that immunopathologic processes and coagulation may be involved in pathophysiologic mechanisms of preterm preeclampsia.\(^{53}\)

Lyall et al in the year 1996 suggested that hyperlipidaemia may be enhanced in preeclampsia, thus abnormal lipid metabolism may have a role in this disorder. Preeclamptic women have increased lipid peroxides products in their serum. Decidual vessels show fibrinoid necrosis of the vessel wall and focal accumulation of lipid laden macrophages similar to that seen in atherosclerosis.\(^{54}\)
Increased mitochondrial damage by lipid peroxidation in trophoblast cells of preeclamptic placentae was a study conducted by Morikawa et al in the year 1997. They suggested that lipid peroxidation byproducts damage the mitochondrial proteins, thus causing dysfunction of trophoblasts that contribute to the pathophysiology of preeclampsia.\(^\text{55}\)

Bax et al conducted a study on energy metabolism and glycolysis of human placental trophoblast during differentiation in the year 1997. Their results showed that energy metabolism in cytotrophoblast is different from that in syncytiotrophoblast.\(^\text{56}\)

In the year 1997 Zhou et al conducted a study on defective endovascular invasion in preeclampsia. They reported that in control pregnancy cytotrophoblasts had two types of interactions with maternal arterioles. In first type of interaction large aggregates of cells were found inside the vessel lumen. These aggregates either lied adjacent to apical surface of endothelium or replaced it in such a way that they appeared to be attached to the vessel wall directly. Thus cytotrophoblasts achieved endovascular invasion. In case of preeclampsia, cytotrophoblast interaction with spiral arteries was very different. Endovascular cytotrophoblastic invasion was limited to vessels that span the superficial decidua. Even if cytotrophoblasts gained access to the lumen they failed to form tight aggregates and remained as individual rounded cells suggesting that they poorly anchored the vessel wall. Thus in preeclampsia cytotrophoblasts displayed altered morphology in their interaction with maternal arterioles and had a limited capacity of endovascular invasion.\(^\text{57}\)

In the year 1998 Knight et al in their study on shedding of syncytiotrophoblast microvilli into the maternal circulation in preeclamptic pregnancies found that higher levels of syncytiotrophoblast microvilli were found in plasma of preeclamptic women. Higher concentrations were found in uterine venous plasma as compared to peripheral venous plasma which confirmed their placental origin. They concluded that high levels of syncytiotrophoblast microvilli shed into maternal circulation may contribute to endothelial dysfunction underlying the maternal syndrome in preeclampsia.\(^\text{58}\)

In the year 1999 Staff et al reported increased total cholesterol, phospholipids and lipid peroxides in decidua basalis of preeclamptic samples as compared to samples from normotensive controls.\(^\text{59}\)
Evidence for peroxynitrite formation in vasculature of women with preeclampsia is a study conducted by Roggensack et al in the year 1999 in which they noted that gestational age at delivery and fetal birth weights was significantly lower in case of preeclamptic group as compared to control group.  

In the year 1999 Dfederico et al in their study on association of preeclampsia with apoptosis of placental cytotrophoblasts found that there was no evidence of apoptotic nuclei in trophoblast cells of floating villi in preeclamptic placentae. Also there was very little apoptosis seen in cytotrophoblast population of control samples. A tissue sample of severe preeclampsia obtained at 26 weeks of gestation showed numerous cytokeratin positive cytotrophoblasts in anchoring villi. Widespread apoptosis of cells that did not express cytokeratin was observed in a sample obtained at 28 weeks of gestation.  

Pathophysiology of hypertension during preeclampsia linking placental ischaemia with endothelial dysfunction is a study by Granger et al in the year 2001 in which they documented that placental ischaemia is an important initiating event in preeclampsia. During early human pregnancy spiral arteries are invaded by cytotrophoblasts, replacing endothelial layer of these vessels by destruction of elastic and muscular tissue. Uterine spiral arteries are exclusively lined by cytotrophoblasts and endothelial cell layer is no longer present by the end of second trimester. This remodelling leads to formation of low resistance arteriolar system with increased blood supply to the fetus. In preeclampsia spiral arteries of placental bed escape endovascular trophoblast remodelling and invasion of spiral arteries is limited. Myometrial segments of these arteries remains anatomically intact. Mean external diameter of uterine spiral arteries in preeclamptic women is less than one half of the diameters of similar vessels from uncomplicated pregnancies. Reduced uteroplacental perfusion and increased ischaemic changes in placenta occur in preeclampsia as a result of failure of trophoblastic invasion. Also increased placental infarcts and increased syncytial knots as a result of abnormal cytotrophoblast proliferation have been found in preeclamptic placentae.  

Studies conducted by Young et al in the year 2002 found lower birth weights and placental weights in hypertensive IUGR cases as compared to normotensive cases. They also found higher incidence of villous infarcts, multifocal lesions, syncytial knots and decidual vasculopathy in hypertensive IUGR cases.
Serdar et al in their study on lipid peroxidation in preeclampsia in the year 2002 reported that lipid peroxidation was enhanced in preeclamptic group.\(^{64}\)

In the year 2002 Hirano et al found in their study that spiral arteries in preeclampsia had thicker wall and narrow lumen as compared to normal pregnancy. Spiral arteries in preeclampsia also showed remnants of elastic fiber at 31 weeks of gestation while normal pregnancy showed hardly any elastic musculature. Observations in their study showed that thin walls had trophoblasts which were not present in thick walls. Thus trophoblastic invasion was related to thinning of walls and decreased elasticity of spiral artery.\(^{65}\)

In the year 2003 Mehew et al in their study to quantify placental morphology in pregnancies complicated by preeclampsia gave particular attention to dimensions and compositions of peripheral villi. In their study they found that fetal weights were reduced in all complicated pregnancies and preeclampsia. Intrauterine growth restriction was associated with placentae having reduced volumes of intervillous space and all types of villi.\(^{66}\)

A non-invasive study on evidence of impaired microvascular function in preeclampsia was conducted by Nyame et al in the year 2003. They suggested that in preeclampsia microvascular dysfunction occurs which is related to alterations in endothelial cell and neutrophil activation. They also noted that fetal birth weight was similar in both normal and preeclamptic groups, however women with preeclampsia had higher systolic and diastolic blood pressures, low platelet counts and higher serum uric acid levels as compared to control group. Also plasma albumin concentrations were lower in preeclampsia as compared to normal pregnancy.\(^{67}\)

In the year 2003 Moldenhauer JS et al in their study on placental lesions with preeclampsia found decidual arteriolopathy, intervillous thrombi and hyper maturity of villi.\(^{68}\)

Endovascular trophoblast invasion was a study conducted by Kaufmann et al in the year 2003 in which they reviewed the routes, mechanism and control of endovascular trophoblastic invasion. They suggested that endovascular trophoblastic invasion involves a side route of interstitial invasion. Impaired interstitial trophoblastic invasion is followed by failure of vascular invasion.\(^{69}\)
Duley et al in the year 2003 conducted a study on preeclampsia and the hypertensive disorders. They reported that preeclampsia is commonly seen among women who have conditions associated with large placenta and in women who have microvascular disease. In preeclampsia there is abnormal trophoblastic invasion with reduced placental perfusion. Endothelial dysfunction occurs due to unknown factors that are released into the maternal circulation and act on endothelial cells. This results in vasospasm.\(^7\)

Udaina et al in the year 2004 conducted a study on relation between placental surface area, infarction and fetal distress in pregnancy induced hypertension with its clinical relevance. They reported that the mean surface area in placentae affected by pregnancy induced hypertension (PIH) decreases with increase in severity of PIH, also cases showing fetal distress have lesser surface area as compared to cases not having fetal distress.\(^7\)

Davidson et al in the year 2004 reported that during normal placental development there is cytotrophoblast invasion into the spiral arterioles. These cytotrophoblasts completely remodel the spiral arterioles into low resistance large capacitance vessels. This endovascular invasion involves replacement of endothelium as well as highly muscular tunica media. The pathogenesis of preeclampsia may involve abnormal cytotrophoblast invasion of spiral arteries, increased oxidative stress, endothelial dysfunction and decreased uteroplacental perfusion.\(^7\)

Angiogenic imbalance in pathophysiology of preeclampsia was a study conducted by Bdolah et al in the year 2004. They noted that normal pregnancy requires balance between pro and anti angiogenic proteins that are made by placenta. They hypothesized that normal physiological increase in anti angiogenic factors at the end of pregnancy occurs too soon or there is excess production of anti angiogenic proteins which results in preeclampsia. They used Periodic acid schiff’s (PAS) stain for histopathologic analysis.\(^7\)

Preeclampsia and the systemic inflammatory response was a study conducted by Redman et al in the year 2004. They stated that the inflammatory response in preeclampsia is exaggerated and increased to the point of decomposition. Some women have placental preeclampsia in which pregnancy is normal but there is ischaemic placenta. In placental preeclampsia there is a stage when the uteroplacental circulation fails to develop fully.\(^7\)
Levy et al in the year 2005 conducted a study on the role of apoptosis in preeclampsia. They reported that excess apoptotic activity in preeclamptic women increases trophoblast apoptosis and inhibits trophoblast invasion into the spiral arteries, thus affecting each step in pathogenesis of preeclampsia. 

In the year 2005 Mangal et al conducted a study which showed bilaminar localisation of placental alkaline phosphatase in syncytiotrophoblast of preeclamptic placentae. They showed that increased activity of placental alkaline phosphatase was directly proportional to maternal blood pressure.

In the year 2005 Mujumdar S et al conducted a study and found significant number of syncytial knots, fibrinoid necrosis, calcified villous spots, endothelial proliferation, cytotrophoblast cellular proliferation in hypertensive group as compared to control group. Stromal and villous histopathological changes like proliferation of tunica media of medium sized blood vessel, stromal fibrosis and calcified areas were significant in hypertensive pregnancies as compared to control group.

According to study conducted by Kos et al in the year 2005 physiological changes were seen in the first and second trimester of pregnancy where spiral arteries of the placenta were converted into the uteroplacenal arteries. These changes lead to losing of muscular elements in the vessel walls and make them unable to respond to vasomotor effects. There was infiltration and replacement of spiral arteries by intermediate trophoblastic cells. These cells which infiltrated the walls of spiral arteries are called migratory, non-villous cells. These cells also penetrated the lumen of the vessels and formed endovascular plugs. Changes were also noticed in basal plate and amniochorionic membranes. Changes like chorionic villitis, intervillous thrombosis, and subchorial thrombosis were found.

Histomorphometric study of placental villi vascular volume in toxemia and diabetes is a study by Maly et al in the year 2005 in which they observed ischaemic changes, branching angiogenesis, prominent syncytial knots, presence of increased immature intermediate villi and decreased number of terminal villi in cases of preeclamptic placentae.
In the year 2005 Boronkai et al found a case of extremely high maternal alkaline phosphatase serum concentration. Histochemical examination of index and control placentae were done and they reported that compared to controls the index placenta showed minimal positivity of alkaline phosphatase enzyme in spite of patient having extremely high serum alkaline phosphatase. They suggested that loss of syncytial membranes in immature villi lead to increased alkaline phosphatase concentrations in maternal circulation.80

In the year 2006 Gupta et al reported that increased lipid peroxidation in preeclampsia leads to formation of lipid hydro peroxides which bind to lipoproteins. These hydro peroxides are then carried to distant sites where they cause ongoing lipid peroxidation and result in systemic oxidative stress which in turn leads to increased super oxide production by placenta. Increased production of lipid peroxides and thromboxane was demonstrated from both villous core components and trophoblast in placentae of preeclamptic patients.81

Goswami et al in the year 2006 conducted a study on syncytiotrophoblast shedding and they concluded that in preeclampsia higher amounts of syncytiotrophoblast microparticles are shed into the maternal circulation as compared to normal pregnancy. These microparticles are believed to be the stimulus for the systemic inflammatory response and endothelial cell damage which characterizes the maternal syndrome.82

In the year 2006 Zhang et al conducted a study on evaluation of frequency and maternal vasculopathy and usefulness of placental examination in pregnancy induced hypertension. They reported that vascular changes such as maternal atherosis, fibrinoid medial necrosis and intervillous thrombosis were observed in preeclampsia.83

In the year 2006 Dokras et al studied feto-placental abnormalities in preeclampsia in mice. They found diminished fetal weights and placental masses in preeclampsia as compared to controls. They reported 40 to 50 percent reduction in placental masses at early and mid-gestational age. Also throughout pregnancy fetuses were significantly smaller. Abnormalities in all placental zones such as proportional depth of placental disc relative to the decidua was markedly diminished in hypertensive strain as compared to that in controls. There was significant reduction in amount of space occupied by the placenta as compared to the decidua in hypertensive mice which suggested restricted
expansion of placenta at early gestational age. Further morphometric analyses showed reduction in placental depth in hypertensive mice. This was largely due to decrease in fractional area occupied by junctional zone in these mice. Histological examination showed PAS positive vacuolated glycogen cells, non-vacuolated eosinophilic cells and trophoblast giant cells in both hypertensive as well as control placentae which indicates that total loss of specific cell type does not contribute to reduction in the size. The labyrinth zone of control placentae showed trophoblast cells that undergo branching morphogenesis and result in large surface area for nutrient and gas exchange between mother and fetus. This labyrinth zone had uniformly elongated fetal vessels with elaborate branching morphogenesis. In contrast to this the labyrinth zone of hypertensive placentae showed attenuated and irregular branching and the extent of expansion of labyrinth towards the junctional zone was reduced. PAS staining showed that fetal vessels of control placentae advanced uniformly towards the trophoblasts with increasing gestational age. In contrast to this in preeclampsia PAS positive broad trabecular columns were seen between the fetal vessels at early gestational age and smaller clusters of trophoblast cells persisted through middle and late gestation. PAS positive fibrinoid deposits that lacked trophoblasts were also prominently observed in fetal labyrinthine blood spaces in hypertensive strain. These fibrinoid deposits were rarely seen in control placentae.

Morphological changes were prominent in control placentae and the spiral arteries were dilated and thin walled while hypertensive placentae had narrow lumen and onion skin appearance which indicated thickening of arterial wall. Areas of linear necrosis and PAS positive fibrinoid deposits were observed within decidual layers of hypertensive mice. These deposits were rarely seen in control placentae. Also compromised maternal placental circulation was observed in hypertensive mice.\textsuperscript{84}

In the year 2006 Coelho et al studied the microvessel density in placental bed among preeclampsia patients. They observed that mean fetal birth weight and microvascular density of decidual segment and myometrial segment was lower in preeclamptic cases as compared to normal controls. They concluded that poor microvascular densities in preeclampsia worsened with increasing hypertension and proteinuria.\textsuperscript{85}

In the year 2007 Hung et al in their study on oxidative stress and antioxidants in preeclampsia reported that there has been increased evidence that oxidative stress in
preeclampsia cause endothelial cell dysfunction. It was hypothesized that deficient trophoblast invasion of endometrial arteries cause intermittent placental perfusion and ischaemia which results in release of free radicles. These free radicles attack fatty acids in cell membranes and form lipid peroxides. Lipid peroxides further cause endothelial cell dysfunction. 

In the year 2006 histochemical techniques such as alcian blue and PAS was used in study conducted by Prieto et al on placental lactogen.

In the year 2008 Huppertz et al conducted a study on placental origins of preeclampsia and concluded that there is aberrant development of villous syncitiotrophoblast which causes impaired maintenance in placental barrier. This leads to necrotic and aponecrotic trophoblast fragments and overall inflammatory response by the mother.

In the year 2008 Peng M et al in their study concluded that there was more superficial depth of invasion of trophoblasts in preeclampsia than normal pregnancy. Superficial myometrial segment showed pathological changes of spiral arteries in placental bed and changes in invasion of trophoblasts which was related to severity of illness. They showed that there is impairment of microvascular development in placental bed in preeclampsia.

In the year 2008 Saleh et al found that nuclei in syncitiotrophoblasts had a tendency of cluster formation in preeclampsia, there was bridging of long syncitial strands in intervillous space which gave the villous tree a pseudolabyrinthine appearance, there was absence of villous core in sectioned syncitial strands, fetal capillaries mostly disappeared few were recognizable, capillaries which were still preserved showed red blood corpuscles in the lumen, in terminal villi there was proliferation of connective tissue which completely replaced fetal blood sinusoids. Fibrosis and endothelial degeneration was seen in basal decidual arterioles, fewer terminal villi and large plaques of fibrin like material between villi was also found.

In the year 2008 Correa et al in their study on placental morphometrical and histopathological changes in different clinical presentations of hypertensive pregnancy observed histological changes such as syncytial knots and fibrin deposits in hypertensive
placentae. They concluded that there could be different types of hypertension but the final pathway leads to microscopic lesions in the placenta which are the same.\textsuperscript{90}

In the year 2008 Guller et al in their study found that some syncytial products are released into maternal blood which negatively impact the function of maternal endothelium and promote manifestations of preeclampsia. They also suggested that placental damage and placental infarction associated with preeclampsia reduces placental transport leading to IUGR.\textsuperscript{91}

Hypertension in pregnancy, abnormal placentation is a hallmark of preeclampsia is a study conducted by Lindheimer et al in the year 2008. They reported that there is failure of normal trophoblastic invasion of the spiral arteries and failing of these vessels to remodel and dilate. This underlies the theory that restriction of placental blood flow leads to hypoxic uteroplacental environment which leads to release of factors that enter the mother’s circulation and initiate the maternal syndrome.\textsuperscript{92}

Study of proportional and absolute volume of placental parenchyma and non parenchyma between normal pregnant women and preeclamptic women was conducted by Kishwara et al in the year 2008. They reported reduction in both proportional and absolute volume in preeclamptic placentae as compared to normotensive placentae.\textsuperscript{93}

Is human placenta proteoglycan remodelling involved in preeclampsia was a study conducted by Warda et al in the year 2008. They used alcian blue stain to find the content of glycosaminoglycans in placental tissue. Their results showed that the content of glycosaminoglycans was markedly less in preeclamptic placentae as compared to normotensive placentae.\textsuperscript{94}

According to study conducted by Kishwara et al in the year 2009 transverse diameter, volume, number of cityledons and size of placentae in preeclampsia were significantly reduced as compared to control placentae.\textsuperscript{95}

A potential role of free fatty acids in the pathogenesis of preeclampsia was a study conducted by Robinson et al in the year 2009. They reported that lipid droplet accumulation was significantly increased in the maternal plasma from pregnancies complicated with preeclampsia as compared to normal uncomplicated controls.\textsuperscript{96}
A study on role of angiogenic factors in pathogenesis of preeclampsia was conducted by Wang et al in the year 2009. They reported that severe preeclampsia is associated with placental hypoperfusion and ischaemia. Acute atherosis, diffuse vascular obstruction, fibrin deposition, intimal thickening, necrosis, endothelial damage and atherosclerosis are findings of preeclampsia. Occlusion of spiral arteries resulting in placental infarcts is also common. In normal placental development uterine spiral arteries of decidua and myometrium are invaded by cytotrophoblasts of fetal origin. These cytotrophoblasts replace the endothelial layer of maternal spiral arteries and transform them from high resistance vessels to high caliber capacitance vessels. In preeclampsia cytotrophoblast invasion is limited to superficial decidua and transformation of spiral arteries is incomplete, thus myometrial segments remain narrow.97

Uteroplacental hemodynamics in the pathogenesis of preeclampsia was a study by Huchinson et al in the year 2009. They observed that in preeclampsia there were increased perfusion rates on maternal side while the fetal side flow rates remained constant. They reported that these elevated flow rates resulted in morphological damage, vacuolation and shedding of cytotrophoblasts and other features which were previously defined in preeclampsia. Biochemical markers of syncytial damage such as alkaline phosphatase was found in maternal perfusates recovered under high flow conditions. They concluded that alterations in intervillous blood flow have the potential to influence the integrity of syncytiotrophoblast as well as the liberation of potentially pathogenic soluble factors.98

Studies conducted by Sammak et al in the year 2009 showed that there was diminished activity of alkaline phosphatase enzyme in syncitiotrophoblast and villous stroma in preeclamptic placentae as compared to control group which showed a very strong reaction to alkaline phosphatase activity. They showed that in preeclamptic placentae alkaline phosphatase activity gradually decreased until it disappeared. This could be as a result of reduced uteroplacental perfusion, endothelial cell damage and placental ischaemia.99

Early onset preeclampsia is characterized by altered placental lipid metabolism is a study by Han et al in the year 2010. They examined total placental fatty acids and reported that fatty acids were significantly decreased preeclamptic placentae as compared to normal
controls. There was no significant difference found between preeclamptic and control placentae after 28 weeks of gestation.100

As documented by Eskild et al in the year 2010 in their study on placental weight and preeclampsia, preeclamptic pregnancies were in the highest decile of placental weights as compared to normotensive pregnancies. They concluded that placental weight is linked to the fetal birthweight, but is not associated with risk of preeclampsia. Thus placental weight is not a useful indicator of placental dysfunction in preeclampsia.101

Lipid peroxidation and antioxidant status in preeclampsia is a study by Kashinakunti et al in the year 2010. They reported that lipid peroxidation product and uric acid level is significantly increased in preeclamptic group as compared to normal.102

Marini et al in the year 2010 conducted a study on distribution of sugar residues in human placentae from pregnancies complicated by hypertensive disorder in the year 2010. They observed that glucose oxidase reactivity was weaker in preeclamptic placentae.103

A study was conducted on histomorphometry of umbilical cord blood vessels in preeclampsia by Blanco et al in the year 2011. Their results showed reduction in mean birth weight and significantly shortened gestation period for preeclamptic group as compared to control group.104

Vinnars et al in the year 2011 carried out a study in which their objective was to correlate the ischaemic changes in placentae with clinical severity of preeclampsia. The placental histopathological changes showed that the amount of infarction increased with severity of preeclampsia.27

Spectrum of changes in placenta in toxemia of pregnancy is a study by Narasimha et al in the year 2011 in which they concluded that striking villous abnormalities such as cytotrophoblast proliferation, thickening of villous basement membrane, fibrinoid necrosis, increase in syncytial knots, villous stromal fibrosis, endarteritis obliterans, paucity of vasculosyncytial membranes and decreased villous vascularity was observed in preeclamptic placentae.105
In the year 2011 Salgado et al in their study on structural changes in preeclamptic placentae observed that chorionic villi of hypertensive placentae showed a complex appearance with many distorted microvilli and frequent cytotrophoblast cells as compared to normal placentae. Chorionic villi showed thickening of basement membrane. Terminal villi of preeclamptic placentae showed patchy necrosis with loss of microvilli and gross thinning of syncytium. Numerous vacuolated mitochondria with loss of cristae, lysosomes, few rough endoplasmic reticulum and glycogen deposits were seen in cytotrophoblast cells where syncytium was absent.106

It was observed that there was absence of vasculosyncytial membrane in villi of hypertensive placentae as compared to normal placentae in a study conducted by Ansari et al in the year 2011 on vasculosyncytial membrane in placental villi of normotensive and hypertensive pregnancies.107

In the year 2011 Londhe et al conducted a morphometric study on placenta and its correlation in normal and hypertensive pregnancy. They reported that mean placental weight, number of cotyledons, mean placental volume and fetal birth weight was lower in hypertensive group as compared to the control group. While mean number of infarcted areas, calcified areas and marginal insertion of cord was higher in case of hypertensive placentae as compared to control placentae.108

In the year 2011 Powe et al in their study on preeclampsia a disease of maternal endothelium reported that there is abnormal placentation in preeclampsia. Cytotrophoblasts of fetal origin invade the maternal spiral arteries in normal placental development and transform them from small caliber resistance vessels to high caliber capacitance vessels. These vessels are then capable of providing adequate placental perfusion to sustain the growing fetus. During this process of vascular invasion the cytotrophoblasts differentiate from epithelial phenotype to endothelial phenotype. This process is referred to as pseudo vasculogenesis. Cytotrophoblasts fail to adopt invasive endothelial phenotype in case of preeclampsia. Instead there is shallow invasion of spiral arteries and they remain small caliber resistance vessels.14

Kishwara et al conducted a study on effects of preeclampsia on perinatal outcome in the year 2011. They reported reduced mean birth weight and mean APGAR score in babies born to preeclamptic mothers as compared to those of controls.109
In the year 2011 Sun Y et al conducted a study which aimed to minimize autofluorescence of renal tissue and demonstrate efficient method to reduce it using sudan black stain.\textsuperscript{110}

Use of PAS and sudan black stain was used in study conducted by Tewari on histological study on placentae of diabetic women in the year 2011.\textsuperscript{111}

In the year 2011 Lima et al studied the serum lipid levels in pregnancies complicated by preeclampsia. Their results showed that very low density lipoproteins and triglyceride values of preeclamptic women were higher than those of healthy women. However there was no significant difference between serum LDL and HDL levels of preeclamptic and healthy controls.\textsuperscript{112}

Lipid peroxidation and antioxidant status was studies by Begum R in the year 2011. They concluded that increased lipid peroxidation may be the important factor in pathogenesis of preeclampsia.\textsuperscript{113}

In the year 2011 Ilie et al reported histological changes like enlargement, atrophy and disruption of endothelium, fibrinoid necrosis, hypertrophy of smooth muscles in the wall of spiral arteries, avascular small villi, hyaline fibrosis of villous stroma, and thrombosis of spiral arterioles were noticed in pregnancy induced hypertensive placentae. Also microscopic changes like heterogenous placental maturation, decreased chorionic villi, decreased density of villous cytotrophoblastic cells and disappearance of fetal capillaries in most villi were observed in pregnancy induced hypertensive placentae as compared to normotensive placentae.\textsuperscript{114}

In the year 2012 use of alcian blue and PAS was done for staining sections and find mucins in placental tissue. This study was conducted by Schefer on normally delivered alpacas and lamalas.\textsuperscript{115}

In the year 2012 Lee et al in their study observed for glycogen phosphorylase isoenzyme plasma concentrations in preeclampsia. They reported that cases of preterm severe preeclampsia had higher glycogen phosphorylase isoenzyme concentration as compared to term preeclamptic cases. Also preeclamptic women had higher concentration of plasma glycogen phosphorylase enzyme as compared to women with normal pregnancy outcome.\textsuperscript{116}
Gheorman et al conducted a study on histochemistry of placenta in the year 2012 using alcian blue and PAS and found glycogen deposits in villous interstitium.\textsuperscript{117}

A study on placental pathology and blood pressure level in women with hypertensive disorders in pregnancy was carried out by Krielessi et al in the year 2012. They observed that placental lesions were seen more often in the severe hypertensive group. Villous fibrinoid necrosis and infarction was significantly increased in severe hypertensive group as compared to mild hypertensive group.\textsuperscript{118}

Nafees et al conducted a study on histopathology of preeclamptic placentae in the year 2012. They concluded that there is improper placental development in preeclampsia. Changes in preeclampsia are due to reduction in maternal uteroplacental blood flow which leads to construction of fetal stem arteries and fetal hypoxia is due to maternal vasospasm.\textsuperscript{119}

Sudan black staining method was used in study conducted by Ravikumar et al on biodiesel production from oleaginous fungi in the year 2012.\textsuperscript{120}

In the year 2012 Kalar et al conducted a study on lipid levels in preeclampsia. Enzymatic calorimetric method was used to determine lipid profile. They concluded that preeclamptic women had deranged lipid profile as compared to normal pregnant women.\textsuperscript{121}

Histological changes like accelerated villous maturation, decidual arteriopathy, placental infarction and intervillous thrombosis in preeclamptic placentae was observed by Mehrabian et al in their study on comparison of placental pathology between severe preeclampsia and HELLP syndrome in the year 2012.\textsuperscript{122}

In the year 2012 Akhlaq et al in their study found that preeclamptic placentae were less in size and thickness as compared to normal placentae. Microscopic features found were hypoplasia of distal villi, smooth muscle hypertrophy of spiral arterioles, villous necrosis, perivillous fibrin deposits and syncytial knots in preeclamptic placentae.\textsuperscript{123}

Histological changes in placentae in pregnancies complicated by preeclampsia and eclampsia and correlation with fetal outcome was a study by Navbir et al in the year 2012. They observed histological changes in preeclamptic placentae such as
cytotrophoblast proliferation in significant villi, vasculosyncytial membrane deficiency, basement membrane thickening, excessive syncytial knotting, stromal fibrosis and fibrinoid necrosis.\[^{124}\]

In the year 2012 Alladin et al in their study have reported that syncytiotrophoblast cells in placenta which are in direct contact with the mother are involved in preeclampsia. There is an extensive remodelling of maternal spiral arteries in normal placenta. Invasion of trophoblasts into the spiral arteries transform small caliber vessels into large capacity vessels. This arterial network is able to access more and more mother’s blood as per the increased requirement of placenta. When placenta is not able to access more nutrients from the mother because of ill developed maternal spiral arteries it can lead to preeclampsia or fetuses that are small for gestational age. Poor placentation and increased oxidative stress causes hypoxia resulting in lack of oxygen is thought to cause clinical signs of preeclampsia. More demand of nutrients from growing fetus is not met by the mother as a result of early poor placentation. Failure of trophoblasts to invade into the decidua prevents remodelling of spiral arteries which finally leads to insufficient blood flow from mother to placenta.\[^{125}\]

Saeed et al studied the histomorphological changes in placentae of preeclamptic mothers in the year 2012. Their results showed that there were increased number of terminal villi, increased number of syncytial knots and increased vasculosyncytial membrane thickness in hypertensive group as compared to normal controls. They concluded that this increase in syncytial knots and vasculosyncytial membrane thickness may be the cause or effect of hypoxia.\[^{126}\]

Immuno histochemical study of the syncytial knots in preeclamptic placentae was studied by Sharma et al in the year 2012. They observed increased number of syncytial knots in preeclamptic placentae as compared to normotensive placentae. They also observed increased number of syncytial knots in peripheral section of preeclamptic placentae as compared to central section.\[^{127}\]

Placental morphology and its correlation with fetal outcome in pregnancy induced hypertension is a study by Navbir et al in the year 2012. They found that the placental weight and volume were much lower in higher proportion of cases eclampsia and moderate to mild preeclampsia. Feto-placental weight ratio was lower in cases of severe
form of disease than in case of milder form of toxaemia. They concluded that lighter placentae usually accompanied low birth weight fetus. Placental infarcts, retroplacental haematoma and calcification were three main gross lesions that were observed, the incidence of which was higher in placentae of hypertensive pregnancies as compared to those of control group. Study group more commonly showed placental calcification while control group showed this feature to a lesser degree.\textsuperscript{128}

Huang et al studied placental phospholipids in preeclamptic pregnancies in the year 2013. They found an increase in total phospholipid content as well as changes in individual classes of phospholipids in preeclamptic placental tissue as compared to control placentae. They reported that these alterations could be due to pathological changes in preeclampsia, such as dysregulation of lipid transport across the syncytiotrophoblast or lipid peroxide insult.\textsuperscript{129}

Role of lipid peroxidation and antioxidant status in pathogenesis of preeclampsia is a study by Phalak et al in the year 2013. They reported that increased level of lipid peroxidation product and decreased levels of antioxidants in preeclamptic women suggested that oxidative role plays a key role in endothelial dysfunction in preeclampsia.\textsuperscript{130}

Studies conducted by Dubova et al in the year 2013 found irregularity in expression of glucose transporters in preeclamptic placentae and concluded that disturbance in glucose transporters could play a major role in IUGR development in severe cases of preeclampsia.\textsuperscript{131}

Nag et al in the year 2013 conducted a study on morphological changes in placenta of hypertensive pregnant women. They concluded that there is increased chance of ischaemic to the placental tissue and maldeveloped villi in patients with pregnancy induced hypertension. As a result of this there may be impaired nutrient transfer and low birth weight babies.\textsuperscript{132}

Effects of pregnancy induced hypertension on placenta was a study conducted by Motwani et al in the year 2013. On gross examination they revealed presence of smaller placentae, calcification foci and infarction in the study group. Microscopic examination showed villous abnormalities, syncytial knots, fibrinoid necrosis, hyalinized villi, stromal
fibrosis, hypo vascularity of villi, cytотrophoblastic cell proliferation and basement membrane thickening. They concluded that pregnancy induced hypertension immensely affected placenta which may be responsible for postnatal outcomes.\textsuperscript{133}

Nahar et al carried out a study on pregnancy induced hypertensive placentae in the year 2013. They observed fibrinoid necrosis, syncytial knots, sclerosis, chorangiosis and calcification were more marked in preeclamptic placentae as compared to control placentae.\textsuperscript{134}

Kaur et al conducted a study on placental weight, birth weight and fetal outcome in preeclampsia and normotensive pregnancies in the year 2013. They observed that fetal outcome was significantly poor in preeclampsia as compared to normotensive pregnancies.\textsuperscript{135}

Placental changes in idiopathic intrauterine growth restriction was a study conducted by Biswas et al in the year 2013. They used PAS stain to observe the chorionic villi in their study.\textsuperscript{1}

In the year 2013 Modi et al studied morphological changes in preeclamptic placentae. Their results showed reduction in placental weight, placental volume, placental thickness and diameter in case of preeclamptic placentae as compared to normotensive placentae. They concluded that preeclamptic placenta underwent a lot of morphological changes which seemed to be the responsible for placental insufficiency in pregnancy induced hypertension.\textsuperscript{136}

Dhabhai et al studied histology of human placenta in normal and pregnancy induced hypertension. They observed increased syncytial knots, increased hyalanized villi, hypovascular villi, fibrinoid necrosis and stromal fibrosis in preeclamptic placentae as compared to normotensive placenta.\textsuperscript{137}

Study of placental changes in pregnancy induced hypertension is a study conducted by Maimoona et al in the year 2013. They reported that the mean placental weight and fetal weight to placental weight ratio reduces as PIH severity increases. They also noted that histological changes like infarction, calcification and syncytial knots were more in severe cases of preeclampsia.\textsuperscript{138}
Placental pathology suggesting that preeclampsia is more than one disease is a study by Nelson et al in the year 2014. They observed chorionic villitis, ischaemic villous necrosis and vascular lesions like decidual arteriolopathy, narrow caliber of spiral arterioles and residual medial smooth muscle cells, fibrinoid necrosis, atherosis of decidual arterioles. Accumulation of foamy lipid filled macrophages with mural fibrinoid necrosis of decidual arterioles was seen. They concluded that women with preeclampsia onset before 34 weeks of gestation had significantly different placental findings as compared to those with preeclampsia at term. Hence these different findings support the hypothesis that preeclampsia is a different disease depending on gestational age at diagnosis.

Stark et al in the year 2014 conducted a study in which they compared the histological differences in placentae of preeclamptic gestations by birth weight, placental weight and time of onset. Their results showed that increased birth weight placentae had increased mural hypertrophy of membrane arterioles and decreased syncytial knots. While decreased birth weight showed increased placenta site giant cells. Increased placental weight had decreased distal villous hypoplasia. Decreased placental weight had increased acute atherosis, increased intervillous fibrin and increased syncytial knots. Early onset disease showed increased syncytial knots, villous agglutination, and infarcts. With all these findings they suggested that preeclampsia is composed of several different processes manifesting a single clinical presentation.

Singh et al in the year 2014 conducted a cross sectional morphological study on preeclamptic placentae. They reported that mean placental weight in case of preeclampsia was less than that of normal placentae. Various pathological changes like retroplacental hematoma, calcification and infarction were noticed in preeclamptic placentae. Decreased placental weight was associated with reduced fetal birth weight with significant increase in severity of hypertension.

Study of structural changes in placenta in pregnancy induced hypertension was conducted by Salmani et al in the year 2014. They observed reduction in thickness, weight and number of cotyledons in preeclamptic placentae as compared to normotensive placentae. Increased areas of calcification, fibrinoid necrosis, hyalinization, increased syncytial knots were observed in preeclamptic placentae as compared to normotensive placentae. They also observed increased areas of medial coat proliferation in blood vessels in preeclamptic placentae.
6. MATERIALS AND METHODS

6.1 STUDY DESIGN: Cross sectional study was conducted in Department of Anatomy of Dr. D.Y. Patil Medical College, Pimpri, Pune.

6.2 METHODS OF DATA COLLECTION: Consecutive convenient sampling method was used. 50 normotensive and 50 preeclamptic placentae were collected immediately after delivery from women who delivered either vaginally or by cesarean section from Department of Obstetrics and Gynecology of Dr.D.Y.Patil hospital and Yashwantrao Chawan memorial hospital, Pimpri, Pune. Institutional ethical committee clearance was obtained. Written informed consent was obtained from all mothers participating in the study.

Inclusion criteria:

- Age from 20-35 years.
- Primigravida.
- Gestational age between 28-38 weeks.
- Willing to participate and signing consent form after explanation of aim and purpose of study.

Exclusion criteria:

- Preexisting hypertension, diabetes mellitus or any other complication.
- Gestational diabetes or any other gestational complications.
- History of maternal diseases such as autoimmune disease or thrombophilic conditions.
- Evidence of any fetal anomalies or intrauterine death.
Samples were divided into two groups as group A and group B:

**Group A: Control group (Normotensive):** Placentae were obtained from pregnant women who did not have any clinically detectable abnormalities. These women had normal blood pressure, no proteinuria and no oedema.

**Group B: Study group (Preeclampsia):** Placentae were obtained from known preeclamptic cases who had no history of hypertension before pregnancy or during first 20 weeks of gestation, who had consistently recorded systolic and diastolic blood pressure of 140/90 mm of Hg or above and proteinuria ≥300 mg per day. The alterations in blood pressure were observed on at least two different occasions, at least six hours apart. Detailed menstrual and obstetric history and past history was obtained to exclude preexisting hypertension and other complications. Fetal weight, sex, any congenital anomaly and APGAR score at 1 and 5 minutes after delivery were recorded as parameters of fetal outcome.

### 6.3 CLASSIFICATION OF SEVERITY OF PREECLAMPSIA

Classification of severity of preeclampsia was done based on the criteria of American College of Obstetricians and Gynecologists. (ACOG)\(^{143}\)

**Mild-moderate**

BP is 140 to 159 mmHg systolic and/or 90 to 109 mmHg diastolic that occurs after 20 weeks of gestation (on 2 occasions at least 6 hours apart) and proteinuria is ≥300 mg/24 hours.

**Severe**

BP is ≥160 mmHg systolic and/or ≥110 mmHg diastolic (on 2 occasions at least 6 hours apart, while the patient is on bed rest) and proteinuria is ≥500 mg/24 hours.
6.4 MORPHOLOGICAL STUDY:

Morphology of placenta was studied under the following:

- Weight
- Diameter
- Thickness
- Number of cotyledons
- Study of maternal and fetal sides
- Presence of necrotic patches

6.4.1 PLACENTAL WEIGHT:
- Placental weight was measured by directly placing the placenta on standardized weighing scale.

6.4.2 PLACENTAL DIAMETER:
- The placenta was placed on a flat surface after trimming and mopping.
- At first the maximum diameter was measured with a metallic scale graduated in centimeters.
- Then second maximum diameter was recorded at right angles to the first one.
- The mean of two diameters was considered as the diameter of placenta.

6.4.3 PLACENTAL THICKNESS:
- Placenta was placed on a flat surface.
- Two circles were drawn from the center of the placenta.
- Thick point needle was inserted to the full thickness of placenta at five points.
- Centre of the inner circle, two different points between inner and outer circle and two different points outside the outer circle.
- Mean of all five points was taken as the thickness of placenta.

6.4.4 NUMBER OF COTYLEDONS:
- Gentle pressure was applied on center of the fetal surface of placenta.
- As a result the cotyledons on the maternal surface became prominent.
- The placenta was then placed on a flat surface with maternal side facing upwards and total number of cotyledons was recorded.
6.4.5 STUDY OF MATERNAL AND FETAL SIDES:

- Maternal and fetal sides of placentae were looked for carefully for any necrotic patches, calcifications and attachment of umbilical cord was noted.
6.5 HISTOPATHOLOGICAL STUDIES

From each placenta whole thickness tissue blocks were taken from center and periphery. Tissue samples from placentae were processed and stained and were observed under light microscope. 100 villi were studied from each of central and peripheral section of placentae for each category of stain. Sections were then photographed by microphotography and transferred to the computer.

6.5.1 HAEMATOXYLIN AND EOSIN STAIN: HISTOPATHOLOGY\textsuperscript{144,145}

Tissues were processed for paraffin blocks as follows:

1. **Fixation**: Each tissue was cut into a small fragment of about 1 cm before fixation. This was to facilitate penetration of fixative and preservation of tissue. Fixative used was 10 % cold formal saline for 48 hours.

2. **Dehydration**: The tissue to be embedded was dehydrated by bathing them in into grades of ascending alcohol (50 -95%)
   - 50% alcohol – 2 hours
   - 70% alcohol – overnight
   - 80% alcohol – 2 hours
   - 95% alcohol – 2 hours
   - Absolute alcohol I – 1 hour
   - Absolute alcohol II – 1 hour

3. **Clearing**: Alcohol was then replaced by xylene.
   - Xylene I – 1 hour
   - Xylene II – 1 hour

4. **Embedding**: Tissue was then placed in melted paraffin at 58 to 60 degree Celsius. Blocks were prepared. Section of 5 micron thickness was taken and was transferred to the glass slide for staining.
6.5.2 PREPARATION OF STAINS:

1% EOSIN STOCK SOLUTION:

Solution was prepared as follows:

- Water soluble eosin Y – 1gm
- Distilled water – 20 ml
- 95% alcohol – 80 ml

i. 1 gm of water soluble eosin Y was added to 20 ml of distilled water.

ii. Eosin was dissolved in water then 80 ml of 95% alcohol was added.

WORKING EOSIN SOLUTION:

Solution was prepared as follows:

- 1% Eosin stock solution – 25ml.
- 80% alcohol – 75 ml.
- Glacial acetic acid – 0.5ml

i. 25ml of 1% Eosin stock solution was added to 75 ml of 80% alcohol.

ii. 0.5 ml of glacial acetic acid was added to 100ml of stain.

HARRIS’S HAEMATOXYLIN:

Stain was prepared as follows:

- Haematoxylin – 1 gm
- Absolute alcohol -10 ml
- Ammonium or Potassium alum – 20 gms
- Distilled water – 200 ml
- Mercuric oxide (red) – 0.5 gms

i. Haematoxylin was dissolved in alcohol.

ii. Alum previously dissolved in hot water was added to it.
iii. The solution was quickly boiled and mercuric oxide was added to it till the solution turned dark purple.

iv. It was cooled rapidly under tap water and filtered before use.

6.5.3 STAINING:

Staining was done routinely with Haematoxylin and Eosin. Haematoxylin is a basic dye which stains the nucleus of the cell and eosin is an acidic dye which stains the components of the cytoplasm.

Technique:

1. **Deparaffinisation**: The slides were treated with two changes of xylene.
   - Xylene I – 15 minutes
   - Xylene II – 15 minutes

2. **Graded hydration**: Slides were passed through following series of alcohol to hydrate the sections.
   - Absolute alcohol I – 2 dips
   - Absolute alcohol II – 2 dips
   - 95% alcohol – 2 dips
   - 80% alcohol – 2 dips
   - 70% alcohol – 2 dips
   - 50% alcohol – 2 dips
   - Rinsed with distilled water.

3. Stained with Haematoxylin for 10 minutes.

4. Washed in running tap water for 10 minutes.

5. Slides were observed under microscope for proper staining. If stained excessively, a dip in acid alcohol was given.

6. Washed in running tap water for 10 minutes.

7. Stained with eosin for 3 minutes.
8. **Dehydration**: The sections were dehydrated in following series of alcohol.

- 50 % alcohol – 2 dips
- 70% alcohol – 2 dips
- 80% alcohol – 2 dips
- 95% alcohol – 2 dips
- Absolute alcohol – 2 dips

9. **Clearing and Mounting**: The slides were dried, cleared in xylene and mounted using DPX.

Positive sites stained – Cytoplasm stained pink

Nuclei stained violet
6.6 PERIODIC ACID SCHIFF’S REACTION (PAS): GLYCOGEN

Tissues were processed for staining as follows: (as explained in H & E staining)

1. Fixation

2. Dehydration

3. Clearing

4. Embedding

5. Blocks were prepared.

Thin sections of 5 microns were taken.

6.6.1 SCHIFF’S REAGENT:

Reagent was prepared as follows:

i. 1gm of basic fucshin was dissolved in 200 ml of boiling distilled water in a stoppered 1 liter flask.

ii. Shaken for 5 minutes

iii. Cooled exactly at 50 degree Celsius, filtered and 20 ml of N/1 HCl was added to the filtrate.

iv. Cooled further to 25 degree Celsius and 1 gm of sodium metabisulfite was added to it.


vi. 2gms of activated charcoal was added and the mixture was shaken for 1 minute.

vii. Charcoal was removed by filtration and the solution was stored in dark at 0-4 degrees.
6.6.2 STAINING:

Periodic acid is an oxidising agent that has the ability to oxidise 1, 2- glycols to formaldehydes. PAS reaction is a commonly used histological technique to localize glycoproteins and glycogen.

**Technique:**

1. Sections were brought to water.

2. Oxidised in 1% periodic acid for 10 minutes.

3. Washed in running water for 5 minutes and rinse in distilled water for 1 minute.

4. Treated with Schiff’s reagent for 30 minutes.

5. Washed in running tap water for 10 minutes.

6. Counterstained with Harris haematoxylin for 6 minutes.

7. Washed in running tap water and rinsed in distilled water.

8. Dehydrated, cleared and mounted in DPX.

PAS positive substances stained – Magenta

  Nuclei stained Blue
6.7 ALCIAN BLUE STAIN (pH 2.5): GLYCOSAMINOGLYCANS (GAGs)

Tissues were processed for staining as follows.

1. Fixation
2. Dehydration
3. Clearing
4. Embedding
5. Blocks were prepared.

6.7.1 PREPARATION OF ALCIAN BLUE STAIN:

Stain was prepared as follows:

- Alcian blue : 0.5gms
- Glacial acetic acid – 3 ml
- Distilled water - 100 ml
  i. 0.5 gms of alcian blue and 3ml of glacial acetic acid was added to 100 ml distilled water and dissolved.
  ii. Filtered and crystals of thymol were added. This gave staining solution of pH 2.5.

Sections : Paraffin sections of 5 micron thickness were taken.

6.7.2 STAINING:

Alcian blue is a copper phthalocyanin dye containing four isothiouronium groups. Alcian blue stain at pH 2.5 stains both sulphated and non sulphated acid glycosaminoglycans.

Technique:

1. Sections were taken to water.
2. Stained in Alcian blue stain for 30 minutes.
3. Rinsed in distilled water.
4. Washed in running water for 5 minutes.

5. Counterstained with fresh light green for 2 minutes.

6. Dehydrated through graded alcohols, cleared in xylene and mounted.

Positive sites stained – Cytoplasm – Light blue

Blood vessels stained Light green.
6.8 MODIFIED GOMORI’S METHOD: PLACENTAL ALKALINE PHOSPHATASE (PALP)\textsuperscript{146}

Serum alkaline phosphatase of both normotensive controls and preeclamptic cases was recorded to correlate with PALP.

Whole thickness tissue was cut out from central and peripheral part of placenta. Frozen sections of 10 micron thickness were taken from this fresh unfixed tissue. Sections were then placed on non albuminised slides. These were further stained by modified Gomori’s method.

6.8.1 INCUBATING MIXTURE:

Was prepared as follows:

- 3 % aqueous Sodium beta glycerophosphate - 20 ml
- 2 % aqueous Sodium barbitone - 30 ml
- 2 % aqueous Calcium chloride - 4 ml
- 2 % aqueous Magnesium sulphate - 2 ml
- Distilled water - 30 ml

6.8.2 STAINING:

Placental alkaline phosphatase activity was demonstrated by using modified Gomori’s method.

Technique:

1. Sections were washed with distilled water and were immersed in incubating mixture for one hour at 37 degree Celsius.

2. Tissues were taken out from the incubator and were brought to room temperature.

3. Stained by 2 % Cobalt nitrate solution for 5 minutes.

4. Then they were stained by freshly prepared Ammonium sulfide solution for 2 minutes. (Ammonium sulfide solution was prepared by adding 50 drops of concentrated solution into 20 ml of water)
5. Tissues were washed with distilled water after every step.

At the end deposition of black precipitate was observed at the sites of enzymatic activity.
Whole thickness tissue was cut out from central and peripheral part of placenta. Frozen sections of 10 micron thickness were taken from this fresh unfixed tissue. Sections were then placed on non albuminised slides.

**6.9.1 SUDAN BLACK STAIN**

Stain was prepared as follows:

i. 0.7 gram of Sudan Black was dissolved in 100 ml of propylene glycol slowly while stirring.

ii. Heated to 100 degree Celsius for a few minutes stirring constantly.

iii. Filtered through Whatman # 2 filter paper.

iv. Cooled and filtered again through frittered glass filter of medium porosity.

v. Stored at 60 degree Celsius.

**6.9.2 STAINING**

**Technique**

1. Slides were fixed in 10% formal saline for 15 minutes.
2. Washed well in tap, rinsed in distilled water, excess water was drained off.
3. Two changes of propylene glycol were given for 5 minutes each.
4. Stained with Sudan Black for 7 minutes and agitated.
5. 85% Propylene glycol for 3 minutes.
6. Rinsed in distilled water.
7. Counterstained with Nuclear fast red for 3 minutes.
8. Washed in tap water and rinsed in distilled water.

Positive sites stained - Fat blue-black
Figure 3

A: Wax bath, B: L molds, C: Semi motorized Microtome, D: Water bath

Figure 4

1. Xylene  
2-6: Series of alcohol  
7. Haematoxylin  
8. Eosin  
9. Periodic acid  
10. Schiff’s reagent  
11. Incubation mixture for PALP  
12. Cobalt nitrate  
13. Ammonium sulphide  
14. Sudan black  
15. Alcian blue
Figure 5

A: Computer, B: Trinocular microscope with microphotographic attachment