CHAPTER 1

INTRODUCTION

1.1 DEFINITION FOR PREECLAMPSIA

Preeclampsia is a pregnancy – specific disorder characterized clinically by new onset of maternal hypertension, proteinuria and edema. It begins after 20 weeks of gestation. Preeclampsia affects 3-5% of pregnant women worldwide [1].

Clinical Features: New onset of maternal hypertension - defined as systolic blood pressure ≥140 mmHg or diastolic blood pressure ≥90mmHg.
Proteinuria - ≥0.3g in a 24 hr urine specimen/protein to creatinine ratio of >0.30.
Edema - sudden onset of severe edema, especially edema of the hands and face.

Preeclampsia is a systemic vascular disorder that may also affect the liver and the brain in the mothers. When the liver is involved, women may present with abdominal pain, nausea, vomiting, and elevated liver enzymes. Pathological examination of liver reveals periportal and sinusoidal fibrin deposition and, in more extreme cases, hemorrhage and necrosis. The severe preeclampsia variant HELLP syndrome (hemolysis, elevated liver enzymes, low platelets) occurs in 20% of women with severe preeclampsia, and is named not only for the liver involvement, but also for the disorder of the coagulation system that develops. Approximately 20% of women with HELLP syndrome develop disseminated intravascular coagulation, which carries a poor prognosis for both mother and fetus [2]. Placental abruption, ascites, hepatic infarction, hepatic rupture, intra-abdominal bleeding, pulmonary edema, and acute renal failure are all severe clinical manifestations associated with preeclampsia that can result in maternal death [3]. Perhaps the most feared complication of preeclampsia is eclampsia. Eclampsia is a very serious
complication of preeclampsia characterized by one or more seizures during pregnancy or in the post-partum period [4]. The brain injury in eclampsia is associated with cerebral edema and characteristic white matter changes of reversible posterior leukoencephalopathy syndrome, which is similar to findings noted in hypertensive encephalopathy and with cytotoxic immunosuppressive therapies [5]. Cerebrovascular complications, including stroke and cerebral haemorrhage, are responsible for the majority of eclampsia-related deaths. Complications affecting the developing fetus include indicated prematurity, intrauterine fetal growth restriction, oligohydramnios, bronchopulmonary dysplasia, and increased risk of perinatal death [6].

1.1.1 Risk Factors for Preeclampsia

- Previous history of preeclampsia. There is a 7-fold risk of recurrence for women who have had the disease in a previous pregnancy [7].
- Multiple gestations: Multiple gestation is an additional risk factor, and triplet gestation carries a greater risk than twin, suggesting that increased placental mass plays some role [8].
- Nulliparity [8].
- Preexisting hypertension, diabetes mellitus, or renal disease [9].
- Obesity, particularly with body mass index (BMI) of 30 or greater [10].
- Maternal age >40 years or under 18 years of age [11].
- Change in paternity from a previous pregnancy [12].
- Increased interpregnancy interval [13].
- Use of barrier contraception [14].
- Conception by intracytoplasmic sperm injection [15].

Consistent with these risk factors, surprisingly, smoking during pregnancy protects against preeclampsia [16].
Preeclampsia is a leading cause of maternal, fetal mortality and morbidity. The only known remedy/treatment is delivery of the placenta. Although it is consider placenta is the origin of the disease, but the target organ is the maternal endothelium.

1.1.2 The Maternal Endothelial Dysfunction caused by Preeclampsia

The maternal vascular endothelium appears to be an important target of factors that are triggered by placental ischemia/hypoxia in preeclampsia. The endothelium is a single - cell lining tissue that covers the luminal side of blood vessels. This strategic location permits it to signal alterations in hemodynamics and humoral factors by synthesizing and releasing vasoactive substances. Thus a critical balance exists between endothelium-derived relaxing and contracting factors that maintain vascular homeostasis. When this delicate balance is disrupted, the vasculature is predisposed to vasoconstriction, leukocyte adherence, mitogenesis, prooxidation, and vascular inflammation [17].

The hypertension of preeclampsia is characterized by peripheral vasoconstriction and decreased arterial compliance. The proteinuria is associated with a renal lesion known as glomerular endotheliosis, in which the endothelial cells of the glomerulus swell and endothelial fenestrations are lost. Podocyturia has been recently associated with preeclampsia during clinical disease. The glomerular filtration rate is decreased compared with normotensive pregnant women, in rare cases, acute renal failure may develop.

1.2 ROLE OF THE PLACENTA IN PREECLAMPSIA

1.2.1 Anatomy of Normal Placenta

The placenta is the organ that facilitates nutrient and gas exchange between the maternal and fetal compartments. As the fetus begins its ninth week of development, the demand for their nutrition increases, where the placenta plays a major role in facilitating the exchange between maternal and fetal components.
The fetal component of the placenta is derived from the trophoblast and extraembryonic mesoderm (chorionic plate); the maternal component is derived from the uterine endometrium.

The development of the placenta begins once the trophoblast establishes close and stable contact with the uterine luminal epithelium and mucosa, as soon as the blastocyst implants. The trophoblast makes up the outer wall of the blastocyst, which surrounds the blastocoel (blastocyst cavity) and the inner cell mass. The trophoblast can be described as the precursor of the placenta. The inner cell mass, the so-called embryoblast, composed of a small group of larger cells. The embryo, umbilical cord and amnion are derived from these cells. In addition to trophoblast, both embryoblast-derived mesenchyme and embryoblast-derived blood vessels contribute to the formation of the placenta. Normally the blastocyst is orientated in a way that the part which contains the embryonic pole attaches to the endometrium.

Implantation in the human begins six to seven days after fertilization and can be divided into three stages. Apposition comes first, which is the beginning of a still unstable adhesion of the blastocyst to the uterine wall. Apposition of the blastocyst is followed by stable adhesion, which is characterized by an increased physical contact between the blastocyst and the uterine epithelium. Now the embryonic pole is orientated towards the uterine epithelium. During attachment and invasion of the endometrial epithelium, the trophoblast cells at the implanting embryonic pole of the blastocyst show increased proliferation resulting in a double-layered trophoblast.

The differentiation of trophoblast into two fundamentally different subtypes—named syncytiotrophoblast and cytotrophoblast begins. The third stage is the invasion process, where the multinucleated syncytiotrophoblast penetrates through the uterine epithelium. As soon as the early embryo has penetrated the epithelium and is surrounded by the endometrial connective tissues, the syncytiotrophoblast forms a complete layer surrounding the early embryo, this layer is encasing the conceptus completely. In the second row beyond the syncytiotrophoblast there are the mononucleated cytotrophoblasts, without any contact to maternal tissues. The
expanding syncytiotrophoblast is continuously fed by dividing and fusing cytotrophoblasts.

Eight days after conception lacunae start to develop out of small intrasyncytial vacuoles on the surface of the implanting blastocyst. This so called lacunar stage of placentation lasts from day 8 to day 13 post conception. The lacunae are separated from each other by syncytial trabeculae, which start to be invaded by cytotrophoblasts after day 12 post conception. At about day 15 post conception mononucleated cytotrophoblasts start to invade the deciduas. Throughout the course of the first trimester they will invade the whole thickness of the endometrium, the inner third of the myometrium and the uterine vasculature the site of the placental bed, i.e. the uterine tissues directly underneath the placental attachment site. This is a key event during early implantation and placentation and responsible for further invasion of the blastocyst, for adaptation of the maternal vessels to pregnancy conditions as well as for anchorage of the developing placenta. Too excessive or too shallow invasion is associated with pathologies of pregnancy.

At about day 13 post conception increased cytotrophoblastic proliferation with subsequent syncytial fusion takes place in the trabeculae. Longitudinal trabecular growth can be observed as well as formation of blindly ending side branches which protrude into the lacunae. These formations are invaded by cytotrophoblasts and as long as they are pure trophoblastic structures, they are called primary villi. Out of these primary villi the branching of villous trees starts. At the same time the lacunar system transforms into the intervillous space. When early villous structures come into contact to the decidual tissues of the developing basal plate, they adhere to the maternal tissues and are called anchoring villi. From the tip of anchoring villi cytotrophoblasts proliferate and start to migrate outwards to trophoblastic cell columns. From these cell columns extravillous trophoblasts migrate into the deciduas, invade the decidual interstitium and the maternal spiral arteries, being subsequently responsible for the establishment of the uteroplacental circulation.
By the beginning of the third week, the trophoblast is characterized by primary villi that consist of a cytotrophoblastic core covered by a syncytial layer. During further development, mesodermal cells penetrate the core of primary villi and grow toward the decidua. The newly formed structure is known as a secondary villus.

By the end of the 3rd week, mesodermal cells in the core of the villous begin to differentiate into blood cells and small blood vessels, forming the villous capillary system. The villus is now known as a tertiary villus or definitive placental villus. Capillaries in tertiary villi make contact with capillaries developing in the mesoderm of the chorionic plate and in the connecting stalk. These vessels, in turn, establish contact with the intraembryonic circulatory system, connecting the placenta and the embryo. Hence, when the heart begins to beat in the 4th week of development, the villous system is ready to supply the embryo proper with essential nutrients and oxygen.

As pregnancy advances, villi on the embryonic pole continue to grow and expand, giving rise to the chorion frondosum (bushy chorion). Villi on the abembryonic pole degenerate, and by the third month, this side of the chorion, now known as the chorion laeve which appears smooth.

The difference between the embryonic and abembryonic poles of the chorion is also reflected in the structure of the decidua, the functional layer of the endometrium, which is shed during parturition. The deciduas over the chorion frondosum, the deciduas basalis, consists of a compact layer of large cells, decidua cells, with abundant amounts of lipids and glycogen. This layer, the decidual plate, is tightly connected to the chorion. The decidual layer over the abembryonic pole is the decidua capsularis. With growth of the chorionic vesicle, this layer becomes stretched and degenerates. Subsequently, the chorion laeve comes into contact with the uterine wall (decidua parietalis) on the opposite side of the uterus, and the two fuse, obliterating the uterine lumen. Hence, the only portion of the chorion participating in the exchange process is the chorion frondosum, which, together with the deciduas basalis, makes up the placenta [18].
The maternal blood is delivered to the placenta by spiral arteries in the uterus. Erosion of these maternal vessels to release blood into intervillous spaces is accomplished by endovascular invasion by cytotrophoblast cells. These cells, released from the ends of anchoring villi, invade the terminal ends of spiral arteries, where they replace maternal endothelial cells in the vessel walls, creating hybrid vessels containing both fetal and maternal cells. To accomplish this process, cytotrophoblast cells undergo an epithelial-to-endothelial transition. Invasion of the spiral arteries by cytotrophoblast cells transforms these vessels from small-diameter, high resistance vessels to larger-diameter, low-resistance vessels that can provide increased quantities of maternal blood to intervillous spaces [19].

1.2.2 Microanatomy of Spiral Arteries

The spiral arteries are small arteries that supply blood to endometrium of the uterus. The histological feature of spiral arteries shows three layers namely, tunica intima, tunica media and tunica adventitia (Figure 1.1). The tunica intima lined by endothelial cells. The tunica media made up of large quantities of smooth muscles with a few elastic fibres in the non-pregnant state.

![Figure 1.1 Tunica Media in Normal Artery 40 X - Van Geison - Control group](image-url)
During pregnancy, the spiral arteries are converted into large dilated vessels for uteroplacental blood flow by losing the media smooth muscle cells and elastic fibres (Figures 1.2, 1.3).

**Figure 1.2** Normal spiral artery showing dilated lumen-40X Van Geison

**Figure 1.3** Normal spiral artery - 10X magnification - Van Geison

**1.2.3 Extravillous Trophoblast**

Extravillous trophoblast are the nonvillous parts of the placenta. In contrast to the villous parts of the placenta, they do not participate in maternofetal exchange since they are never vascularized by both the maternal and fetal circulations. These cells are large, intensely basophilic stained cells. Extravillous trophoblast cells can be found in chorionic plate, basal plate and uteroplacental arteries. Extravillous trophoblast is derived from the trophoblastic wall of the blastocyst.

The extravillous trophoblast is divided into two types i.e. extravillous syncytiotrophoblast and cytotrophoblast. The extravillous syncytiotrophoblast is remainders of syncytiotrophoblast seen mostly at the intervillus surfaces of all non-villous parts of the placenta. Extra villous cytotrophoblast are mononuclear trophoblast cells present outside the villi. The extravillous cytotrophoblast are classified into proliferative phenotype and invasive phenotype. The proliferative phenotype is proliferative stem cells resting on or near the basal lamina facing the
villous or chorionic stroma and are equals to proximal extravillous trophoblast cells. Invasive phenotype is non-proliferative disseminated daughter cells equals to distal extravillous trophoblast cells. The invasive phenotype has two types of cells namely the interstitial trophoblast and endovascular trophoblast cells. The invasive types which prevails in early stages of pregnancy, are highly invasive cells that migrate from the superficial basal plate until the myometrium. The endovascular trophoblast cell invades the walls and lumina of uteroplacental vessels. The endovascular trophoblast has two subtypes namely, intramural trophoblast that are invasive trophoblast cells infiltrating the walls of uteroplacental vessels and intraarterial trophoblast are invasive trophoblast cells that replace endothelium and forms intraluminal plugs in uteroplacental arteries (Figure 1.4).

**Figure 1.4 Subpopulation of Extravillous Trophoblast**

1.2.4 Remodelling of Spiral Arteries by Trophoblast Invasion

During early pregnancy endovascular extravillous trophoblast cells migrate down the lumens of the spiral arteries, while interstitial trophoblast cells migrate
through the endometrial stroma and penetrate the vessel walls from their outside (Figure 1.5).

**Figure 1.5 Normal placentation [20]**

In normal pregnancy, the interstitial trophoblast cells invade as deep as the inner third of the myometrium, where they progressively transform into immotile giant extravillous trophoblast cells (Figure 1.6). Both endovascular and interstitial invasion are associated with the physiological conversion of the spiral arteries.

**Figure 1.6 The spiral artery surrounded and thoroughly infiltrated by extravillous trophoblast cells with destroyed muscular coat – arrow denotes extravillous trophoblast - 40 X Van Geison - Control group**
During this process the arteries lose the smooth muscle in their walls and their elastic laminas, results in vessels dilatation and are converted into flaccid conduits (Figure 1.7). This creates a high-flow, low-resistance circulation that increases maternal blood flow to the placental villi at the maternal-fetal interface. The extent of conversion varies across the placental bed, and is greatest in the central region where trophoblast invasion is most extensive [21].

Figure 1.7  Lumen of normal spiral artery – 40 X Van Geison – Control group

The remodeling of uteroplacental or spiral arteries is also called physiological changes of uteroplacental arteries. According to structural criteria the process of physiological changes can be divided into three stages: [22]

1. **Trophoblast invasion – independent vascular changes:** The initial changes to uteroplacental arteries involve a generalized perturbation of these arteries, endothelial basophilia and vacuolation, disorganized vascular smooth muscle, and lumen dilation. The pregnancy – induced changes in uteroplacental arteries are independent of direct trophoblast invasion and are considered to involve maternal activation of local decidual artery rennin- angiotensin systems [23].

2. **Vascular remodeling induced by perivascularly located interstitial trophoblast:** Following trophoblast invasion - independent changes, the uteroplacental arteries within the implantation region are invaded by extravillous trophoblast cells. In a first step, extravillous trophoblast cells in juxtaposition against uteroplacental artery structures are
associated with further vascular remodeling. The physiological dilatations of uteroplacental arteries comprise reduction of media smooth muscle cells and deposition of fibrinoid material before infiltration of the media by trophoblast.

3. **Trophoblast infiltration of vessel walls:** The third stage of uteroplacental vascular remodeling is characterized by infiltration of the arterial wall by endovascular trophoblast. The uteroplacental arteries undergo further dilation up to several times the original diameter of the lumen. Trophoblast infiltration of the media smooth muscle coincides with loss of elastic fibres [24, 25].

Thus the physiological changes produce a significant reduction in peripheral vascular resistance in the placental bed allowing a much greater blood flow into and through the intervillous space of the placenta.

### 1.2.5 Abnormal Remodelling of Spiral Arteries in Preeclampsia

Preeclampsia is associated with a generalized impairment of trophoblast invasion that includes both reduced interstitial and reduced endovascular trophoblast invasion. Therefore the transformation of spiral arteries remains incomplete (Figure 1.8).

![Figure 1.8 Abnormal placentation in Preeclampsia](image-url)
The cytotrophoblast invasion of the spiral arteries is limited to the superficial deciduas, and the myometrial segments remains narrow [27]. The wall of the spiral arteries will be thick with the smooth muscle and elastic lamina without trophoblast invasion (Figure 1.9).

Figure 1.9 Thickened Tunica Media in Preeclamptic Artery – 40 X Van Geison – Preeclampsia group

As the luminal diameter of uterine spiral arteries remains narrow (Figure 1.10), the uteroplacental perfusion reduces and the placenta becomes ischemic as gestation progresses. This causes morphological and histological changes in the placenta.

Figure 1.10 Obliterated lumen of spiral artery in preeclampsia  40 X - Van Geison – Preeclampsia group
1.3 GROSS MORPHOLOGY OF PLACENTA

The placenta is a specialized extraembryonic tissue in which blood vessels of the foetus are brought into close intimacy with the maternal blood for the purpose of nutrition, respiration, excretion and other metabolic activities during intrauterine life of the fetus.

By the beginning of the fourth month, the placenta has two components: (1) A fetal portion, formed by the chorion frondosum and (2) A maternal portion, formed by the decidua basalis.

On the fetal side, the placenta is bordered by the chorionic plate; on its maternal side it is bordered by the decidua basalis, of which the decidual plate is most intimately incorporated into the placenta. Between the chorionic and decidual plates are the intervillous spaces, which are filled with maternal blood. They are derived from lacunae in the syncytiotrophoblast and are lined with syncytium of fetal origin. The villous trees grow into the intervillous blood lakes.

During the fourth and fifth months, a number of septa, from decidua project into intervillous spaces but do not reach the chorionic plate. As a result of this septum formation, the placenta is divided into a number of compartments, or cotyledons. Because the decidual septa do not reach the chorionic plate, contact between intervillous spaces in the various cotyledons is maintained.

As a result of the continuous growth of the fetus and expansion of the uterus, the placenta also enlarges. Its increase in surface area roughly parallels that of the expanding uterus, and throughout pregnancy, it covers approximately 15% to 30% of the internal surface of the uterus. The increase in thickness of the placenta results from arborization of existing villi and is not caused by further penetration into maternal tissues.

At full term, the placenta is discoid with a diameter of 15 to 25 cm, approximately 3 cm thick and weighs about 500 to 600 gms. At birth, it is torn from the uterine wall and, approximately 30 minutes after birth of the child, is expelled.
from the uterine cavity. When the placenta is viewed from the maternal side, 15 to 20 slightly bulging areas the cotyledons, covered by a thin layer of decidua basalis, are clearly recognizable. Grooves between the cotyledons are formed by decidual septa. The fetal surface of the placenta is covered entirely by the chorionic plate. Attachment of the umbilical cord is usually eccentric and occasionally even marginal (Figure 1.11). In general, the length of pregnancy for a full-term fetus is considered to be 280 days or 40 weeks after onset of the last menstruation or more accurately, 266 days or 38 weeks after fertilization.

Figure 1.11 Morphology of normal placenta showing fetal and maternal surfaces - control group

The fetal or birth weight of an infant is one of the most important determinant of its chances of survival, healthy growth and development. By international agreement low birth weight has been defined as a birth weight of less than 2.5kg (up to and including 2499grams) the measurement taken preferably within the first hour of life, before significant postnatal weight loss has occurred.

The status of the newborns is estimated by APGAR score in 1&5 min. A score of less than 7 is usually considered abnormal and indicate underlying placental pathology, such as chronic uteroplacental vascular disease.
1.4 STRUCTURE OF CHORIONIC VILLI

The chorionic villi are the functional unit of placenta which provides oxygen and nourishment to the fetus. The histological appearance of chorionic villi varies with the gestational age and with the stage of development and maturation of villous tree. It is made up of trophoblast layer that surrounds the innermost embryonic mesodermal connective tissue with blood vessels. Each chorionic villi are separated by intervillous space in which maternal blood circulate (Figure 1.12).

![Microanatomy of placenta showing chorionic villi – 10 X PAS – Control group](image)

- The chorionic villi are covered by syncytiotrophoblast, an epithelial surface layer that separates the villous interior from the maternal blood, which flows around the villi. Unlike other epithelial surfaces, the syncytiotrophoblast is not composed of individual cells but represents a continuous, uninterrupted, multinucleated surface layer without separating cell borders.
- Beneath the syncytiotrophoblast is a layer of single or aggregated cells, cytotrophoblast, which are the stem cells of the syncytiotrophoblast; they support the growth and regeneration of the syncytiotrophoblast.
- The trophoblastic basement membrane separates syncytiotrophoblast from the stromal core of the villi.
- The stroma is composed of varying numbers and types of connective tissue cells, connective tissue fibres and ground substance.
- Additionally, the stroma contains fetal vessels of various kinds and calibers. In the larger stem villi, the vessels are mainly arteries and veins; in the peripheral branches, most fetal vessels are capillaries or sinusoids.

### 1.4.1 Classification of Chorionic Villi

The ramification of the villous trees can be subdivided into segments that differ mainly in caliber, stromal structure, vessel structure and position within the villous tree. Five villous types have been described, they are:

1. **Stem villi** are characterized by a condensed fibrous stroma, arteries and veins, or arterioles and venules with a media or adventitia (Figure 1.13). The villous tree is formed by stem villi. The stem villi branches are represented by main stem villi, also called villous trunk. It represents the basal area of the villous tree, which ensures the connection to the chorionic plate. These villi generate four generations of branches, from first order to fourth order branches. These four generations are short, thick, detached from the trunk and situated in the proximity of the chorionic plate. Fourth order branches result in 2 to 30 (generally 10) unequal thinner dichotomizations, which are situated at the periphery of the villous tree. Part of these peripheral branches is divided and forms the so-called anchoring villi, which are connected to the basal deciduas. The villous trunk (trunchi chorii), the thick (rami chorii) and thin branches (ramuli chorii) and the anchoring villi are considered stem villi, which differ in caliber and position. In the mature placenta, they represent 20-25% of the volume of villi. These villi have...
fibrous stroma. Large stem villi contain an artery and a vein, which are centrally located [28].

Figure 1.13  Stem villi – 40 X H&E – Control group

2. Immature intermediate villi are bulbous, peripheral, immature continuations of stem villi with Hofbauer cells in the stroma. Normally this type persists in small groups within the centres of the villous trees (placentones) and represents the immature forerunners of stem villi. This type of villi prevails in immature placentas (Figure 1.14). Immature intermediate villi appear in the first and second trimesters of pregnancy. These are villi with an increased caliber. The central axis is represented by a reticular stroma, in which blood vessels and intercommunicating stromal channels occur. These channels allow the circulation of hofbauer cells, fetal macrophages. These cells are involved in the remodeling of the stromal connective tissue, as well as in the morphogenesis and angiogenesis of the villi. The immature intermediate villi ensure the rapid and effective growth of the villous tree. The first immature intermediate villi appear during gestational week 8. Between gestational weeks 14 and 20, these are the most
numerous. The transformation of these immature intermediate villi into stem villi is initiated during the first trimester of pregnancy. The process will continue until term. As a result, the number of intermediate villi decreases as pregnancy advances. Sometimes, they completely disappear near term, other times they persist as small areas in the center of the villous tree, serving as growth areas. The transformation of these immature intermediate villi into stem villi is progressive. Initially, stromal fibrosis occurs in the proximity of the vascular wall. An increase in the number of collagen fibres takes place, which will compress stromal channels, causing them to disappear. Immature intermediate villi represent the site of fetal-maternal exchange during the first two trimesters of pregnancy, when stem villi are not yet completely formed [29].

![Figure 1.14 Immature intermediate villi showing Hofbauer cell (H)-arrow denotes the cell – 40 X Immunostain - CD 68 - Control group](image)

3. Mature intermediate villi are long, slender, peripheral ramifications characterized by the absence of vessels with a light-microscopically identifiable media and adventitia (Figure 1.15). The transformation of
mesenchymal villi into immature intermediate villi is replaced by the formation of mature intermediate villi. This aspect is determined by angiogenesis, which starting with this moment no longer forms branches, the vessels only increasing in length. Mature intermediate villi are continued with terminal villi. It can be said that mature intermediate villi represent an intermediate development stage between mesenchymal and mature villi. Mature intermediate villi are long, thin, with peripheral branches. They have a zigzag trajectory. They generate terminal villi, representing a matrix for these structures. At the level of mature intermediate villi there are arterioles, collecting venules and numerous capillaries. These are structures that actively participate in fetal-maternal exchange, due to the increased degree of vascularization [30].

Figure 1.15  Mature intermediate villi – 40X H&E - Control group

4. Terminal villi are the final, grape-like ramifications of the mature intermediate villi, characterized by their high degree of capillarization and the presence of highly dilated sinusoids. They represent the main
sites of fetomaternal exchange (Figure 1.16). Terminal villi represent the final ramifications of the villous tree, in the third trimester of pregnancy. They have an alveolar appearance, being compared to a grape grain. They are connected to the mature intermediate villi through a narrowed portion. During the third trimester of pregnancy, the villous maturation phenomena prevail over the villous tree growth processes. The formation of terminal villi is closely related to the increase in length of the capillaries, to the formation of capillary loops that will press the trophoblast and will form alveolar prominences in the intervillous space. Terminal villi are formed by the trophoblast and the connective axis in which there are peripheral capillaries with sinusoidal dilatations.

The fusion of the basal capillary membrane with the trophoblast membrane results in the formation of a metabolic membrane, at the level of which fetal-maternal exchange takes place. This membrane is also called the vasculosyncytial membrane of terminal villi. At the level of terminal villi, the degree of vascularization is increased, and
5. Mesenchymal villi are the most primitive, they prevail during the first stage of pregnancy, where they are the forerunners of immature intermediate villi. During later stages of the surfaces of immature intermediate villi or at the tips of mature intermediate villi at this stage they also act as zones of villous proliferation and further branching. The mesenchymal villi are formed by an axis represented by the mesenchyma with vascular structures in the connective axis, covered by the trophoblast, with the two layers, the cytotrophoblast and syncytiotrophoblast. In the mesenchymal villi, the capillaries are poorly developed and do not have sinusoidal dilations. Between gestational weeks 5 and 7, these are the only vascularized villi. During the first gestational weeks, mesenchymal villi represent the proliferation area with the formation of villous branches. The differentiation of the other types of villi starts from mesenchymal villi. During gestational weeks 7-8, the mesenchymal villi become immature intermediate villi. The transformation of mesenchymal villi into immature intermediate villi continues until the end of the second trimester of pregnancy. As pregnancy advances, these villi are reduced, being replaced by the other types of villi. Small islets in the centre of the villous tree may persist, which represent areas that will ensure the proliferation of new villous branches [32].

1.4.2 Villous Histopathology

1.4.2.1 Syncytial knots

Syncytiotrophoblast covers the outer surface of the chorionic villi (Figure 1.17). It is a multinucleated layer with indistinct cell margins. The syncytiotrophoblast produces progesterone, oestrogen, HCG and other placental hormones. On the surface of the syncytiotrophoblast layer, the aged and late apoptotic syncytiotrophoblast nuclei are packed into apical protrusions called
syncytial knots. These syncytial knots are released into the maternal circulation and are transported to the lungs through maternal venous system. In the lungs, the syncytial knots are engulfed by lung macrophages. Increased numbers of syncytial knots are called Tenny Parker changes (Figure 1.18). In a placental section, when group of small terminal villi with increased syncytial knots were seen, then preeclampsia is a prominent possibility [33].

![Figure 1.17 Thickness of trophoblast – Normal – 40X PAS – Control group](image1)

![Figure 1.18 Increased number of syncytial knot in preeclamptic placenta – 40X Immuno stain- sFLT-1 – Preeclampsia group](image2)
1.4.2.2  Cytotrophoblastic proliferation

Cytotrophoblast are round cells with basophilic cytoplasm arranged in a row between the syncytiurn and the trophoblast basal membrane. The cytotrophoblast layer is initially continuous, but then it becomes discontinuous with areas in which the syncytiotrophoblast comes into direct contact with the basal membrane. Starting with months 3-4 of pregnancy, the cytotrophoblast is reduced and finally disappears towards the end of pregnancy. Disappearance of cytotrophoblast cells progresses from the smaller to larger villi, and although some always persist in large villi, they do not participate in the exchange between the two circulations.

Langhan’s cells are present at about 20% of the villous surface at term, only one clearly identifiable langhan’s cell per cross section of any peripheral villus. Increased numbers of langhans cells (in routine paraffin histology: more than two visible per peripheral villous cross section) are found in preeclampsia (Figure 1.19). It is said that their number increases with the severity and duration of preeclampsia [34].

Figure 1.19  Cytotrophoblastic Proliferation – 40 X – PAS – Preeclampsia group
1.4.2.3 **Paucity of Vasculosyncytial Membrane**

Vasculosyncytial membranes are the sinusoidal dilatation of the terminal villous capillaries, which bulge against the trophoblastic surfaces and attenuate them to thin lamellae (Figure 1.20). The placental exchange takes place in the villi at the vasculosyncytial membrane where the fetal vessels are in intimate contact with the covering syncytial membrane.

![Vasculosyncytial Membrane](image)

**Figure 1.20** Vasculosyncytial Membrane – Normal – 40 X PAS – Control group

1.4.2.4 **Trophoblastic basement membrane thickening**

The trophoblastic basement membrane is the layer which separates the trophoblastic mantle from mesenchymal core.

In routine paraffin sections of normal villi, the trophoblastic basement membrane is seen in special stains like periodic acid – Schiff (PAS). The constituents of the basal lamina are produced by the villous trophoblast.

Marked thickening of the basement membrane becomes clearly visible in various pathologic conditions, such as preeclampsia and IUGR (Figure 1.21). The
increased thickness indicates an altered trophoblastic activity which may be increased secretion or decreased turnover of basal lamina molecules [35].

1.4.2.5 Fibrinoid necrosis

The fibrinoid necrosis also called as intravillous fibrinoid. It is a fibrinoid patch that replaces villous stroma and vasculature underneath an intact trophoblastic cover (Figure 1.22).

Figure 1.21 Trophoblast basement membrane thickening – 40 X PAS – Preeclampsia group

Figure 1.22 Fibrinoid necrosis in higher magnification – 40 X PAS – Preeclampsia group
1.4.2.6 Stromal fibrosis

Fibrosis of stem villi is a good indicator of placental maturity (Figure 1.23). Fibrosis starts on about the 15th week postmenstruation, begins around the stem vessels and should be complete a few weeks before term. When reticular, unfibrosed connective tissue persists under the trophoblastic membrane, it signifies immaturity.

![Stromal fibrosis in higher magnification - 40 X Van Geison – Preeclampsia group](image)

1.4.2.7 Stem vessels

The composition of arterial and venous walls of the stem villi largely corresponds to that of the vessels of the chorionic plate and of the umbilical cord. In both vessel types of stem villi, the elastic membranes are largely absent. The muscular coats are thinner and the muscle cells are more dispersed than in corresponding arteries and veins of other organs.

In preeclampsia, the fetal stem arteries shows three lesions namely, obliterative endarteritis, fibromuscular sclerosis and thrombosis.
i) Obliterative endarteritis is a severe proliferating endothelium results in an occlusion of the lumen of the artery (Figure 1.24).

Figure 1.24  Obliterative endarteritis – 40 X Van Geison – Preeclampsia group

ii) Fibromuscular sclerosis is a marked thickening of the intimal and medial layers of the stem vessels with luminal obliteration (Figures 1.25 and 1.26).

iii) Thrombosis of fetal stem vessels is the presence of an organized occluding or partly occluding thrombus adhering to the endothelium of the fetal vessels.

Figure 1.25  Hyperplastic arteriolosclerosis- onion skin, concentric laminated thickening of the wall - 40 X - Van Geison – Preeclampsia group
1.4.2.8 Chorangiosis

Chorangiosis refers to a numerical increase of capillaries within the peripheral placental villi. In normal placenta, chorionic villi rarely contain more than 5 vascular channels. The diagnostic criteria for chorangiosis was described by Altshuler in 1984, as the presence of a minimum of 10 terminal villi, containing more than 10 capillaries per villus in 10 medium power fields in at least 3 or more random, non infarcted placental areas when using a × 10 ocular. The severity of chorangiosis can be assessed by determining the number of vessels within each villus and the placental area throughout which the vasculature is seen [36].

Chorangiosis occurs in women who have pregnancies at very high altitude and in preeclampsia. It is an adaptation to chronic oxygen deficiency (Figure 1.27).
1.4.2.9 Hofbauer cells

Hofbauer cells are fetal macrophages residing in the stroma of the chorionic villi of human placenta (Figure 1.28). It was first described by Hofbauer in 1903. These cells are large round or spherical shaped cells with eccentric nuclei. Their length vary from 10-30µm in diameter. The hofbauer cells are of fetal origin, and can be detected as early as day 10 post conception in the human chorionic villous stroma until term [37]. They are large granulated cells, categorized as placental M2/ alternatively activated macrophages. The hofbauer cells have features in common with macrophages, such as cytoplasmic processes, large vacuoles, many pinocytotic vesicles and intracytoplasmic granules, which enables micropinocytotic activity and phagocytosis. These macrophages are more likely involved in preventing the transmission of pathogens from the mother to the foetus [38, 39].

Figure 1.27 Chorangiosis - 40X H&E - Preeclampsia group
The Hofbauer cells are found close to trophoblast cells, near fetal capillaries or between both. Their population remains constant throughout gestation, occupying around 40% of villous stromal cells. Hofbauer cells originate from mesenchymal cells during the early stages of pregnancy, before the fetal circulation is established. Later, once fetal circulation is established, the Hofbauer cells may additionally originate from fetal bone marrow-derived monocytes, as macrophages in other organs do. They play a major role in the process of vasculogenesis and angiogenesis by expressing vascular endothelial growth factor (VEGF) [40], villous tree development and promotion of the placental mesenchyme.

1.5 IMMUNOHISTOCHEMICAL STUDY

1.5.1 Soluble fms-like Tyrosine Kinase-1 (sFLT-1)

Soluble fms-like tyrosine kinase also referred to as sVEGFR-1, an antiangiogenic protein. It is a soluble form of the VEGF/PLGF receptor FLT-1 produced by alternative splicing. sFLT-1 was initially identified as a product of cultured human endothelial cells and subsequently shown to be produced by the placenta and released into the maternal circulation.
fms–like tyrosine kinase (FLT-1) is a receptor for VEGF. It is composed of seven extracellular immunoglobulin homology domain, a single transmembrane region and an intracellular tyrosine kinase sequence that is interrupted by a kinase-insert domain. The FLT-1 gene is localized to chromosome 13q12-q13 [41]. FLT-1 binds VEGF-A, VEGF-B and PLGF with high affinity and is expressed in many human tissues including monocytes/macrophages and placental trophoblasts, and its expression is up regulated by hypoxia.

Alternative splicing of the pre-mRNA which encodes FLT-1 results in the production if sFLT-1 comprising the ligand-binding domain of FLT-1 but lacking the membrane spanning and intracellular domains. Soluble FLT-1 is secreted by endothelial cells, monocytes and the placenta. Soluble FLT-1 acts as a potent antagonist of VEGF-A and PLGF, by inhibiting their binding to cell surface receptors as well as by forming heterodimers with kinase insert domain receptor (KDR), and is considered as an anti-angiogenic factor [42].

1.5.2 Vascular Endothelial Growth Factor (VEGF)

Vascular endothelial growth factor (VEGF) is a dimeric glycoprotein involved in vasculogenesis (the process by which new blood vessels are formed in embryonic life) and angiogenesis (the process by which blood vessels branch to form new blood vessels). It is a proangiogenic factor that promotes the proliferation and survival of endothelial cells and induces vascular permeability [43]. VEGF binds to its receptors FLT-1(VEGFR-1) and KDR (VEGFR-2) to promote the vascular endothelial function. It also has a direct vasodilatory effect on the systemic vasculature by inducing nitric oxide.

The human VEGF gene has been assigned to chromosome 6p12 - p21.1 and is organized as eight exons separated by seven introns [44]. Ferrara et al first reported VEGF in 1989. They demonstrated that medium exposed to bovine pituitary cells was capable of initiating a mitogenic effect that was specific to endothelial cells. When it was first described VEGF has been thought to act as a vascular permeability factor (VPF) [45].
So far, four different VEGF subtypes have been described, VEGF-A to VEGF-D. The first identified VEGF-A is a disulfide-linked homodimeric glycoprotein that plays an important role in the vasculogenesis, angiogenesis, vascular permeability and vasodilatation. VEGF-A is a heparin binding growth factor known to bind directly to its surface receptors (fms-like tyrosine kinase-1 (FLT-1) and (KDR) Kinase insert domain receptor) which was expressed on vascular endothelial cells. VEGF-B has structural similarities to VEGF–A and PLGF, and VEGF-B gene is localized to chromosome 11q13. It is expressed as two isoforms VEGF-B_{167} and VEGF-B_{186} and is abundant in heart and skeletal muscle. The VEGF-C and VEGF-D has been recognized as a lymphangiogenic growth factor and their gene has been localized to chromosome 4q34 and Xp22.31 respectively.

Vascular endothelial growth factor is a disulfide-linked homodimeric protein of between 24 and 46 kDa with six differentially spliced forms. They are VEGF-A_{121}, VEGF-A_{145}, VEGF-A_{165}, VEGF-A_{183}, VEGF-A_{189} and VEGF-A_{206} having 121, 145, 165, 183, 189 and 206 amino acids respectively. VEGF-A_{165} is the predominant isoform and native VEGF-A closely resembles VEGF-A_{165}.

The key molecules of VEGF family, namely VEGF-A and PLGF (placental growth factor homologous to VEGF based on amino acid and cDNA sequences but is a very weak stimulator) and the receptors VEGFR-1 and VEGFR-2 are expressed in the human placenta throughout gestation, and it regulates placental angiogenesis and maternal spiral artery remodeling [46].

1.5.3 Endothelial Nitric Oxide (eNOS)

The nitric oxide (NO) is a gas that is generated in the endothelium through the metabolic conversion of L-arginine to L-citrulline by an enzyme nitric oxide synthase (NOS). There are three distinct isoforms of NOS, two of which are constitutive calcium/calmodulin dependent (the endothelial and neuronal types) and the third is inducible and is not dependent upon calcium / calmodulin for its enzymatic action. Among these synthases, only the endothelial type of NOS is
transcribed in the human placenta under normal conditions. eNOS also known as nitric oxide synthase 3 (NOS3) or constitutive NOS (cNOS), is an enzyme that in humans is encoded by the NOS3 gene. Nitric Oxide generated by eNOS has been demonstrated to contribute to the regulation of vascular tone as a vasorelaxing factor. Loss of eNOS results in vascular abnormalities, including vasoconstriction, smooth muscle proliferation, activation of blood elements, and an increased extracellular matrix synthesis [47].

1.5.4 Role of sFLT-1/sVEGFR-1 in Preeclampsia

In preeclampsia, the soluble fms-like tyrosine kinase receptor mRNA is generated by alternative splicing of the same pre-mRNA. This recombinant soluble human receptor binds the vascular endothelial cell growth factor with high affinity and inhibits its mitogenic activity for vascular endothelial cells. It acts as an efficient specific antagonist of vascular endothelial cell growth factor [48].

sFLT-1 is a soluble form of the vascular endothelial growth factor receptor that lacks the cytoplasmic tail and transmembrane domain but retains the extracellular ligand-binding domain (sFLT-1 is also named sVEGFR1). sFLT-1 prevents circulating vascular endothelial growth factor and placental growth factor interactions with their proangiogenic receptors and functions as an antiangiogenic factor (Figures 1.29, 1.30). The level of sFLT-1 in the plasma of women with preeclampsia is elevated in comparison with that in women with uncomplicated pregnancies, a feature consistent with microarray data showing that sFLT-1 mRNA levels are elevated in placentas from women with preeclampsia compared with placentas of normotensive pregnant women.

In preeclampsia, sFLT-1 levels begin to rise at least 5 weeks before the onset of clinical disease and remain elevated compared with unaffected women [49].
In view of the critical contributions of sFLT1 to disease pathogenesis, it is important to identify factors/cells responsible for increased synthesis and secretion of sFLT-1 by the placentas of women with preeclampsia.

Figure 1.29 Normal action of vascular endothelial growth factor [50]

Figure 1.30 The action of sFLT-1 cause endothelial dysfunction by antagonizing vascular endothelial growth factor [50]
1.5.5 **Aim of the Study**

- To study the alterations in the placental morphology of preeclampsia compared to normal.
- To observe histomorphometrical changes in preeclamptic placenta compared to normal.
- To compare the histopathological changes in the placenta of preeclampsia with that of normal.
- To determine the effect of preeclampsia on the vasculature of placenta compared to control.
- To study the potent activity of hofbauer cells in the pathogenesis of preeclampsia.
- To delineate the immunolocalization of angiogenic and anti-angiogenic proteins in the placental cells of preeclampsia.
- To identify the placental cells that produce sFLT-1 in preeclampsia.