IMMUNOHISTOCHEMICAL EXPRESSION OF CD 24 IN CYTOTROPHOBLAST, SYNCYTIOTROPHOBLAST, BLOOD VESSELS, VILLOUS STROMA IN PLACENTA OF PRE-ECLAMPSIA

BY
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DISSERTATION SUBMITTED TO
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DOCTOR IN MEDICINE
IN
PATHOLOGY

UNDER THE GUIDANCE OF
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IMMUNOHISTOCHEMICAL EXPRESSION OF CD 24 IN CYTOTROPHOBLAST, SYNCYTIOTROPHOBLAST, BLOOD VESSELS, VILLOUS STROMA IN PLACENTA OF PRE ECLAMPSIA INTRODUCTION Pre eclampsia is a multifactorial hypertensive disorder of pregnancy and a cause of preventable maternal and fetal mortality and morbidity1. Pre eclampsia is diagnosed by sudden onset hypertension (>20weeks of gestation) and atleast one associated complication including proteinuria, maternal organ dysfunction or uteroplacental dysfunction like fetal growth restriction.1 According to WHO incidence of pre eclampsia ranges from 2% and 10% of pregnancies worldwide.2 Pre eclampsia is a multifactorial disease involving various etiological factors that is genetic factor, environmental factors, immunological factors and vascular factors. While the exact pathogenesis isn't fully understood,it is seen that pre eclampsia is linked to immune tolerance during pregnancy .Its believed that abnormal immune responses, particularily related to maternal –fetal interface play a role in development of pre eclmapsia.3 CD24 is a glycosylated protein with a small protein core linked to plasma membrane through glycosyl-phosphatidylinositol anchor. CD 24 is primarly expressed in immune cells such as hematopoietic stem cells, B and T lymphocytes, epithelial cells and neural cells.4 High expression of CD 24 can be detected by immunohistochemistry and is found to be increasingly expressed in epithelial ovarian(83%), breast (85%), non-small cell lung(45%), prostate (48%) and pancreatic cancer (72%).5 CD24 shows expression on villous tissues and circulating immune cells. Normally in normal pregnancy CD24 blocks macrophages and supports the growth of the placenta by inhibiting action of immune tolerance . Therefore, CD24 reduction in early Pre eclampsia may be linked to increased rejection of the placenta, its reduced expression may be associated with early cases of Pre eclampsia.6 However its expression in placenta as a marker of immunotolerance in preeclampsia is not well researched. OBJECTIVES To determine the immunohistochemistry expression of CD 24 in placenta of pre eclampsia To compare the Expression of CD24 in normal placenta and pre eclampsia placenta REVIEW OF LITERATURE HISTORICAL ASPECTS7 The placenta, unique to mammalian pregnancy, is distinct because it forms through the interaction of fetal and maternal tissues. Its structure varies greatly among species. Throughout history, the placenta has been revered across cultures, symbolizing health, fortune, and protection, often seen as a mystical entity and even as a talisman against danger. Matteo Renaldo Colombo , a pupil of Vesalius and his successor as professor at the University of Padua, first used the term placenta. Pre-Eighteenth Century In ancient Egypt,

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LIST OF ABBREVIATIONS

PE - Pre-eclampsia

IHC - Immunohistochemistry

CD - Cluster of Differentiation

Syn - Synctiotrophoblast

Cyt - Extraembryonic mesoderm

FC - Fetal capillaries

EVT - Extravillous trophoblast cells

VC - Villous Chorangiosis

H&E - Haematoxylin and Eosin

BP - Blood Pressure

HDP - Hypertensive disorders of Pregnancy

WHO - World Health Organisation

ACOG - American College of Obstetricians and Gynaecologists

VEGF - Vascular endothelial growth factors

PIGF - Placental growth factors

BMI - Body Mass Index

qRT - PCR - Quantitative Real-Time Polymerase Chain Reaction

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ABSTRACT

INTRODUCTION

Pre eclampsia is a multifactorial hypertensive disorder of pregnancy and a cause of preventable maternal and fetal mortality and morbidity. Pre eclampsia is diagnosed by sudden onset hypertension (>20weeks of gestation) and atleast one associated complication including proteinuria, maternal organ dysfunction or uteroplacental dysfunction like fetal growth restriction. CD24 shows expression on villous tissues and circulating immune cells. Normally in normal pregnancy CD24 blocks macrophages and supports the growth of the placenta by inhibiting action of immune tolerance. Therefore, CD24 reduction in early Pre eclampsia may be linked to increased rejection of the placenta, its reduced expression may be associated with early cases of Pre eclampsia.

AIM AND OBJECTIVE

1. To determine the immune histochemistry expression of CD 24 in placenta of pre clampsia.
2. To compare the Expression of CD24 in normal placenta and pre eclampsia placenta

MATERIALS AND METHODS

This study was performed in Department of Pathology in association with Department of Obstetrics & Gynaecology, Sri Devaraj Urs Medical College attached to RLJH and Research Center, Tamaka, Kolar during the period of September 2022 to December 2023. The study included a total of 154 placenta cases that included 77 pre-eclampsia placentas and 77 normal healthy controls. IHC was performed using antibodies against CD 24 and expression of CD 24 n was analysed and interpreted. CD 24 was compared among the cases and the controls and was also correlated with clinicopathological parameters. Statistical analysis was performed

RESULTS

Low expression of C D 24 was seen in majority of pre clampsia cases and control group in syncytiotrophoblast ,cytotrophblast ,villous stroma and blood vessels

CONCLUSION

Pre eclampsia is evolving as an important pregnancy complication as it is multifactorial in nature and linked to many factors like immunotolerance. CD 24 marker of immunotolerance is low in syncytiotrophoblast, cytotrophoblast, blood vessels and villous stroma of both pre eclampsia and normal placenta. More studies using CD24 -Siglec-10 markers is needed to confirm the same.

IMMUNOHISTOCHEMICAL EXPRESSION OF CD 24 IN CYTOTROPHOBLAST, SYNCYTIOTROPHOBLAST, BLOOD VESSELS, VILLOUS STROMA IN PLACENTA OF PRE ECLAMPSIA

INTRODUCTION

Pre eclampsia is a multifactorial hypertensive disorder of pregnancy and a cause of preventable maternal and fetal mortality and morbidity[1]. Pre eclampsia is diagnosed by sudden onset hypertension (>20weeks of gestation) and atleast one associated complication including proteinuria, maternal organ dysfunction or uteroplacental dysfunction like fetal growth restriction.[1] According to WHO incidence of pre eclampsia ranges from 2% and 10% of pregnancies worldwide.[2]

Pre eclampsia is a multifactorial disease involving various etiological factors that is genetic factor, environmental factors, immunological factors and vascular factors. While the exact pathogenesis isn't fully understood,it is seen that pre eclampsia is linked to immune tolerance during pregnancy .Its believed that abnormal immune responses, particularily related to maternal –fetal interface play a role in development of pre eclmapsia.[3]

CD24 is a glycosylated protein with a small protein core linked to plasma membrane through glycosyl-phosphatidylinositol anchor. CD 24 is primarly expressed in immune cells such as hematopoietic stem cells, B and T lymphocytes, epithelial cells and neural cells.[4]

High expression of CD 24 can be detected by immunohistochemistry and is found to be increasingly expressed in epithelial ovarian(83%), breast (85%), non-small cell lung(45%), prostate (48%) and pancreatic cancer (72%).[5]

CD24 shows expression on villous tissues and circulating immune cells. Normally in normal pregnancy CD24 blocks macrophages and supports the growth of the placenta by inhibiting action of immune tolerance . Therefore, CD24 reduction in early Pre eclampsia may be linked to increased rejection of the placenta, its reduced expression may be associated with early cases of Pre eclampsia.[6]

However its expression in placenta as a marker of immunotolerance in preeclampsia is not well researched.

OBJECTIVES

To determine the immunohistochemistry expression of CD 24 in placenta of pre eclampsia

To compare the Expression of CD24 in normal placenta and pre eclampsia placenta

REVIEW OF LITERATURE

HISTORICAL ASPECTS[7]

The placenta, unique to mammalian pregnancy, is distinct because it forms through the interaction of fetal and maternal tissues. Its structure varies greatly among species. Throughout history, the placenta has been revered across cultures, symbolizing health, fortune, and protection, often seen as a mystical entity and even as a talisman against danger.

Matteo Renaldo Colombo , a pupil of Vesalius and his successor as professor at the University of Padua, first used the term placenta.

Pre-Eighteenth Century

In ancient Egypt, the placenta was thought to house the "External Soul." A sculpture found on an Egyptian ceremonial slate from Hierakonpolis portrays a Pharaoh in a ceremonial procession, preceded by five attendants. One of these attendants is depicted carrying a standard believed to symbolize the Royal placenta along with its umbilical cord, representing the Pharaoh's "soul" or "secret helper". In the folklore of numerous Pacific Islands, Australasia, and African cultures, the placenta is viewed as the infant's sibling, companion, or soul, often believed to possess supernatural properties.

The Greeks acknowledged the significance of the placenta, referring to it as the "flat cake" in fetal nutrition. They named the outermost embryonic membranes the chorion (meaning "membrane") and the innermost membrane surrounding the fetus the amnion (meaning "bowl"). It's believed that the Greek philosopher-biologist Aristotle might have been the first to use the term "chorion," and he also identified the yolk sac in lower vertebrates.

Galen, in the 2nd century AD, proposed that fetal and maternal blood vessels were interconnected through the placenta, providing vital and alimentary blood to the fetus.

In the Renaissance, anatomists like Leonardo da Vinci and Andreas Vesalius contributed detailed illustrations of the fetal anatomy and placental structure, with da Vinci correctly noting that fetal and maternal blood circulations were separate. Harvey’s observations about

fetal circulation were revolutionary, though he could not explain the role of respiration in utero. The discovery of oxygen and its role in respiration later clarified how fetuses could thrive without breathing air.

In the 17th century, John Mayow suggested that the placenta acted like a "uterine lung," providing essential gases to the fetus, a concept that foreshadowed later discoveries by scientists like Joseph Priestley and Antoine Lavoisier.

By the 18th century, the development of placental biology was increasingly based on observation and experimentation, leading to a clearer understanding of the fetus's nutritional and respiratory needs.

Eighteenth and early Nineteenth Centuries

In the early 18th century, it was widely believed that the maternal and fetal circulations were interconnected. However, in 1734, Alexander Monro primus, a professor at the University of Edinburgh, argued that they were separate after studying in pregnant women. He initially mistook a tissue-like substance between the uterus and placenta for the placenta itself but later recognized the placenta as a distinct structure with fetal vessels extending into the uterine tissue to connect with maternal blood vessels.

A few years later, Wilhelm Noortwyck incorrectly suggested that some uterine vessels were fetal and connected to maternal vessels. In his 1774 work *The Gravid Uterus,* anatomist William Hunter clarified the distinction between maternal and fetal circulations. By injecting colored wax into the uterine vessels, he demonstrated that there was no direct connection to the umbilical vessels. Hunter also observed that the absence of one umbilical artery could be a sign of fetal malformations

John Hunter, William's brother, acknowledged the discovery of separate circulations to a 1754 dissection by Colin Mackenzie, which confirmed the findings. Despite these discoveries, some, like Ebenezer Sibly in 1794, continued to believe in a vascular connection between maternal and fetal blood supplies, and debates on the subject persisted into the mid-1800s.

In the early nineteenth century, two theories about implantation and decidua emerged. Karl Friedrich Burdach's *Einstülpung* theory suggested the decidua covered the fallopian tube

openings, allowing the ovum to insinuate between its layers. In contrast, William Hunter and Gabriel Valentin's *Einssat* theory proposed that the blastocyst "pushed aside" the decidua to implant, similar to the modern histolytic theory where the decidua overgrows and embeds the ovum.

Latter Nineteenth and Early Twentieth Centuries.

In the mid-19th century, researchers were still working to understand the complexity of maternal-fetal blood exchange through the placenta. One major point was circulation of maternal blood in intervillous space. Some, like William Benjamin Carpenter, suggested that the fetal vascular tufts were suspended in a cavity surrounded by maternal tissue, while others, like Arthur Farre, described maternal blood vessels opening directly into the placenta. Although many acknowledged that maternal blood occupied this space, some doubtly about its free flow through an open cavity, as no similar structures were observed in other mammals. However, these findings were later confirmed in primates.

During this period, the function of the placenta was not fully understood. Sir William Turner challenged the prevailing belief that fetal nourishment occurred through diffusion across a placental barrier, suggesting that more complex processes were involved. At the same time, researchers such as Theodor Langhans and Ambrosius Hubrecht made significant advancements in understanding placental structure. They identified the syncytiotrophoblast and cytotrophoblast layers and highlighted the important role of the trophoblast in implantation and the development of the placenta.

In terms of placental anatomy, several classifications were proposed, including those by Thomas Huxley and Hans Strahl, which sought to categorize placental types based on their structure and the maternal-fetal interface. Otto Grosser's classification of placental types (e.g: epithelio-chorial, endothelio-chorial, hemochorial) became influential, though later studies, especially using electron microscopy, revealed the complexities and limitations of these classifications.

In the early 20th century, advancements in microscopy, including the use of electron microscopes, greatly enhanced our understanding of placental fine structure and led to a more detailed view of placental development and the mechanisms of maternal-fetal exchange. Despite these advances, understanding placental biology remained an evolving field.

Mid-Twentieth Century to the Present

Mid-20th century research on placental development and function was shaped by pioneering figures such as Harland Winfield Mossman, who in 1937 and later in 1987, explained placental morphology and its biological functions in mammals. Along with Mossman, researchers like Boyd and Hamilton studied human placental development, tracing its growth from implantation to pregnancy's end. Contributions from scientists like Flexner, Corner, and Reynolds also advanced understanding of placental nutrient exchange, blood flow patterns, and hormonal regulation.

In the latter part of the 20th century, further discoveries highlighted the complexity of placental function, such as the non uniform blood flow within the placenta and its role in respiratory gas exchange. Research also explored how hormonal interactions between the placenta, maternal body, and fetus are essential for fetal growth and pregnancy progression. Advances in techniques such as radioactive isotope tracing and studies of uterine and placental blood flow provided deeper insights of physiological processes. This combined approach is used to deeper insights into the placenta's role in fetal development and its influence on pregnancy outcomes, providing a more thorough understanding of this vital organ.

FUNCTIONS OF PLACENTA [8]

Placental metabolism: During early pregnancy, the placenta synthesizes glycogen, cholesterol and fatty acids providing nutrients and energy for the fetus. These metabolic functions support its roles in transport and hormone secretion.

Placental Transfer: The expansive surface area of the placental membrane facilitates bidirectional transport of substances between fetal and maternal circulation.

a. Transfer of Gases

Oxygen, carbon dioxide, and carbon monoxide diffuse across the placental membrane.

b. Nutritional Substances

Nutrients such as glucose, water, cholesterol, triglycerides, and phospholipids are transferred from the mother to the fetus.

c. Hormones

Hormones like thyroxine (T4) and triiodothyronine (T3) are transferred across placental slowly while some protein hormones like insulin and pituitary hormones have limited passage to the fetus.

d. Electrolytes

Electrolytes are freely exchanged across the placental membrane, each type at its own rate. Intravenous fluids with electrolytes administered to the mother also pass to the fetus through placenta.

e. Maternal Antibodies and Protein

The fetus produces limited antibodies due to its immature immune system. Maternal antibodies, particularly Immunoglobulin G (IgG) gamma globulins, are transferred to the fetus via the placenta through transcytosis, peaking around 26 weeks.

f. Drugs and Drug Metabolites

Maternal drug intake can directly or indirectly impact the fetus by interfering with maternal or placental metabolism. The amount of drug or metabolite reaching the placenta is regulated by maternal blood levels and placental blood flow

g. Infectious Agents

Viruses like cytomegalovirus, rubella, coxsackieviruses, and those linked with variola, varicella, measles, herpes, and poliomyelitis can breach the placental membrane, resulting in fetal infection.

DEVELOPMENT OF PLACENTA[9]

The placenta arises from the outer cell layer of blastocyst, the trophectoderm. The embryo develops from the inner cell mass. The extraembryonic mesenchyme and trophoblast make up the most of the placental tissues. Around the blastocyst, the trophoblast layer divides into two layers. Later trophoblast cells begin to small lacunae which fill the maternal blood. As lacunae enlarges, they connect to the mother fetus. The layer of cytotrophoblast multiples and forms primary villi in the surrounding tissue. These primary villi grow into secondary villi. The fetal blood arteries inside villi begin to create circulation and bond between the mother and fetus as the villi grow and form complex structure.

Pre lacunar Stage: Day 1–8 Post conception

Following fertilization, the zygote matures into a flattened structure consisting of 107–256 cells called a blastocyst. The trophoblast, the blastocyst's outer layer, will eventually give rise to the placenta. The inner cell mass, a little collection of bigger cells found inside, gives rise to the embryo, umbilical cord, and amnion.

Around days 6 or 7 after conception, the blastocyst adheres to the endometrium, the lining of the uterus, at the embryonic pole, marking the beginning of the implantation process. For procedures like in vitro fertilization, a certain period of time known as the implantation window is essential to successful implantation.

The trophoblast cells divide and create two layers while implantation goes on. While the outer layer of syncytiotrophoblast fuses together to form a continuous layer, the inner layer of cytotrophoblasts does not initially come into contact with maternal tissues. The trophoblastic shell is formed when this syncytiotrophoblast develops finger-like projections that infiltrate the endometrium.[9]

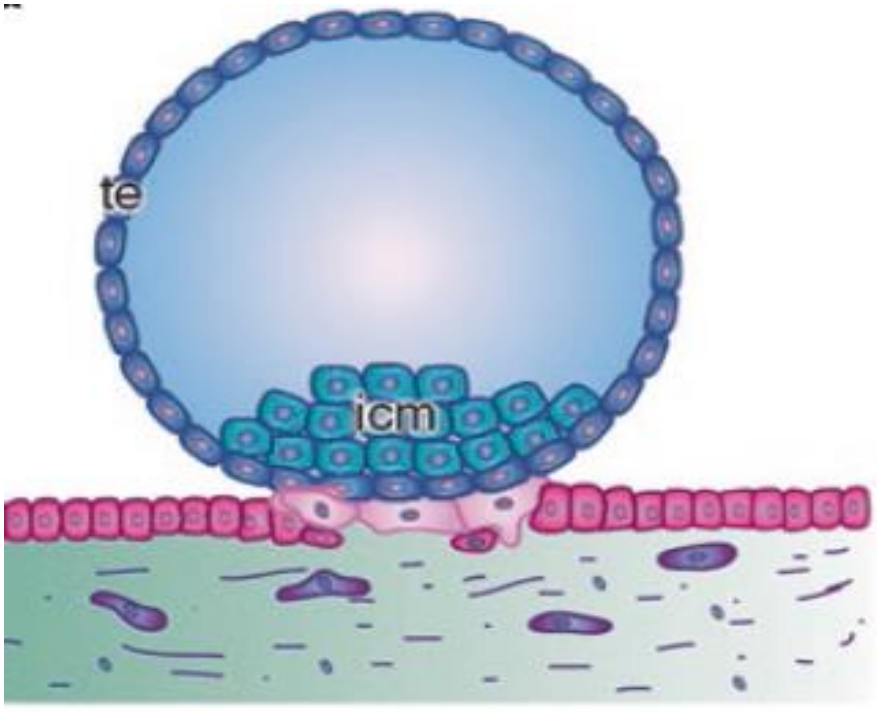


Figure 1: The polar trophectoderm overlying the inner cell mass first attaches to the uterine epithelium.[10]

Te –polar trophectoderm, icm-inner cell mass

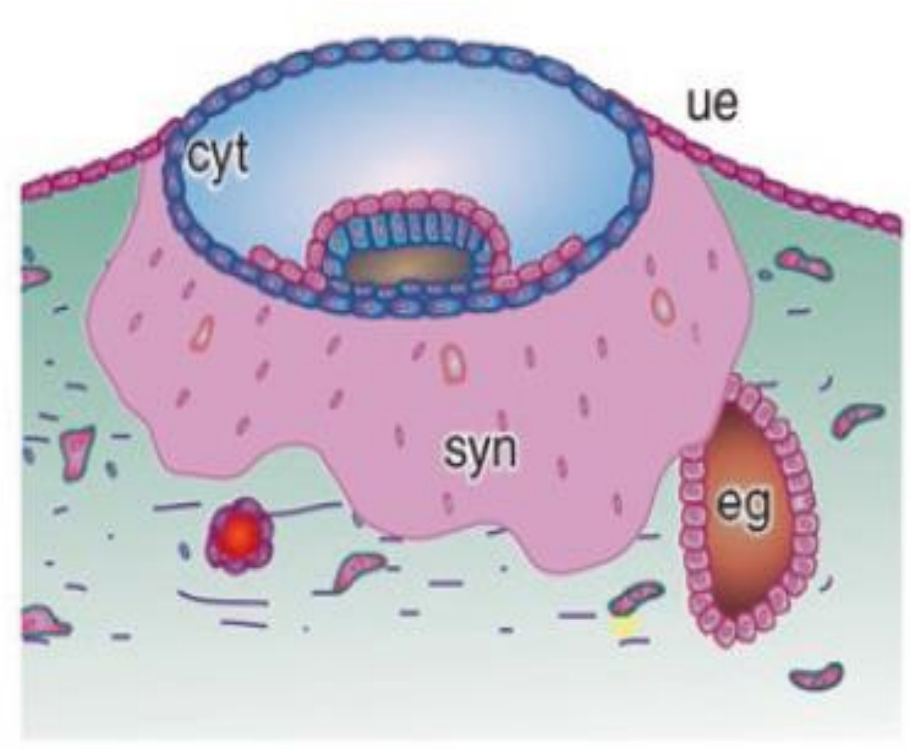


Figure 2: shows prelacunar stage in which the syncytial masses have fused to form the primary syncytiotrophoblast that invades into the maternal endometrium [10]
Syncytiotrophoblast (syn), Endometrial gland (eg), Cytotrophoblast (cyt).

Lacunar Stage: Day 8–13 Post conception

On day 8 after conception small vacuoles begin to form in the syncytiotrophoblast and they combine to form a network of spaces known as lacunae. Trabeculae, which are bands of syncytiotrophoblast, divide these lacunae. By day 12, the uterine lining covers the location and the blastocyst is entirely implanted.

After developing into trabeculae, the cytotrophoblast cells eventually reach the outer trophoblastic layer and come into touch with the endometrium on day 13.

The smooth chorion (chorion laeve) is formed by the thinner outer layer of the trophoblast, which thickens at the implantation site and creates the placental disk. Trophoblastic covering of the blastocyst consist of three layers.[9]

a. **Primary chorionic plate**, facing the blastocystic cavity
b. **Lacunar system** including the trabeculae
c. **Trophoblastic shell**, facing the endometrium

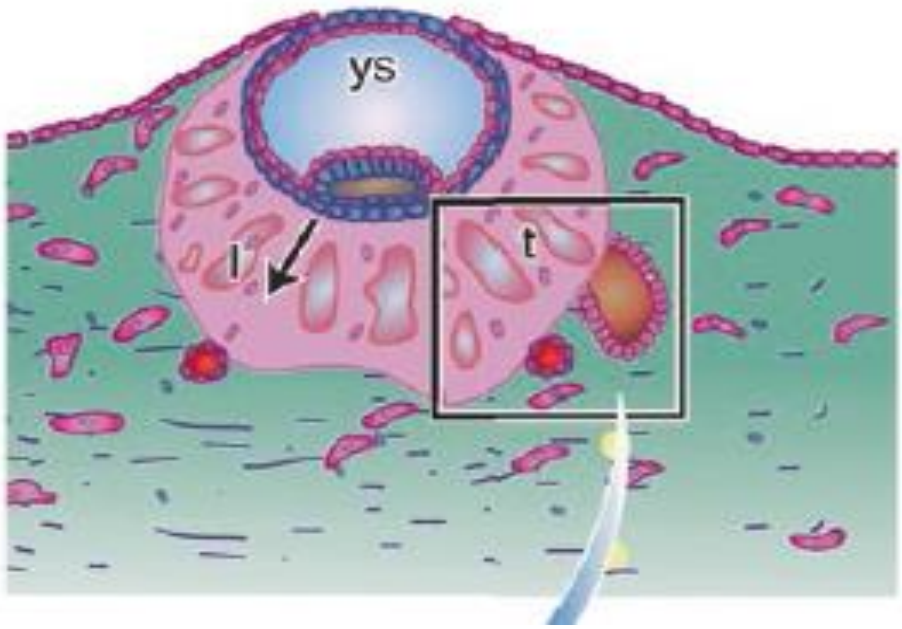


Figure 3: shows lacunar stage during which spaces, the lacunae, appear within the syncytiotrophoblast and divide it into a series of pillars or sheets referred to as trabeculae.[10]

Lacunae (l), Trabeculae (t), Primary yolk sac (ys)

Early Villous Stage: Day 13–28 Post conception[9]

In early villous stage, primary villi which are made up of an inner core of cytotrophoblast and an outside layer of syncytiotrophoblast, are formed when cytotrophoblast invades the trabeculae. The villous stage of placentation begins at this point.

Primitive villous trees are formed when the villi spread out. Anchoring villi are those that remain attached to the trophoblastic shell. The main villi are next invaded by mesenchymal cells, transforming them into secondary villi with an inner cytotrophoblast layer, an outer syncytiotrophoblast layer, and a connective tissue core..

The transition to tertiary villi occurs by days 18–20, when the first fetal capillaries show up in the villi. By the start of the fifth week, a full fetoplacental circulation is established when these capillaries network together.

Certain cytotrophoblast cells produce syncytial sprouts that resemble early primary villi as villous trees grow. Some of them form secondary villi after being invaded by mesenchyme, although many of these degenerate. Fetal vasculature can develop inside the villi as a result of this process.

Throughout this process, the placental barrier which consists of several layers such fetal endothelium and syncytiotrophoblast maintains the separation of fetal and maternal blood. The cytotrophoblast discontinues by the final trimester, but the fetal endothelium retains its own basal lamina.

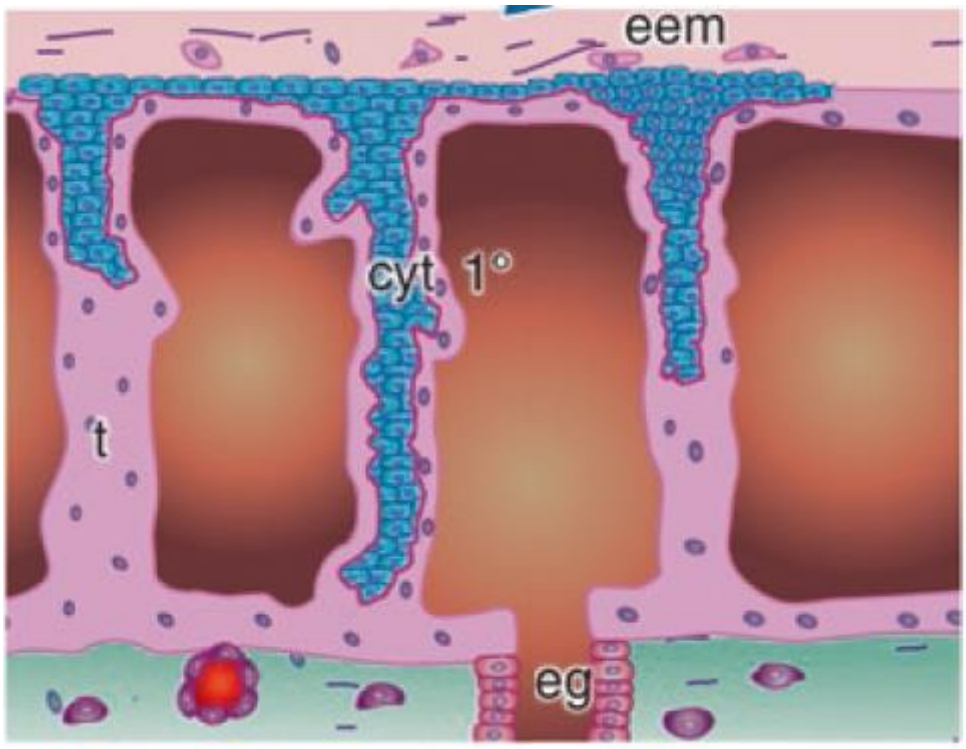


Figure 4: shows transition from lacunar to primary villous stage. Cytotrophoblast cells penetrate into the trabeculae, converting them into primary villi.[10]
Extraembryonic mesoderm (eem)

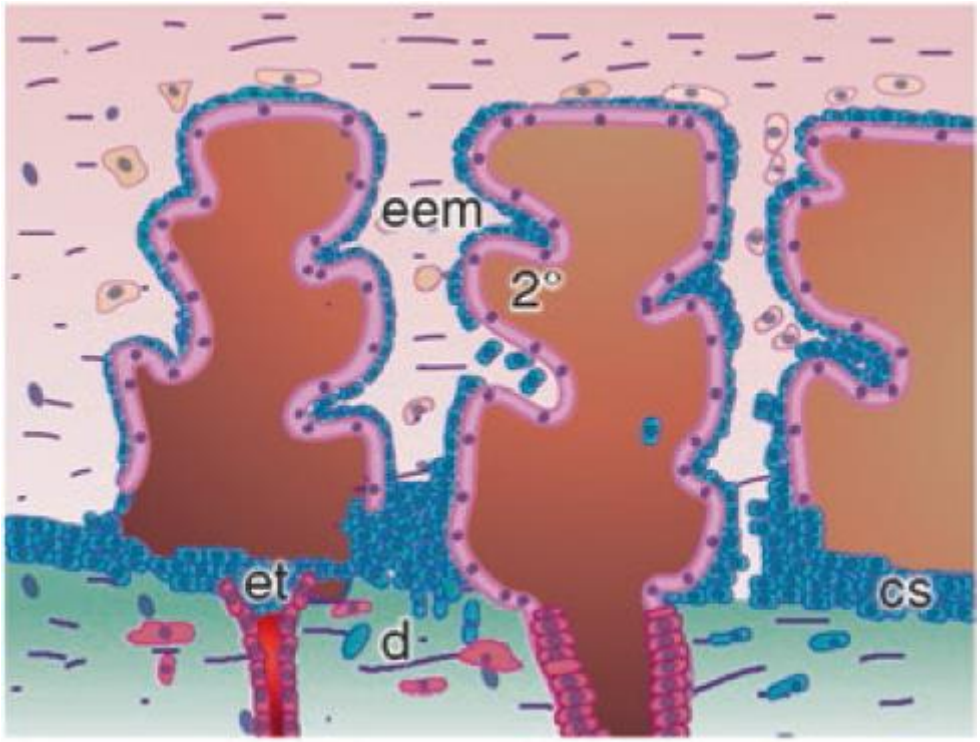


Figure 5: shows secondary villous stage in which extraembryonic mesoderm has penetrated into the cytotrophoblast core, converting primary villi into secondary villi.[10]
Cytotrophoblastic cell column (ccc), Cytotrophoblastic shell (cs), Decidua (d), Endovascular trophoblast cells (et)

Second Month and Beyond[9]

Starting in the second month after conception the chorionic plate's connective tissue thickens and fibrous tissue spreads into the villous stems. After then, tertiary villi differentiate to produce a variety of villi with distinct structures and purposes.

The cytotrophoblast decreases in frequency and the syncytiotrophoblast thins out as the placenta develops. While the number of fetal capillaries increases and approaches the villous surfaces, the average diameter of the villi shrinks. As a result, the placental barrier becomes more thinner, reducing the distance for maternofetal diffusion.

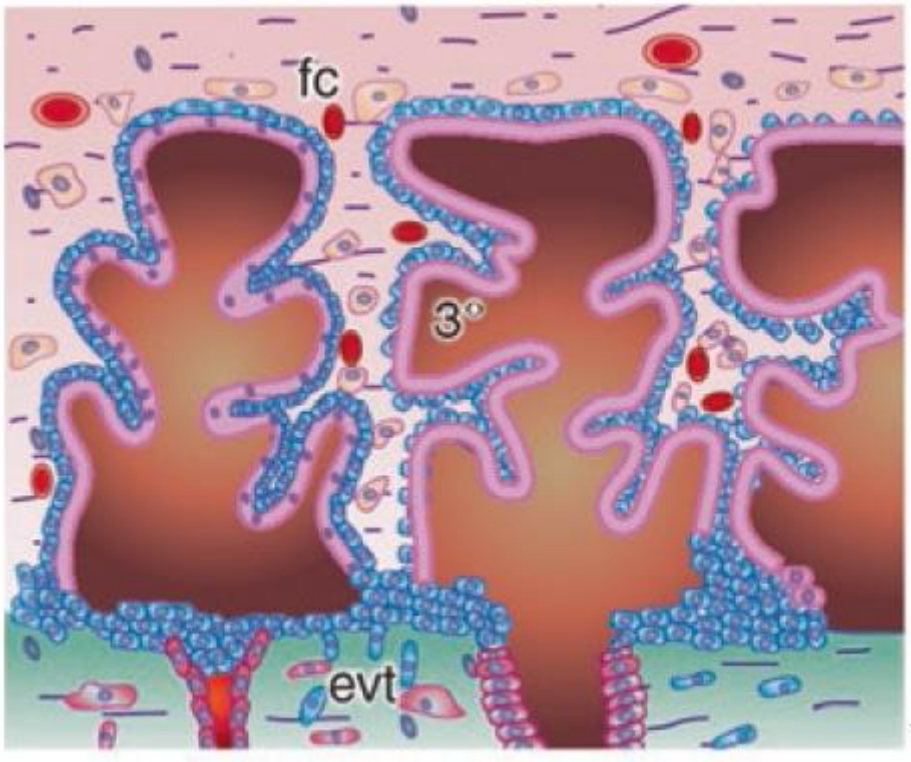


Figure 6: Tertiary villous stage. Fetal capillaries differentiate within the mesoderm core, transforming secondary villi into tertiary villi. Lateral branches arise from the surface of anchoring villi, increasing complexity of villous tree. Extravillous trophoblast cells differentiate from the outer surface of the shell and invade the maternal tissues.[10]
Fetal capillaries (fc), Extravillous trophoblast cells (evt)

SPIRAL ARTERY REMODELING

Spiral artery remodeling is a one of the maternal adaptation during pregnancy, delivers of high volumes of blood to the placenta to support fetal growth. When this process fails, it is linked to major obstetric complications such as late miscarriage, fetal growth restriction, and preeclampsia.

1. Anatomy of spiral artery remodeling

The uterine artery branches into the arcuate arteries, which further divide into radial arteries that ultimately form the spiral arteries. These highly muscularized vessels penetrate through the myometrium into the endometrium/decidua. The terminal parts of the spiral arterioles in the endometrium functionalis are shed during menstruation and regenerated from the basal layer (endometrium basalis) in each cycle.[11] This remodeling of spiral arterioles is one of the few examples of physiological angiogenesis in the adult. Abnormal development of these

vessels outside of pregnancy has been linked to conditions such as heavy menstrual bleeding (endometrial origin) and recurrent pregnancy loss.[12]

2. In pregnancy

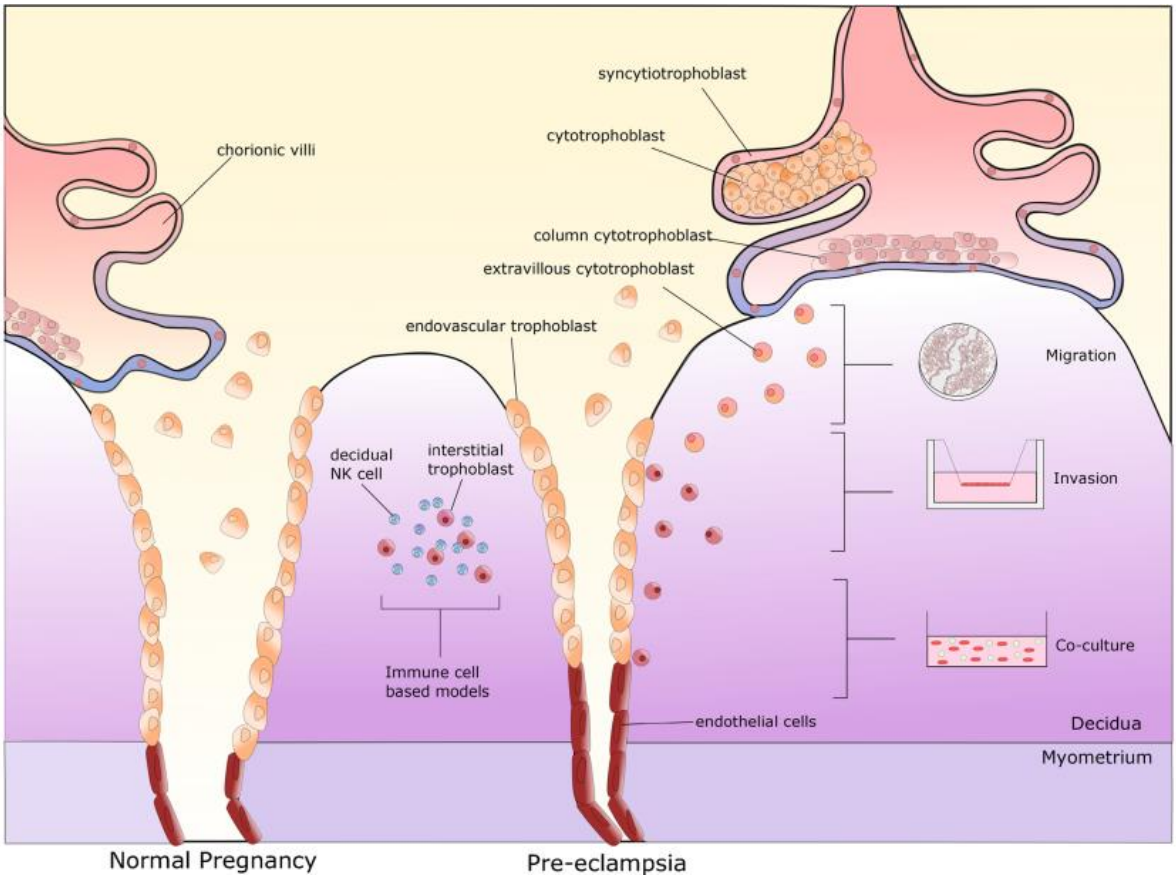
During a pregnancy, the trophoblastic cells that form the outer layer of the developing placenta invade the maternal tissues. The trophoblasts invade not only the endothelial lining of these vessels but also the underlying muscular tunica media. As the trophoblastic cells penetrate deeper into the spiral arteries, they remodel these vessels by replacing the smooth muscle cells with cytotrophoblasts and modifying the structure of the vessel walls.

This remodeling process transforms the spiral arteries from small, muscular arterioles with high vascular resistance into large, more compliant, and low-resistance vessels with a much greater capacitance. This trophoblast invasion results in the arteries becoming wider and less constricted, allowing for significantly increased blood flow to the placenta.[13]

3. In pre eclampsia

In preeclampsia, trophoblastic cells invade the decidual portion of spiral arteries but do not penetrate the myometrial segment. This incomplete invasion prevents proper remodeling of the arteries, resulting in higher vascular resistance and reduced blood flow to the placenta.[14] Here trophoblasts fail to invade myometrial segment of the spiral arteries, preventing the necessary transformation. As a result, the arteries remain narrow and less compliant, causing reduced blood flow to the placenta leading to placental hypoperfusion. This inadequate remodeling leads to hypoxic trophoblast tissue within the placenta, as the blood supply is insufficient to meet the metabolic demands of the growing fetus and also reduced placental blood flow contributes to complications, including fetal growth restriction and this impaired placentation is associated with several pregnancy outcome such as second-trimester fetal death, placental abruption, preterm labor, and preterm premature rupture of membrane all of these are associated with increased the risks to both maternal and fetal health.[15]

Figure 7: shows the spiral artery remodeling in normal, pregnancy and pre eclampsia [16]



VILLOUS DEVELOPMENT AND VILLOUS TYPES [9]

Placental villi is classified into subtypes based on their caliber, stromal, vessel architecture, position in villous tree and function.

1. Mesenchymal Villi: Mesenchymal villi usual begins in fifth week of gestation (counted from menstrual period) as villous vascularisation starts. Syncytial outgrowths (syncytial sprouts) formed from villi after sixth week. Trophoblastic sprouts are produced from cytotrophoblast, whereas villous sprouts produced by proliferation of connective tissue. Additionally new mesenchymal villi formed by development of fetal capillaries within connective tissue core. Mesenchymal villi develop into immature intermediate villi later in the second trimester. These villi develop into mature intermediate villi throughout the third trimester.

2. **Intermediate villi:** are interposed between stem villi.

• **Immature intermediate villi** are bulbous, peripheral and immature continuation of stem villi These represents the immature precursors of stem villi, typically persists in small groups within centers of villous trees.

Morphology of immature intermediate villi is bulbous with prominent cytotrophoblast, a thick trophoblastic cover and characteristic reticular stroma with fluid filled stromal channels. There are Hofbauer cells in the stroma. The stroma contains Hofbauer cells. The capillaries of fetus are underdeveloped.

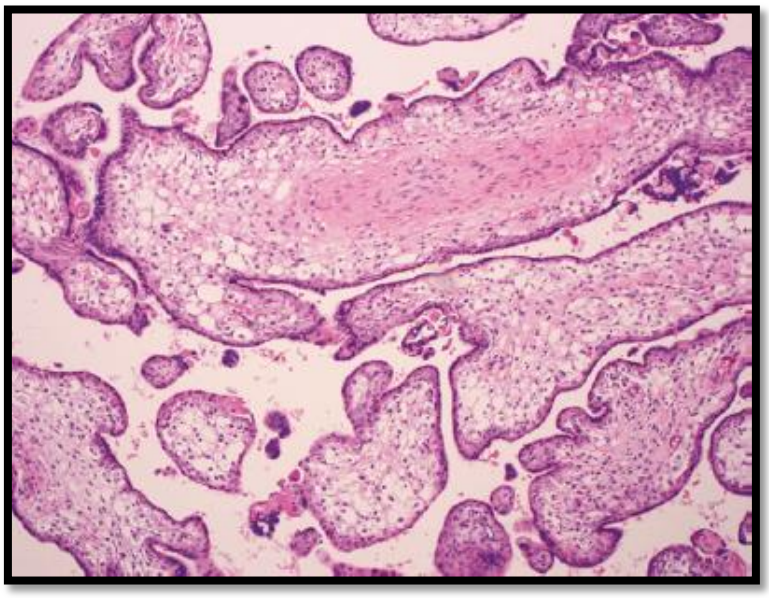


Figure 8: shows central stromal fibrosis, originating from the larger fetal vessels

• **Mature intermediate villi** are long, slender, peripheral ramifications characterized by the presence of vessels without adventitia. The stroma is made up of loose, unoriented bundles of connective tissue fibres and cells and they are many capillaries, tiny terminal arterioles and collecting venules

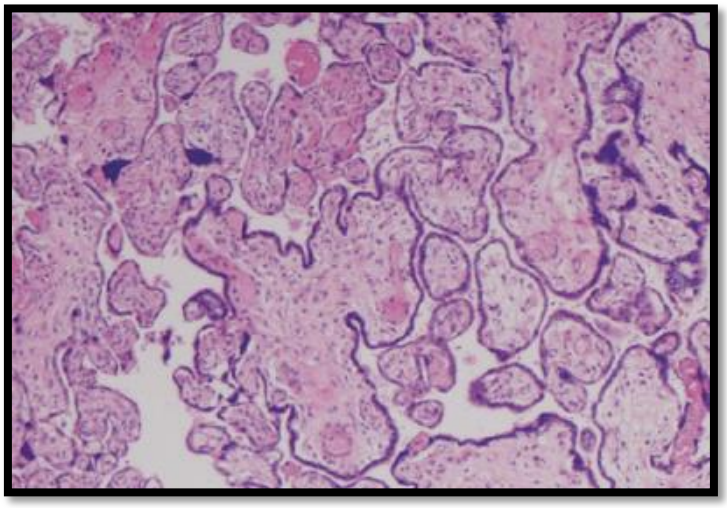


Figure 9. Immature intermediate villi with typical reticular stroma

3.Stem villi gives the villous tree structural support. It characterized by presence of arteries and veins, arterioles and venules, depending on their location, as well as condensed fibrous stroma**.**

Histology: 20% villous surface include cytotrophoblast covered by trophoblastic layer .The surfaces of villi, especially the big stem villi, are degenerative and largely replaced with fibrinoid in mature placenta. Condensed bundles of collagen fibres make up the stroma, and occasionally contains mast cells, macrophages and fibroblasts**.**

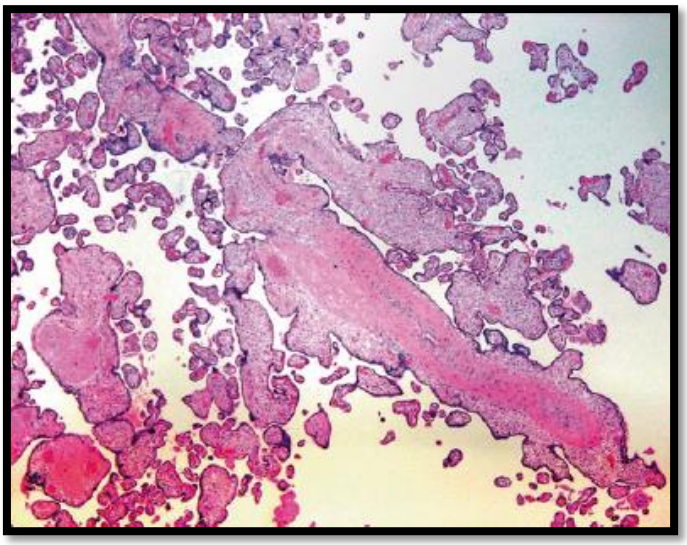


Figure 10: Note that the adventitias of the right and vein directly continue into the surrounding dense fibrous stroma of the villus. Superficially, numerous smaller vessels of the paravascular capillary net are seen

4.Terminal villi: Terminal are the villous tree's last branches. They may be single structure or side branch that resemble bunches of grapes. A thin neck connects these villi to large intermediate villi. They are strongly linked to dilated capillaries, have a thin layer of trophoblasts covering them, have connective tissue and few macrophages. These capillaries have a basal layer and a continuous linning.[9]

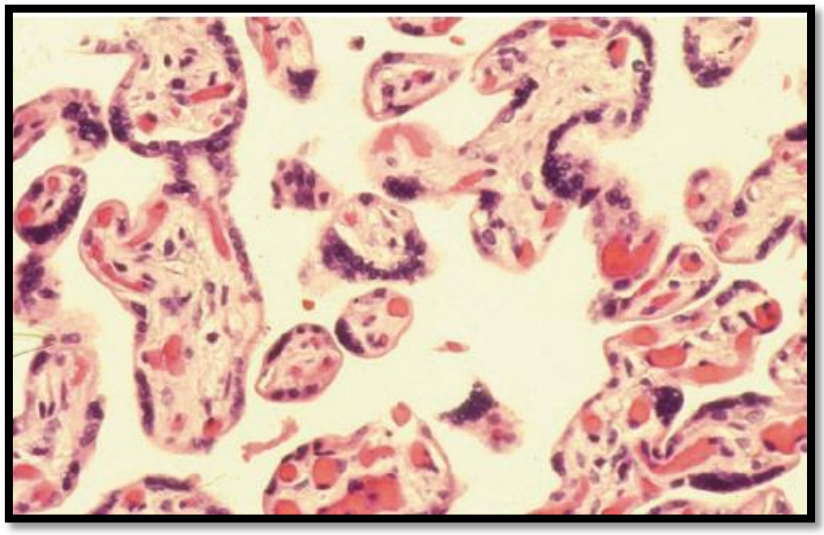


Figure 11: Mature intermediate and terminal villi, both of modest caliber, are the predominant villous forms. In term placentas, the stroma is fully fibrosed and the trophoblastic coating of the stem villi is partially replaced by fibrinoid. Cellular connective tissue or reticular stroma

NORMAL ANATOMY OF PLACENTA [3]

Human placenta is a circular disc that is 3 cm thick in center and 25-20 cm in diameter. It weighs roughly 500g and has spongy texture. At term, it makes up 30% of the uterine wall and has a 1:6 weight ratio to the infant. The umbilical cord has three vessels (two arteries and one vein) and inserted in center.

Fetal surface

It is has smooth and glistening amnion covering with umbilical cord in the midline. Umbilical cord vessels radiating from insertion to cord are visible beneath amnion. About four fifth of placenta is fetal origin.

Maternal surface

It is rough and spongy. Its dull red color comes from maternal blood. It is possible to see a thin, shaggy, grayish layer (remaining decidua basalis) that has separated from the placenta. The lobes or cotyledons, which are 15–20 rather convex polygonal regions bounded by fissures, make up the maternal surface.

There are numerous grayish specks to be seen. Less than one-fifth of the placenta is made up of the maternal component. Only the blood in the intervillous gap and decidu basalis are maternally derived.

NORMAL HISTOLOGY OF PLACENTA

Structure of Umbilical cord [9]

The umbilical cord contains two arteries and one vein, present within Wharton's jelly and is continuous with the surface of both the placenta and fetal skin. Its surface is lined with amnionic epithelium which is made up of stratified, unkeratinized squamous cells close to the umbilicus. This creates a transitional layer that give rise to keratinized stratified squamous epithelium of abdominal wall. The epithelium changes from stratified columnar epithelium to simple columnar epithelium upon reaching fetal surface. The basal cells are similar to those of the amnion, but the stratified layers' superficial cells are squamous and may appear pyknotic. A fine network of microfibrils contains the ground substance (collagen, laminin, heparan sulfate, hyaluronic acid, and glycosylated carbohydrates) that makes up Wharton's jelly. These extracellular matrix molecules accumulate near "stromal clefts." The stromal spaces and surrounding meshwork of contractile cells help control the turgor of the cord by preventing compression of the umbilical veins and kinking or bending of the chord. The cord's low cellular composition and high water content prevent the umbilical vessels from compressing

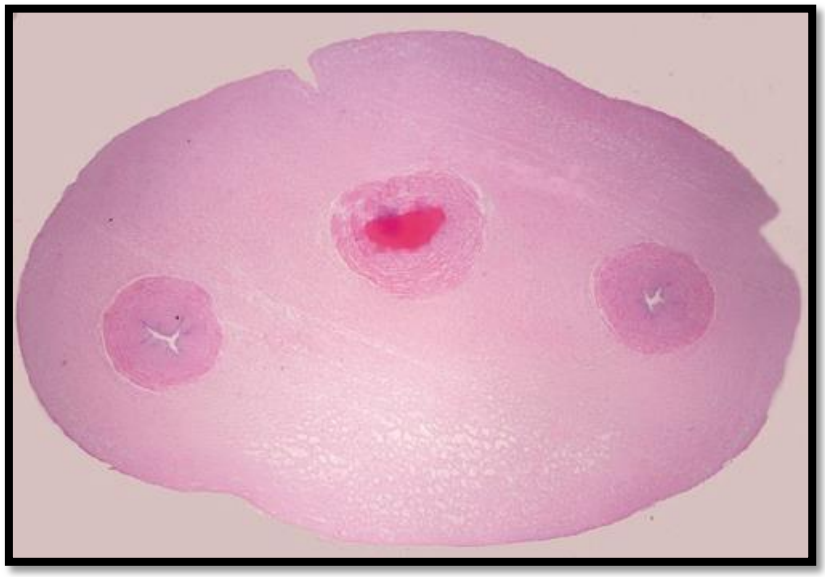


Figure 12 : shows mature umbilical cord, near its placental insertion showing a sparsely cellular Wharton's jelly, an umbilical vein and two arteries.

Structure of Chorion [17]

Chorion lies outside the amnion and is primarily made up of stroma and trophoblastic cells. The chorion is made up of several layers. The amnion and chorion are separated by the spongy layer, which is comparatively acellular. Below the spongy layer, fibroblasts and macrophages can be found in scattered form. The reticular zone comes next, and then a false basement membrane. The decidua and trophoblastic cells are located beneath this. There may be artifactual subamniotic clefts and subchorionic fibrin, although they are not clinically significant.

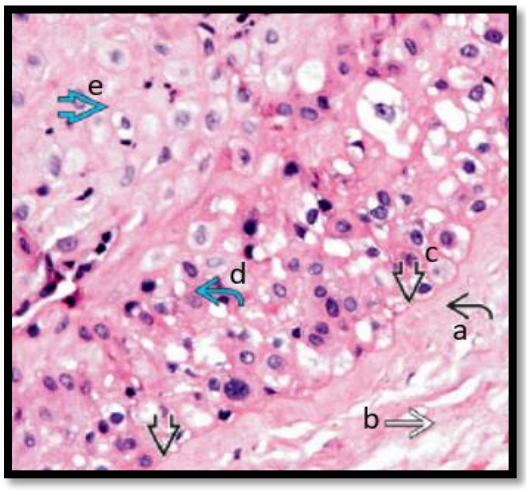


Figure 13 : shows a:Reticular zone, b:Acellular layer, c :Basement membrane, d: Decidua

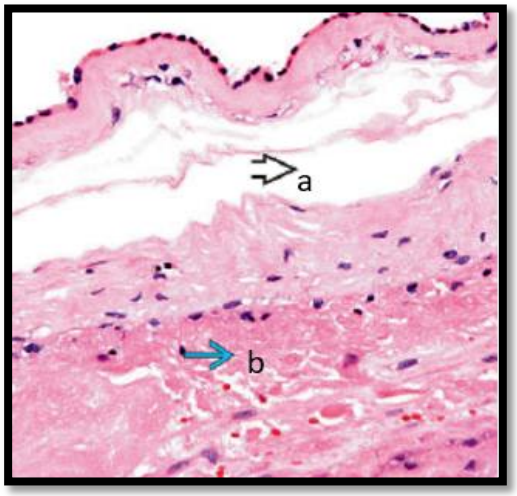


Figure 14 : shows a: Subamniotic clefts , b: Subchorionic fibrin

Structure of amnion [9]

Amnion is a transparent structure that separates from the chorion underneath with ease. It never merges with chorion as it is passively linked by the amniotic fluid's internal pressure, it never really merges with chorion. Since amnion lacks its own blood arteries, it receives nourishment and oxygen from the surrounding amniotic fluid and fetal surface vessels. The fetal ectoderm is the source of the amnionic epithelium, which covers the fetal skin and umbilical cord. The epithelium is composed of single layer of flat, cuboidal to columnar cells. While flatter cells are typically found in the periphery, taller, columnar cells are typically found close to the placental border where the membranes implantation occurs. The basement membrane that the amnionic epithelium rests on has a small layer of connective tissue termed the amnionic mesoderm attached to it. Amionic mesoderm is composed of fibroblast layer and compact stromal layer. The amnion has several functions in the placenta. It is essential for the structural integrity and junctional permeability of the membranes and contributes to the turnover of amniotic fluid and pH maintenance.

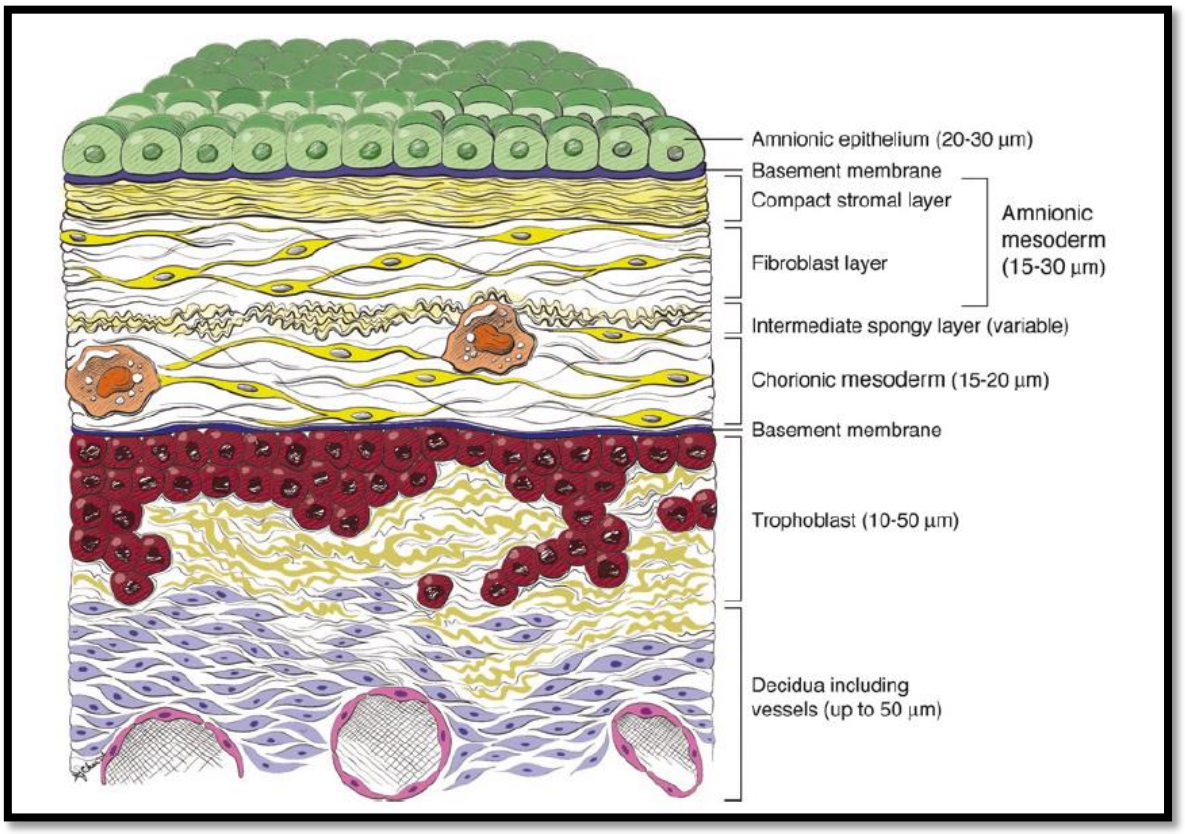


Figure15 : shows layers of fetal membranes

Structure of placental villi [18]

Once maternal blood enters the trophoblastic lacunae in the placenta, the tissue between them becomes stronger, and stem villi develop to attach to the cytotrophoblastic shell. Complex structure is created by side branches develop into lacunae. The mesenchymal core of each villus contains capillaries from embryonic blood arteries. Supported by the layer of dividing cytotrophoblast cells, a continuous layer of syncytiotrophoblast separates the villous capillaries from maternal blood, Cytotrophoblast layers begins to thin out about fourth month. The villi of the villous tree decrease in size as more branches grow, lowers barrier between mother blood and fetal capillaries. The decidua basalis, or maternal tissue, regresses as the placenta develops, leaving behind linked septa that reach into the cytotrophoblastic shell. The former locations of the maternal septa indicate the about 20 irregular segments known as cotyledons that make up the ejected placenta after childbirth.s

Early placenta

Numerous villi (V) extend into the lacuna system (L), typically filled with maternal blood in vivo. Some villi exhibit branching, with solid cores of cytotrophoblast and intermediate trophoblast (I) extending from the villi to form new branches.

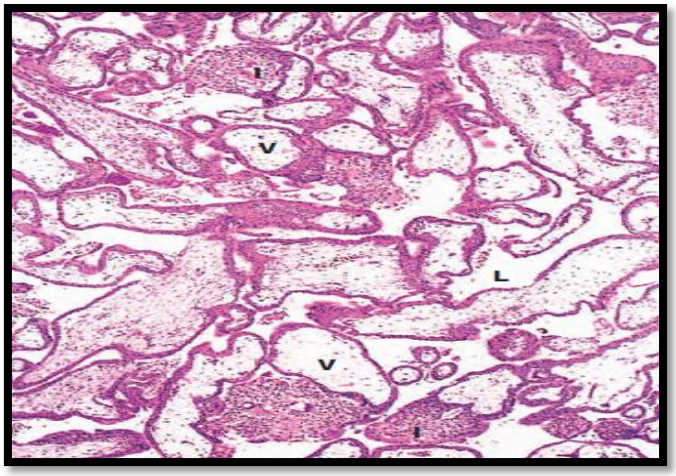


Figure 16: shows the villi contain a core of primitive mesenchyme (M) and are surrounded by trophoblast. This trophoblast consists of an inner layer of cytotrophoblast cells (C) and a broader outer layer of syncytiotrophoblast (S). In certain regions, solid buds of trophoblast are visible, indicating the formation of new branches.

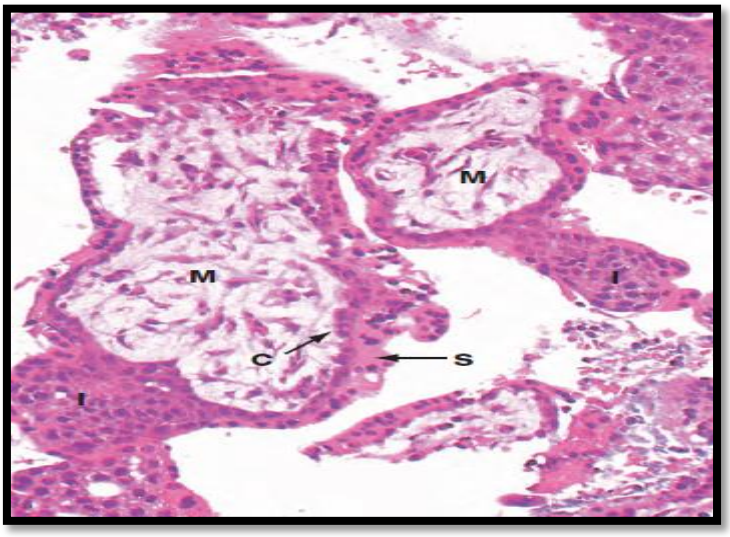


Figure 17: The syncytiotrophoblast layer (S) is distinguishable from the smaller, single layer of cytotrophoblast cells (C). Mesenchymal cells (MC) are large and feature extensive branching cytoplasmic processes. The intercellular matrix appears myxoid due to its high content of glycosaminoglycans.

Term Placenta

Numerous villi are observed, cut at different angles and ranging in diameter from large mainstem villi to very small terminal branch villi. Compared to the early placenta, the villous pattern is significantly more intricate, with a much smaller average villous diameter. This reflects the extensive branching growth of the villi as the placenta expands. Notably, large blood vessels (V) are visible within the largest villi.

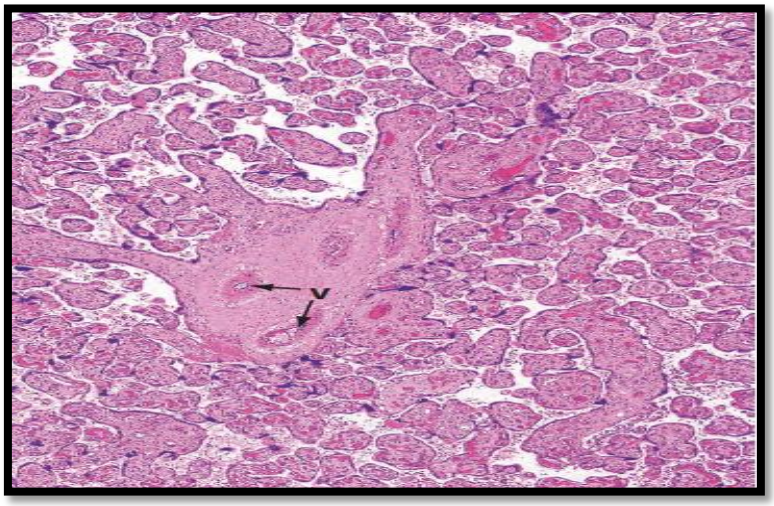


Figure 18 :shows numerous villi and shows branching growth of the villi as the placenta enlarges

Contrast the pronounced vascularity of the villous cores with that of the earlier placenta highlighting the significantly augmented villous surface area interacting with lacunae (L) filled with maternal blood. A notable characteristic of the term placenta is the presence of syncytial knots (K), where syncytiotrophoblast nuclei aggregate into clusters, leaving regions of thin cytoplasm devoid of nuclei in between.

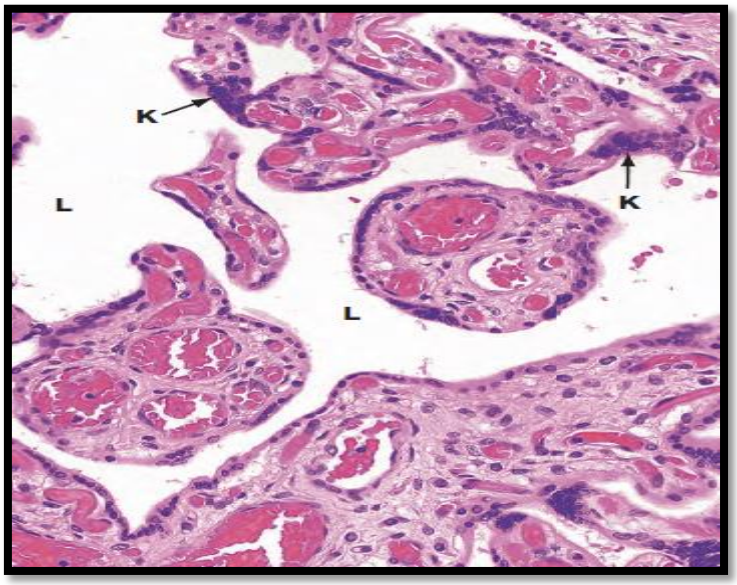


Figure 19 : shows branching nature of villi and presence of syncytial knot is the feature of the term placenta

The focus is on a small branch villus, emphasizing the proximity of fetal capillaries (C) to maternal blood in the surrounding lacuna (L). In this context, the trophoblast layer is reduced to a thin layer of syncytiotrophoblast only, and the capillaries tend to be situated at the periphery of the core. The diffusion barrier separating maternal and fetal circulations consists of five layers: trophoblast, trophoblast basement membrane, villous core supporting tissue, capillary endothelial basement membrane, and endothelium. In some instances, fetal capillaries are positioned so closely to the trophoblast that their basement membranes fuse (F), diminishing the diffusion barrier to just three layers.

HISTOPATHOLOGICAL FINDINGS IN ABNORMAL PLACENTA

MACROSCOPIC FINDINGS [19]

1.Perivillous fibrin deposition: observed in marginal angle and in peripheral area of placenta. The plaques are hard and have irregular outline . Cut surface is white with brown or yellow tinge.

2.Subchorionic fibrin plaque: Laminated white plaque, typically in the shape of a triangle. There is a clear separation between the fibrin plaques and healthy placental tissue.

3.Infract: occurs anywhere in the placental tissue, although it is more frequently observed in the periphery than the center. Triangular in shape, infracts are only seen in placental tissue and do not make contact with the basal plate. The infract extends from the basal plate to the placenta's entire thickness.

4.Calcification: Small, hard, scattered flecks seen on maternal surface of placenta. There are flecks of calcium seen in basal plate and calcification in chorionic plate is unusual.

5. Retroplacental hematoma: It is large occupies most of maternal surface of placenta and hematoma lies between basal plate of placenta and uterine wall. Freshly formed hematoma is soft, red and separated from maternal surface.

MICROSCOPIC FINDINGS[20]

1. **Increased Syncytial Knot Formation**: Haemochorial placentas frequently adapt by forming synchytium at the maternofetal interface. Small clusters of syncytiotrophoblast nuclei that protrude marginally beyond the villous surface are known as true syncytial knots. They usually have highly compacted chromatin in their nuclei. An average of 28% of villi at term contain a syncytial knot on their surface, according to a recent study of healthy placentas.

2.Villous Oedema: An accumulation of fluid in the villous stroma and between capillaries and the trophoblast layer is a characteristic of villous oedema. Congenital nephrotic syndrome, fetal cardiac abnormalities, fetal anemia, and adenomatoid malformation are among the conditions that can cause villous edema.

3. Extramedullary Haemopoiesis: The development and maturation of blood cells outside the medullary compartments in the bone marrow is known as extramedullary hemopoiesis. They are located in the villous stroma of the placenta. Chronic fetomaternal hemorrhage linked to intervillous or subchorionic thrombosis may manifest as placental extramedullary hemopoiesis.

4. Intravillous haemorrhage: It is an early stage of placental parenchymal infarction and is a sign of an acute hypoxic event of the placenta. Retroplacental hemorrhage is linked to it.

The decidua is separated from the placenta by blood, which causes the parenchyma to become indented. Placental arteries dilate as a result of the extreme acute hypoxia, resulting in severe capillary congestion in the fetus. This reaction is similar to that seen in pulmonary ventilation-perfusion mismatch

5. Villous Chorangiosis: Increases in capillary cross sections per villus that are patchy-diffuse and often measured in terminal villi but may also extend into intermediate villi. A single layer of flattened endothelium, a continuous linear basement membrane, and the absence of surrounding pericytes line the capillaries in VC. Although VC can affect the entire placenta, it often affects the lower two thirds of the placental parenchyma in a patchwork manner. Although VC is most noticeable around the edges of parenchymal regions where capillaries are frequently dilated, it's crucial to check for impacted villi elsewhere to prevent overdiagnosis.

6.Stromal fibrosis: As stromal collagen progressively build up during pregnancy, the stroma exhibits a fine network of fibrous tissue at term. Placenta from diabetes mothers, prolonged pregnancies, and pre eclampsia are linked to stromal fibrosis of greater than 3%, which is a morphological sign of reduced villous perfusion.[19]

HYPERTENSIVE DISORDER OF PREGNANCY [21]

1. **GESTATIONAL HYPERTENSION** is defined as elevated blood pressure more than 140/90 mmHg occurring after 20 weeks of gestation, in absence of proteinuria or organ damage.

2. PREECLAMPSIA is characterized by newly diagnosed hypertension along with proteinuria (excretion of ≥300 mg of protein in a 24-hour urine collection) or signs of end-organ damage after 20 weeks of gestation. Also pre eclampsia can manifest without hypertension if gestational proteinuria is associated with organ damage. End-organ damage affects the kidneys, liver, brain, and hemostatic systems, with conditions like HELLP syndrome.

3. PREECLAMPSIA WITH SEVERE FEATURES is characterized by a systolic blood pressure (SBP) of ≥160 mmHg or a diastolic blood pressure (DBP) of ≥110 mmHg, a platelet count below 100 × 103/µl, liver transaminase levels twice the upper limit of normal, serum

creatinine level above 0.285 mmol/l, severe persistent right upper-quadrant pain, pulmonary edema, or new-onset cerebral visual disturbances. Approximately half of women diagnosed with preeclampsia progress to this severe form.

4. ECLAMPSIA develops when preeclampsia leads to new-onset tonic-clonic seizures. These seizures can occur before, during, or after labor, termed antepartum, intrapartum, or postpartum eclampsia, should not be of any other cause.

5. CHRONIC HYPERTENSION is characterized by hypertension (≥140 mmHg systolic BP and/or ≥90 mmHg diastolic BP) existing prior to pregnancy or diagnosed before the 20th week of gestation.

6.PREECLAMPSIA SUPERIMPOSED ON CHRONIC HYPERTENSION occurs when a woman has baseline hypertension before or during pregnancy, along with pre eclampsia. This is defined as newly diagnosed proteinuria after 20 weeks of gestation, sudden worsening of hypertension, and/or signs of end-organ damage.

RISK FACTORS [22]

1. Nulliparity
2. Age >40 years
3. Pregnancy with assisted reproduction
4. Interpregnancy interval >7 years
5. Family history of preeclampsia
6. Woman born small for gestational age
7. Obesity/gestational diabetes mellitus
8. Multifetal gestation
9. Pre eclampsia in previous pregnancy
10. Poor outcome in previous pregnancy
11. Fetal growth restriction, placental abruption, fetal death
12. Pre existing medical- genetic conditions
13. Chronic hypertension
14. Renal disease
15. Type 1 (insulin-dependent) diabetes mellitus

16. Antiphospholipid antibody syndrome
17. Factor V Leiden mutation

PATHOGENESIS OF PRE ECLAMPSIA

Pre eclampsia likely results from a combination of maternal and fetal/placental factors. Reduced blood flow, oxygen deprivation, and ischemia within placenta can result from abnormalities in the development of placental blood arteries in early pregnancy. This can prompt the release of antiangiogenic substances into the mother's bloodstream, affecting her systemic endothelial function and resulting in hypertension and various other symptoms of the condition, including issues related to the blood, nervous system, heart, lungs, kidneys, and liver. Despite these observations, it is still unknown what specifically caused the abberant placental development and the series of events that followed

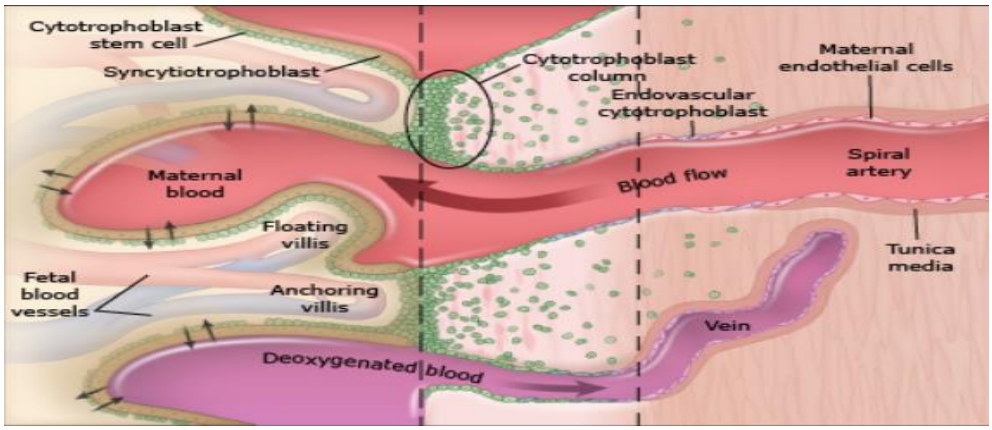
1. ABNORMAL DEVELOPMENT OF THE PLACENTA

Abnormal remodeling of spiral arteries:

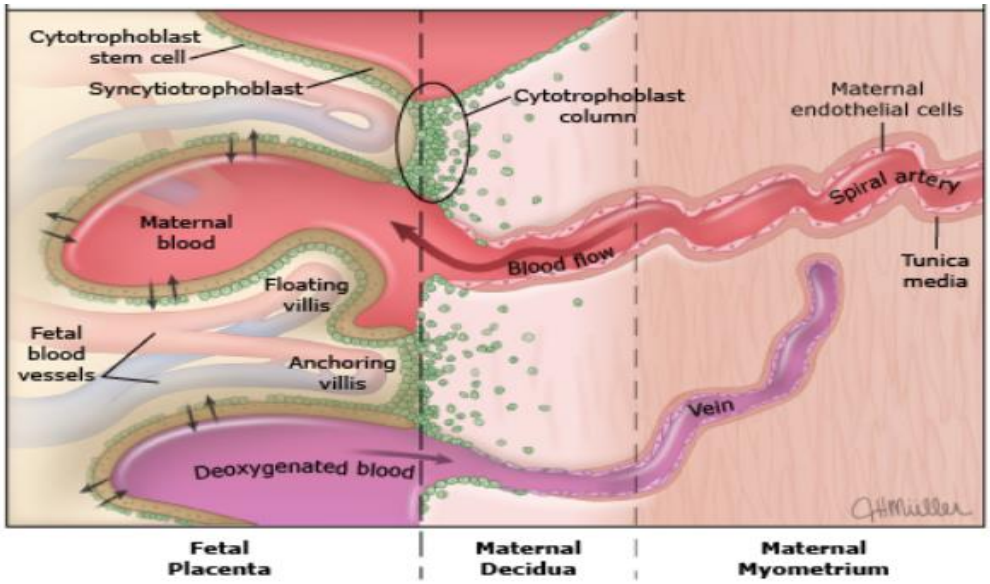
During normal Pregnancy, Cytotrophoblast cells from the developing placenta go through the decidua and into a portion of the myometrium. In both endothelium and muscular tunica media, they infiltrate the maternal spiral arteries, the final branches of the uterine artery that supply blood to the developing fetus or placenta. These arteries undergo a change as a result of this invasion, going from tiny muscle arterioles to bigger vessels with higher capacitance and lower resistance. As a result, the placenta receives substantially more blood flow than other parts of the uterus. Although it is unclear exactly when trophoblast invasion of these arteries stops, spiral arterial remodeling usually starts in the latter part of first trimester and is finished by 18 to 20 weeks of pregnancy.[13]

In preeclampsia, cytotrophoblast cells manage to infiltrate the decidual portion of the spiral arteries but are unable to penetrate the myometrial segment.[14] As a result, the spiral arteries do not develop into the big, winding vascular channels that are usually seen when fibrinoid material replaces the muscle-elastic wall. Instead, these arteries stay narrow, which results in comparatively hypoxic trophoblast tissue and decreased blood supply to the placenta. Numerous unfavorable pregnancy outcomes, such as fetal death in the second trimester, placental abruption, preeclampsia with or without intrauterine growth restriction, intrauterine

growth restriction without maternal hypertension, preterm labor, and premature membrane rupture, have been connected to this failure in deep placentation..[15]



NORMAL



PRE ECLAMPSIA

Figure 20 : a shows mechanism of spiral artery remodeling in normal

b shows mechanism of spiral artery remodeling in pre eclampsia

Defective trophoblast differentiation

One of the cause for defective invasion of spiral arteries may be the inadequate differentiation of trophoblast. The process of trophoblast differentiation during endothelium invasion involves the changes in expression of several molecules such as metalloproteinases, adhesion molecules, extracellular matrix components, cytokines and HLA-G molecule from class 1d major histocompatibility complex .[23] The process by which invasive trophoblasts alter the expression of adhesion molecules characteristic of endothelial cells (integrin alpha1/beta1,

alpha v/beta3, and VE-cadherin) to those characteristic of epithelial cells (integrin alpha6/beta1, alpha v/beta5, and E-cadherin) during normal differentiation is known as pseudo-vasculogenesis. However, preeclamptic individuals trophoblasts do not exhibit pseudo-vasculogenesis or elevated adhesion molecule expression.[13]

Placental hypoperfusion, hypoxia, ischemia

Hypoperfusion, which is exacerbated as pregnancy progresses because the uterine vasculature is unable to support the usual increase in blood flow to the fetus/placenta, is a result of abnormal placental development. Atherosis (lipid-laden cells in arteriole walls), fibrinoid necrosis, thrombosis, arteriolar constriction, and placental infarction are examples of late placental abnormalities suggestive of ischemia. Although not all of these lesions are always seen in women with preeclampsia, there appears to be a relationship between the degree of these lesions and the disease's severity and early onset. [13]

The development of preeclampsia is significantly influenced by hypoperfusion, hypoxia, and ischemia. During pregnancy, these variables probably cause the placenta to create different chemicals that, when released into the mother's bloodstream, prevent angiogenesis. In particular, endoglin and soluble fms-like tyrosine kinase-1 (sFlt-1) bind to placental growth factor (PlGF) and vascular endothelial growth factor (VEGF), causing endothelial dysfunction, vascular damage, and extensive maternal vascular inflammation. In the end, this cascade leads to proteinuria, hypertension, and other preeclampsia symptoms.[24]

Decidual pathology

According to research, inadequate decidualization may be a factor in some people's reduced cytotrophoblast invasion. A pattern that is compatible with poor decidualization has been seen in microarray examinations of samples of chorionic villus. Interestingly, decidual cells from preeclamptic women express more sFLT1, suggesting that shallow implantation could be caused by inadequate inhibition of anti-angiogenic factors during implantation. To completely understand how endometrial variables contribute to the development of preeclampsia, more research is necessary.[25]

2. IMMUNOLOGIC FACTORS

Women with preeclampsia have been found to exhibit immunological abnormalities similar to those seen in organ rejection. HLA-C, HLA-E, and HLA-G are among the unusual HLA class I antigens seen in extravillous trophoblast (EVT) cells. Natural killer (NK) cells enter the maternal decidua and interact closely with EVT cells because they express different receptors that detect class I molecules, such as CD94, KIR, and ILT. It is believed that this connection controls placental implantation.[26] Furthermore, preeclampsia patients tend to have less regulatory T cells (Tregs), a subpopulation of CD4 T cells that are essential for reducing inflammatory immune responses and safeguarding the fetus, both systemically and in the placental bed. Maternal-paternal gene conflict in preeclampsia is thought to cause aberrant placental implantation through decreased Tregs, increased NK cell activity, and other mediators of the immune response.

Furthermore, meta-analyses have demonstrated that women who conceive naturally are four times less likely to develop pre eclampsia than those who use oocyte donation and an incidence that is more than double that of those who use other assisted reproductive procedures. This pattern supports the idea that preeclampsia may develop as a result of immunological intolerance between the mother and fetus.[27]

3.GENETIC FACTORS

While most of instancs preeclampsia occur sporadically, genetic factors are thought play a role in the disease in about one third cases.

Observations suggesting a genetic predisposition to preeclampsia include:

a. Pre eclampsia is two to five times more likely to occur in primigravid women with family history of the condition than those without
b. Imprinted genes can partially explain the maternal contribution to the development of pre eclampsia
c. Women with a history of preeclampsia in a previous pregnancy face a more than sevenfold increased risk
d. The pre eclampsia is more likely in Spouses of men whose pregnancies were complicated by preeclampsia

e. Women pregnant by men with a history of preeclampsia in previous partners face a higher risk compared to pregnancies with normotensive partners.[28]

4. ENVIRONMENTAL AND MATERNAL SUSCEPTIBILITY FACTORS

Low calcium intake - Calcium supplementation has promise in avoiding preeclampsia in high-risk women, and epidemiological studies indicate a correlation between low dietary calcium consumption and increased risks of preeclampsia. Although the exact mechanism underlying this connection is unknown, it may be related to modifications in calcium regulating hormones that impact either vascular or immunological function in preeclampsia.

High body mass index- A study found a direct correlation between increasing body mass index (BMI) and the risk of developing preeclampsia. The odds ratio for preeclampsia increased from 1.65 in women with a BMI of 25 to 30 kg/m² to 6.04 in women with a BMI ≥40 kg/m². Obesity is susceptible to preeclampsia by triggering chronic inflammation and endothelial dysfunction, potentially synergizing with placental angiogenic factors to induce the microangiopathic features of the condition.[29]

In vitro fertilization-Compared to spontaneous conception, pregnancies following in vitro fertilization (IVF) show a heightened risk of adverse pregnancy outcomes, such as preeclampsia and fetal growth restriction.[30]

5. INFLAMMATION

Placental cell-free DNA and circulating syncytiotrophoblast debris may be contributing factors to maternal inflammation, which is particularly noticeable in preeclampsia. Necrosis and apoptosis brought on by placental hypoxia release cell-free DNA into the mother's bloodstream. Elevated trophoblast cell-free DNA levels are seen in preeclamptic women as early as 17 weeks of pregnancy, peaking three weeks prior to the beginning of symptoms. Higher levels of sFlt1 are correlated with this increase. Hazardous syncytial proteins, including sFlt1, are present in synchytial microparticles that transport cell-free fetal DNA. Although there is insufficient proof, the inflammatory state may increase vascular endothelial sensitivity to harmful substances like sFlt1 and sEng.[31]

A meta-analysis investigating maternal infection's link to preeclampsia found that pregnant women with urinary tract infection (pooled odds ratio [OR] 1.57, 95% CI 1.45-1.70) and periodontal disease face an elevated risk of developing the condition.[32]

6. INCREASED SENSITIVITY TO ANGIOTENSIN II

Increased sensitivity to angiotensin II has been observed in preeclampsia, which may be related to increased bradykinin (B2) receptor overexpression in afflicted individuals. Patients with preeclampsia have higher concentrations of agonistic antibodies that target the angiotensin AT-1 receptor. Given that angiotensin II is the AT-1 receptor's natural ligand, increased receptor activation caused by auto-antibodies may be a factor in the condition's hallmark hypertension and vascular damage.[33]

7.COMPLEMENT ACTIVATION

Complement activation or dysregulation may play a important role in preeclampsia development. Preeclampsia is more likely to occur in pregnant women with autoimmune diseases, such as systemic lupus erythematosus and antiphospholipid antibody syndrome, which frequently cause the placenta's classical complement pathway to become activated.[34]

According to early clinical research, severe preeclampsia is associated with higher markers of alternative complement pathway activation. Mutations in complement regulatory proteins can put women at risk for preeclampsia even if they do not have autoimmune disorders. Furthermore, germline abnormalities in alternative complement pathway connected to HELLP syndrome, a serious consequence of preeclampsia.[35]

CD 24

Cluster of differentiation 24 (CD24), known as heat-stable antigen (HSA), is glycosylated 31–34α amino acid protein attached to the cell surface by a glycosylphosphatidylinositol anchor.[4] The CD 24 gene, found on chromosome 6p21.3, encodes the CD 24 protein, which has a molecular weight of 27 kDa and is composed of 80 amino acids.[36] It is expressed on B and T lymphocytes ,monocytes and granulocytes as well as epithelial, neural and muscle cells.[37]

X-ray crystallography has been used to establish the crystal structure of CD 24. Four antiparallel β-strands combine to produce a compact, globular shape with a β-barrel fold.

Cys53 and Cys73 form a disulfide link that stabilizes the β-barrel. The protein's N-terminal region includes a flexible loop region and a brief α-helix.[38]

CD 24 is a key regulator in the immune evasion strategies of cancer cells, acting as a new phagocytosis that helps immune system in identifying and destroying malignant cells.[39]

By interacting with macrophages inhibitory receptor Siglec-10, CD 24 successfully sends a "don't eat me" signal, which stops cancer cells from being phagocytosed. This interaction complicates tumour-immune dynamics and makes therapeutic interventions in immune responses against tumours.[40]

CD24 AND ITS RECEPTORS

CD 24 is a cell surface protein that interacts with a variety of cell surface receptors, including P-selectin, Siglec-10, and β1 integrin, which are important in controlling cell adhesion, migration, differentiation, and death. Despite having no inherent enzyme function, CD24 interacts with a variety of receptor proteins, including the NKG2D receptor, Siglec-10, and Siglec-15.[6]

ROLE OF CD24 IN BENIGN CONDITIONS

1.Autoimmune diseases: CD24 has been connected to autoimmune disorders and plays a role in regulation of immune response. The link between CD24 and autoimmune disorders is strongly supported by clinical data. CD24 polymorphisms are linked to the onset of autoimmune disorders, including multiple sclerosis, rheumatoid arthritis, and systemic lupus erythematosus .[41] Zhou et al. were the first to link CD24 V/V genotype with an increased risk and development of multiple sclerosis and also found that CD24V/V genotype patients had higher levels of CD24 expression on peripheral blood T cells than patients with the CD24A/A genotype. [42]

2. Inflammation diseases: The body's natural reaction to infections or injuries is inflammation. Numerous compounds that fall into two main categories are the cause of it. The primary and most important category includes pathogen-related molecular patterns, or PAMPs, whereas the secondary and less important group includes damage-related molecular patterns, or DAMPs.[43] The link between CD24 and Siglec 10/G specifically inhibits the inflammatory response to tissue damage. CD24 binds with Siglec-10 to reduce inflammatory responses induced by danger-associated molecular patterns (DAMPs), but it has no influence on inflammatory responses produced by pathogen-associated molecular patterns

(PAMPs).[44]It has been discovered that a variety of pathogens can break the CD24-Siglec G/10 relationship by either removing sialic acids from CD24 or by lowering the expression of Siglec G/10.

3. Neurological disorders: CD24 stimulates myelination and axonal development. CD 24 is important for cellular communication and function, especially for activities like neurogenesis, neurite expansion, and neuronal migration.[45] In the dentate gyrus of the hippocampus and the rostral migratory stream, which is the route taken by freshly generated neurons in the direction of the olfactory bulb, CD24 expression persists until adulthood. According to these findings, CD24 is active at various stages of neuronal migration and connection formation. The results give the information about how CD24 controls neuronal migration and synapse development. Additionally, non-neuronal ependymal cells with cilia that border the ventricles were shown to express CD24. [46]Furthermore, CD24 has been connected to the genesis of neurological disorders such neuronal injury. This suggests that after being triggered by several other contributing factors, CD24 expression facilitates the onset of Experimental Autoimmune Encephalitis and may be Multiple Sclerosis.[47]

4. Metabolic disorder Obesity, diabetes, dyslipidemia, nonalcoholic fatty liver disease, and nonalcoholic steatohepatitis are metabolic diseases that have a serious threat to global health. [48]According to Yang L. et al. CD24Fc helps with metabolic issues associated with obesity, but the CD24-Siglec-E linkage is disrupted, making these conditions worse.[49] Through interactions based on sialosides, Siglec-E recognizes CD24, lowering inflammation and preventing metabolic syndrome. For diseases including obesity, dyslipidemia, insulin resistance, and nonalcoholic steatohepatitis, these findings provide a viable immunotherapeutic strategy by revealing the inhibitory effect of the CD24-Siglec-E axis in metabolic dysfunctions and metaflammation.

ROLE OF CD24 IN MALIGNANCY

Studies have shown that CD 24 is highly expressed in various tissues. [50] Increased blood levels of CD24 have been shown in recent research to be a potential biomarker for early cancer diagnosis and a new prognostic indication.[51] Cancers such as breast, ovarian, pancreatic, and bladder cancer have demonstrated a correlation between CD24 and cancer stem cells. includes leukemia, multiple myeloma, colon cancer, hepatocellular carcinoma, and melanoma.[4]

CD 24 is one of the most important markers for cancer diagnosis and prognosis. For instance, aggressive carcinoma exhibits significantly greater CD24 expression in breast cancer than precancerous lesions. Furthermore, poor prognosis, histology grades, tumor size, and lymph node positivity are all linked to the presence of CD24 on cell surface and in the cytoplasm.[52]

CD24 –ROLE AND FUNCTION DURING PREGNANY AND PRE ECLAMSPIA

The role of CD24 in promoting immune tolerance was first explored within the immune system, and later, its involvement in tumors was also studied.[37]

Barkal et al.stated that anti-phagocytic " don't eat me " like CD 24 can protect cancer cells from attacks by macrophages that express siglec-10. Monoclonal antibodies that block CD24-siglec-10 signalling improves removal of CD 24 malignancies and suggesting CD24 inhibition in immunotherapy.[53]

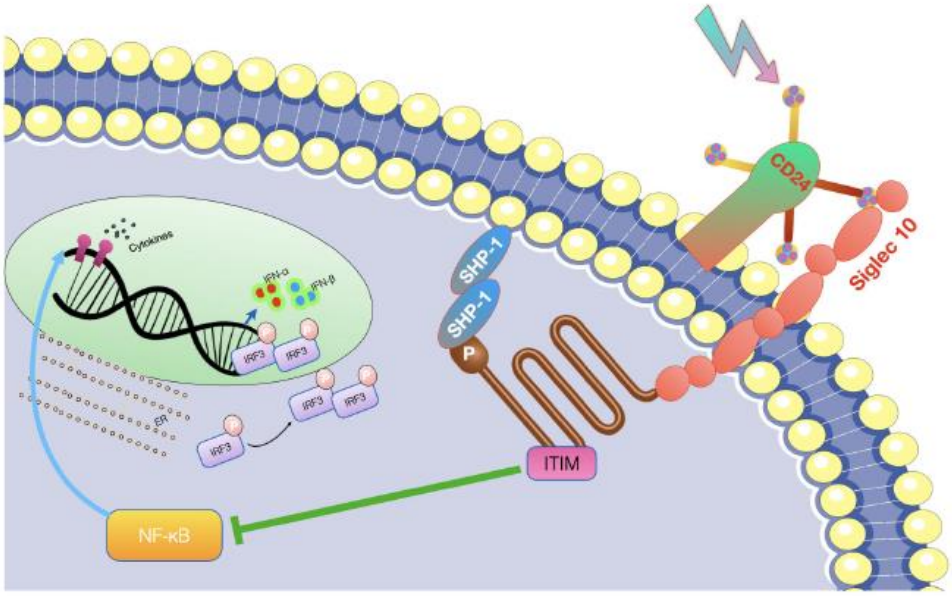


Figure 21: shows how CD24 antibodies can prevent the activation of the CD24-Siglec-g/10 signal, which enables macrophages to identify and destroy tumor cells as part of the immune clearance process.[40]

CD24 shows expression on villous tissues and circulating immune cells. It is also seen in placenta there is blockage of CD 24 leading to blocking of macrophage in placenta villi and increased immunotolerance. It is seen that in normal pregnancy CD24 blocks macrophages and supports the growth of the placenta. Therefore, CD24 reduction in early Pre eclampsia may be linked to increased rejection of the placenta, and its reduced expression may be associated with early cases of Pre eclampsia.[6]

MATERIALS AND METHODS

STUDY DESIGN: Cross sectional study

DURATION OF STUDY: 2 years (September 2022- December 2023)

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

SAMPLE SIZE

This cross sectional study was conducted to compare the Expression of CD24 in normal placenta and pre-eclampsia placenta. A study conducted by Blint Nagy et al, shows that the average CD24 concentration, ng/µl in preeclampsia group was 18.94±26.86 and for control group it was 53.58±92.05.[54] Thus with 95% C.I and 95% power, the minimum sample size required for the present study was calculated as follows.

$$N= \frac{(Z_{1-\alpha/2} + Z_{1-\beta})^2 \, 2 \, \sigma^2}{(\mu 1 - \mu 2)^2}$$

$\mu 1 = 18.94$

$\mu 2 = 53.58$

Average Standard Deviation = 59.45

Z Table value = 1.96

Power = 95%

Therefore. n= 76.56

The minimum sample size required for each group of the present study will be 77 and the total sample size is 154.

COLLECTION OF DATA

A total of 77 placentas was collected from >28 weeks of gestational age from pre eclampsia which includes mild, moderate, severe type obeying the criterias of pre eclampsia either through vaginal delivery or cesarean section and 77 placentas from normal antenatal case either through vaginal delivery or cesarean section at R L Jalappa Hospital& Research Centre in Sri Devaraj Urs Medical College, Kolar from September2022 -December2023 was included in this study.

INCLUSION CRITERIA

All patients diagnosed with pre eclampsia > 28 weeks of gestation age complied with criteria of pre eclampsia who had delivery at R L Jalappa Hospital & Research Centre between September 2022-December 2023

EXCLUSION CRITERIA

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

METHODOLOGY

METHOD OF COLLECTION:

1. The placenta was collected immediately after delivery from mild, moderate, severe pre-eclampsia cases and healthy groups and is washed in tap water to eliminate any blood clots.
2. Gross inspection was done noting weight, diameter, thickness, number of cotyledons on the maternal surface, calcification, infarction and umbilical cord vessels and knots.
3. The placenta is sliced at regular intervals of 0.5cm and gross abnormalities were detected (Bread and slice method)
4. The whole specimen was left for fixation in 4% formalin for more than 48 hrs.

SAMPLING PROCEDURE:

1. Five sections were taken from each central and peripheral areas.
2. Additional sections were taken from abnormal areas.
3. Tissue sections 5 micrometre thickness were cut from paraffin embedded blocks and stained by conventional H&E stain.
4. Placental changes were compared with the severity of the pre-eclampsia (mild, moderate and severe) with that of control group.

TISSUE PROCESSING:

Table 1: Tissue processing steps

Formalin	7 Hours
Water	20 minutes
60 % Isopropyl alcohol	1 Hour
70 % Isopropyl alcohol	1 Hour
80 % Isopropyl alcohol	1 Hour
90 % Isopropyl alcohol	1 Hour
100 % Isopropyl alcohol	1 Hour
100 % Isopropyl alcohol	1 Hour
Chloroform I	1 Hour
Chloroform II	1 Hour
Paraffin wax I	1 Hour
Paraffin wax II	1 Hour
TOTAL	**17 Hours 20 Minutes**

After processing the sections were embedded and tissue section of 5 um were cut by the routine histopathological procedure of our department. The slides were stained with hematoxylin and eosin using by following steps:

1. The section was deparaffinized and hydrating through grading of alcohols to water
2. It was kept in Harris hematoxylin for 4 mins
3. Rinsed in running tap water for 5 mins
4. Differentiated in 1% acid alcohol for 5 secs
5. Washed well in Tap water for 5 mins (Until Bluing)
6. Dipped in Eosin for1 min
7. Rinsed in tap water for 5 mins.
8. Dehydrated in grading of alcohols in ascending order.

9. Dipped in Xylene (Clearing)
10. Mounted in DPX

HAEMATOXYLIN AND EOSIN STAINING:

In H&E staining, calcification appeared as areas of dense, thick dark blue staining. Fibrosis is a result from the formation of excess fibrous connective tissue due to chronic inflammation or injury and this is seen as regions of increased collagen deposition. Necrosis is identified as acellular, eosinophilic areas with a loss of tissue architecture. Excessive syncytial knots, clusters of syncytial cells, are indicative of placental hypoxia. Peri-villous edema is seen as widened spaces between the villi, caused due to fluid accumulation.

IMMUNOHISTOCHEMICAL STAINING:

PROTOCOL:

IHC staining was performed on 4% formalin-fixed (fixed for >48hrs at 25 degree Celsius) paraffin-embedded 4 micro meter tissue sections which were taken on coated slides. Tissue sections were deparaffinized in xylene and were rehydrated through a descending ethanol series at room temp for 5 minutes, followed by a wash in distilled water after allowing to cool for 10 mins.

Table 2: Antibody, clone, species used in IHC staining

Antibody	Clone	Species	Producer	Control	Stain
CD 24	Monoclonal	Rabbit	Quartett	tonsil	Membrane

THE PROCEDURE OF IHC IS AS FOLLOWS:

1. Tissue blocks were fixed in 10% formalin were used to create sections that were 3-5 µm thick.
2. These sections were placed on organo silane slides that had a positive charge.
3. Glass slides were incubated overnight at 58 degrees temperature on hot plate.
4. Deparaffinization was conducted by immersing the sections in Xylene I and Xylene II for 15 mins each.

5. Dexylnization was carried out using pure alcohol I and II with each step lasting for 1 min.

6. Dealcoholisation is done by immersing the slides in 90% and 70% alcohol for 1 minute each.

7. The slides were rinsed with distilled water to make sure that the sections did not dry at any point during the staining process.

8. Antigen Retrieval was achieved through enzymatic treatment in a microwave set at power 10 for 6 minutes, using TRIS EDTA buffer at a pH of 6.0, and repeated for two cycles.

9. The slides were rinsed with distilled water for 5 minutes.

10. The sections were washed in Tris Buffer Solution (TBS) at a pH of 7.6 for two consecutive 5-minute periods.

11. A peroxidase block was applied for 10-15 minutes to inhibit endogenous peroxidase enzyme activity.

12. TBS buffer washes were performed for three separate 5-minute intervals.

13. A power block was applied for 10-15 minutes to prevent non-specific reactivity with other tissue antigens.

14. The sections were incubated with a specific primary antibody for 45 minutes to detect tissue markers by antigen-antibody reaction.

15. The sections were rinsed in Tris buffer (pH 7.6) for 5 mins before processing. This was repeated 3 times with gentle agitation to remove unbound antibodies.

16. A super enhancer was introduced for 20 mins to enhance the interaction between the primary and secondary antibodies.

17. TBS buffer washes were performed for 5 mins intervals to remove unbound antibodies.

18. A highly responsive polymer horseradish peroxidase (poly HRP) was applied for 30 minutes to extend the chain and mark the enzyme.

19. The inclusion of DAB for 5-8 minutes leads to the production of a chromogen, leading to antigen coloration.

20. TBS buffer washes were performed for three 5-minute intervals.

21. The sections were rinsed with tap water for 5 minutes, then counterstained with hematoxylin for 1 minute.

22. The slides was dehydrated with 90% alcohol and absolute alcohol for 2 minutes each.

23. The slides were then cleared with a mixture of alcohol and xylene for 2 minutes each.

24. Finally, the slides were mounted with DPX.

Table 3: After the process of IHC the slides were interpreted and documented as below

Basement Membrane	GRADING	
Cytotrophoblast	+	++
Syncytiotrophoblast	+	++
Villous stroma	+	++
Blood vessels	+	++

The expression was graded as

- + = 1
- ++ = 2

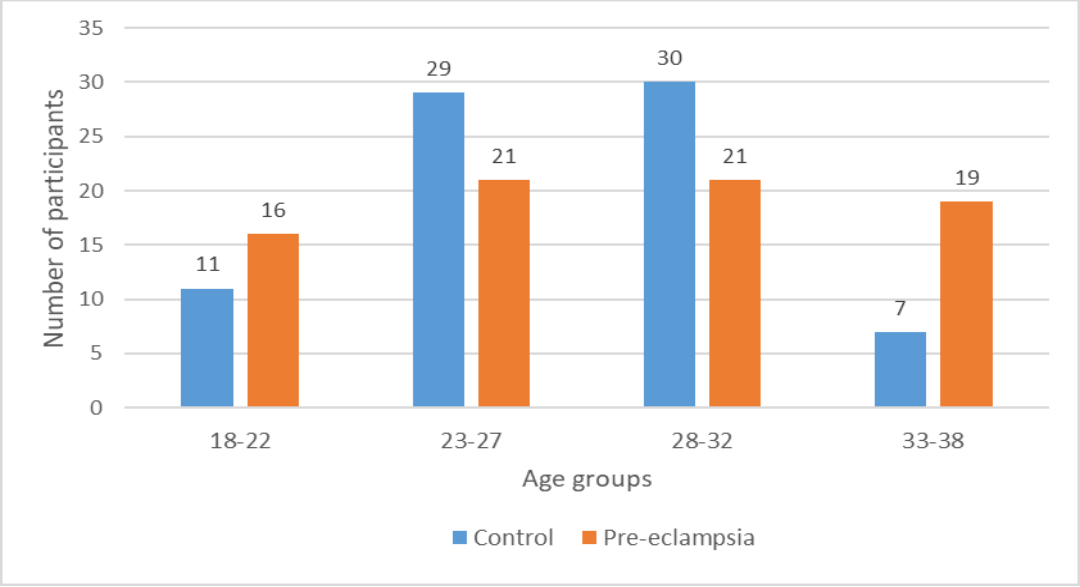
RESULTS

A total of 154 placentas were included out of which 77 (50%) placentas were from normal pregnancy (without pre eclampsia) which formed healthy control group and 77 (50%) placentas were from pregnancy with pre-eclampsia (BP>140/80). Of these 20 cases were of mild preeclampsia, 18 cases of moderate preeclampsia and 39 cases were of severe pre-eclampsia. All the mothers in the control group and the pre-eclampsia group fulfilled the selection criteria.

Table 4: Age categorization among healthy controls and cases groups

Age group	Control (n=77)	Pre-eclampsia (n=77)	Pre-eclampsia		
			Mild (n=20)	Moderate (n=18)	Severe (n=39)
18-22	11	16	4	6	6
23-27	29	21	9	4	8
28-32	30	21	6	6	9
33-38	7	19	1	2	16
Grand Total	**77**	**77**	**20**	**18**	**39**

Chart 1: Age categorization among healthy control groups and cases groups

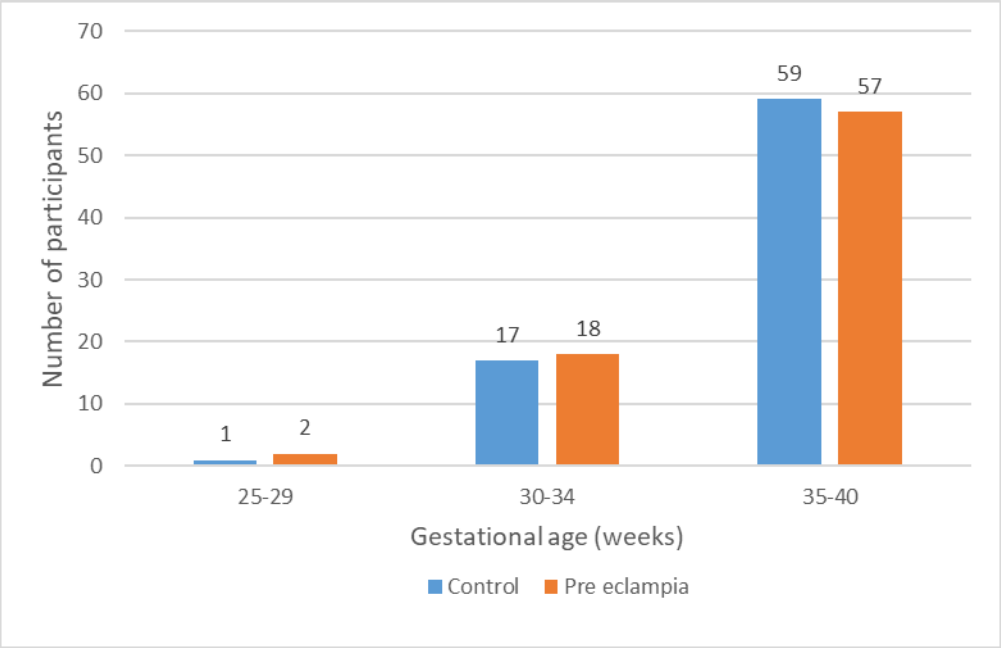


Mean age of the mothers was 27.1 ± 3.8 years in the control group and 27.9 ± 5.3 in pre-eclampsia.

Table 5 : Gestational age among control groups and cases groups

Gestational age	Control (n=77)	Pre-eclampsia (n=80)	Pre-eclampsia		
			Mild (n=20)	Moderate (n=18)	Severe (n=39)
25-29	1	2	0	1	1
30-34	17	18	4	5	9
35-40	59	57	16	12	29
Grand Total	77	77	20	18	39

Chart 2: Gestational age among healthy control and cases groups

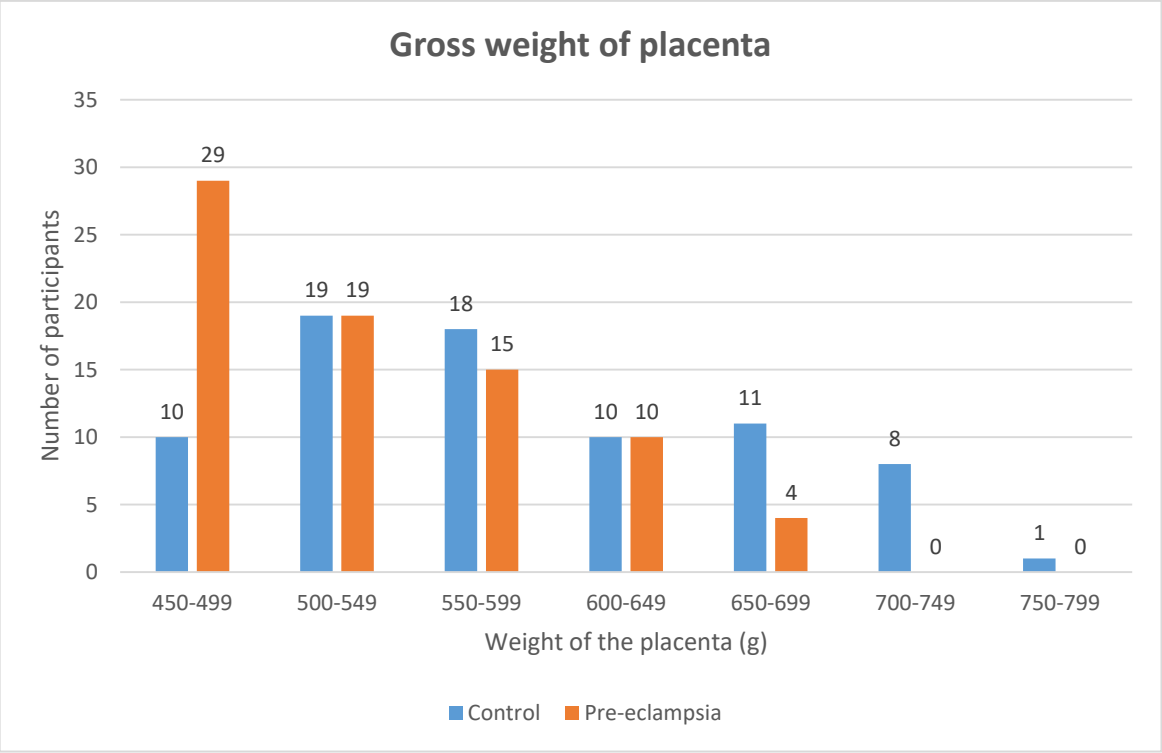


Most of the cases and controls had a gestational age of 28-40 weeks. The median gestational age was 38 weeks (Interquartile range: 4 weeks) in the control group and was 36 weeks (Interquartile range: 4 weeks) in pre-eclampsia cases. The gestational age of the patient was significantly different in case and control groups (Mann Whitney U= 2373.0, p=0.0314).

Table 6: Gross weight of placenta among control groups and cases groups

Gross weight of placenta	Control (n=77)	Pre-eclampsia (n=77)	Pre-eclampsia		
			Mild (n=20)	Moderate (n=18)	Severe (n=39)
450-499	10	29	10	5	14
500-549	19	19	5	6	8
550-599	18	15	4	3	8
600-649	10	10	0	3	7
650-699	11	4	1	1	2
700-749	8	0	0	0	0
750-799	1	0	0	0	0
Grand Total	77	77	20	18	39

Chart 3: Gross weight of placenta among control and cases groups



The weight of the placenta in most of the cases and controls was between 450-499 g and control was between 500-549. The median gross weight of placenta was 560 grams (IQR:140g) in the control group and 550 g (IQR:80g) in pre-eclampsia. There was no significant difference in the gross weights of the placenta in control and pre eclampsia cases (Mann Whitney U= 2610.0, p = 0.2002).

The gross image of placenta is shown in figure 22 and 23

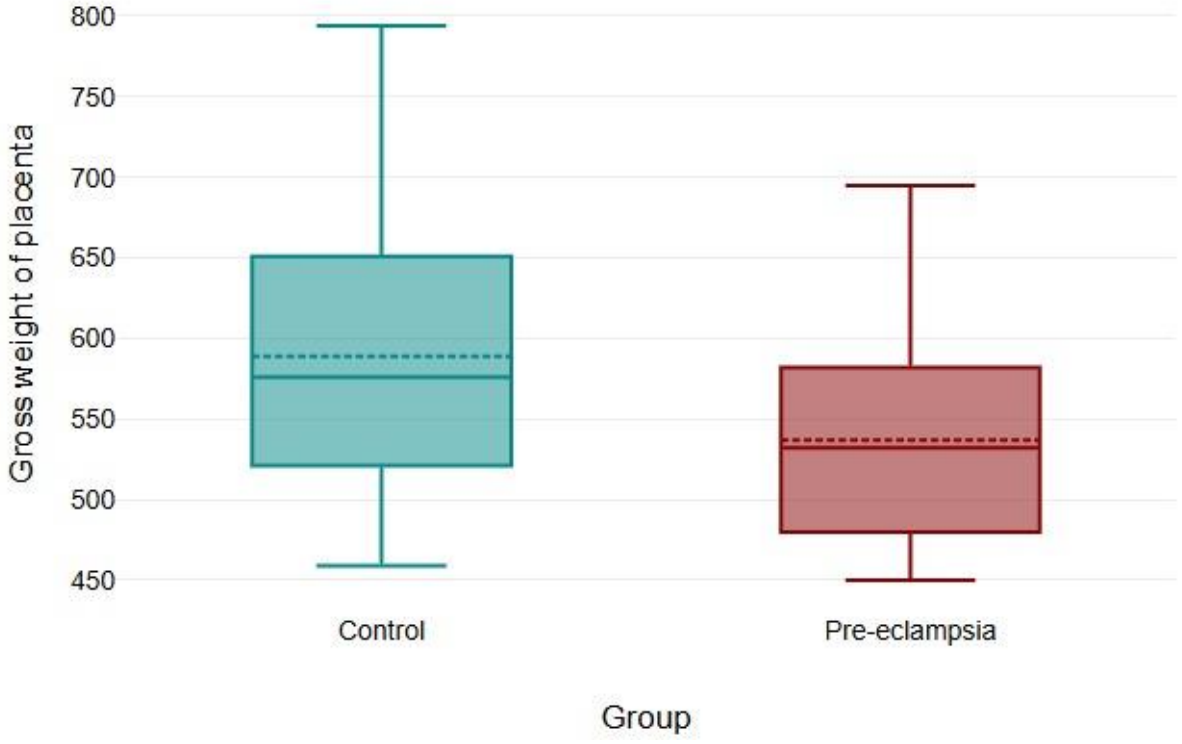
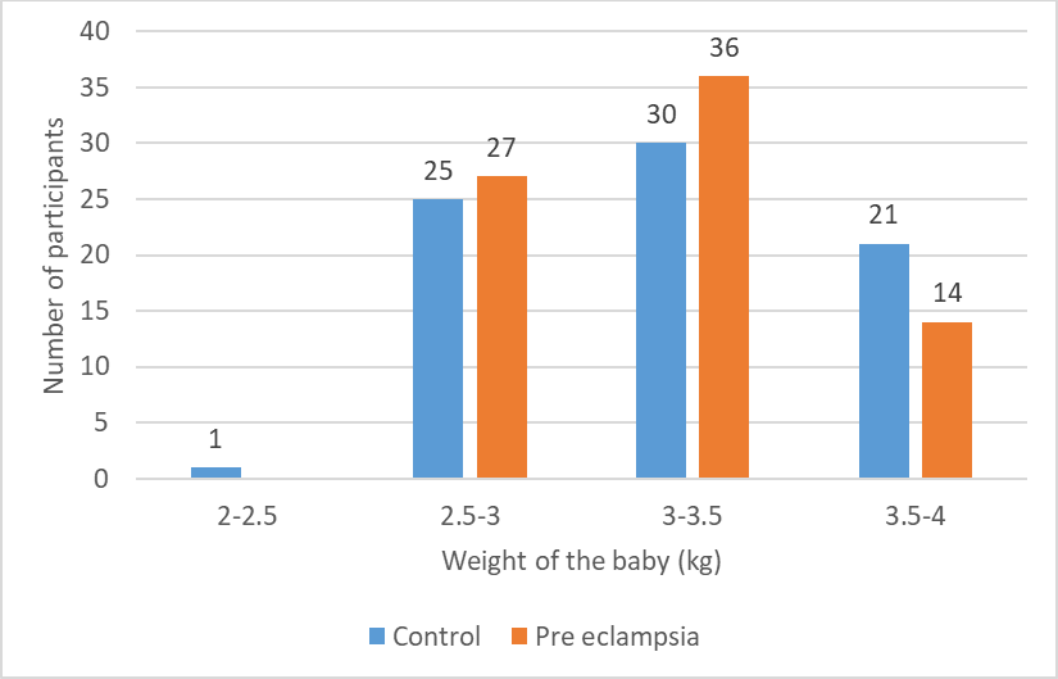


Table 7: Weight of baby among control groups and cases groups

Weight of baby (kg)	Control (n=77)	Pre-eclampsia (n=77)
2-2.5	1	0
2.5-3	25	27
3-3.5	30	36
3.5-4	21	14
Grand Total	77	77

Chart 4: Weight of baby among control and cases groups



Birth weights of most of the babies in cases and control groups were between 3-3.5kg . The median birth weight of baby is 3.2 kgs (IQR= 0.65) in the control group and 3.1 kg (IQR=0.52) in pre-eclampsia. There was no significant difference in the birth weights of the babies in control and pre eclampsia (Mann Whitney U= 2705.00, p=0.348).

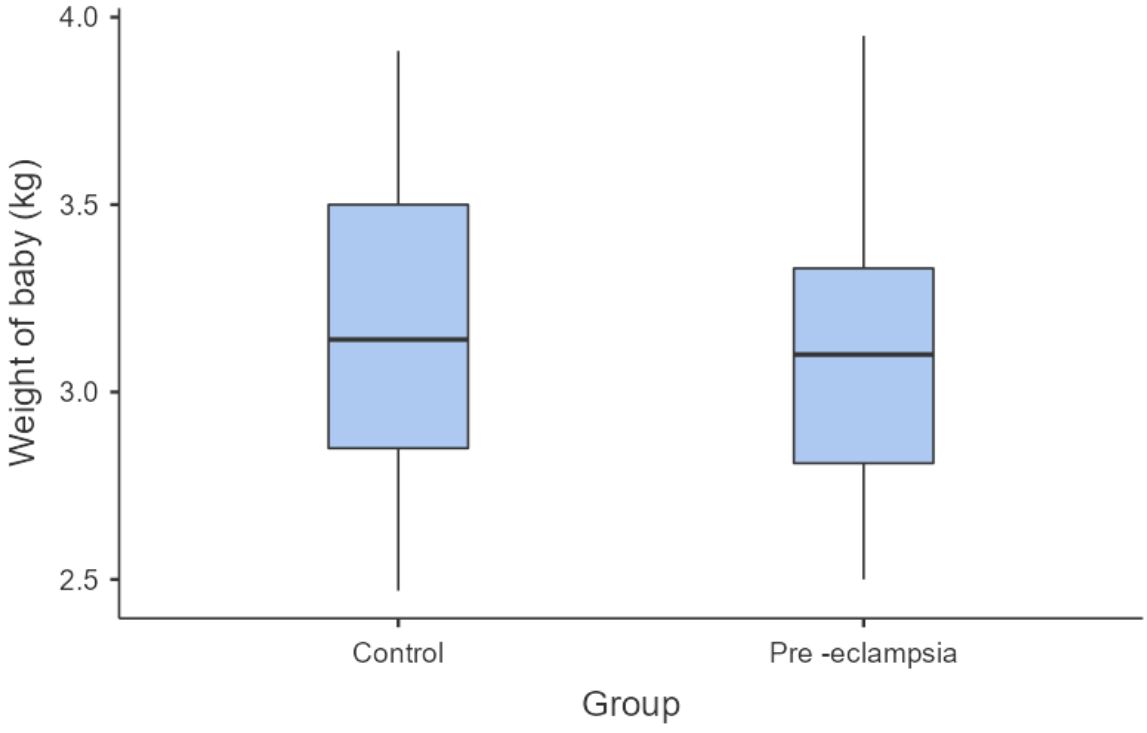
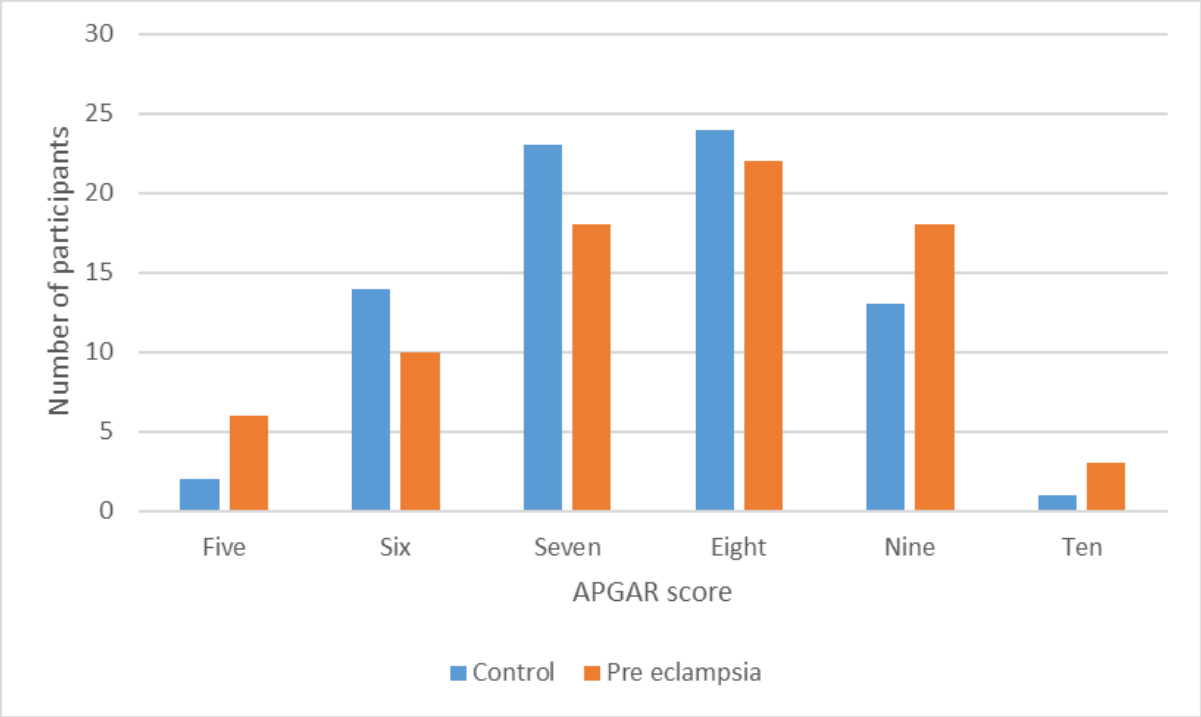


Table 8: Apgar score among control groups and cases groups

Apgar score of babies	Control (n=77)	Pre-eclampsia (n=77)
5	2	6
6	14	10
7	23	18
8	24	22
9	13	18
10	1	3
Grand Total	77	77

Chart 5: Apgar score among control groups and cases groups

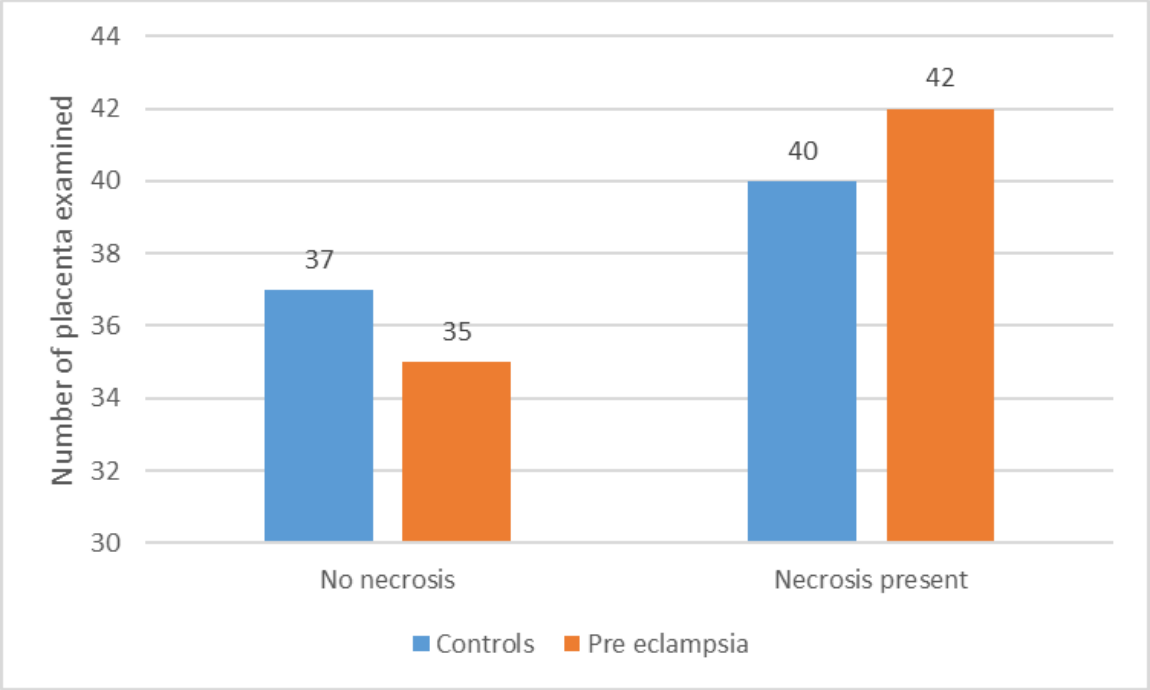


Most of the new born babies had an APGAR score of 7 and above in both the groups.

Table 9: Gross and microscopic findings – Necrosis among cases and control groups

Necrosis	Control (n=77)	Pre-eclampsia (n=77)	Pre-eclampsia		
			Mild (n=20)	Moderate (n=20)	Severe (n=40)
Present	40	42	8	5	29
Absent	37	35	12	13	10
Total	77	77	20	18	39

Chart 6: Necrosis among healthy controls and cases groups

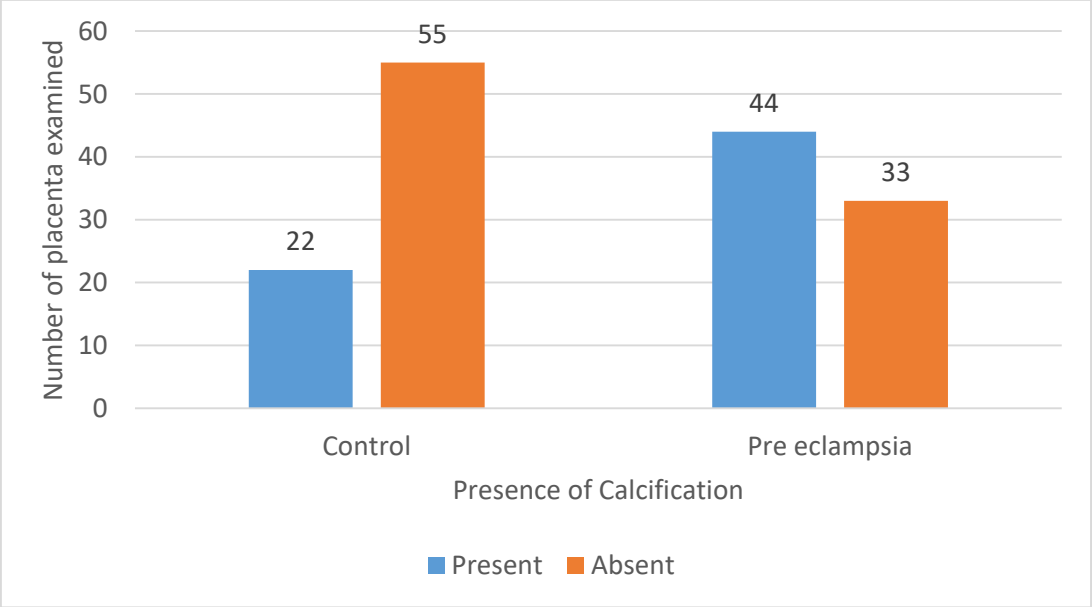


Majority of the cases and controls showed that necrosis. There was no significant difference in the presence of necrosis in cases and controls (χ^2 =0.104, df=1, p=0.747).

Table 10: Gross and microscopic findings – Calcification among cases and healthy control groups

Calcification	Control (n=77)	Pre-eclampsia (n=77)	Pre-eclampsia		
			Mild (n=20)	Moderate (n=18)	Severe (n=39)
Present	22	44	9	8	27
Absent	55	33	11	10	12
Total	77	77	20	18	39

Chart 7: Calcification among controls and cases groups

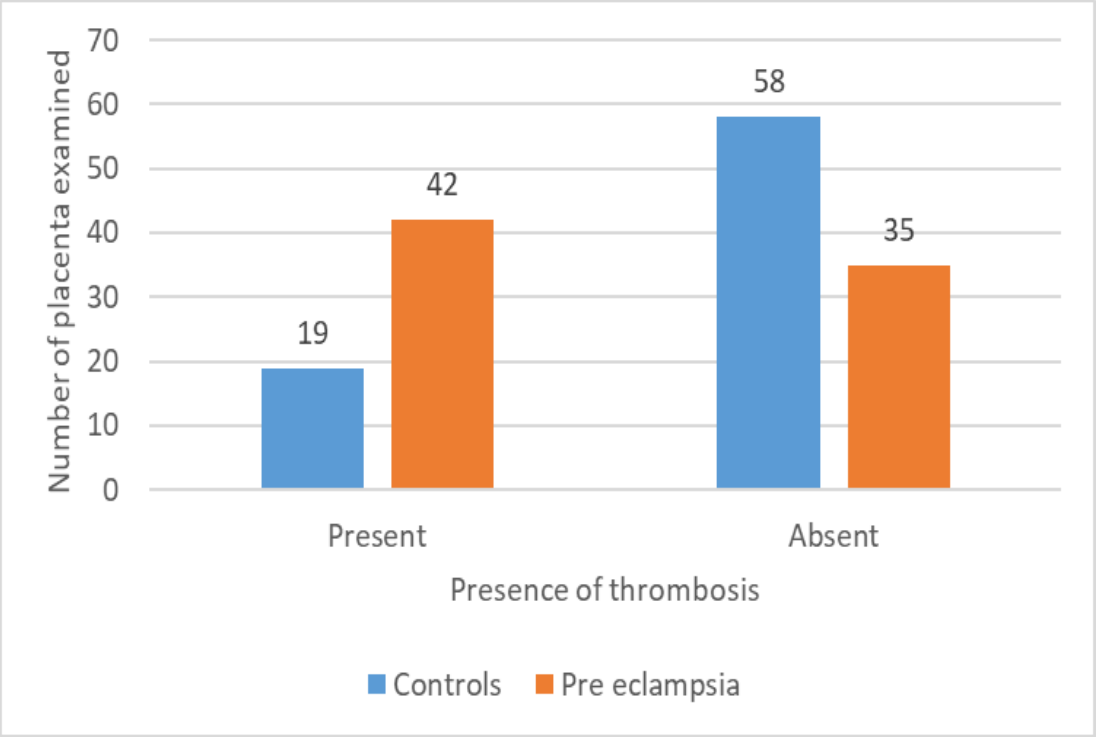


Significantly higher proportion of placenta of participants with pre eclampsia showed calcification compared to the control group (χ^2 =12.833, df=1, p=0.000).

Table 11: Gross and microscopic findings – Thrombosis among cases and control groups

Thrombosis	Control (n=77)	Pre-eclampsia (n=77)	Pre-eclampsia		
			Mild (n=20)	Moderate (n=18)	Severe (n=39)
Present	19	42	7	8	27
Absent	58	35	13	10	12
Total	77	77	20	18	39

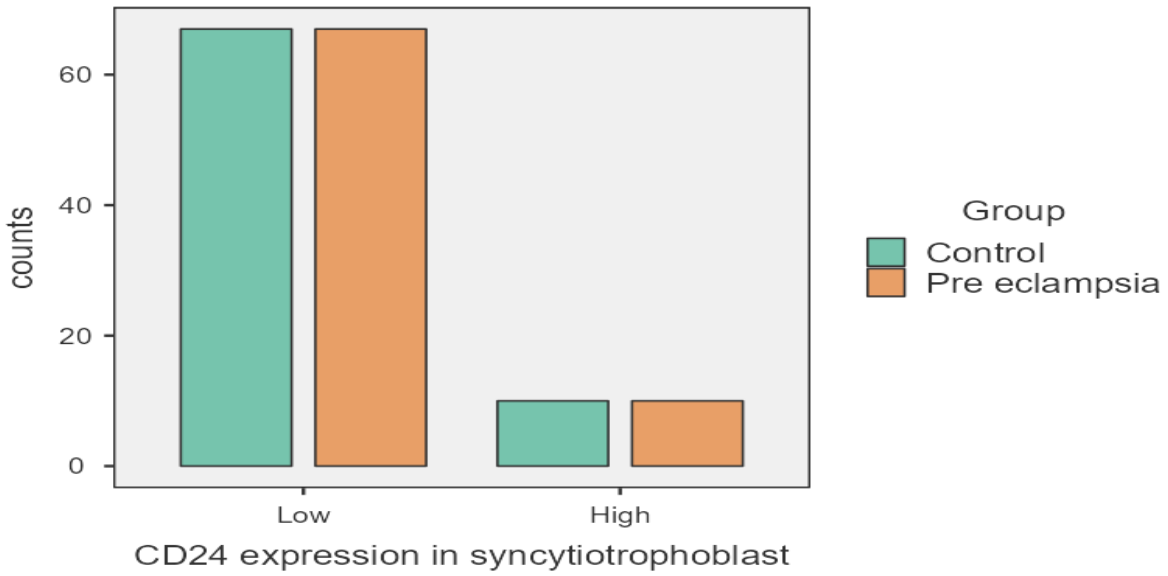
Chart 8: Thrombosis among controls and cases groups



Significantly higher proportion of placenta of participants with pre eclampsia showed thrombosis compared to the control group (χ^2 =14.36, df=1, p=0.000).

Table : 12- Frequencies of CD 24 expression in syncytiotrophoblast

Frequencies of CD24 expression in syncytiotrophoblast			
CD24 expression in syncytiotrophoblast	**Group**	**Counts**	**% within group**
Low	Control	67	87%
	Pre -eclampsia	67	87%
High	Control	10	13%
	Pre -eclampsia	10	13%



High CD24 expression in syncytiotrophoblast was found in 13% of controls, and 13% of cases. This difference was not statistically significant (χ^2 p value= 1).

Table:13 -Expression of CD 24 among control and pre eclampsia group in syncytiotrophoblast

	Severity of Pre eclampsia					
CD24 expression in syncytiotrophoblast	**Control**	**Mild**	**Moderate**	**Severe**	**Total (Pre eclampsia)**	**Grand Total**
Low	67	16	15	36	67	134
High	10	4	3	3	10	20
Grand Total	**77**	**20**	**18**	**39**	**77**	**154**

There was no significant association between the severity of pre eclampsia and degree of CD24 expression in syncytiotrophoblast (Fischer's Exact p value= 0.495)

Low expression and high expression of CD 24 in syncytiotrophoblast in normal is seen in figure 24 and figure 25

Low expression and high expression of CD 24 in syncytiotrophoblast in PE is seen in figure 26 and 27

Chart 9:CD 24 expression among control and pre eclampsia cases in syntiotrophoblast

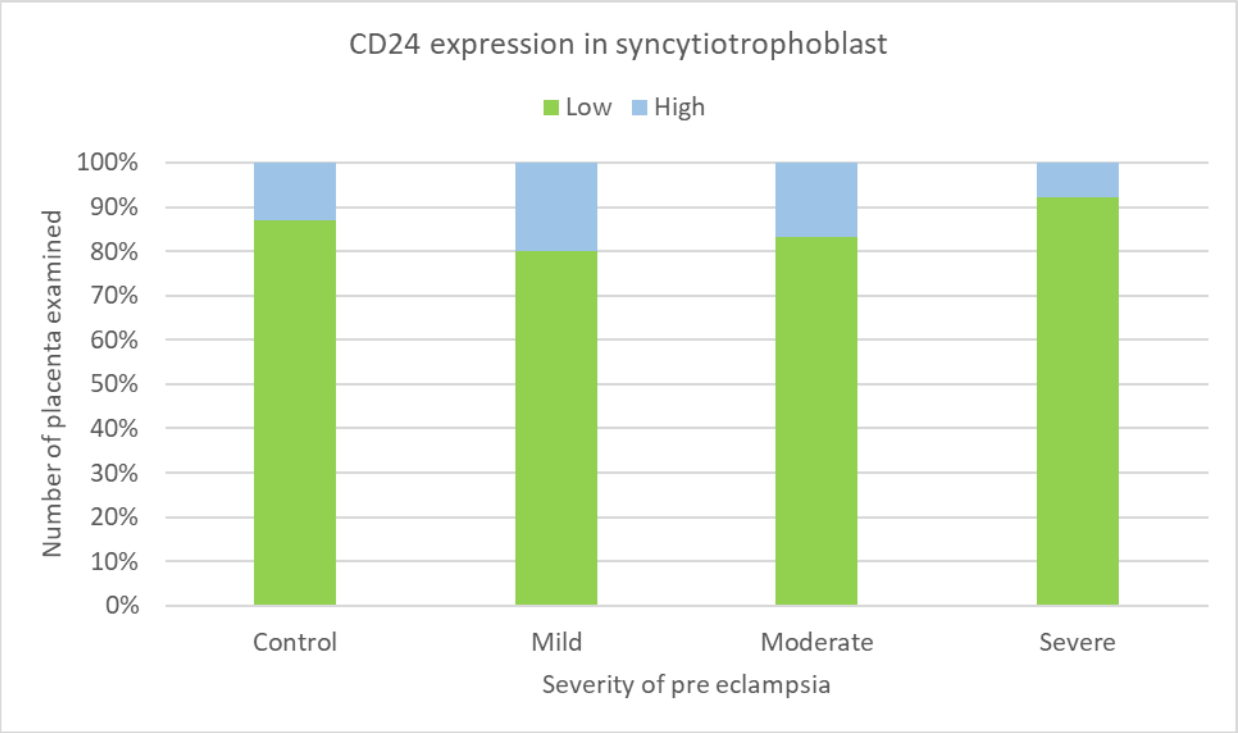
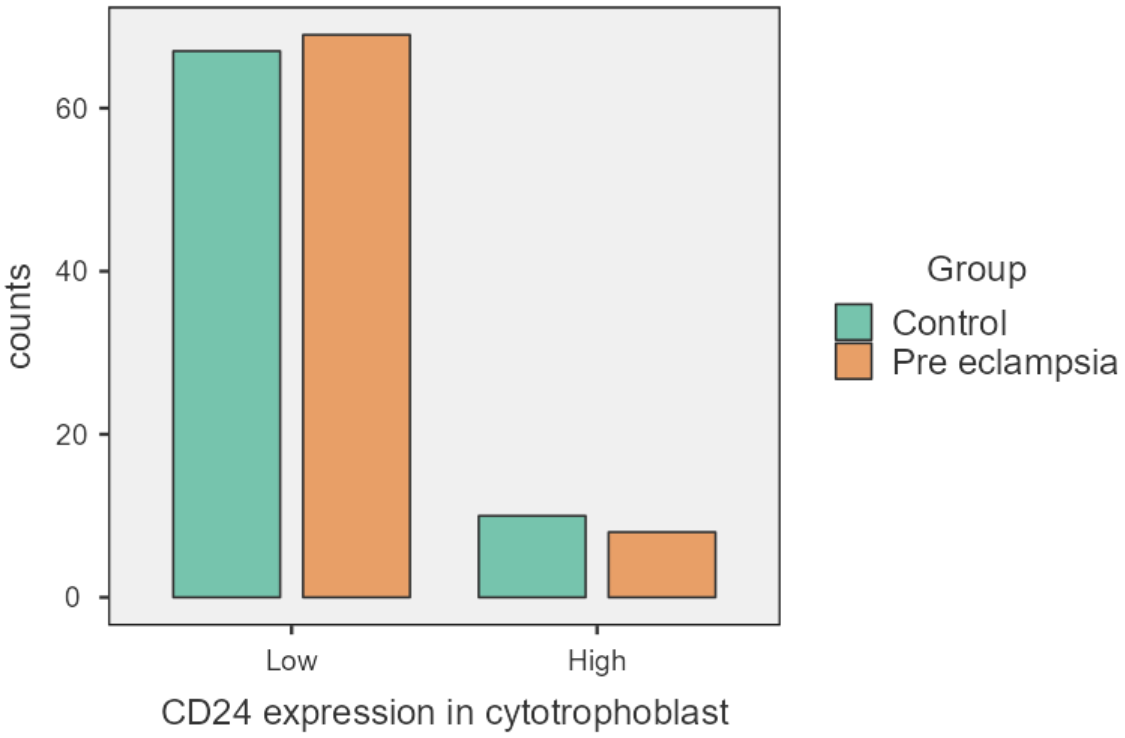


Table : 14- Frequencies of CD 24 expression in Cytotrophoblast

Frequencies of CD24 expression in cytotrophoblast			
CD24 expression in cytotrophoblast	**Group**	**Counts**	**% within group**
Low	Control	67	87%
	Pre -eclampsia	69	90%
High	Control	10	13%
	Pre -eclampsia	8	10%



CD24 expression of varying degrees in cytotrophoblast was found in 13% of controls, compared to 10% of cases. This difference was not statistically significant (χ^2 = 0.252, df=1, p=0.616).

Table:15 -Expression of CD 24 among control and pre eclampsia group in Cytotrophoblast

			Severity of Pre eclampsia (%)			
CD24 expression in cytotrophoblast	**Control**	**Mild**	**Moderate**	**Severe**	**Total (pre eclampsia)**	**Grand Total**
Low	67	17	16	36	69	136
High	10	3	2	3	8	18
Grand Total	**77**	**20**	**18**	**39**	**77**	**154**

There was no significant association between the severity of pre eclampsia and degree of CD24 expression in cytotrophoblast (Fischer's Exact p value= 0.780)

Chart 10:CD 24 among expression control and pre eclampsia cases in Cytotiotrophoblast

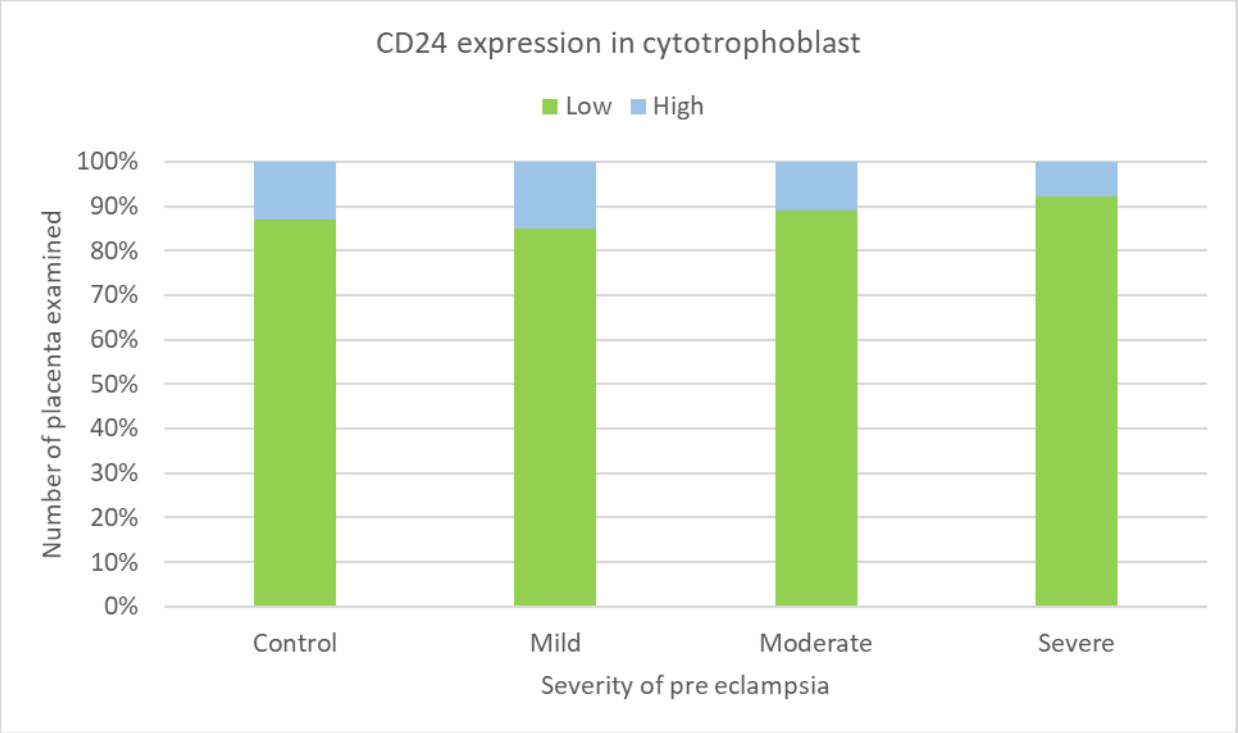
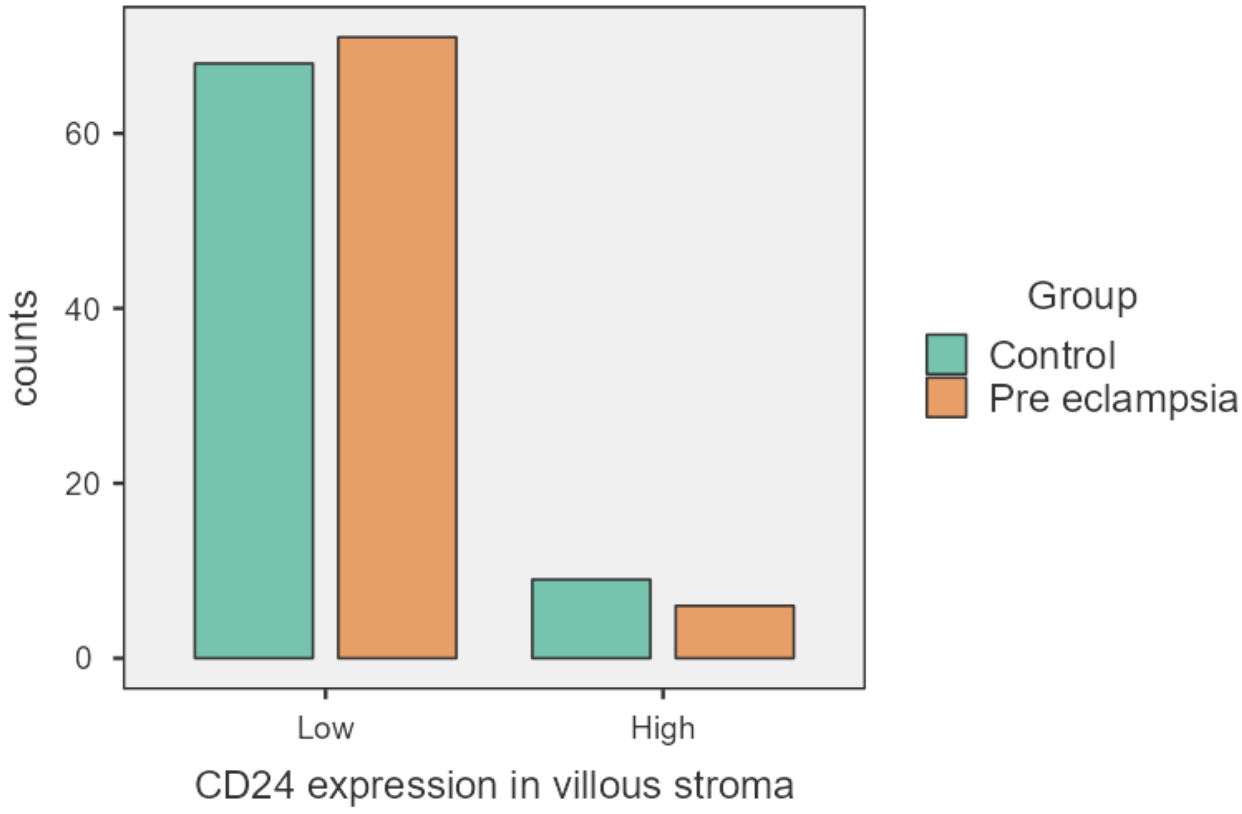


Table : 16- Frequencies of CD 24 expression in Villous stroma

Frequencies of CD24 expression in villous stroma			
CD24 expression in villous stroma	**Group**	**Counts**	**% within group**
Low	Control	68	88%
	Pre -eclampsia	71	92%
High	Control	9	12%
	Pre -eclampsia	6	8%



CD24 expression of varying degrees in villous stroma was found in 12% of controls, compared to 8% of cases. This difference was not statistically significant (χ^2 = 0.665, df=1, p=0.415).

Table:17 -Expression of CD 24 among control and pre eclampsia group in Villous stroma

		Severity of Pre eclampsia				
CD24 expression in villous stroma	**Control**	**Mild**	**Moderate**	**Severe**	**Total (pre eclampsia)**	**Grand Total**
Low	68	18	16	37	71	139
High	9	2	2	2	6	15
Grand Total	**77**	**20**	**18**	**39**	**77**	**154**

There was no significant association between the severity of pre eclampsia and degree of CD24 expression in villous stroma (Fischer's Exact p value= 0.7166)

Chart 11 :CD 24 among expression control and pre eclampsia cases in Villous stroma

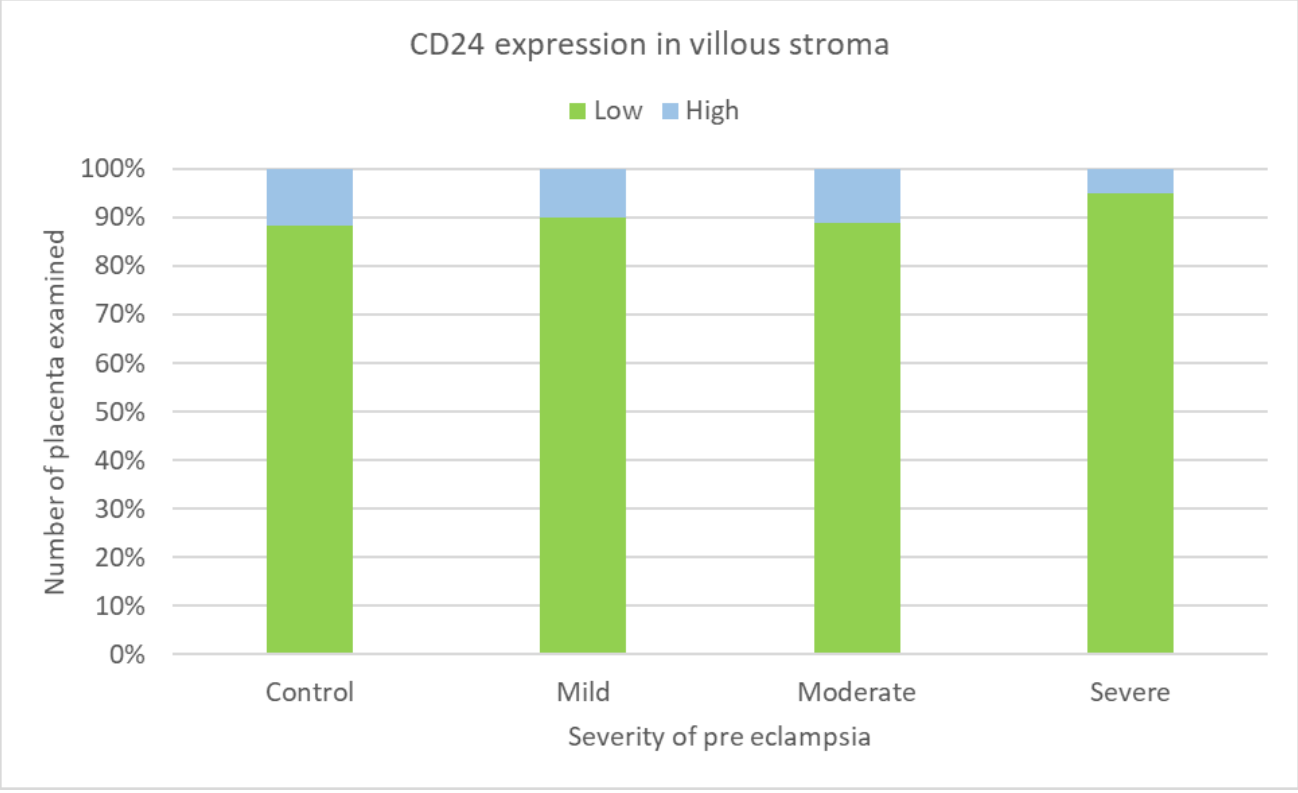
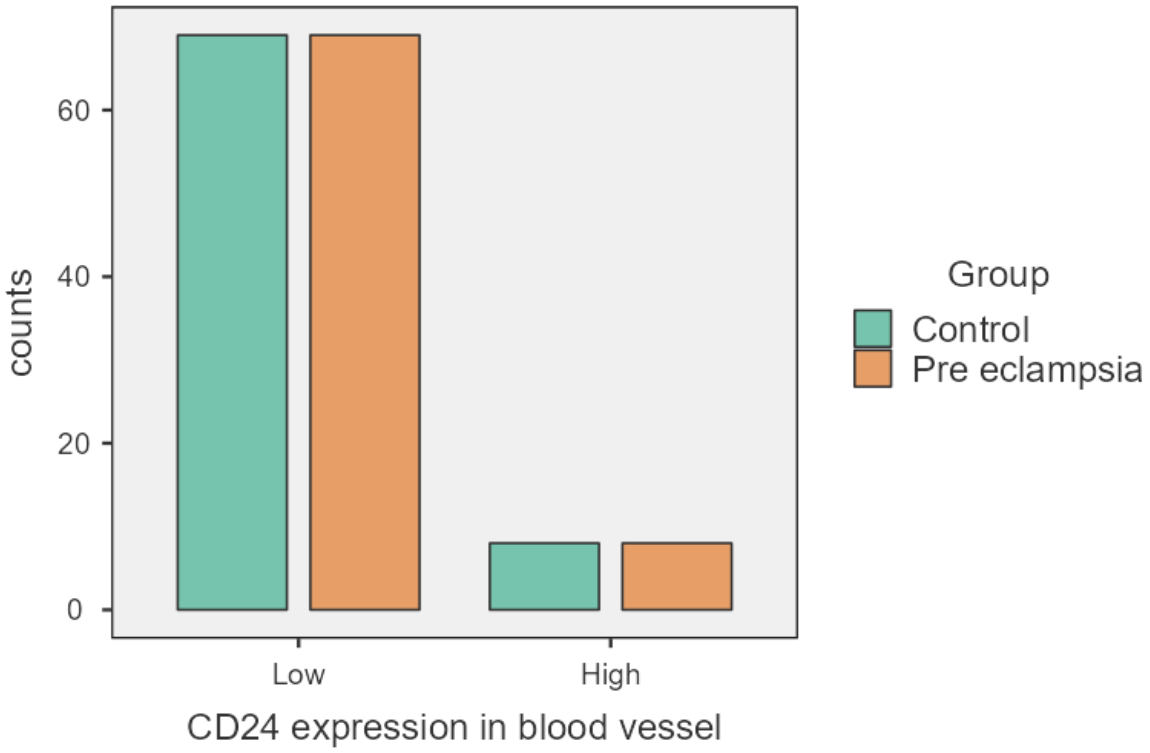


Table :18- Frequencies of CD 24 expression in Blood vessels

Frequencies of CD24 expression in blood vessel			
CD24 expression in blood vessel	**Group**	**Counts**	**% within group**
Low	Control	69	90%
	Pre -eclampsia	69	90%
High	Control	8	10%
	Pre -eclampsia	8	10%



CD24 expression of varying degrees in blood vessels was found in 10% of controls, and 10% of cases. This difference was not statistically significant (χ^2 = 0.000, df=1, p=1.000).

Table:19 -Expression of CD 24 among control and pre eclampsia group in Blood vessels

		Severity of Pre eclampsia				
CD24 expression in blood vessel	**Control**	**Mild**	**Moderate**	**Severe**	**Total (pre eclampsia)**	**Grand Total**
Low	69	17	16	36	69	138
High	8	3	2	3	8	16
Grand Total	**77**	**20**	**18**	**39**	**77**	**154**

There was no significant association between the severity of pre eclampsia and degree of CD24 expression in blood vessel(Fischer's Exact p value= 0.812)

Chart 12 :CD 24 among expression control and pre eclampsia cases in blood vessels

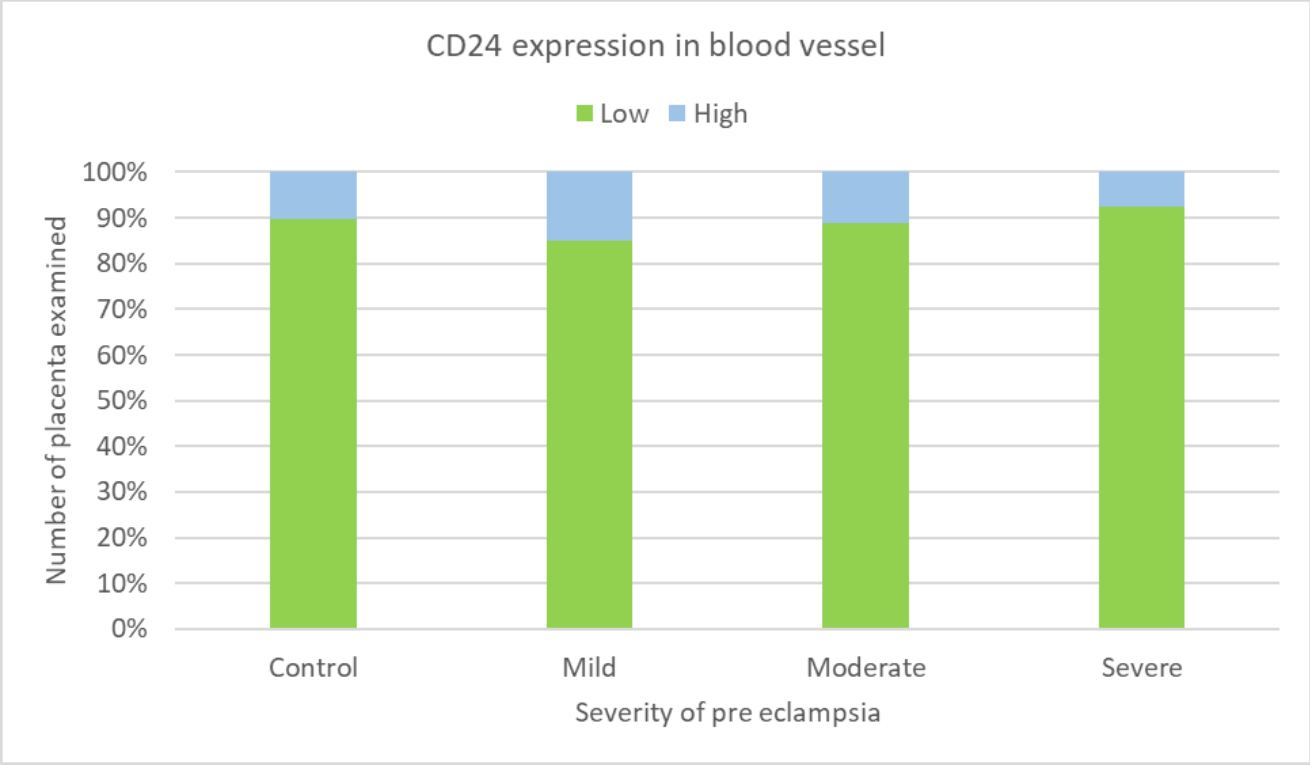


Figure 22: Shows gross image of placenta. The shiny glistening surface is the fetal surface with centrally placed umbilical cord

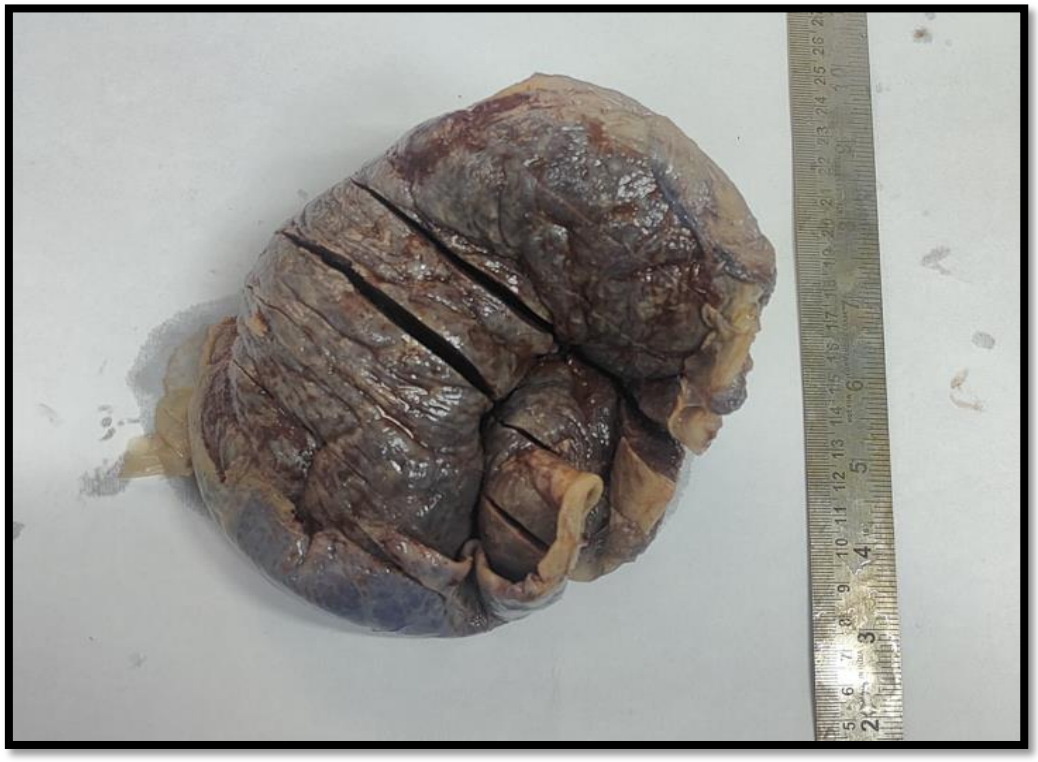


Figure 23: Shows the gross image of placenta. The ragged surface is the maternal surface

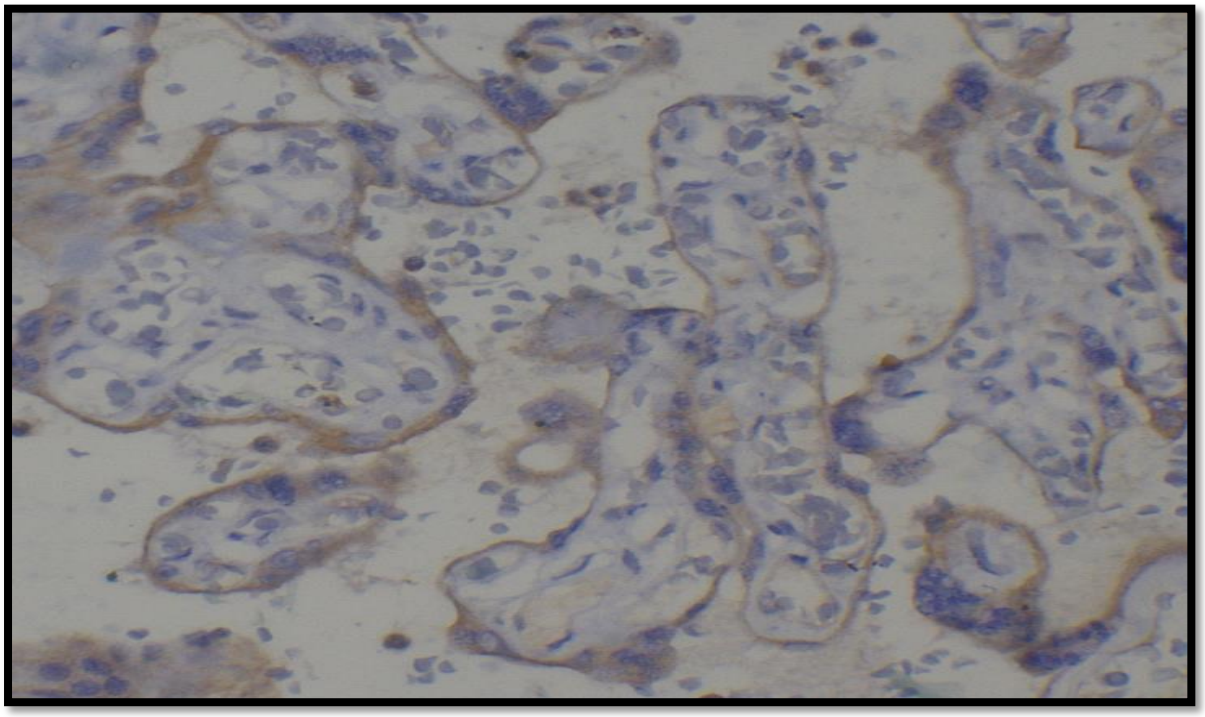


Figure 24: shows low expression of CD 24 in syncytiotrophoblast -normal under 400X magnification

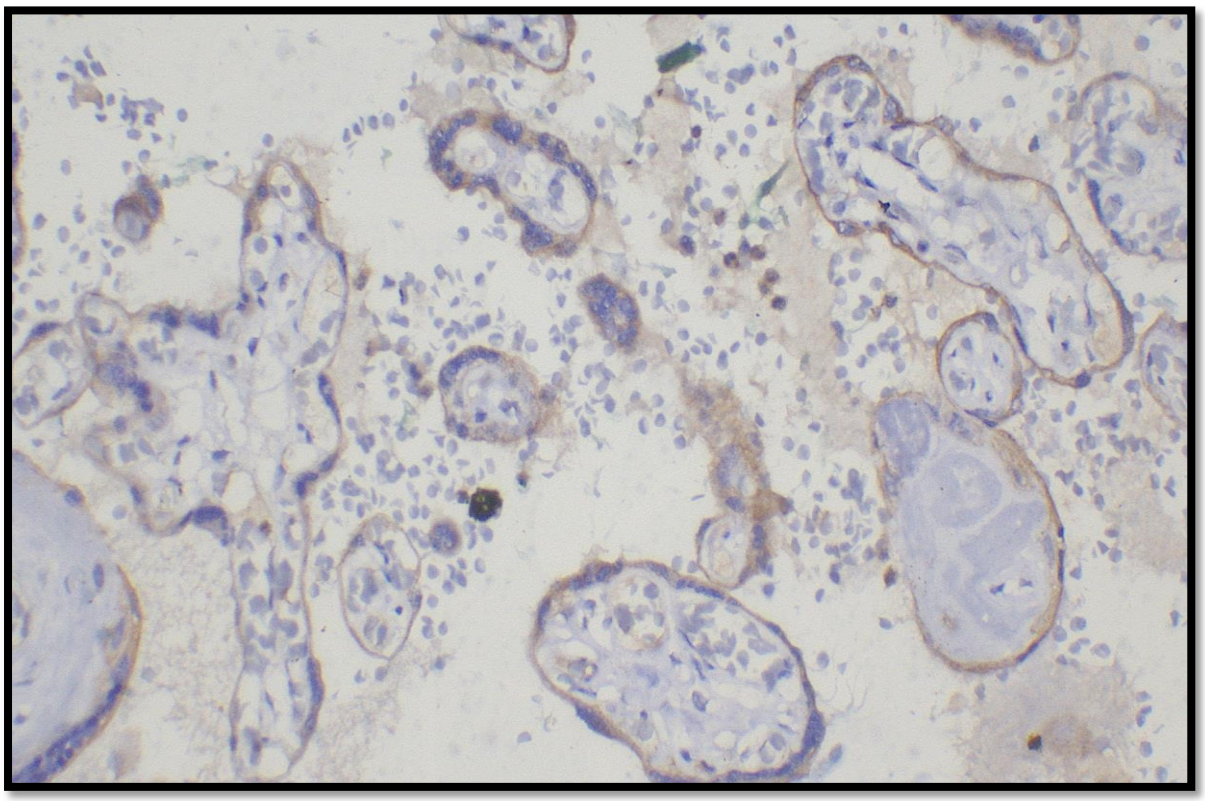


Figure 25 : shows high expression of CD 24 in syncytiotrophoblast - normal under 400X magnification

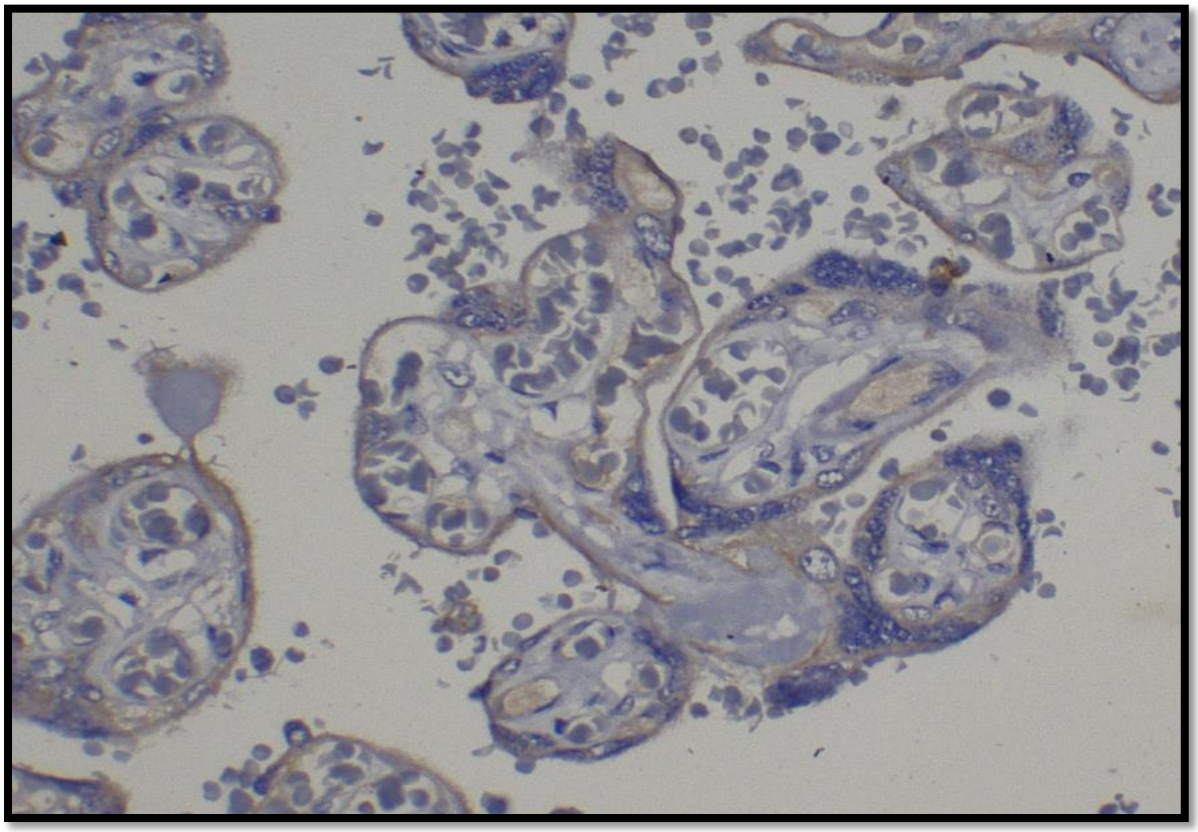


Figure 26: Shows low expression of CD 24 in syncytiotrophoblast- PE under 400X magnification

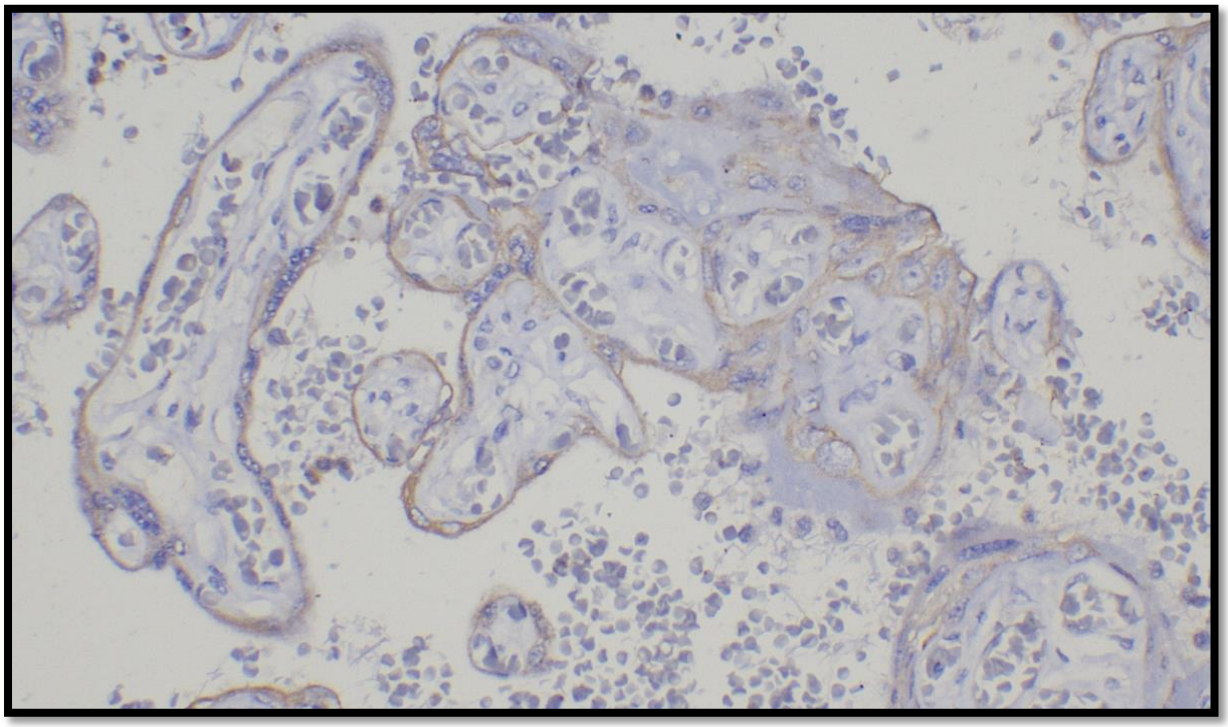


Figure 27: shows high expression of CD 24 in syncytiotrophoblast -PE under 400X magnification

DISCUSSION

The placenta is a specialised organ of pregnancy, performing many important functions. It grows and changes significantly throughout pregnancy, developing alongside the fetus from the early stages till delivery.[55] When the placenta doesn't function properly, it can cause various pregnancy complications, which may have serious effects on both the mother and the baby's health in turn leading to complications one among which is pre eclampsia.[56]

Pre eclampsia is a multifactorial hypertensive disorder of pregnancy and a leading cause of preventable maternal and fetal mortality and morbidity.[1] It is caused by various factors in which one of the mechanism is immunotolerance where CD 24 acts as a natural immunosuppressant by preventing rejection of placenta during pregnancy and supports the growth of placenta. It is also expressed on the surface of hematopoietic cells and diverse tumour cells which functions through its interaction with sialic acid-binding immunoglobulin -type lectins (SIGLECS).This interaction plays a very important role in immunosupression response of placenta .During the first trimester CD24 showes high expression in villous and extravillous cytotrophoblasts, mild expression in stromal cells and negative in syncytiotrophoblasts. Co-localization of CD24 with Siglec-10 was observed in endometrial glands and in first trimester decidual cells in close vicinity to extracellular trophoblasts. The presence of the CD24-Siglec-10 in these regions of fetal-maternal interactions suggests a possible role in mediating immune tolerance at the fetal-maternal interface. Therefore, CD24 reduction has been linked to early pre eclampsia. [57]

In our study, we focused on evaluating the immunohistochemistry expression of CD24 in Syncytitorophoblast, Cytotrophoblast, Villous stroma and Blood vessels of placentas from preeclampsia cases, comparing it with placentas from normal pregnancies. A total of 154 placentas were included in the study, with 77 from normal pregnancies as the control group and 77 from preeclampsia cases which were further divided into mild, moderate, and severe categories of preeclampsia. The other parameters that were considered for comparsion included the age group of the participants, gestational age at delivery, and the gross weight of the placenta. Additionally, we examined the Apgar scores of the babies to assess neonatal health. The study also included both gross and microscopic findings, features of necrosis, thrombosis, and calcification in the placental tissues.

In our study, the majority of participants in both the case and control groups were in the age range of 28-32 years, but most participants with severe preeclampsia were in the 33-38 years age group. In a study done by **Malames** et al in which the age range was between 18-40year. [58]Another study by **Ocal E et al** showed age group of 27-36 years in control group and 26-48 years in of pre eclampsia patient. [59] **Kishwara et al** found age of the mother was 21-31 years in both control and Pre eclampsia group. [60] Though not statistically significant as increase trend of severe pre eclampsia in older individuals seen in our study is due to fact that planning pregnancy later in life, hence study of pathogenesis of pre eclampsia becomes relevant to the present scenario

Regarding gestational age, most participants in both the case and control groups were between 35 and 40 weeks of gestation, and median gestational age was 38 weeks in control group and was 36 weeks in pre eclampsia cases, with a higher prevalence of severe preeclampsia observed in this age range thus suggest that pre-eclampsia is seen more in third trimester of pregnancy in our study .The gestational age of the patient was significantly different in case and control groups as compared to study done by **CR Gore** shows gestation age of 38-40 weeks in control group and 39 weeks in pre eclampsia group. [61]Another study by **Kishwara et al.** shows the mean gestational age of the mother was 37-39 weeks and 35-37weeks in control and pre eclampsia respectively. [60]This shows that female with pre eclampsia in our population deliver at an earlier gestational age hence there is a need for early diagnosis and comprehensive targeted treatment of these patients.

The normal weight range for the placenta is between 400 and 750 grams. The majority of cases in our study's control had 500-549 and pre eclampsia groups had placental weights between [62]450 and 499 grams with higher incidence of severe pre eclampsia observed in this group , and there was no significant difference between the gross weights of the placentas in the control and pre eclampsia groups. When compared with study done by **Rohini Motwani** shows mean weight of the placenta was significantly lower in pregnancy induced hypertension groups is 395gms than in the control group is 462gms.[63] In another study done by **Punia N et al** showed placental weight of 482 gms in pregnancy induced hypertension and control shows placental weight of 476 gms and here weight of placenta in case group and control are almost similar. The overall reduction in weight of placenta in pre eclampsia can be related to hypoxic changes.

The baby's birth weight ranged from 3 to 3.5 kg for the cases and control groups, with a median weight of 3.2 kg for the control group and 3.1 kg for the pre-eclampsia group which is within normal range. Babies in control and pre-eclampsia had significantly different birth weights. When compared with study done by **Punia N et al** shows birth weight 2.5 kg in pregnancy induced hypertension and 2.8 kg in control group.[62] Another study done **CR Gore et al**. shows mean birth weight in pregnancy induced hypertension is 2.5 kg and control is 2.8 kg indicating low birth weight in pregnancy induced hypertension.[61] Regarding Apgar scores, we found that an Apgar score of 7 and above was seen in a larger number of participants in both the case and control groups when compared with study done by **Uzunov AV** most of the newborns from adolescents with preeclampsia had an Apgar score of 9 and control group the majority of newborns of adults with pre eclampsia had an Apgar score of 7. [64]The variation of weight may be due to geographical and cultures differences

On comparing the gross and microscopic features of the placentas, necrosis seen in pre eclampsia cases and control group Whereas in study done by **CR Gore et al.** shows infarction in 1 case out of 30 in control group and 20 cases out 30 in Pregnancy induced hypertension and calcification in 11 cases out of 30 in control group and 26 cases out of 30 in pregnancy induced hypertension .

CD 24 expression in Syncytiotrophoblast

67 cases of control group and 67 cases of pre eclampsia had low expression of CD 24 and 10 cases of control group and 10 cases of pre eclampsia had high expression. Out of 67 cases of low expression in pre eclampsia, 36 of them had severe pre eclampsia ,15 had moderate pre eclampsia, 16 had mild pre eclampsia. A study done by **Sammar M** in pre term and normal term placentas showed variable levels of cytoplasmic staining. In same study consisting of early pre eclampsia and late pre eclampsia cases showed slight syncytiotrophoblast staining seen in early pre eclampsia group and extremely high staining was seen in late pre eclampsia cases.[65]

Cytotrophoblast

67 cases of control group and 69 cases of pre eclampsia had low expression of CD 24 and 10 cases of control group and 8 cases of pre eclampsia had high expression. Out of 69 cases of low expression in pre eclampsia, 36 of them had severe pre eclampsia,16 had moderate pre eclampsia, 17 had mild pre eclampsia. In a study done by **Sammar M** in both pre term and

normal term placentas (control group) showed trophoblastic staining while early pre eclampsia and late pre eclampsia cases showed strong villous trophoblast staining, moderate staining in pre term cases of pre eclampsia.[65]

Villous stroma

68 cases of control group and 71 cases of pre eclampsia had low expression of CD 24 and 9 cases of control group and 6 cases of pre eclampsia had high expression. Out of 71 cases of low expression in pre eclampsia, 37 of them had severe pre eclampsia ,16 had moderate pre eclampsia, 18 had mild pre eclampsia .In a Study done by **Sammar M** showed light staining of CD 24 in the villous stroma.[65]

Blood vessels

69 cases of control group and 69 cases of pre eclampsia had low expression of CD 24 and 8 cases of control group and 8 cases of pre eclampsia had high expression. Out of 69 cases of low expression in pre eclampsia, 36 of them had severe pre eclampsia ,16 had moderate pre eclampsia, 17 had mild pre eclampsia .In a Study done by **Sammar M** showed light staining of CD 24 in the blood vessels.[65]

Expression of CD 24 in Syncytitorophoblast, Cytotrophoblast, Villous stroma and Blood vessels is quite enigmatic. Various studies have been done to look into CD24 expression in various trimesters using various techniques. In study done by **Nagy B** using RT-PCR opined that significantly low levels of CD 24 expression was in pre eclampsia cases and high expression was in control group. These findings were consistent with our findings of low expression of CD 24 in pr eclampsia using IHC.[54]Anothe study by **Sammar M** using Invitro models by qRT- PCR and Western blot analysis which also showed reduced expression of CD 24 in pre eclampsia cases.[66]

Another study regarding role of CD24 in pre eclampsia has revealed that no expression of CD 24in syncytiotrophoblast and variable expression of same molecule in cytotrophoblast, stroma and blood vessels was in first trimester placentas using Immunohistochemistry.[57]

In a study done by, **Sammar M** using Western blot analysis to identify CD24 protein in placental tissue lysates from preterm preeclamptic controls and term delivery controls. These immunoblots showed diffuse bands of CD24 with molecular weights between 30 and 60 kDa.

Semi-quantitative research utilizing densitometry revealed that CD24 protein expression was considerably lower in the placentas of preterm PE patients than in term controls.[65]

The patho mechanism action of CD24 is quite complex as CD24 requires siglec-10 for its action .This has been explained by a study **Lin H** who demonstrated that serum levels of siglec-10and CD24elevated in early pregnancy and also in missed abortion ,however the loss of CD24 In pre eclampsia may be the reason for loss of immunosuppression.[67] Invitro models of Pre eclampsia using BeWo cells and JEG-3 cells with overexpressing STOX1 and mutant variants of STOX1 A and STOX1 B lead to reduction of CD24/siglec-10 complex expression, when extracellular vesicles were added to BeWo cells ,expression of CD 24 increased.[66]

These variations of CD 24 in pre eclampsia placenta has been well documented in various studies using different methods. However the low expression of CD 24 in our study both in control and cases leads to another hypothesis that low CD 24 expressio may lead to loss of immnosupression that may induce labourin normal preganancies. However more studies are needed in this regard.

LIMITATIONS OF OUR STUDY

1. Our study used only Immunohistochemistry, instead we could have used simultaneously western blot and PCR technique
2. It is conducted only on term pre eclampsia cases
3. CD 24 as it acts through siglec-10, we could have used siglec-10 for immunohistochemistry

CONCLUSION

Pre eclampsia is evolving as an important pregnancy complication as it is multifactorial in nature and linked to many factors like immunotolerance. CD 24 marker of immunotolerance is low in syncytiotrophoblast, cytotrophoblast, blood vessels and villous stroma of both pre eclampsia and normal placenta. More studies using CD24 -Siglec-10 markers is needed to confirm the same.

Our study has used only IHC for CD 24. The physiochemical properties of CD 24 such as its solubility, chemical reactions with various reagents used in histopathology, is not well established. Hence use of fresh tissue and tests such as western blot, PCR techniques may establish better results.

Further it is proved that CD 24 need Siglec-10, STOX 1A and STOX 1B for it expression studies using multiple molecules with better techniques is advisable.

SUMMARY

1. A total of 154 placenta were studied. 77 from pre eclampsia group and 77 from control group. Preeclampsia group was further divided into mild pre-eclampsia(20), moderate(18) and severe pre-eclampsia (39)
2. Most of our preeclampsia cases were in the age group of 33-38 years and control cases were in 28-32 years
3. The gestational age was 35-40 weeks in both pre eclampsia and control group and weight of baby was 3-3.5 kg in both control and pre eclampsia group
4. Low expression of CD 24 was seen in majority of cases in pre eclampsia and control group in syncytiotrophoblast, cytotrophoblast, villous stroma and blood vessels

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PATIENT PROFORMA

Name:
Age:

Hospital Number:

Biopsy Number:

Chief Complaint:

History of Presenting Illness:

Past History:

Personal History:

Local Examination:

Histopathological Diagnosis:

Gross:

Microscopy:

Mild/Moderate/Severe Pre-eclampsia:

Proteinuria:

Weight of Baby:

APGAR Score:

INFORMED CONSENT FORM

STUDY TITLE: IMMUNOHISTOCHEMICAL EXPRESSION OF CD24 IN CYTOTROPHOBLAST ,SYNCYTIOTROPHOBLAST ,BLOOD VESSELS ,VILLOUS STROMA IN PLACENTA OF PRE ECLAMPSIA

I, _____have read or have been read to me the patient information sheet and understand the purpose of the study, the procedure that will be used, the risk and benefits associated with my involvement in the study and the nature of information will be collected and disclosed during the study.

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

I, the undersigned, agree to participate in this study and authorize the collection and disclosure of my personal information for the dissertation.

Name and signature / thumb impression Date:

Place:

Name and signature / thumb impression Date:

Place:

(Witness/Parent/ Guardian/ Husband)

ತಿಳಿಸಲಾದಒಪ್ಪಿಗೆನಮೂನೆ

ಅಧ್ಯಯನದಶೀರ್ಷಿಕೆ: ಪ್ರೀಎಕ್ಲಾಂಪ್ಸಿಯಾಜರಾಯುವಿನ (placenta)ದಲ್ಲಿನಸಿಂನ್ಸಿಷಟಿಯೋಫೋಬ್ಲಾಸ್ಟ್ , ಸೈಟೊಟಿಯೋಫೋಬ್ಲಾಸ್ಟ್, ರಕ್ತನಾಳಗಳುಮತ್ತುವಿಲಸೊಸ್ಟ್ರೀಮಾಧಲ್ಲಿ ಇಮ್ಯುನೊಹಿಸ್ಟೋಕೆಮಿಸ್ಟ್ರಿಬಳಸಿ CD24 ಅಭಿವ್ಯಕ್ತಿಯನ್ನುಕಂಡುಹಿಡಿಯುವುದು.

ನಾನು ಮಾಹಿತಿ ಹಾಳೆಯನ್ನು ಓದಿದ್ದೇನೆ ಅಥವಾ ನನಗೆ ಓದಿತಿಳಿಸಿದ್ದಾರೆ ಮತ್ತು ಅಧ್ಯಯನದ ಉದ್ದೇಶ, ಬಳಸಲಾಗುವವಿಧಾನ,ಅಧ್ಯಯನದಲ್ಲಿ ನನ್ನಪಾಲ್ಗೊಳ್ಳುವಿಕೆಗೆಸಂಬಂಧಿಸಿದಅಪಾಯಮತ್ತುಪ್ರಯೋಜನಗಳುಮತ್ತುಮಾಹಿತಿಯಸ್ವರೂಪವನ್ನುಸಂಗ್ರಹಿಸಲಾಗುತ್ತದೆಮತ್ತುಅಧ್ಯಯನದಸಮಯದಲ್ಲಿಬಹಿರಂಗಪಡಿಸಲಾಗುತ್ತದೆ.ಅಧ್ಯಯನದ ವಿವಿಧಅಂಶಗಳಿಗೆಸಂಬಂಧಿಸಿದಂತೆನನ್ನಪ್ರಶ್ನೆಗಳನ್ನುಕೇಳಲುನನ್ನಅವಕಾಶವನ್ನುನಾನುಹೊಂದಿದ್ದೇನೆಮತ್ತುನನ್ನಪ್ರಶ್ನೆಗಳಿಗೆನನ್ನತೃಪ್ತಿಗೆಉತ್ತರಿಸಲಾಗಿದೆ.ಈಕೆಳಗೆಸಹಿಮಾಡಿರುವನಾನುಈಅಧ್ಯಯನದಲ್ಲಿಭಾಗವಹಿಸಲುಒಪ್ಪಿರುತ್ತೇನೆಮತ್ತುಪ್ರಬಂಧಕ್ಕಾಗಿನನ್ನವೈಯಕ್ತಿಕಮಾಹಿತಿಯಸಂಗ್ರಹಣೆಮತ್ತು ಬಹಿರಂಗಪಡಿಸುವಿಕೆಯನ್ನುಅಧಿಕೃತಗೊಳಿಸುತ್ತೇನೆ.

ಹೆಸರುಮತ್ತುಸಹಿ / ಹೆಬ್ಬೆರಳಿನಗುರುತು

ಸ್ಥಳ:

ದಿನಾಂಕ

ಹೆಸರುಮತ್ತುಸಹಿ / ಹೆಬ್ಬೆರಳಿನಗುರುತು

(ಸಾಕ್ಷಿ/ಪೋಷಕ/ಗುರು/ಪತಿ)

ಸ್ಥಳ:

ದಿನಾಂಕ

ಮತ್ತಷ್ಟುಸ್ಪಷ್ಟೀಕರಣಕ್ಕಾಗಿನೀವುಅಧ್ಯಯನಶೋಧಕವನ್ನುಸಂಪರ್ಕಿಸಬಹುದು

ಡಾIIದೀಪಿಕಾಸಿ

PATIENT INFORMATION SHEET:

STUDY TITLE: IMMUNOHISTOCHEMICAL EXPRESSION OF CD 24 IN CYTOTROPHOBLAST ,SYNCYTIOTROPHOBLAST ,BLOOD VESSELS ,VILLOUS STROMA IN PLACENTA OF PRE ECLAMPSIA

PLACE OF STUDY: Department of Pathology, Sri Devaraj Urs Medical College, Kolar.

The main aim of the study is to find the immunohistochemistry expression of CD24 in cytotrophoblast ,syncytiotrophoblast ,blood vessels,villous stroma in placenta of pre eclampsia .The specimens will be collected from the department of pathology, SDUMC,Kolar. This study will be approved by the institutional ethical committee. The information collected will be used only for dissertation and publication. There is no compulsion to agree to participate. You are requested to sign / provide thumb impression only if you voluntarily agree to participate in the study. All information collected from you will be kept confidential and will not be disclosed to any outsider. Your identity will not be revealed. You will not receive any monetary benefits to participate in this research. This informed consent document is intended to give you a general background of study. Please read the following information carefully and discuss with your family members. You can ask your queries related to study at any time during the study. If you are willing to participate in the study you will be asked to sign an informed consent form by which you are acknowledging that you wish to participate in the study and entire procedure will be explained to you by the study doctor. You are free to withdraw your consent to participate in the study any time without explanation and this will not change your future care.

For any clarification you are free to contact the investigator.

All the cost incurred for collection of data, performing the immunohistochemistry tests, analysis, printing publication will be borne by the post graduate student (**Dr. Deepika C**)

PRINCIPAL INVESTIGATOR: Dr Deepika C. Mobile No: +918197599325

ರೋಗಿಯಮಾಹಿತಿಹಾಳೆ:

ಕ್ರಮಸಂಖ್ಯೆ :

ರೋಗಿಯಹೆಸರು :

ಮೊಬೈಲ್ನಂಬರ್ :

ಅಧ್ಯಯನದಶೀರ್ಷಿಕೆ: ಪ್ರೀಎಕ್ಲಾಂಪ್ಸಿಯಾಜರಾಯುವಿನ (placenta)ದಲ್ಲಿನಸಿಂನ್ಸಿಷಟೊ್ರಫೋಬ್ಲಾಸ್ಟ್ , ಸೈಟೊಟೊ್ರಫೋಬ್ಲಾಸ್ಟ್, ರಕ್ತನಾಳಗಳುಮತ್ತುವಿಲಸೊ್ಟ್ರೀಮಾಧಲ್ಲಿ ಇಮ್ಯುನೊಹಿಸ್ಟೋಕೆಮಿಸ್ಟ್ರಿಬಳಸಿ CD24 ಅಭಿವ್ಯಕ್ತಿಯನ್ನುಕಂಡುಹಿಡಿಯುವುದು

ಅಧ್ಯಯನದ ಸ್ಥಳ: ರೋಗಶಾಸ್ತ್ರ ವಿಭಾಗ, ಶ್ರೀದೇವರಾಜ್ಅಸ್ವ್ಯೆದ್ಯಕೀಯಕಾಲೇಜು, ಕೋಲಾರ.

ಪ್ರೀಎಕ್ಲಾಂಪ್ಸಿಯಾಜರಾಯುವಿನ (placenta) ದಲ್ಲಿನಸಿಂನ್ಸಿಷಟೊ್ರಫೋಬ್ಲಾಸ್ಟ್, ಸೈಟೊಟೊ್ರಫೋಬ್ಲಾಸ್ಟ್, ರಕ್ತನಾಳಗಳುಮತ್ತುವಿಲಸೊ್ಟ್ರೀಮಾಧಲ್ಲಿ ಇಮ್ಯುನೊಹಿಸ್ಟೋಕೆಮಿಸ್ಟ್ರಿಬಳಸಿCD24ಅಭಿವ್ಯಕ್ತಿಯನ್ನುಕಂಡು ಹಿಡಿಯುವುದುಅಧ್ಯಯನದಮುಖ್ಯಗುರಿಯಾಗಿದೆ.ಕೋಲಾರದಎಸ್ ಡಿಯುಎಂಸಿಯಪ್ರಸೂತಿಮತ್ತುಸ್ತ್ರೀರೋಗವಿಭಾಗದಿಂದಜರಾಯುವನ್ನುಸಂಗ್ರಹಿಸಲಾಗುವುದು.ಈಅಧ್ಯಯನ ವನ್ನುಸಾಂಸ್ಥಿಕನೈತಿಕಸಮಿತಿಯುಅನುಮೋದಿಸುತ್ತದೆ.ಸಂಗ್ರಹಿಸಿದಮಾಹಿತಿಯನ್ನುಪ್ರಬಂಧಮತ್ತುಪ್ರಕಟಣೆಗೆ ಮಾತ್ರಬಳಸಲಾಗುತ್ತದೆ.ಭಾಗವಹಿಸಲುಒಪ್ಪಿಕೊಳ್ಳಲುಯಾವುದೇಒತ್ತಾಯವಿಲ್ಲ.ಅಧ್ಯಯನದಲ್ಲಿಭಾಗವಹಿಸಲು ನೀವುಸ್ವಯಂಪ್ರೇರಣೆಯಿಂದಒಪ್ಪಿದರೆಮಾತ್ರಹೆಬ್ಬೆರಳಿನಗುರುತನ್ನುಸಹಿಮಾಡಲು/ಒದಗಿಸಲುನಿಮ್ಮನ್ನುವಿ ನಂತಿಸಲಾಗಿದೆ.ನಿಮ್ಮಿಂದಸಂಗ್ರಹಿಸಿದಮಾಹಿತಿಯನ್ನುಮತ್ತುನಿಮ್ಮಗುರುತನ್ನುಗೌಪ್ಯವಾಗಿಇರಿಸಲಾಗುತ್ತದೆಮ ತ್ತುಹೊರಗಿನವರಿಗೆಬಹಿರಂಗಪಡಿಸುವುದಿಲ್ಲ.ಈಸಂಶೋಧನೆಯಲ್ಲಿಭಾಗವಹಿಸಲುನೀವುಯಾವುದೇಹಣಕಾಸಿನ ಪ್ರಯೋಜನಗಳನ್ನುಸ್ವೀಕರಿಸುವುದಿಲ್ಲ.ಈಮಾಹಿತಿಯುಕ್ತಸಮ್ಮತಿಡಾಕ್ಯುಮೆಂಟ್ನಿಮಗೆಸಾಮಾನ್ಯಅಧ್ಯಯನದ ಹಿನ್ನೆಲೆಯನ್ನುನೀಡಲುಉದ್ದೇಶಿಸಿದೆ.ದಯವಿಟ್ಟುಕೆಳಗಿನಮಾಹಿತಿಯನ್ನುಎಚ್ಚರಿಕೆಯಿಂದಓದಿಮತ್ತುನಿಮ್ಮಕು ಟುಂಬಸದಸ್ಯರೊಂದಿಗೆಚರ್ಚಿಸಿ.ಅಧ್ಯಯನದಸಮಯದಲ್ಲಿನೀವುಯಾವುದೇಸಮಯದಲ್ಲಿಅಧ್ಯಯನಕ್ಕೆಸಂ ಬಂಧಿಸಿದನಿಮ್ಮಪ್ರಶ್ನೆಗಳನ್ನುಕೇಳಬಹುದು.ನೀವುಅಧ್ಯಯನದಲ್ಲಿಭಾಗವಹಿಸಲುಸಿದ್ಧರಿದ್ದರೆ,ತಿಳುವಳಿಕೆಯುಳ್ಳ ಸಮ್ಮತಿಯನಮೂನೆಗೆಸಹಿಹಾಕಲುನಿಮ್ಮನ್ನುಕೇಳಲಾಗುತ್ತದೆಮತ್ತುಅದರಮೂಲಕನೀವುಅಧ್ಯಯನದಲ್ಲಿಭಾಗ ವಹಿಸಲುಬಯಸುತ್ತೀರಿಎಂದುಒಪ್ಪಿಕೊಳ್ಳುತ್ತೀರಿಮತ್ತುಸಂಪೂರ್ಣಕಾರ್ಯವಿಧಾನವನ್ನುಅಧ್ಯಯನವೈದ್ಯರು ನಿಮಗೆವಿವರಿಸುತ್ತಾರೆ.ವಿವರಣೆಯಿಲ್ಲದೆಯಾವುದೇಸಮಯದಲ್ಲಿಅಧ್ಯಯನದಲ್ಲಿಭಾಗವಹಿಸಲುನಿಮ್ಮಸಮ್ಮತಿ ಯನ್ನುಹಿಂಪಡೆಯಲುನೀವುಸ್ವತಂತ್ರರಾಗಿದ್ದೀರಿಮತ್ತುಇದುನಿಮ್ಮಭವಿಷ್ಯದಚಿಕಿತ್ಸೆಯನ್ನುಬದಲಾಯಿಸುವುದಿ ಲ್ಲ.ಯಾವುದೇಸ್ಪಷ್ಟೀಕರಣಕ್ಕಾಗಿನೀವುತನಿಖಾಧಿಕಾರಿಯನ್ನುಸಂಪರ್ಕಿಸಲುಮುಕ್ತರಾಗಿದ್ದೀರಿ.ದತ್ತಾಂಶಸಂಗ್ರಹ ಣೆ, ಇಮ್ಯುನೊಹಿಸ್ಟೊಕೆಮಿಸ್ಟ್ರಿಪರೀಕ್ಷೆ, ವಿಶ್ಲೇಷಣೆ, ಮುದ್ರಣ, ಪ್ರಕಟಣೆಗೆತಗಲುವಎಲ್ಲಾವೆಚ್ಚವನ್ನುಸ್ನಾತಕೋತ್ತರವಿದ್ಯಾರ್ಥಿಯುಭರಿಸಬೇಕಾಗುತ್ತದೆ.**(ಡಾIIದೀಪಿಕಾಸಿ)**

ಪ್ರಮುಖಸಂಶೋಧಕರಹೆಸರುಮತ್ತುರುಜು:**ಡಾIIದೀಪಿಕಾಸಿ,**ದೂರವಾಣಿಸಂಖ್ಯೆ:8197599325

KEYS TO MASTER CHART

Sev of PE	Severity of Pre-Eclampsia
Ges	Gestational weeks
Gross – Wt of Pla	Gross – Weight of Placenta
Gross – Meas	Gross – Measurements
Wt of baby	Weight of baby
G&M – Nec	Gross & Microscopy – Necrosis
G&M – Cal	Gross & Microscopy – Calcification
G&M – Throm, mas	Gross & Microscopy – Thrombi, masses
IHC CD 24	Immunohistochemistry expression of CD 24
Syncytio	Syncytiotrophoblast
Cyto	Cytotrophoblast
BV	Blood vessels
Clin Dia	Clinical diagnosis
Mi	Mild
Mod	Moderate
Sev	Severe

Biopsy no	Age	Hosp no	Sev of PE	Ges	Gross - Wt of Pla (in gms)	Gross - Meas	APGAR Score (Out of 10)	Wt of baby (in kgs)	G&M - Nec	G&M - Cal	G&M - Throm, mas		IHC CD 24		
												Syncytio	Cytotrpho	Villous stroma	BV
B/2778/22	26	154845	Mi	39	580	15x12x8cm	9	3	No	No	Yes	1	1	1	1
B/2955/22	26	163169	Sev	36	460	12x10x77cm	5	3.5	Yes	Yes	Yes	1	1	1	1
B/2971/22	22	163391	Sev	38	500	13x11x9cm	6	3.94	No	Yes	No	1	1	1	1
B/537/22	25	165166	Mi	30	560	17x15x9cm	6	3.32	Yes	No	No	1	1	1	1
B/3146/22	25	167824	Sev	33	460	10x8x5cm	5	3.66	Yes	Yes	Yes	2	2	1	2
B/537/23	30	198787	Mi	40	480	11x10x8cm	8	2.97	No	No	No	1	1	1	1
B/397/22	20	194608	Sev	38	500	12x10x9cm	5	2.6	Yes	Yes	Yes	1	1	1	1
B/449/23	28	186797	Sev	34	470	11.5x10x9cm	7	3.27	Yes	No	No	1	1	1	1
B/426/23	36	196384	Sev	29	510	13x12x10cm	6	3.8	Yes	Yes	Yes	1	1	1	1
B/427/23	23	194601	Sev	38	560	11x10x9cm	8	3.33	No	Yes	No	1	1	1	1
B/606/22	19	200175	Mi	36	500	12x11x9cm	9	3.52	No	Yes	No	1	1	1	1
B/915/23	35	21176	Sev	35	480	11.5x10x9cm	6	2.81	Yes	Yes	Yes	1	1	1	1
B/932/23	33	212592	Sev	34	520	13x11x10cm	7	2.5	Yes	No	No	1	1	1	1
B/905/23	22	211045	Sev	38	600	17x15x12cm	8	3.95	No	No	Yes	1	1	1	1
B/965/23	35	213270	Sev	34	550	15x13x10cm	6	2.6	Yes	Yes	No	1	1	1	1
B/967/23	26	213735	Mi	35	500	12x10x10cm	8	2.8	No	No	No	1	1	1	1
B/987/23	29	214158	Sev	39	530	14x11x10cm	7	3.73	Yes	No	No	1	1	1	1
B/1522/23	30	230638	Mi	35	650	12x10x5cm	7	3.2	No	Yes	Yes	1	1	1	1
B/1555/23	23	236742	Sev	36	580	12x10x7cm	9	3.25	Yes	No	No	1	1	1	1
B/1560/23	30	230695	Mod	39	530	15x9x7cm	7	2.5	No	No	No	1	1	1	1
B/1567/23	28	231834	Sev	38	520	13x8x5cm	5	3	No	Yes	No	1	1	1	1
B/1732/23	31	236742	Mi	34	530	15x10x8cm	8	2.85	Yes	No	Yes	1	1	1	1
B/1771/23	27	238124	Mod	35	620	15x9x6cm	9	2.75	No	Yes	Yes	1	1	1	1
B/1768/23	23	237178	Mi	39	580	14x9x6cm	9	2.95	No	Yes	No	1	1	1	1
B/1782/23	24	238451	Mi	40	650	15x9x5cm	7	3.25	Yes	No	No	1	1	1	1
B/1856/23	28	237985	Sev	37	530	14x9x6cm	9	3.3	No	No	Yes	1	1	1	1
B/1868/23	37	241436	Sev	35	550	15x9x5cm	7	3.2	No	Yes	No	1	1	1	1
B/2028/23	25	246418	Mi	35	630	15x9x4cm	6	3.4	No	No	No	2	2	2	2
B/2040/23	33	247563	Mi	39	590	16x10x7cm	7	3.25	Yes	Yes	No	1	1	1	1
B/2051/23	30	242368	Mi	37	540	13x8x5cm	9	3.3	No	No	Yes	1	1	1	1
B/2042/23	20	247668	Mi	39	550	15x10x7cm	8	2.7	No	Yes	No	2	2	2	2
B/2195/23	36	251989	Sev	34	580	13x9x4cm	7	3.1	No	Yes	Yes	1	1	1	1
B/2217/23	28	246202	Mod	40	600	13x10x6cm	9	2.5	No	No	No	2	2	2	2
B/2219/23	36	253216	Sev	39	550	15x8x5cm	8	3.2	No	No	No	1	1	1	1
B/2226/23	19	244834	Sev	38	540	13x10x4cm	8	3.45	Yes	Yes	No	1	1	1	1
B/2236/23	35	254484	Sev	39	540	15x9x5cm	6	3.2	Yes	Yes	No	1	1	1	1
B/2238/23	18	254759	Mod	37	520	15x11x7cm	9	2.5	No	Yes	Yes	2	2	2	2
B/2243/23	28	254609	Mi	36	510	15x9x7cm	7	3.2	Yes	No	Yes	1	1	1	1
B/2251/23	27	255055	Mod	39	580	12x9x6cm	7	2.5	No	No	Yes	1	1	1	1
B/2268/23	32	255565	Mod	37	550	15x10x6cm	7	2.75	No	No	No	1	1	1	1
B/2280/23	25	251090	Mi	38	590	14x10x8cm	8	3	Yes	Yes	No	2	2	1	2
B/2287/23	20	255289	Mod	39	510	15x11x6cm	8	3	No	No	No	1	1	1	1
B/2293/23	22	255704	Mi	38	480	12x8x6cm	7	3.2	No	Yes	Yes	1	1	1	1
B/2300/23	20	256644	Mod	37	530	15x11x7cm	8	2.75	No	Yes	Yes	1	1	1	1
B/2105/23	26	225747	Mod	39	510	15x12x8cm	8	3	No	No	Yes	1	1	1	1
B/2108/23	23	249663	Sev	38	590	13x10x5cm	9	3.45	No	No	Yes	1	1	1	1
B/2340/23	36	257662	Sev	35	520	15x12x9cm	10	2.85	No	Yes	Yes	1	1	1	1
B/2215/23	34	253958	Mod	34	530	12x10x7cm	8	3.6	No	No	No	1	1	1	1
B/2304/23	32	256688	Sev	36	610	14x12x10cm	8	3.5	Yes	Yes	Yes	1	1	1	1
B/95/23	30	126227	Sev	37	460	13x11x9cm	7	3.75	Yes	Yes	Yes	1	1	1	1
B/822/23	33	207994	Mod	32	630	15x12x9cm	7	2.75	No	Yes	No	1	1	1	1
B/718/23	31	203945	Mod	35	550	18x14x10cm	8	3.1	No	No	No	2	1	1	1
B/1458/23	28	228032	Mod	36	670	14x11x10cm	8	3	Yes	Yes	Yes	1	1	1	1
B/1446/23	25	227117	Mi	34	540	11x9x7cm	5	2.6	No	No	No	1	1	1	1
B/1444/23	27	227215	Sev	37	500	18x15x12cm	9	2.8	Yes	No	Yes	1	1	1	1
B/1050/23	22	216113	Mi	38	570	17x14x10cm	9	3.35	Yes	Yes	No	2	1	1	1
B/1439/23	33	228397	Sev	39	550	12x10x8cm	8	3	Yes	Yes	Yes	1	1	1	1
B/3117/23	24	275668	Mod	33	600	16x8x10cm	10	3.7	Yes	Yes	Yes	1	1	1	1

B/3460/23	30	253041	Mi	40	620	13x10x8cm	9	3	Yes	Yes	Yes	1	1	1	1
B/3253/23	32	284782	Sev	35	600	15x8x7cm	6	3.6	Yes	No	Yes	1	1	1	1
B/3368/23	33	287961	Sev	36	560	14x7x9cm	8	3.3	Yes	Yes	Yes	1	1	1	1
B/3348/23	20	287866	Sev	37	580	16x9x7cm	9	2.95	Yes	No	Yes	1	1	1	1
B/3631/23	26	295857	Mi	32	590	12x9x7cm	9	3.1	No	No	No	1	1	1	1
B/3628/23	35	295898	Sev	30	580	17x14x10cm	6	3.3	Yes	No	Yes	1	1	1	1
B/3735/23	32	299211	Sev	31	620	15x12x8cm	5	3.65	Yes	Yes	Yes	1	1	1	1
B/3927/23	30	303564	Mod	33	620	12x10x7cm	7	3.75	Yes	No	Yes	1	1	1	1
B/4013/23	38	307140	Sev	35	610	13x11x10cm	8	3	Yes	Yes	Yes	1	1	1	1
B/4167/23	27	311730	Sev	36	680	15x13x11cm	9	3.4	Yes	Yes	Yes	1	1	1	1
B/4255/23	22	314695	Mod	32	550	16x14x12cm	9	2.9	Yes	Yes	No	1	1	1	1
B/4662/23	32	311469	Sev	30	640	14x9x8cm	6	3.1	Yes	Yes	Yes	1	1	1	1
B/636/23	22	201177	Mod	35	530	15x13x11cm	8	2.7	No	No	No	1	1	1	1
B/847/23	36	207106	Sev	37	680	14x13x11cm	8	2.65	Yes	Yes	Yes	1	1	1	1
B/1039/23	26	215802	Sev	38	480	11x9x8cm	10	2.8	Yes	Yes	Yes	1	1	1	1
B/1713/23	35	236195	Severe	39	600	12x10x9cm	7	2.6	Yes	Yes	Yes	2	2	2	2
B/1072/23	21	238457	Mod	28	570	15x8x7cm	8	2.9	Yes	Yes	No	1	1	1	1
B/1768/23	20	237178	Sev	35	500	16x14x11cm	9	3	Yes	Yes	Yes	1	1	1	1
B/1793/23	33	227392	Sev	30	630	17x15x12cm	7	3.1	Yes	Yes	Yes	2	2	2	2

Biopsy no	Age	Hosp no	Clin diag	Ges	Gross - Wt of Pla (in gms)	Gross - Measurments	APGAR Score	Wt of Baby	G&M - Nec	G&M - Cal	G&M - Throm, mas		IHC CD 24		
												Syncytiotropho	Cytotropho blast	Villous stroma	Blood vessels
B/376/23	27	154845	Normal Placenta	40 weeks	520	18x15x7 cm	8	3.91	No	No	Yes	1	1	2	1
B/388/23	23	194920	Normal Placenta	39 weeks	490	15x11x8 cm	7	3.05	Yes	Yes	Yes	1	1	1	1
B/406/23	29	195225	Normal Placenta	39 weeks	500	13x10x8 cm	6	3.03	No	No	No	1	1	1	1
B/612/23	24	200226	Normal Placenta	40 weeks	500	13x11x7cm	7	3.3	Yes	No	No	1	1	1	1
B/535/22	24	198612	Normal Placenta	38 weeks	540	17x14x9 cm	7	2.62	Yes	Yes	Yes	2	2	2	1
B/408/23	21	83582	Normal Placenta	28 weeks	490	10x9x8 cm	9	3.65	No	No	No	2	2	1	2
B/605/23	28	210660	Normal Placenta	40 weeks	600	10x9x5 cm	7	3.29	Yes	Yes	Yes	1	1	1	1
B/857/23	28	208962	Normal Placenta	30 weeks	450	10x9x5 cm	6	3.88	Yes	No	No	2	2	2	1
B/897/23	21	206483	Normal Placenta	39 weeks	520	10x9x4 cm	8	2.88	Yes	No	No	1	1	1	1
B/836/23	24	208005	Normal Placenta	39 weeks	480	12x10x7 cm	7	3.84	No	No	No	1	1	1	1
B/848/23	21	20849	Normal Placenta	40 weeks	650	18x14x10 cm	7	2.47	No	No	No	1	1	1	1
B/849/23	22	209053	Normal Placenta	40 weeks	560	15x14x9 cm	6	3.42	Yes	Yes	Yes	1	1	1	1
B/834/23	30	208097	Normal Placenta	39 weeks	700	18x16x10 cm	6	3.75	Yes	No	No	1	1	1	1
B/833/23	23	207835	Normal Placenta	39 weeks	550	12x10x5 cm	8	2.53	No	No	Yes	1	1	1	1
B/846/23	26	205804	Normal Placenta	40 weeks	650	18x13x9cm	7	2.67	Yes	Yes	No	2	2	2	2
B/888/23	23	207380	Normal Placenta	40 weeks	760	20x18x12cm	9	3.69	No	No	No	1	1	1	1
B/923/23	28	209982	Normal Placenta	40 weeks	510	14x12x17 cm	9	3.14	Yes	No	No	2	2	2	2
B/933/23	22	212711	Normal Placenta	32 weeks	600	14x11x7cm	5	3.72	No	Yes	Yes	2	2	2	2
B/930/23	27	212674	Normal Placenta	36 weeks	500	12x10x6cm	7	3.33	Yes	No	No	2	2	2	2
B/919/23	24	212011	Normal Placenta	33 weeks	530	14.5x10x5cm	8	2.89	No	No	No	1	1	1	1
B/904/23	25	199613	Normal Placenta	38 weeks	680	18x15x9cm	8	3.75	No	No	No	2	2	2	2
B/908/23	24	211218	Normal Placenta	35 weeks	650	17x12x8cm	5	2.81	Yes	No	No	1	1	1	1
B/934/23	27	188741	Normal Placenta	38 weeks	550	13x11x5cm	10	3.77	Yes	Yes	Yes	1	1	1	1
B/966/23	26	213732	Normal Placenta	38 weeks	600	12x10xcm	9	3.5	No	No	No	1	1	1	1
B/968/23	27	212573	Normal Placenta	34weeks	470	15x10x9cm	8	2.75	Yes	No	No	1	1	1	1
B/1521/23	25	222983	Normal Placenta	32 weeks	500	11x9x7cm	7	3.25	No	No	Yes	1	1	1	1
B/1523/23	30	229892	Normal Placenta	32 weeks	650	13x9x6cm	8	3.5	No	No	No	1	1	1	1
B/1527/23	28	230732	Normal Placenta	32 weeks	570	12x8x6 cm	6	2.85	No	No	No	1	1	1	1
B/1537/23	31	231221	Normal Placenta	31 weeks	540	12x10x9cm	7	3.1	Yes	Yes	No	1	1	1	1
B/1543/23	23	231165	Normal Placenta	33 weeks	520	13x9x6cm	8	3.25	No	No	Yes	1	1	1	1
B/1539/23	27	231232	Normal Placenta	39 weeks	560	12x8x5cm	9	3.5	No	No	No	1	1	1	1
B/1551/23	31	226523	Normal Placenta	33 weeks	550	15x11x6cm	8	3.25	No	Yes	No	1	1	1	1
B/1553/23	28	231254	Normal Placenta	31 weeks	630	13x10x9cm	8	2.95	Yes	No	No	2	2	1	2

B/1571/23	23	224188	Normal Placenta	31 weeks	720	10x9x6cm	6	3.5	No	No	No	1	1	1	1
B/1577/23	30	231802	Normal Placenta	32 weeks	590	13x10x8 cm	7	2.85	Yes	No	No	1	1	1	1
B/1579/23	36	227278	Normal Placenta	39 weeks	570	16x9x5cm	6	3.01	Yes	Yes	No	1	1	1	1
B/1591/23	31	232378	Normal Placenta	33 weeks	550	12x9x6 cm	8	3.5	No	No	Yes	1	1	1	1
B/1592/23	31	232412	Normal Placenta	40 weeks	610	11x9x7cm	8	2.75	Yes	No	No	1	1	1	1
B/1594/23	33	229613	Normal Placenta	38 weeks	700	13x10x8cm	8	3.25	yes	No	No	1	1	1	1
B/1599/23	22	232701	Normal Placenta	36 weeks	580	12x9x7cm	9	2.75	No	No	No	1	1	1	1
B/1582/23	32	208237	Normal Placenta	38 weeks	690	13x10x7cm	8	3.5	Yes	No	No	1	1	1	1
B/1569/23	27	231803	Normal Placenta	39 weeks	480	15x10x7cm	7	3.8	No	No	No	1	1	1	1
B/1564/23	26	228179	Normal Placenta	36 weeks	700	11x9x5cm	6	2.95	No	No	No	1	1	1	1
B/1584/23	24	232242	Normal Placenta	38 weeks	570	13x11x9cm	7	3.25	Yes	Yes	Yes	1	1	1	1
B/1566/23	31	197264	Normal Placenta	39 weeks	550	13x11x9cm	8	2.85	No	No	No	1	1	1	1
B/1600/23	34	196338	Normal Placenta	36 weeks	680	15x12x11cm	7	3.6	No	No	Yes	1	1	1	1
B/1603/23	29	145038	Normal Placenta	37 weeks	710	13x9x7cm	9	2.95	No	Yes	Yes	1	1	1	1
B/1726/23	30	236797	Normal Placenta	32 weeks	680	13x10x6cm	8	2.5	Yes	No	No	1	1	1	1
B/1727/23	21	236794	Normal Placenta	38 weeks	470	15x10x6cm	6	3.65	Yes	No	Yes	1	1	1	1
B/1729/23	28	237223	Normal Placenta	37 weeks	580	15x9x6cm	9	3	No	Yes	No	1	1	1	1
B/1740/23	30	23684	Normal Placenta	38 weeks	630	13x9x5cm	8	3.45	No	No	No	1	1	1	1
B/1743/23	35	226925	Normal Placenta	40 weeks	530	12x10x7cm	7	3.36	No	No	No	1	1	1	1
B/1760/23	27	234139	Normal Placenta	38 weeks	730	13x10x9cm	9	3.3	Yes	Yes	Yes	1	1	1	1
B/1762/23	30	235036	Normal Placenta	37 weeks	660	11x8x5cm	7	2.95	No	No	No	1	1	1	1
B/1764/23	22	225588	Normal Placenta	39 weeks	570	14x11x9cm	6	3.7	Yes	No	No	1	1	1	1
B/1773/23	28	238098	Normal Placenta	40 weeks	510	12x10x5cm	9	3.3	Yes	No	No	1	1	1	1
B/856/23	29	240945	Normal Placenta	37 weeks	550	11x9x5cm	8	3	No	Yes	No	1	1	1	1
B/2038/23	28	247619	Normal Placenta	39 weeks	490	13x11x7cm	7	3.2	Yes	Yes	No	1	1	1	1
B/2063/23	25	244752	Normal Placenta	37 weeks	510	13x10x8cm	6	2.8	Yes	No	Yes	1	1	1	1
B/2050/23	23	229406	Normal Placenta	38 weeks	560	12x9x5cm	7	3	Yes	No	No	1	1	1	1
B/2097/23	28	241254	Normal Placenta	38 weeks	660	15x9x5cm	8	3.25	No	Yes	No	1	1	1	1
B/2041/23	30	247596	Normal Placenta	37 weeks	560	12x9x6cm	7	2.75	No	No	No	1	1	1	1
B/2095/23	25	249336	Normal Placenta	39 weeks	610	13x11x7cm	6	2.8	Yes	No	No	1	1	1	1
B/2096/23	33	249479	Normal Placenta	40 weeks	500	12x10x6cm	7	2.8	Yes	No	No	1	1	1	1
B/2062/23	24	238452	Normal Placenta	38 weeks	490	13x9x5cm	6	2.75	Yes	Yes	No	1	1	1	1
B/2045/23	31	247958	Normal Placenta	36 weeks	520	15x10x7cm	8	3	Yes	No	No	1	1	1	1
B/2107/23	33	239397	Normal Placenta	37weeks	500	12x8x5cm	7	3.2	No	Yes	No	1	1	1	1
B/2113/23	34	249884	Normal Placenta	35 weeks	710	11x9x5cm	8	3	Yes	No	No	1	1	1	1

B/2119/23	29	250253	Normal Placenta	38 weeks	670	13x8x5cm	6	3.1	Yes	No	No	1	1	1	1
B/2128/23	25	250455	Normal Placenta	32 weeks	620	11x7x5cm	9	2.85	Yes	Yes	No	1	1	1	1
B/2143/23	30	251498	Normal Placenta	36 weeks	480	15x7x5cm	8	3.5	No	No	No	1	1	1	1
B/2144/23	31	239053	Normal Placenta	38 weeks	500	12x8x5cm	7	3.9	No	Yes	No	2	2	2	2
B/2156/23	22	225488	Normal Placenta	40 weeks	550	11x9x6cm	8	3	No	No	No	1	1	1	1
B/2171/23	28	252558	Normal Placenta	34 weeks	720	15x10x7cm	9	2.75	Yes	Yes	Yes	1	1	1	1
B/2176/23	22	252022	Normal Placenta	39 weeks	630	15x9x5cm	8	3.2	Yes	No	No	1	1	1	1
B/2178/23	23	252577	Normal Placenta	38 weeks	520	13x9x5cm	9	3	No	No	No	1	1	1	1
B/2184/23	21	252754	Normal Placenta	36 weeks	630	15x9x7cm	7	2.75	Yes	No	Yes	1	1	1	1