8. DISCUSSION

8.1 MORPHOLOGY:

Placenta is an essential organ for maintenance of pregnancy and for promoting normal growth and development of fetus.\textsuperscript{71} It gives the most accurate record of infant’s prenatal experience.\textsuperscript{142} Placenta undergoes different changes in weight, structure, volume, shape and function continuously throughout gestation to support the prenatal life.\textsuperscript{147} Intrauterine growth of fetus mainly depends on adequate function of placenta. Placenta is a fetal organ. It shares same stress and strain to which the fetus is exposed. Thus any disease which affects the mother has a great impact on placenta. Anatomical structure of placenta greatly influences its function. Thus study of placial morphology is considered essential.\textsuperscript{136} Pregnancy complications like preeclampsia are reflected on placenta both macroscopically and microscopically. The fetus depends on placenta for normal development, thus pathological changes in placenta result in reduced blood flow across placenta and uteroplacental insufficiency.\textsuperscript{138,142}

Weight of placenta is functionally significant as it is related to villous surface area and fetal metabolism.\textsuperscript{71} In present study it was observed that mean placental weight was less in preeclamptic placentae as compared to control placentae. In present study the mean placental weight was 502.26 gms in control placentae and 430.38 gms in preeclamptic placentae. Similar results were reported by Udaina et al, Mallik et al and Mujumdar et al\textsuperscript{71,148,77} who found reduced placental weight in preeclamptic cases as compared to normotensive controls. Comparative studies were also done by Aparna et al.\textsuperscript{149} However they concluded that gross abnormalities were significant in control placentae. Mayhew et al\textsuperscript{66} reported that preeclampsia was not associated with main effects on placial morphology. The present study noted that the mean placental diameter and thickness was 18.7 cms and 2.3 cms in control placentae and 17.2cms and 1.8cms in preeclamptic placentae respectively. Preeclamptic placentae were smaller and thinner as compared to control placentae. Similar findings have been reported by Modi et al, Londhe et al and Salmani et al.\textsuperscript{13,108,142} Teasdale et al\textsuperscript{45} found significant reduction in transverse diameter of preeclamptic placentae. This reduction may be due to small size of placenta in preeclampsia. Ciblis et al\textsuperscript{150} and Kishwara et al\textsuperscript{95} reported that preeclamptic placentae were smaller than normal placentae indicating an underlying pathological process which
interferes with normal growth of placenta. Number of cotyledons were also less in preeclamptic placentae as compared to control placentae. The mean number of cotyledons was 18.9 in control placentae and 16 in preeclamptic placentae. Similar findings were reported by Londhe et al, Nag et al and Kishwara et al. Rath et al reported that intercotyledonous vasculature is altered in hypertensive placentae resulting in low birth weight babies.

In present study it was noted that all placental dimensions that is mean placental weight, thickness and diameter show significantly lower values in preeclamptic placentae as compared to control placentae. The main reason for reduced placental weight in preeclampsia could be uteroplacental insufficiency. Preeclampsia adversely affects placental morphology. It has been recorded that maternal vasospasm in preeclampsia leads to decreased maternal uteroplacental blood flow. Hypoxia and reduction in blood flow could be responsible for morphological alterations of placenta in preeclampsia. Rath et al reported that intercotyledonous vasculature is altered in hypertensive placentae resulting in low birth weight babies.

The mean external diameter of uterine spiral arterioles in preeclamptic women is less than half of the diameter of these arterioles in normotensive controls. This results in reduced uteroplacental blood flow due to which placenta shows more and more ischemic changes as gestation continues. Placenta tries to compensate for reduced supply however these compensatory changes are insufficient and thus fails to develop adequate placental mass. Kishwara et al and Teasdel et al have reported reduced parenchymal components such as intervillous space, trophoblasts, mass of peripheral villous tissue and capillaries in preeclamptic placentae as compared to control placentae. Sankar et al reported reduced villous diameter, surface area and vessel densities in preeclamptic placentae. Thus reduced proportional and absolute volume in preeclampsia could contribute to reduced morphological dimensions in preeclamptic placenta. Preeclampsia is a primary cause of placental insufficiency. Abnormal cytotrophoblastic invasion leads to placental ischemia and endothelial dysfunction which characterizes preeclampsia. The main impact on fetus is undernutrition due to uteroplacental vascular insufficiency which leads to growth retardation. Odegard et al reported that preeclampsia was associated with 5% reduction in fetal weight. The risk of small for gestational age was four times higher in infants born to preeclamptic women as compared to normotensive controls. Kishwara et al reported that the mean fetal weight in control group and preeclamptic cases was 2.80 kg and 2.26 kg respectively. Boyd and Scott et al reported
that the mean fetal weight was 2714 gms and 1998 gms in control group and preeclamptic cases respectively.

**In present study** the mean fetal birth weight in control group was 2.8 kg and preeclamptic cases it was 2.1 kg. It was observed that increased incidence of IUGR and reduced placental weight increased with severity of hypertension. Also smaller placentae usually accompanied low birth weight babies. Similar findings have been reported by Londhe et al, Udaina et al, Salmani et al, Sankar et al and Meyhew et al.\textsuperscript{108,71,142,153,66}

**Present study** noted that the mean fetal APGAR score was 8.9 and 6.5 in control group and preeclamptic cases respectively. Similar findings were reported by Navbir et al\textsuperscript{128}, Kishwara et al\textsuperscript{109} who reported lower APGAR score in preeclampsia than normotensive controls. Placenta seems to adapt well to hypoxic condition in preeclampsia, although the compensatory changes that occur are insufficient. Thus, leading to inadequate placental mass causing placental dysfunction and consequent chronic fetal hypoxemia. Myatt et al, Soma et al, Eskild et al \textsuperscript{156,157,101} suggested that placental insufficiency and impaired placental function in hypertensive pregnancy leads to low fetal birth weight.
8.2 HISTOLOGY

8.2.1 SYNCYTIAL KNOTS:

Syncytiotrophoblast plays a major role throughout pregnancy and is a major site for nutrient exchange. Aggregates of syncytial nuclei on the surface of terminal villi are called as syncytial knots. The cells of both cytotrophoblast and syncytiotrophoblast divide ultimately and undergo apoptosis. The number of syncytiotrophoblast nuclei increases nine fold from 13 weeks of gestation to term and can be used to evaluate villous maturity. Most of these nuclei are dispersed within the syncytioplasm, while old apoptotic nuclei aggregate and form membrane sealed bodies that are referred as syncytial knots.

Increased syncytial knots are associated with conditions of uteroplacental malperfusion and hold importance in placental examination. The syncytiotrophoblast loses its power of division due to high degree of differentiation. Thus it depends on cytotrophoblast throughout pregnancy. To counterbalance the continuous input this material is extruded continuously in the maternal circulation.

In present study it was observed that the mean number of syncytial knots in both central and peripheral sections of preeclamptic placentae was significantly higher as compared to control placentae. Similar results have been observed by Saeed et al, Sharma et al, Navbir et al, Dhabhai et al, Rajkumar et al and Nafeez et al. They found higher number of syncytial knots in preeclamptic placentae as compared to control placentae. Khalid et al, Narsimha et al, Tomas et al and Tenney et al have reported that knots were present in nearly all the terminal villi of preeclamptic placentae while only in 10-15% of villi in control placentae.

Sharma et al observed higher number of syncytial knots in peripheral zone of preeclamptic placentae as compared to central zone. Altered rate of apoptosis has been linked to preeclampsia. Pathogenic mechanism proposed behind preeclampsia is placental ischemia and hypoxia which may affect the trophoblast functioning and lead to stimulation of apoptotic pathway thus increasing rate of trophoblast turnover. Central areas of placenta are more oxygenated compared to peripheral areas due to normal differential placental perfusion. Peripheral zones are less oxygenated and are
Histology and Histochemistry of Placenta in Pregnancies Complicated by Preeclampsia

more prone to hypoxic changes which causes increased syncytial knot formation in peripheral zone as compared to central zone.\textsuperscript{127} However present study did not show any significant difference in number of syncytial knots in central and peripheral sections of preeclamptic placentae.

Excessive formation of syncytial knots is a feature of preeclampsia.\textsuperscript{105,164,165} Diminished fetal blood flow in cases like preeclampsia cause an accelerated ageing of syncytial nuclei.\textsuperscript{168} Kos et al, Haezel et al, Maly et al, Motwani et al, Navbir et al\textsuperscript{78,169,79,133,124} found increased syncytial knots associated with ischemic change in placenta. Genset et al\textsuperscript{170} reported that stromal fibrosis and excessive syncytial knotting are results of overall reduction in fetal perfusion of placenta. Failure of vascular remodelling and hypoxia is implicated in pathophysiology of preeclampsia.\textsuperscript{170} Fox et al\textsuperscript{171} suggested that syncytial knots indicate compromised fetal circulation. Excessive number of syncytial knots are found on villi which are inadequately perfused by the fetal circulation. This response to villous hypovascularity indicates accelerated sequestration of aged nuclei to optimally utilize the amount of trophoblasts available for transfer of oxygen and nutrient supply across the syncytium.\textsuperscript{168,171}

Release of apoptotic material into maternal circulation has been suggested as the mechanism for maternal endothelial disruption in preeclampsia. Apoptosis occurs within syncytiotrophoblast particularly in those areas associated with damage and fibrin deposits. Increased placental apoptosis may be due to external factors such as hypoxia and reactive oxygen species which may be the underlying cause in preeclampsia.\textsuperscript{172}

Haezel et al\textsuperscript{169} found increased number of syncytial knots in preeclamptic placentae following exposure to hypoxia and reactive oxygen species. Increased number of syncytial knots in preeclampsia may result from exposure to a form of oxidative stress.\textsuperscript{169} Syncytiotrophoblast is vulnerable to oxidative stress in vivo because it is intimately related to maternal blood. Many complications of pregnancy such as preeclampsia which show increased syncytial knotting are associated with oxidative stress.\textsuperscript{160}

Hypoxia triggers apoptosis. As mentioned earlier, oxidative stress may be associated with enhanced apoptosis and increased turnover of syncytiotrophoblast. Thus human term placenta exposed to hypoxia and oxidative stress may exhibit increased apoptosis.
Apoptosis at the syncytiotrophoblastic tips causes increased syncytial deportation in preeclampsia affecting the integrity of the tissue. Apoptotic nuclei are found in syncytial knots and may contribute to the shedding of syncytial fragments into the maternal circulation. Increased syncytial knotting represents a mechanism of sequestering damaged nuclei into the areas of syncytiotrophoblast where they do not interfere with maternal fetal exchange.

Previously many authors have confirmed uteroplacental insufficiency in preeclampsia. Thus it is concluded that reduced uteroplacental blood flow, consequent hypoxia and oxidative stress may lead to increased syncytial knot formation in preeclampsia.

**8.2.2 CYTOTROPHOBLAST PROLIFERATION:**

Terminal villi of placenta consists of stroma containing fetal capillaries beneath the layer of progenitor cytotrophoblast cells which are in turn covered by multinucleate syncytiotrophoblast. Syncytiotrophoblast has no proliferative capabilities. Cytotrophoblasts are stem cells from which syncytiotrophoblast is derived.

Present study showed significantly increased cytotrophoblast proliferation in both central and peripheral sections of preeclamptic placentae as compared to control placentae. Jones et al found significant increase in number of cytotrophoblast cells in preeclamptic placentae. Narasimha et al, Arnholdt et al, Motwani et al, Nafeez et al, Maqueo et al and Sodhi et al also found similar results.

One of the histological changes secondary to uteroplacental ischemia is proliferation of villous cytotrophoblast cells. Cytotrophoblast can be considered as a regenerative zone in mature placenta which is quiescent normally, but becomes activated when syncytiotrophoblast suffers ischemic damage. Ischemia leads to damaged syncytiotrophoblast. Cytotrophoblast cells proliferate in an attempt to repair and replace damaged syncytiotrophoblast. It has been suggested that decreased uterine circulation leads to mild anoxia which brings about the proliferation and increased prominence of cytotrophoblasts.

Oxidative stress is evident in preeclampsia. Syncytiotrophoblast is transcriptionally inactive. It has to depend on cytotrophoblast to derive cytoprotective enzymes at times of...
oxidative stress. Thus increased cytotrophoblast proliferation in preeclampsia could be interpreted as fetal defense mechanism initiated in response to vulnerable syncytiotrophoblast.178

Hypoxia changes the balance between proliferation and differentiation of cytotrophoblast. Thus hypoxic conditions have been reported to result in proliferation of cytotrophoblasts.179 Before the cytotrophoblasts reach a supply of maternal blood near the uterine lumen of placenta proper, they proliferate in hypoxic environment. Within the uterine wall they stop dividing and are directed towards maternal arterioles along the gradients of increasing oxygen tension. It was hypothesized that this scenario could be prolonged in preeclampsia and the cytotrophoblasts continue to proliferate abnormally in hypoxic environment.61

It has been proposed that true syncytial knots are accumulation of aged nuclei.160 Excessive syncytial knotting in preeclampsia have been reported by previous workers like Kos et al, Haezel et al, Maly et al and Motwani et al.78,169,79,133

Thus in present study it can be hypothesized that increased cytotrophoblast proliferation could be a response to hypoxia to maintain syncytiotrophoblast for efficient exchange of nutrients.

8.2.3 THICKENING OF TROPHOBLASTIC BASEMENT MEMBRANE :

In present study significantly increased number of villi showing basement membrane thickening were observed in both central and peripheral sections of preeclamptic placentae as compared to control placentae.

Various workers like Fox et al, Costa et al, Motwani et al, Sodhi et al and Salgado et al180,181,133,176,106 have previously demonstrated thickening of trophoblastic basement membrane in preeclamptic placentae. Marked thickening of villous basement membrane is associated with various pathological conditions like preeclampsia.180 Basement membrane thickening in more than 3% of villi is considered abnormal and is a common feature of toxemic pregnancies.105 Navbir et al124 observed villous basement membrane thickening and poor fetal outcome in hypertensive placentae. Narsimha et al105 reported incidence of basement membrane thickening to be 95% in placentae of toxemic pregnancies.
Previously many workers have reported increased cytotrophoblast proliferation in preeclamptic placentae.\textsuperscript{105,124,133} Cytotrophoblast cells which secrete basement membrane protein as a response to placental ischemia. Thus thickened basement membrane is a byproduct of cytotrophoblast cell proliferation. This leads to high percentage of villi with thickened basement membrane.\textsuperscript{107,105,180,133}

### 8.2.4 VASCULOSYNCTIAL MEMBRANES:

Vasculosyncytial membrane is a primary site of feto-maternal exchange. It is formed when syncytiotrophoblast surrounds the terminal villi and makes close contact with the fetal capillaries.\textsuperscript{153} Sinusoidal dilatation of terminal villous capillaries bulging against the trophoblastic surfaces and their alteration to a thin lamella results in vasculosyncytial membrane. It is closely related to fetal villous vascularization. Immature placenta show reduced villous capilarisation and paucity of vasculosyncytial membrane.\textsuperscript{182}

**In present study** significantly reduced number of vasculosyncytial membranes were observed in villi of both central and peripheral sections of preeclamptic placentae as compared to control placentae. Dimitrovskia et al\textsuperscript{183} and Fox et al\textsuperscript{184} reported low vasculosyncytial membrane count in preeclamptic placentae. Becker et al\textsuperscript{185} and Sodhi et al\textsuperscript{176} have claimed that pregnancies complicated by preeclampsia have unduly low proportion of placental villi with vasculosyncytial membranes. Navbir et al\textsuperscript{124} reported significant vasculosyncytial membrane deficiency in preeclamptic placentae as compared to control placentae. Low birth weight and birth asphyxia was observed in babies born to hypertensive mothers with vasculosyncytial membrane deficiency. Ansari et al\textsuperscript{107} observed that vasculosyncytial membranes were absent in hypertensive placentae. According to Fox et al\textsuperscript{184} less than 5\% of villi showed vasculosyncytial membrane in placentae of hypertensive pregnancies. Failure of trophoblastic differentiation can be related to this paucity of membranous area. They also reported inverse relationship between incidence of villous vasculosyncytial membrane and fetal hypoxia.\textsuperscript{184} Dimitrovskia et al\textsuperscript{183} suggested low vasculosyncytial membrane count as a manifestation of villous regression. Narasimha et al\textsuperscript{105} reported that paucity of vasculosyncytial membrane is an index of hypoxia. They observed paucity of membranes was higher in toxemic pregnancies correlating with the severity of disease. Kher et al\textsuperscript{186} and Mathews et al\textsuperscript{187} have reported association of deficient vasculosyncytial membrane and poor fetal outcome.
Previous workers have reported cytotrophoblast proliferation in preeclampsia.\textsuperscript{105, 175, 133, 119, 32, 176} \textbf{Present study} also showed cytotrophoblast proliferation in preeclamptic placentae. A layer of cytotrophoblasts is observed between syncytiotrophoblast and fetal blood vessels. Thus it can be suggested that increased cytotrophoblast proliferation also could be the reason for paucity of vasculosyncytial membranes in preeclamptic placentae. However Horkey et al\textsuperscript{188} suggested that there was increased formation of these membranes in fetal hypoxia in an attempt to increase surface available for gaseous transfer.

It was also observed that thickness of vasculosyncytial membranes was increased in villi of preeclamptic placentae as compared to control placentae. Previous workers have reported basement membrane thickening in preeclampsia.\textsuperscript{180} Thickening of vasculosyncytial membrane in preeclamptic placentae was reported by Saeed et al.\textsuperscript{126} Sankar et al\textsuperscript{153} reported increased syncytial knot density and increased thickness of vasculosyncytial membranes in preeclamptic placentae. Oxygen in the intervillous space diffuses into the fetal capillaries through vasculosyncytial membrane and reaches the fetus via fetal vascular network. Increased thickness of vascular syncytial membrane reduces feto-placental circulation resulting in intrauterine distress of fetus. Previous studies revealed that smooth muscle actin showed a few contractile cells in normal terminal villi stroma. The presence of these processes and also thickening of trophoblastic membrane was confirmed by ultrastructural observation. Presence of myofibroelastic system was demonstrated in terminal villi which is remodeled in preeclampsia.\textsuperscript{181} \textbf{In present study} also increased basement membrane thickening was observed in preeclamptic placentae leading to thick vasculosyncytial membranes.

\textbf{8.2.5 STROMAL FIBROSIS AND FIBRINOID NECROSIS:}

Motwani et al\textsuperscript{133} reported increased incidence of stromal fibrosis in preeclamptic placentae which may be related to reduced uteroplacental blood flow due to obliterative endarteritis. Mehrotra et al\textsuperscript{189} reported increased villous fibrosis in placentae of mothers having anaemia. Reduced blood flow and normal aging is thought to be responsible for formation of stromal fibrosis.\textsuperscript{190} Previously many workers like Myatt et al, Soma et al and Esklid et al\textsuperscript{156,157,101} have confirmed uteroplacental insufficiency in preeclampsia. Hypoxia and fetal malnutrition is evident in preeclampsia. Thus reduced placental blood flow may be the cause of increased stromal fibrosis and fibrinoid necrosis in preeclamptic
placentae. **In present study** increased stromal fibrosis was observed in both central and peripheral sections of preeclamptic placentae as compared to control placentae.

However, insignificant association between stromal fibrosis and fetal outcome was reported by Kher et al.\textsuperscript{186} Navbir et al\textsuperscript{124} found stromal fibrosis commonly in control placentae as well as preeclamptic placentae. According to them stromal fibrosis is also commonly found in control placentae and does not seem to affect the fetal outcome.

The villous fibrinoid necrosis is a form of senile amyloid material being deposited as a result of immune attack on trophoblastic cells which contain misspecified proteins because of ageing process. It appears as a homogenous mass of fibrinoid material which shows a few degenerate syncytial nuclei at the periphery.\textsuperscript{191,124} Fibrinoid material in affected villi contains considerable quantity of immunoglobins.\textsuperscript{192} Many workers believe that this lesion is due to replacement of villus by fibrin either by maternal blood from intervillous space or fetal blood in villous capillaries.\textsuperscript{193,194} Mirchandani et al\textsuperscript{195} have shown a linear relationship between thickening of basement membrane and fibrinoid necrosis. Both these findings have been considered as a manifestation of antigen antibody reaction in the body. **In present study** increased fibrinoid necrosis was observed in both central and peripheral sections of preeclamptic placentae as compared to control placentae. Similar results have been reported by Fox et al, Salmani et al, Narasimha et al, and Nafeez et al.\textsuperscript{196,142,105,119} They found increased fibrinoid necrosis in preeclamptic placentae.

**8.2.6 HYPOVASCULAR VILLI :**

**In present study** increased villous stroma and reduced number of vessels and hypovascular villi were observed in significant number of preeclamptic placentae. Kalra et al\textsuperscript{197}, Motwani et al\textsuperscript{133} and Narsimha et al\textsuperscript{105} reported reduced villous vascularity in preeclamptic placentae as compared to control placentae. Saleh et al\textsuperscript{89} observed that villi of preeclamptic placentae showed condensed villous connective tissue core and regression of villous capillaries upto complete disappearance. Krielessi et al\textsuperscript{118} observed reduced number of vessels in preeclamptic placentae. Reduced number of vessels may be due to their reduced formation or secondary to stromal fibrosis.\textsuperscript{105,133} This leads to impaired nutrient transfer. Sankar et al and Krielessi et al observed avascular terminal villi in preeclamptic placentae. These changes in preeclamptic placentae eventually cause
functional disturbances resulting in oxidative stress and hypoxic conditions. Coelho et al\textsuperscript{85} observed poor microvessel density in hypertensive pregnancies which worsened with increasing levels of hypertension and proteinuria. This shows decreased vascularization with increased severity of disease which consequently leads to lower nutritional status of fetus.\textsuperscript{85}

### 8.2.7 ENDARTERITIS OBLITERANS:

Swelling and proliferation of intimal cells together with thickening and reduplication of the basement membrane characterize obliterative endarteritis of fetal stem arteries.\textsuperscript{133} Present study showed endarteritis obliterans in significant number of preeclamptic placentae. Similar results were reported by Motwani et al, Narasimha et al, Kalra et al, Davey et al and Mhasodkar et al.\textsuperscript{133,105,197,198,199} Ilie et al\textsuperscript{114} found enlarged and disrupted endothelium in preeclamptic placentae. Endothelial proliferation of arteries and hyalinization are probably the aftermath of hypertension.\textsuperscript{71,45} Saleh et al\textsuperscript{89} and Sankar et al\textsuperscript{153} reported that villi of preeclamptic placentae showed endothelial degeneration, smooth muscle hypertrophy in tunica intima and atheromatous formation. These changes were caused due to hypoxia. Fibrinisation, stenosis and atherosis leads to reduction in blood vessel density in terminal villi in preeclampsia. This consequently results in reduced perfusion and oxidative stress. Ischemic damage of placental tissue occurs with mal developed villi.\textsuperscript{89}

### 8.2.8 TUNICA MEDIA PROLIFERATION:

In present study tunica media proliferation was observed in significant number of preeclamptic placentae. Motwani et al, Mhasodkar et al, Narasimha et al and Salmani et al\textsuperscript{133,199,105,142} previously reported medial coat proliferation and hypertrophy of tunica media in preeclampsia. Mamuri et al\textsuperscript{200} observed medial coat proliferation of medium sized blood vessels in placentae of hypertensive group as compared to control group. Krielessi et al\textsuperscript{118} noted avascular villi and thickened vessels in hypertensive placentae.

Present study noted histopathological changes in preeclamptic placentae like increased syncytial knots, cytotrophoblast proliferation, basement membrane thickening, paucity of vasculosyncytial membranes, stromal fibrosis, fibrinoid necrosis, hypovascular villi,
endoarteritis obliterans and tunica media proliferation. These changes may affect the
gaseous exchange and fetal nutrient supply and consequently lead to IUGR.
8.3 PLACENTAL ALKALINE PHOSPHATASE (PALP)

Human alkaline phosphatase is found in higher concentrations in liver, bile duct, bone, intestines and placenta. Alkaline phosphatase is one of the important enzymes secreted by placenta. Placental alkaline phosphatase (PALP) is a polymorphic and heat stable enzyme and high levels of this enzyme is found in trophoblast of placenta. It is localized in apical and basal cells of syncytiotrophoblast plasma membrane. It is synthesized from placental syncytiotrophoblast from the twelfth week of pregnancy and is released into the maternal blood. In early pregnancy PALP activity is low. Measurable levels of PALP appear in maternal serum by the end of first trimester and increases progressively with gestational age and normally peaks at term.

PALP is said to be involved in nutrient transport from mother to fetus and also in transport of maternal IgG to the fetus. PALP appears to fulfil the requirements for mechanism to transport functional antibodies to fetus because of its affinity for free IgG molecules. It has a role in active transport of phosphates, absorption of nutrients and uptake mechanism through the plasma membrane, calcification and bone resorption and may be connected to protein synthesis in the cell. Suggestions have been made that this enzyme is involved in transfer of glucose and fatty acids across the cell membrane. Alkaline phosphatase is reported to be concerned with carbohydrate and phospholipid metabolism. Thus PALP contributes to the maintenance of fetal health by being involved in defense from toxic substances and nutrient mobilization.

Alkaline phosphatase enzyme has been used as a biochemical marker in bone diseases and conditions such as prostatic carcinoma and myocardial infarction. PALP has been extensively studied by various workers. Elevated PALP levels may indicate premature delivery. Various studies have shown that alkaline phosphatase levels in maternal serum can be used as a marker for idiopathic preterm delivery. Studies have also shown that pregnant women with decreased serum levels of alkaline phosphatase could be associated with intrauterine growth restriction (IUGR) and premature labour.

In several studies this enzyme has been used as a biochemical marker of metabolic bone disease in neonates.

In present study it was found that PALP activity is more intense in preeclamptic placentae as compared to control placentae. This finding was similar to Mangal et al,
Curzen P, Jeacock et al and Dempsey et al\textsuperscript{76,31,30,28} who found high amount of PALP localisation in preeclamptic placentae as compared to control placentae. In present study majority of villi in control placentae showed PALP activity localized over the basal membrane of syncytiotrophoblast, moderate activity was seen on the microvillous surface and cytoplasm of syncytiotrophoblast. PALP activity on basal syncytial membrane has been reported by Matsubara et al\textsuperscript{7} and Lister et al.\textsuperscript{209} This could be because placental alkaline phosphatase is produced from the basal membrane of syncytiotrophoblast.\textsuperscript{99} Matsubara et al, Kameya et al and Jones et al\textsuperscript{7,210,211} have earlier reported PALP activity on cytoplasm and microvillous surface of syncytiotrophoblast. This could be because alkaline phosphatase enzyme is mainly produced by the syncytiotrophoblast and by microvilli of syncytium.\textsuperscript{203,76,211}

Preeclamptic placentae showed a very strong localization of PALP in both central and peripheral sections suggesting increase in the placental alkaline phosphatase activity. In majority of villi in preeclamptic placentae very strong PALP activity was seen on basal membrane, apical microvillous surface of syncytiotrophoblast, cytoplasm of syncytiotrophoblast, cytotrophoblast and connective tissue stroma. This observation is in accordance with previous workers like Mangal et al,\textsuperscript{76} Curzen P\textsuperscript{31} who observed strong PALP activity in syncytiotrophoblast of preeclamptic placentae as compared to control placentae. Dempsey et al\textsuperscript{28} reported PALP activity in connective tissue stroma of preeclamptic placentae. However the findings in the present study differ from previous workers like Sammak et al, Boronkai et al and Francis et al\textsuperscript{99,80,40} who observed decreased alkaline phosphatase activity in preeclamptic placentae as compared to control placentae.

Placental ischemia is evident in preeclampsia. There is decrease in synthetic activity of trophoblast and cellular respiration is also probably depressed.\textsuperscript{212} PALP appears to be moderately resistant to hypoxia. Preeclamptic placentae showed considerable increase in lysosomal activity. This is presumably a response to placental ischemia, which leads to alteration in the pH of trophoblast and stimulates lysosomal activity. Thus syncytial damage is apparent in preeclamptic placentae. Syncytial damage and destruction in preeclampsia leads to release of PALP from vesicles into cytoplasm.\textsuperscript{211} Increased amount of placental alkaline phosphatase enzyme was found in toxaemic placentae at 34-37 weeks. Ischemic changes in trophoblast during this period could be the reason for marked
rise in PALP activity in toxaemic preeclampsia. Increased PALP in turn leads to rise in serum alkaline phosphatase as it occurs in cases of hepatitis in which it is attributed to destruction of liver cells.\(^{34}\) Premature placental ageing in toxemia is characterized histochemically by accumulation of PALP in syncytiotrophoblast.\(^{30}\)

Alkaline phosphatase is said to be tissue specific and often occurs in human non trophoblastic tumors and is considered as a marker for malignant transformation of normal tissue.\(^{213}\) It has been reported to play a role in cell division in both normal and transformed cells.\(^{214}\) PALP is said to be expressed in human placenta and many types of cancers.\(^{215}\) In present study cytotrophoblast cell proliferation was observed. Previous workers have also reported cytotrophoblast proliferation in preeclampsia.\(^{105,175,133,119}\)

Increased activity of PALP in preeclamptic placentae might be the consequence of cytotrophoblast cell proliferation.

Various authors have studied variable number of placentae and reported their findings. Most of them having a small sample size of 2 preeclamptic placentae by Dempsey et al\(^ {28}\), 10 preeclamptic cases by Sammak et al\(^ {99}\) and 8 placentae by Fox et al.\(^ {216}\). In present study 50 preeclamptic placentae were studied comparing them with 50 control placentae. This larger sample size could give us a better understanding of localization of alkaline phosphatase activity in placental tissue.

The other parameter recorded in present study was serum alkaline phosphatase. Serum alkaline phosphatase has been studied by many workers earlier. Various studies have reported significant increase in serum alkaline phosphatase in late pregnancy.\(^ {80,40}\) Serum alkaline phosphatase in pregnancy is mainly of placental origin.\(^ {203,40,217,218}\) Placental alkaline phosphatase activity is localized to the external surface of syncytial villi and are in close relation with maternal circulation. This suggests that the enzyme is released from the trophoblast and major proportion of it enters the maternal blood.\(^ {209, 219}\) Thus, its increased level in maternal serum might be due to increased PALP. In present study it was observed that serum alkaline phosphatase levels were significantly high in preeclamptic cases as compared to normotensive controls. This finding is in accordance to previous workers like Curzen P, Mangal et al\(^ {76}\) and Kapoor et al.\(^ {220}\) Women with high maternal blood pressure had higher levels of serum alkaline phosphatase and increased intensity of PALP localization in placentae. Similar findings were reported by Mangal et al\(^ {76}\) and Curzen P.\(^ {31}\) Abnormally high serum alkaline phosphatase levels
represent placental damage and failing placental function.\textsuperscript{34,220,221} Placental ischemia, necrosis, damage of chorionic villi and infarction can increase the levels of PALP in maternal serum.\textsuperscript{202,76} Histochemically alkaline phosphatase enzyme is found in association with osteoblasts which may be the source of elevated levels of alkaline phosphatase in growing children.\textsuperscript{222} PALP shows rising levels in maternal serum in second and third trimester of pregnancy. This coincides with the period of fetal osteogenesis and calcification of fetal skeleton. Mobilization of calcium from maternal system for fetal calcification process is facilitated by PALP. This confirms placental origin of alkaline phosphatase in maternal serum.\textsuperscript{76,34}

Rate of secretion and production of alkaline phosphatase has been observed to correlate strongly with the increasing nutritional demands of growing fetus.\textsuperscript{223} PALP is said to be involved in nutrient transport from mother to fetus.\textsuperscript{201} It is believed that alkaline phosphatase plays a role in transfer mechanisms across membranes thus rich content of this enzyme is found in absorptive epithelia such as renal tubules or gut.\textsuperscript{215,224} In placenta, abundant alkaline phosphatase content of trophoblastic invaginations and pinocytic vesicles indicate importance of PALP in endocytosis and trophoblastic transfer.\textsuperscript{211} PALP is said to be involved in metabolism and further breakdown of glycogen and energy production.\textsuperscript{33} In present study it was observed that the mean fetal birth weight in preeclamptic cases was lower than those of normotensive controls. This finding is similar to Udaina et al\textsuperscript{71}, Salmani et al\textsuperscript{142} and Sankar et al.\textsuperscript{153} Many workers like Myatt et al\textsuperscript{156}, Soma et al\textsuperscript{157} and Eskild et al\textsuperscript{101} suggested that placental insufficiency and impaired placental function in hypertensive pregnancy leads to low fetal birth weight. There is some evidence that compensatory changes which limit the effects of ischemic damage are brought into play in preeclampsia.\textsuperscript{212} Since PALP has major role in transport and energy production increased PALP activity in preeclamptic placentae could be a compensatory mechanism to provide nutrition to the fetus in ischemic conditions.
8.4 PLACENTAL GLYCOGEN

Fetal growth is a complex process that involves interaction of mother, placenta and fetus. The growth and development of fetus depends on adequate nutrient supply to the fetus such as glucose, lipids and amino acids.\textsuperscript{225} Glucose is the primary source of energy for the growing fetus.\textsuperscript{226,227} Glycogen storage is considered as an index of glucose availability to the growing fetus which in turn is the key factor controlling the fetal growth.\textsuperscript{228} Glycogen is among the substances to be described earliest in the placenta.\textsuperscript{229} Glucose transfer by placenta in early pregnancy is determined by amount of glucose stored as glycogen.\textsuperscript{227} Placental glycogen exists in cells chiefly as a readily available source of energy for growing fetus in early pregnancy.\textsuperscript{229,230} Alteration in placental and fetal glycogen storage not only alters the fetal growth but also affects fetal survival.\textsuperscript{231}

Lot of animal studies have been conducted on placental glycogen. Placental glycogen activity in human placenta is still doubtful. Limited studies have been carried out on human placental glycogen in normotensive controls, also negligible data is available on placental glycogen in preeclamptic cases as there are no comparative studies done. Placental glycogen has been correlated with both high and low rates of metabolism.\textsuperscript{229} Claude Bernard\textsuperscript{232} first demonstrated glycogen in rabbit placenta. They suggested that placenta produced glucose for the fetus in early pregnancy as functional maturity of fetal liver was pending. Placental glycogen levels are higher in early pregnancy than at term.\textsuperscript{230,227} It is reported that in early gestation placenta has a marked ability to synthesise glycogen but this decreases steadily to term. The early placenta has high rate of glucose uptake due to its rapid growth rate. At term however placental growth rate is slowed and glucose uptake is less. There is decrease in both glucose production and glucose utilisation as the gestation proceeds.\textsuperscript{29,230} Dempsey et al\textsuperscript{233} reported presence of glycogen in human placenta in early gestation. As gestation advanced glycogen was seen to diminish. Christi et al\textsuperscript{33} observed small concentrations of placental glycogen in fetal stroma of control placentae. Villi C\textsuperscript{29} reported that maximum concentration of placental glycogen is reached by eight week of gestation and the amount decreases till 18 to 20 weeks. There after concentration remains constant until term. They reported that placental glycogen concentration decreases as fetal tissue concentration increases.

**In present study** increased and widespread glycogen deposits were observed in preeclamptic placentae as compared to control placentae. High intensity glycogen
Histology and Histochemistry of Placenta in Pregnancies Complicated by Preeclampsia

deposits were observed mainly in syncytium, basement membrane, connective tissue stroma and around fetal blood vessels. Similar results were observed by Arkwrith et al.\textsuperscript{50} They observed more glycogen per gram wet weight in preeclamptic placentae as compared to control placentae. Histochemically they observed increased glycogen in syncytium of preeclamptic placentae as compared to control placentae. Also activity of glycogen synthase was 16 fold higher in preeclamptic placentae as compared to control placentae.

In a normal pregnant woman hepatic glucose production is increased by 16 to 30 % to meet the increasing needs of placenta and growing fetus.\textsuperscript{235} Glucose uptake is estimated to be 33mmol/kg/min during third trimester of pregnancy.\textsuperscript{236} Maternal blood glucose is known to be the primary energy substrate for the fetal growth and development and the fetus almost entirely depends on glucose from maternal circulation.\textsuperscript{237,238} Fetal glucose levels are maintained by transplacental transport of glucose from mother to fetus.\textsuperscript{226,231} Thus under normotensive conditions placenta and hence the fetus can obtain glucose from maternal circulation at any stage of pregnancy as long as the mother is alive. Maternal glucose levels are well maintained in fetal circulation thus there in no need for placenta to store glycogen.\textsuperscript{230}

Placental supply of nutrients majorly determines the intrauterine growth of the fetus. This mainly depends on morphology of placenta, its blood supply and its synthesis and metabolism of nutrients.\textsuperscript{226} In normal pregnancy glucose production resulting from liberation of glucose from stored glycogen is small in comparison to amount of glucose directly received from maternal blood.\textsuperscript{230} Hugget et al\textsuperscript{239} observed that glycogen rich placentae are associated with glucose in fetal blood sugar. In preeclampsia however placental ischemia is evident. Previous workers have reported uteroplacental insufficiency an placental ischemia in preeclampsia.\textsuperscript{156,157,101} It has been hypothesised that placental ischemia is an early event in preeclampsia which leads to placental production of soluble factors that cause maternal endothelial dysfunction.\textsuperscript{240} Increased incidence of placental infarction in preeclampsia provides evidence for local placental ischemia.\textsuperscript{50} With placental dysfunction there is insufficient nutrient supply to the fetus despite adequate nutrient availability in the mother. Also placental dysfunction may contribute to increased consumption of glucose by placenta itself.\textsuperscript{238} Brett et al\textsuperscript{238} and Wislocki et al\textsuperscript{241} reported that glycogen is present in areas of placenta with sluggish
oxidative metabolism and excess glycogen deposition is related to altered circulation. Thus it may be possible that placental glycogen is increased in preeclampsia to compensate for lack of nutrition to the fetus due to placental ischemia.

In IUGR fetal glucose uptake rates and levels of glucose transporters are increased. Preeclampsia is associated with increased glucose uptake as compared to normal pregnancy. This suggests that placental glycolysis is increased in preeclampsia. Placenta also has high metabolic demand. It consumes half of the glucose delivered from uterine circulation. Placenta requires this energy to carry out various functions. Placenta maintains its own glucose supply at the expense of fetal stores. This may occur when placental blood flow decreases which may cause fetal hepatic glycogenolysis and a positive umbilical arteriovenous glucose concentration difference.

In normal pregnancies glycogen accumulation is observed in first trimester of pregnancy. It is mainly confined to earlier stages of trophoblast development. Increased glycogen content is associated with malignant transformation and cellular immaturity. Increased glycogen content in preeclampsia thus indicates more towards immature trophoblast. Previous workers have reported increased cytotrophoblast proliferation in preeclampsia. In present study also increased cytotrophoblast proliferation is observed in preeclamptic placentae. As reported by Chau et al there is increased shedding of these cells in maternal circulation. Thus cytotrophoblast proliferation and cytotrophoblast deportation lead to increased trophoblast turnover in preeclampsia. This results in decrease in average age and level of maturity of these cells. Thus there is increase in glycogen content.

It is evident that abnormal carbohydrate metabolism is associated with trophoblast related pregnancy disorders such as preeclampsia. There is evidence that glycolytic enzymes are associated with actin filaments of cytoskeleton of cells which possibly provide local energy supply for cytoskeletal functions. Cytoskeletal reorganisation is presumed to be a fundamental part of syncytium formation. Previous workers have reported abnormal cytotrophoblast proliferation in preeclampsia. Thus abnormal cytotrophoblast differentiation seen in preeclampsia could be accompanied by abnormal glycogen metabolism.
The main purpose of glycogen breakdown is production of energy. Breakdown of glycogen to pyruvate further proceeds to carbon dioxide and water via Krebs cycle. Christi et al\textsuperscript{33} reported decrease in Krebs cycle enzymes at term in control placentae. Arkwrith et al\textsuperscript{50} observed increased glycogen phosphorylase activity in preeclamptic placentae as compared to normotensive controls. Glycogen degradation may provide required energy for continuous amino acid transfer to the fetus even in anoxia. Thus placental glycogen is considered as an energy source for placental metabolic activity.\textsuperscript{230}

Placental ischemia in preeclampsia may lead to glycogen storage by the placenta for its own metabolism as well as to supply nutrition to the growing fetus.
8.5 PLACENTAL GLYCOSAMINOGLYCANS (GAGs):

Placental connective tissue stroma is made up of collagen fibres, ground substance and cellular elements. Acid mucopolysaccharides also known as glycosaminoglycans (GAGs) are mainly present in connective tissue stroma and walls of blood vessels. GAGs are abundantly expressed in placenta. Transport of metabolic products, regulation of water metabolism, maintenance of balance between water and electrolytes as well as defence mechanisms involved in bacterial and viral infections of tissues depend on the mucopolysaccharides. They also have anticoagulant, inflammatory and proangiogenic properties and are involved in angiogenesis and blood homeostasis.\(^{247,248,249}\)

Lee et al\(^{250}\) reported that higher amounts of GAGs are present in control placenta at term. Changes in levels of GAGs with maturation of placenta would be related to their role in placental transport. Wasserman et al\(^{251}\) reported that placental GAGs are involved in maintaining the structural integrity and possibly have a role in blood homeostasis. It has been suggested that GAG synthesis in endothelial cells in turn leads to increased vascularity.\(^{248}\)

In present study reduced GAGs were observed in preeclamptic placentae as compared to control placentae. Similar results were reported by Warda et al\(^{94}\) and Gunatillake et al.\(^{248}\) Warda et al\(^{94}\) observed that the average content of placental GAGs was lower in preeclamptic placentae as compared to control placentae. They reported alterations in placental GAGs in preeclampsia. They suggested that structural changes in GAGs directly contribute to the development of preeclampsia. Wasserman et al\(^{252}\) reported alterations in GAGs content in toxemic placentae. However, our study differs from Fama et al\(^{253}\) who observed increased GAGs content in preeclamptic placentae.

There is very little known about placental GAGs as a contributing variable in developing preeclampsia. Preeclampsia induced alterations in composition of GAGs may impair biological functions of placenta.\(^{94}\) Changes in placental GAGs may alter molecular transport through placenta’s connective tissue and may affect the rate of fetal growth.\(^{250}\) Normotensive uncomplicated pregnancies represent hypercoagulable state. It has been suggested that excessive thrombin production may originate in the placenta. In spite of this placental thrombosis in normotensive pregnancies is rare. This suggests that thrombin generation must be tightly regulated within the uteroplacental circulation.
Preeclampsia however shows exaggerated increase in pro-coagulant activity which leads to thrombosis in utero-placental circulation. It has been suggested that GAGs may play a key role in preventing thrombotic events in placenta. Reduced expression of placental GAGs in preeclampsia may contribute to increased placental thrombin generation.\textsuperscript{254,248}

Thus reduced distribution of GAGs may directly contribute to the development of preeclampsia and also may affect placental transport.
8.6 PLACENTAL LIPIDS:

Lipids are the major structural components of cell membrane. The role of placental lipid metabolism is therefore relevant to cell replication and immunological responses which may further be affected by changing pattern of blood lipids during gestation. Placental lipid metabolism may also be involved in mechanism conferring immune status on the conceptus. Changes in lipid metabolism in pregnancy ensure a continuous supply of nutrients to the growing fetus. Despite the importance of lipids in cell function their metabolism still remains doubtful in placental investigation.\(^{230,121}\) Physiologic pregnancy is associated with broad spectrum of metabolic adaptations which includes increased lipid metabolism and lipoproteins. Elevated lipid levels in first to third trimester may serve as energy store to fulfil maternal and fetal metabolic needs. Maternal hypertriglyceridemia in late gestation has an important role as a source of triglycerides for milk formation just before parturition.\(^{121}\) During early pregnancy there is increased body fat accumulation and lipogenesis. In late pregnancy however, there is accelerated breakdown of fat depots which play a key role in fetal development. Accumulation of lipids in maternal tissues and maternal hyperlipidaemia are consistent manifestations of altered lipid metabolism occurring during gestation.\(^{255}\) Fatty acids are used for placental oxidation and membrane formation and cholesterol is used by placenta for steroid synthesis.\(^{235}\)

Placental transport of lipids to the fetus involves metabolic alterations in the placenta and release into fetal plasma.\(^{256}\) During early gestation maternal cholesterol is important source of cholesterol for the fetus, however its importance becomes minimal during late pregnancy due to high capacity of fetal tissues to synthesise cholesterol.\(^{255}\) The concentration of phospholipids is about 75% of total placental lipids. It has been suggested that placental phospholipids are involved in transport of amino acids across placenta.\(^{230}\)

Previous workers mainly focused on maternal lipid profile in pregnancy. Kalar et al\(^{121}\) found high lipid levels in maternal circulation of preeclamptic cases as compared to normotensive controls. Lima et al\(^{112}\) reported higher levels of triglycerides in maternal serum of preeclamptic patients as compared to healthy controls. Robinson et al\(^{96}\) reported that elevation of free fatty acids in maternal circulation in preeclampsia may be involved in pathogenesis of preeclampsia. Negligible data is available on lipids in placental tissue.
So far limited numbers of studies have investigated the fat content of placental tissue in preeclampsia. Huang et al\textsuperscript{129} are the only one to study the distribution of lipids in placental tissue of control and preeclamptic placentae. In present study fat accumulation was identified histochemically using sudan black stain. Increased and widespread reactivity of sudan black was observed in preeclamptic placentae as compared to control placentae. Increased fat deposition was observed on syncytial basement membrane, cytoplasm and villous connective tissue stroma. Similar results have been reported by Huang et al.\textsuperscript{129} They reported 30\% increase in fat deposition in preeclamptic placental tissue as compared to control placentae.

Preeclampsia is a pregnancy specific disorder that adversely affects maternal vascular function and fetal intrauterine growth. It is a major cause of maternal and fetal morbidity and mortality.\textsuperscript{4} In preeclampsia placental damage appears to trigger the maternal syndrome. Hypertension and organ damage are mediated by endothelial dysfunction. Endothelium seems to be the target of factors produced by damaged placenta. Thus abnormal placentation in preeclampsia induces endothelial dysfunction and is responsible for maternal syndrome.\textsuperscript{257} Dyslipidemia is the hallmark of preeclampsia.\textsuperscript{258} Association of altered serum lipid profile and preeclampsia has been reported earlier . Functions of various organs involved in lipid and lipoprotein metabolism are known to be affected by preeclampsia and related disorders. Several studies have shown that endothelial dysfunction is related to hyperlipidemia.\textsuperscript{121,259} Present study showed abnormal fat deposition in preeclamptic placentae. Thus abnormal lipid metabolism seems to be a source of endothelial dysfunction and pathogenesis of preeclampsia.

Hererra et al\textsuperscript{260} reported that enhanced oxidative stress in pregnancy may be related to maternal hyperlipidaemia . Previous workers like Siddiqui et al\textsuperscript{261}, Maarten et al\textsuperscript{262} have reported enhanced oxidative stress and lipid peroxidation in preeclampsia. Increased oxidative stress produces lipid peroxides which are highly reactive compounds that can have direct interactions with cell membrane and cause cellular dysfunctions.\textsuperscript{263} It has been demonstrated that placental production of lipid peroxides is abnormally increased in preeclampsia which cause endothelial injury and dysfunction.\textsuperscript{102} Staff et al\textsuperscript{59} reported increased total cholesterol, phospholipids and lipid peroxides in decidua basalis of preeclamptic samples as compared to samples from normotensive controls. They suggested that maternal endothelial dysfunction in preeclampsia could be due to elevated
lipid content in decidua basalis tissue. Preeclamptic women present arterial lesions at uteroplacental implantation sites. Changes in lipid metabolism in preeclampsia may contribute towards endothelial lesions. Increased phospholipids in preeclamptic placentae could be a source of lipid compounds that cause oxidative damage within placental tissue and may be associated with pathophysiology of placenta in preeclampsia.

Transplacental nutrient transport from mother to fetus is mediated across basal plasma membrane of syncytiotrophoblast. Placental activities such as transport, permeability, activities of enzyme and stability are greatly influenced by the physical state of membrane lipid bilayer and protein lipid interactions. Cholesterol helps to maintain the fluidity of cell membranes. Changes in cholesterol to phospholipid ratio in placenta affects placental function and transport. Cholesterol can effectively modulate the physical state of phospholipid bilayer and can lower membrane fluidity. Previous workers have reported that under normal physiological conditions cholesterol phospholipid ratio of syncytial membranes decreases during pregnancy, thus membrane fluidity increases. In preeclampsia however it has been reported that syncytiotrophoblast has decreased fluidity. Huang et al reported increased mean cholesterol in preeclamptic placentae as compared to normal control placentae. Solomon et al have reported that high cholesterol levels are associated with preeclampsia. Thus these increased levels in preeclampsia can reduce membrane fluidity by altering cholesterol phospholipid ratio and therefore disrupt transport across placental trophoblast. There is evidence that suggests major involvement of maternal lipid metabolism in fetal growth exists, however exact mechanism is unknown. Impaired lipid metabolism and placental transport seem to contribute to decreased fetal birth weight.

In present study increased reactivity of sudan black was observed in preeclamptic placentae as compared to control placentae. This demonstrates increase in phospholipids in preeclamptic placental tissue. Presence of hypercoagulation in the placental circulation has been one of the hypothesis associated with pathogenesis of preeclampsia. It has been suggested that phospholipids may be involved in the intravascular coagulation associated with toxaemia of pregnancy. Thus increased phospholipids may activate blood coagulation and may contribute to the development of preeclampsia.
9. CONCLUSION

In present study it is concluded that:

- There is strong association with low placental weight and low fetal birth weight. This data indicates that low placental weight is associated with placental dysfunction. Thus it can be conclude that severity of preeclampsia has adverse effect on morphology of placenta and consequently affects the fetal weight.

- All the histopathological changes in preeclamptic placentae like increased syncytial knots, cytotrophoblast proliferation, basement membrane thickening, paucity of vasculosyncytial membranes, stromal fibrosis, fibrinoid necrosis, hypovascular villi, endarteritis obliterans and tunica media proliferation are due to uteroplacental insufficiency. These histopathological changes account for impaired gaseous exchange and affect the fetal nutrient supply which consequently leads to IUGR.

- Syncytial damage due to placental ischemia may lead to abnormally high PALP and consequent increase in serum and alkaline phosphatase. Ischemic placental damage affects the transport of important materials between mother and fetus eventually leading to poor nutrition of fetus and low fetal birth weight. Another probable reason could also be that increase in alkaline phosphatase is to compensate for lack of nutrients supplied to the fetus due to placental ischemia as PALP has a major role in nutrient supply and maintenance of fetal health. PALP could be used as a marker for detecting ongoing placental damage and IUGR and further damage could be arrested.

- Placental ischemia in preeclampsia may lead to glycogen storage by the placenta for its own metabolism as well as to supply nutrition to the growing fetus.

- Preeclampsia is associated with reduced distribution of placental GAGs. Thus decreased levels of GAGs may directly contribute to the development of preeclampsia and also may affect placental transport. Increased thrombus formation in uteroplacental circulation in turn leads to uteroplacental insufficiency and consequent fetal growth restriction.

- Present study demonstrated higher fat content in preeclamptic placentae as compared to control placentae. Thus it is suggested that abnormal lipid metabolism may be involved in pathogenesis of preeclampsia. Altered lipid
profile in preeclampsia can reduce membrane fluidity and disrupt transport across placental trophoblast. This could compromise the transport of nutrients to the fetus and may be one of the causes of low fetal birth weight. However this needs further investigation.
10. SUMMARY

Cross sectional study was conducted in Department of Anatomy of Dr. D.Y. Patil Medical College, Pimpri, Pune. Consecutive convenient sampling method was used. 50 normal and 50 preeclamptic placentae were collected immediately after delivery from women who delivered either vaginally or by cesarean section from Department of Obstetrics and Gynecology of Dr.D.Y.Patil hospital and Yashwantrao Chawan memorial hospital, Pimpri, Pune. Institutional ethical committee clearance was obtained. Written informed consent was obtained from all mothers participating in the study.

Samples were divided into two groups as group A and group B:

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

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

Morphology of placenta was studied under the following:

- Weight
- Diameter
- Thickness
- Study of maternal and fetal sides
- Presence of necrotic patches
Histopathological studies:

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

Following stains were used:

- Haematoxylin and Eosin – Histopathology
- Modified Gomori’s method – PALP
- Periodic acid Schiff’s reaction – Glycogen
- Alcian blue – Glycosaminoglycans
- Sudan black- Lipids

Morphology: All the morphometric parameters were significantly reduced in preeclamptic placentae as compared to control placentae. (p value < 0.05)

- Mean blood pressure of Normotensive controls and Preeclamptic cases was 118/78 mmHg and 156/98 mmHg respectively.
- Mean placental weight of control and preeclamptic placentae was 502.26 gms and 430.38 gms respectively.
- Mean placental diameter and thickness was 18.7 and 2.3 for control placentae and 17.2 and 1.8 in preeclamptic placentae.
- Mean number of cotyledons was 18.9 in control placentae and 16 in preeclamptic placentae.
- Marginal insertion of cord was observed in 3(6%) of control placentae and 12(24%) of preeclamptic placentae.
- Placental infarcts were observed in 4(8%) of control placentae and 33(66%) of preeclamptic placentae.
Histopathology:

In present study it was observed that histopathological changes like syncytial knotting, cytotrophoblast proliferation, basement membrane thickening, paucity of vasculosyncytial membranes, stromal fibrosis and fibrinoid necrosis were significantly increased (p value <0.05) in preeclamptic placentae.

Vascular changes such as hypovascular villi, endarteritis obliterans and tunica media proliferation in fetal blood vessels were also significantly increased (p value< 0.05) in preeclamptic placentae.

- Mean number of syncytial knots were 43.5 and 49 in central and peripheral sections of control placentae and 70.7 and 73.1 in central and peripheral sections of preeclamptic placentae.
- Mean number of villi showing cytotrophoblast proliferation were 18.9 and 21 in central and peripheral sections of control placentae and 74 and 71.1 in central and peripheral sections of preeclamptic placentae.
- Mean number of villi showing basement membrane thickening were 2.5 and 2.8 in central and peripheral sections of control placentae and 53.6 and 38.4 in central and peripheral sections of preeclamptic placentae.
- Mean number of villi showing vasculosyncytial membranes were 86.4 and 81.4 in central and peripheral sections of control placentae and 28.2 and 25.3 in central and peripheral sections of preeclamptic placentae.
- Mean number of villi showing stromal fibrosis were 0.7 and 1.6 in central and peripheral sections of control placentae and 7.7 and 7.2 in central and peripheral sections of preeclamptic placentae.
- Mean number of villi showing fibrinoid necrosis were 1.6 and 2.1 in central and peripheral sections of control placentae and 15.8 and 14 in central and peripheral sections of preeclamptic placentae.
Hypovascular villi were not observed in control placentae. Central sections of 27(54%) and peripheral sections of 30 (60%) placentae showed hypovascular villi.

Endarteritis obliterans was not observed in control placentae. Central sections of 13(26%) and peripheral sections of 14(28%) placentae showed endarteritis obliterans.

Tunica media proliferation was not observed in control placentae. Central sections of 27(54%) and peripheral sections of 13(26%) placentae showed tunica media proliferation.

Histochemistry:

100 villi were studied from each of central and peripheral section of placentae for distribution of enzymatic activity for each category of stain.

Quantification of enzymatic activity was assessed visually and was classified based on the extent of distribution of enzymatic activity from + to ++++

Placental Alkaline phosphatase activity (PALP) – Modified Gomori’s method:

Control Placentae:

Out of 50 placentae majority of villi in central section of 14(28%) and peripheral section of 15(30%) placentae showed PALP localisation only on the basal syncytial membrane. Cytoplasm, microvillous surface of syncytiotrophoblast, cytotrophoblasts and connective tissue stroma did not show any localisation.

Out of 50 placentae majority of villi in central section of 36(72%) and peripheral section of 35(70%) placentae showed strong PALP localisation on the basal syncytial membrane, slightly low intensity PALP localisation on cytoplasm and microvillous surface of syncytiotrophoblast. Cytotrophoblasts and connective tissue stroma did not show any localisation.

Preeclamptic placentae:

Out of 50 placentae majority of villi in central section of 10(20%) and peripheral section of 11(22%) placentae showed high intensity of PALP localisation on the basal syncytial membrane, cytoplasm and microvillous surface of
syncytiotrophoblast. Slightly low intensity of PALP activity was observed on cytotrophoblast cells. Connective tissue stroma did not show any localisation.

- Out of 50 placentae majority of villi in central section of 38(76%) and peripheral section of 36(72%) placentae showed high intensity of PALP localisation on the basal syncytial membrane, cytoplasm and microvillous surface of syncytiotrophoblast. Slightly low intensity of PALP activity was observed on cytotrophoblast cells. Connective tissue stroma also showed strong PALP localisation.

- Majority of villi in central section of remaining 2(4%) placentae and peripheral section of remaining 3(6%) placentae showed PALP distribution similar to that of control placentae.(+++)

**Glycogen – Periodic acid Schiff's reaction (PAS)**

**Control placentae:**

- Out of 50 placentae majority of villi in central section of 33(66%) placentae and peripheral section of 36(72%) placentae showed PAS positive material around fetal blood vessels and basement

- Out of 50 placentae majority of villi in central section of 17(34%) placentae and peripheral section of 14(28%) placentae showed PAS positive material around fetal blood vessels, basement membrane and syncytiotrophoblast.

**Preeclamptic placentae:**

- Out of 50 placentae majority of villi in central section of 47(94%) placentae and peripheral section of 48(96%) placentae showed PAS positive material around the fetal blood vessels, basement membrane, syncytiot and connective tissue stroma.

- Majority of villi in central section of remaining 3(6%) placentae and peripheral section of remaining 2(4%) placentae showed distribution of glycogen similar to that of control placentae that is around fetal blood vessels, basement membrane and syncytium.
Glycosaminoglycans (GAGs) distribution : Alcian blue

Control Placentae:
- Central and peripheral sections of all 50(100%) control placentae showed GAGs distribution in syncytiotrophoblast, basement membrane, connective tissue stroma and around fetal blood vessels.

Preeclamptic Placentae:
- Out of 50 placentae majority of villi in central section of 29(58%) placentae and peripheral section of 27(54%) placentae showed GAGs distribution only in syncytium and basement membrane.
- Out of 50 placentae majority of villi in central section of 6(12%) placentae and peripheral section of 6(12%) placentae showed GAGs distribution only in syncytium.
- However, central section of remaining 15(30%) placentae and peripheral section of remaining 17(34%) placentae showed GAGs distribution similar to that of control placentae in syncytiotrophoblast, basement membrane, connective tissue stroma and around fetal blood vessels.

Lipids : Sudan black

In both control and preeclamptic placentae fat deposition was observed in syncytiotrophoblast, basement membrane and connective tissue stroma.

Number of villi showing fat distribution were counted to differentiate between control and preeclamptic placentae.

Control placentae:
- (+) : Fat deposition was observed in central section of 30(60%) and peripheral section of 29(58%) placentae.
- (++) : Fat deposition was observed in central section of 17(34%) and peripheral section of 20(40%) placentae.
- (+++) : Fat deposition was observed in central section of 3(6%) and peripheral section of 1(2%) placentae.
Histology and Histochemistry of Placenta in Pregnancies Complicated by Preeclampsia

Preeclamptic placentae:

- (+++): Fat at deposition was observed in central section of 12(24%) and peripheral section of 10(20%) placentae.
- (++++) Widespread and increased fat deposition was observed in central section of 34(68%) and peripheral section of 37(74%) placentae.
- (++) However, central section of 4(8%) placentae and peripheral section of 3(6%) placentae showed lipid distribution similar to control placentae.
11. LIMITATIONS OF STUDY

- Present study required use of many chemicals for preparation of various stains. Few chemicals were expensive and difficult to procure.
- Illiteracy and resultant lack of awareness regarding the study made it difficult to convince mothers to consent to participate in the study.
12. REFERENCES


143. ACOG Committee on Obstetric Practice. ACOG Practice Bulletin. Diagnosis and management of preeclampsia and eclampsia. Obstet Gynaecol 2002; 99:159-167


152. Harbinder JS. Preeclampsia: is it all in the placenta? Malaysian journal of Medical sciences, 16(1), 2009, 8


214. Telfer JF, Green CD. Placental alkaline phosphatase activity is inversely related to cell growth rate in HeLa S3 cervical cancer cells. Federation of European Biochemical Societies. 1993;329(3):238-244.


Histology and Histochemistry of Placenta in Pregnancies Complicated by Preeclampsia


