CHAPTER 2

LITERATURE REVIEW

2.1 STUDIES RELATED TO GROSS MORPHOLOGY OF PLACENTA

Ramesh et al [51] reported that the mean weight of placenta, birth weight of the fetus, diameter of placenta and numbers of cotyledons were reduced with increasing grades of hypertension.

Pradeep and Abhay [52] stated that the morphometric parameters of placenta like weight, volume were significantly lower in hypertensive group compared to normal and it had significant correlation with the birth weight of new born. Preeclampsia is associated with substantial changes in placental morphology due to reduced uteroplacental blood flow.

Segupta kishwara et al [53] found that the values related to diameter, thickness, number of cotyledons and volume of placentae in preeclampsia are less than that of control group. According to them these changes have occurred due to insufficient blood supply to the placenta in preeclampsia.

Udainia and Jain [54] suggested that there is a significant lowering of placental weight in pregnancy induced hypertension. Placentae weighing less than 250gms are found only in pregnancy induced hypertension. As severity of hypertension increases, placental weight decreases as confirmed by minimum placental weight of 250gms in mild hypertension and 200gms in severe hypertension.

Garg et al [55] showed that there is a linear correlation between placental weight, foetal weight and the site of umbilical cord insertion in hypertensive mothers.
Damania et al [56] had studied sixty placentae of hypertensive disorder of pregnancy and reported that birth weight, placental weight and fetoplacental ratio were less in hypertensive than in the normotensive controls.

Chakravorty [57] in his study on pregnancy induced hypertension found the mean placental weight of 410gms in mild hypertension and 350gms in severe hypertension. He found mean foetal birth weight 2805gms in the normal term pregnancy, 2724 gms in mild hypertension and 1759 gms in severe hypertension.

Rath et al [58] studied the site of insertion of umbilical cord of placenta in pregnancy with hypertensive disorder and calculated the insertion percentage. The umbilical cord insertion percentage was correlated with the birth weights of infants in normal, mild, moderate and severe hypertensive mothers. They have noticed that only in severe hypertensive cases, the marginal attachment of umbilical cord with 0-25 insertion percentage, scored the highest in the series with 42%. They attributed that this type of cord insertion hampers the growth of fetus to a maximum level resulting in low birth weight babies. The weight of the placenta and infants in hypertensive group were found to be lower than the normal group. The mean fetoplacental ratio of the hypertensive groups were less than those of the normal group.

Redman [59] stated that preeclampsia is unique to pregnancy, but occurs even in the absence of a fetus and resolves with termination of pregnancy and removal of the placenta, and appears to be associated with a placental pathology.

Shanklin [60] who after studying 5000 placentae, observed a high degree of correlation between anomalous cord insertion and low birth weight.

Nobis and Das [61] in their study have shown that the placental weight in toxemic cases varies from 279 to 407g.

Bhatia et al [62] have shown reduced placental weight in severe toxemia, the lowest weight recorded being 280g.
Cibils [63] reported that the placentae from hypertensive patients were significantly smaller than the normal suggesting that the pathologic process interferes with the normal placental growth.

Sultana et al [64] reported reduced mean number of cotyledons, diameter and thickness in eclampsia placenta compared to control. The morphological changes in placenta are possibly due to reduced uteroplacental blood flow in eclampsia. They also observed statistically significant decreases in weight, volume, diameter and thickness in eclamptic placentas than in normotensives.

Barton et al [65] observed that the mean birth weight of the neonate was significantly lower in the group with preeclampsia than that of gestational hypertension.

Xiong et al [66] observed that the overall mean birth weight of the babies was markedly lower among babies born to mothers with preeclampsia than among babies born to normotensive mothers. According to them the birth weights were significantly lower in mothers with preeclampsia who delivered at ≤ 37 weeks of gestational age.

Lamminpaa et al [67] reported that women of advanced maternal age were 1.5 times more likely to have preeclampsia compared to women under 35 years of age. Women of advanced maternal age were significantly more likely to have preterm deliveries before 34 and 37 weeks and to have small for gestational age (SGA) infants. The risk increase being 70% in preterm delivery before 34 weeks and 40% in both preterm delivery before 37 weeks and SGA. Secondly women of advanced maternal age (AMA) had a twofold increased risk of required cesarean deliveries.

Gruslin and Lemyre [68] found women of AMA had preeclampsia more often than younger women, and their pregnancies were more likely to be complicated by preterm deliveries and impaired fetal growth. Premature infants are at higher risk of neonatal mortality and morbidity as well as neurodevelopmental impairments, these tend to be inversely proportional to gestational age.

Bujold et al [69] demonstrated that low-dose aspirin started in early pregnancy may reduce the incidence of preeclampsia and intrauterine growth restriction.
Miranda et al [70] stated that chronic and pregnancy-related hypertension increase risk for low birth weight and preterm birth as well as older maternal age was resulting in shorter gestations.

Verkauskiene et al [71] in their study compared the weight of newborns and placentas in cases of intrauterine growth retardation. They suggested that these newborns had a low mean placental weight but a high placental ratio. Placental ratio had a strongly negative relationship with birthweight, and it was a strong indicator of impaired prenatal linear growth.

Fox [72] has stressed the importance of analyzing the placental pathology quantitatively and has stated that the importance of the lesions could be realized only when assessed in relation of foetal growth and maturation.

Das et al [73] showed the relations between birth weight, placental area and placental volume in hypertensive disorders of pregnancy. They correlated the reduced placental area and volume with the fetal outcome in hypertensive pregnancies. They suggested that reduced placental volume leads to low birth weight of the baby.

Masodkar [74] observed a low APGAR score in toxemia of pregnancy. In toxemia pregnancy, the placental weight, volume and gestational age decreased with reduced birth weight of the baby. The low birth weight and APGAR score in toxemia of pregnancy may be due to placental ischemia.

Odegard et al [75] found that preeclampsia was associated with a 5% reduction in birth weight. In severe preeclampsia, the reduction was 12% and in early onset disease birth weight was 23% lower than control.

Maham akhlaq et al [76] stated that mean gestational age of preeclampsia group was 34.6 weeks compared with that of control group was 37.9 weeks.

Duley [77] reported low birth weight and decreased APGAR score in hypertensive pregnancy. In developing as well as in developed countries the impact
of hypertension in pregnancy is heavy, primarily on the fetus and it results in low birth weights and decreased APGAR score.

Sharmishtha and Sandhya [78] found placentae were small with irregular surface area and volume showed reduction in the placentae of pregnancy induced hypertensive mothers. Thus, the severity of hypertension adversely affects both fetal and placental outcome.

2.2 STUDIES RELATED TO HISTOMORPHOMETRY OF CHORIONIC VILLI

Ducray et al [79] studied the fetal component of the placenta in normotensive and preeclampsia groups using morphometric image analysis. The features examined showed significant difference between normotensive and preeclampsia placentae like reduced carrying capacity of stem villi and relative thickness of stem villi arterial walls with increased extent of fibrosis.

Devishankar et al [80] found histomorphometrically, the placentas in preeclampsia group had lesser villous surface area and smaller diameter, the density of the terminal villi was significantly higher compared to control groups. The density of fetal blood vessels in terminal villi was significantly decreased in the preeclampsia group compared to controls, but the diameters of terminal villi were more or less similar between the groups and were significant.

Egbor et al [81] studied the placental villous and vascular abnormalities in early and late - onset preeclampsia with and without fetal growth restriction. Early onset preeclampsia was associated with a reduction in placental weight, volume of the intervillous space, terminal villous volumes and surface areas of terminal villi. Late onset preeclampsia resulted in a significant reduction in stem vein volume, intervillous space volume, terminal villi volume and intermediate villi capillary volume were all significantly reduced in the presence of FGR.

Nakamura [82] found the intervillous space and the volume of the terminal villi decreased in proportion to the degree of toxemia. The area of terminal villus,
the area of the capillary lumina and the number of capillaries per villous were reduced in the placenta of toxemia pregnancy.

Ahmed Khairy Makled et al [83] observed the villous diameter and villous surface area were significantly reduced in pregnancy complicated by preeclampsia with and without IUGR.

Arnholdt et al [84] suggested that there was an increase in the proliferation rate of the trophoblast in preeclampsia. The mean numbers of villi count in gestational hypertensive placentas and preeclamptic placentas are significantly higher than those is normotensive placentas. The reason behind this increase of number of villi in diseased placentas could be that hypertensive placentas might be trying to increase the total surface area of nutrient and gas exchanging sites(by increasing the number of villi) to compensate the hypoxic state of the placenta due to the lack of trophoblastic invasion into the maternal decidua.

Barua [85] stated that mean absolute volume of placenta was significantly lower in eclampsia group as compared to control group.

Teasdale [86] found that the volume of placenta was significantly decreased in preeclamptic mothers and there was a reduction of parenchymal tissue due to significant reduction in peripheral villi, fetal capillaries and intervillous space volume. All parenchymal tissue components i.e intervillous space, peripheral villi, trophoblast and capillaries, were decreased by approximately 50% in the placentae of preeclamptic mothers. Despite of 52% reduction in the mass of peripheral villous tissue, the stem villous tissue was shown to be increased in the placentae of the mother with growth retarded foetus. Intervillous space volume was significantly reduced in relation to the total villous tissues in the placentae of the preeclamptic compared to the controls. He also found significant reduction of transverse diameter in preeclampsia group, this reduction seems to be due to the small size of placenta in preeclampsia group.
Kishwara et al [87] reported that intervillous space volume was significantly reduced in relation to the total villous tissue volume. There was proportionally more stem and less peripheral villous tissues in the placentae of the preeclampsia compared to the controls. The volume of the intervillous space is also reduced slightly by the deposition of fibrin.

Jones and Fox [88] suggested that some of the morphological changes in preeclampsia could represent the compensatory mechanism in the placenta eg., an increase in stem villous volume, marked reduction of total villous volume and intervillous space volume etc. that are helpful to some extent to overcome the unfavourable maternal environment, in spite of the reduction in volume (both proportional and absolute).

Pijnenborg et al [89] stated that during establishment of fetoplacental circulation, uterine spiral arteries undergo remodeling: spiral artery endothelial cells are replaced by endovascular extrvillous trophoblasts cells (EVT) and the arterial smooth muscle and elastic is lost and replaced by fibrinoid. This process terminates in low- resistance, high output vessels.

Nasiell et al [90] stated that placental blood flow is dependent on humoral and endothelial derived factors because placental tissue and vessels lack autonomic innervations.

Behrendt and Kuhnel [91] reported in preeclampsia that disruption of basement membrane was attributed to a deposition of collagen like tissue in the basement membrane which compromises its strength and hence fails to withstand the mechanical pressure exerted by increased diastolic blood pressure.

Meekins et al [92] demonstrated hypertrophy of tunica media in severe preeclampsia with mean diastolic blood pressure 110mm Hg. This hypertrophy may be secondary to the development of hypertension and may act as a protective mechanism against high pressure.
Rodica et al [93] reported the structural modifications of placenta in the pregnancies with pregnancy induced hypertension versus normal. They observed the changes in endothelium-76.47%, fibrinoid necrosis-73.52%, the hypertrophy of tunica media-67.64%, bridging syncytial knots-32.35%, avascular small terminal villi with hyaline fibrosis of the stroma-41.17% and the thrombosis of the spiral arterioles-26.47%.

Peng Mei et al [94] stated that the reduction of the vascular dimensions is constantly accompanied by significant structural disorders which have an impact upon the lumen of spiral arteriole with changes in its tunica intima, media and fibrillary structures. These structural modifications are associated quasi-constantly with the pregnancy induced hypertension cases versus the normotensive cases, in which they are quite rare.

Bokari et al [95] demonstrated a significant reduction of luminal diameter of spiral arterioles and increased disruption of basement membrane at multiple sites, hypertrophy of tunica media with fibrinoid necrosis as a feature of preeclamptic placentae in groups with diastolic blood pressure 108 and 123mm Hg.

Kadyrov et al [96] found that the trophoblast invasion into the placental bed in early-onset preeclampsia/intrauterine growth restriction is limited by increased apoptosis, resulting in narrower spiral arteries. The trophoblast invasion into spiral arteries was severely impaired with reduced spiral artery lumen ratio in preeclampsia/intrauterine growth restriction pregnancies compared with normal.

Delmis [97] showed the basic alteration in preeclampsia occurs due to inadequate trophoblast migration and lack of spiral artery physiological transformation. This leads to the persistent of musculoelastic layer of spiral arterioles, therefore their lumen stays narrow during the entire pregnancy, and their wall sensitive to vasoconstrictive factors.

Hirano et al [98] observed that the wall of the spiral artery, with the invasion of trophoblast was thin, but spiral arteries without trophoblasts invasion was thick in
width. The wall width was thick in preeclampsia compared with normal stating that the trophoblastic invasion controls the function of spiral arteries.

Starzyk et al [99] demonstrated that in preeclampsia the spiral and basal arteries are more tortuous or densely distributed than normal placental bed arteries, with smaller-caliber lumens and thicker walls. Failure of proper placentation may result in abnormal spatial anatomy in the placental bed. Alternatively, an anatomic variant of spiral and basal arteries may be more susceptible to hemodynamic stresses and endothelial damage and may predispose to preeclampsia.

2.3 STUDIES RELATED TO VILLOUS HISTOPATHOLOGY

Boyd and Scott [100] observed microscopic abnormalities in the villi like decreased villous vascularity, basement membrane thickening, stromal fibrosis, cytotrophoblastic proliferation, syncytial knot formation and villous fibrinoid necrosis have been reported. These are thought to represent a response, often of a compensatory nature to disturbances in blood flow.

Aparna Narasimha and Vasudeva [101] reported the villous abnormalities in preeclampsia like cytotrophoblastic proliferation (86%), thickening of the villous basement membrane (95.23%), increase in syncytial knots (90.4%), villous stromal fibrosis (92%), fibrinoid necrosis (97.85%), endarteritis obliterans (53.96%), paucity of vasculosyncytial membrane (93.65%).

Majumdar et al [102] found that mother with moderate to severe PIH (pregnancy induced hypertension) had smaller, irregular placentae with marginal insertion of umbilical cord with deviation in respect of foci of calcification, infarction and histological findings like cytotrophoblast cellular proliferation, syncytial knot formation, fibrinoid necrosis, hyalinised villous spots, stromal fibrosis were present in greater amount in hypertensive placentae. The changes in the placentae may be the cause/effect or both of hypertension in pregnancy of mothers who were normotensive.
Soma et al [103] observed microscopically placental abnormalities like increased syncytial knots, cytotrophoblastic proliferation, thickening of the trophoblastic basement membrane, obliterative endarteritis in toxemia. These placental abnormalities are due to the occlusion or narrowing of the uteroplacental vasculature as well as placental ischemia.

Lic et al [104] observed significant difference between normal term pregnancy and severe pregnancy induced hypertension groups like proliferation of cytotrophoblast, numbers of the placental villi with syncytial knots, thickness of basal lamina, fibrinoid necrosis, stromal fibrosis of villi and the vascular number of villi.

Fox [105] studied fetal stem arteries from 682 placentas, 36 of which were from stillbirths. He observed three lesions: obliterative endarteritis, fibromuscular sclerosis and thrombosis. These lesions were common in patients with hypertension (about 30%) and diabetes (23%).

Troll Mann et al [106] have demonstrated upregulation of the fetal vasculature endothelial growth factor in preeclampsia, speculating that it may be a ‘potential early indicator of severe birth asphyxia’. It is further relevant because fetal villous obliteration and capillary of sequelae of ‘poor maternal intervillous perfusion’.

Evensen et al [107] showed increased number of Hofbauer cells in severe preeclampsia with HELLP syndrome. These increased Hofbauer cells may be associated with increased inflammation or may have an adaptive mechanism at the fetal site of the placenta in patients with HELLP syndrome.

Anteby et al [108] in their study showed that the hofbauer cells express sprout (spry) proteins, which are important regulators of branching morphogenesis and growth factor signaling. They concluded that, placenta expression of spry imply an important role of hofbauer cells in placental villous branching. This study suggests that hofbauer cells might be involved in the development of chorionic villi.
Wetzka et al [109] reported an isolation procedure for hofbauer cells that yields a very pure population of placental macrophages which are viable and can be stimulated by LPS (lipopolysaccharide). They also cultured hofbauer cells in low oxygen (5% O$_2$) to imitate the conditions of placental hypoxia showed a decreased production of PGE$_2$ (prostaglandin E2) with TXA2 (thromboxane) synthesis remaining unchanged.

Altshuler [110] stated that chorangiosis is a condition in which placental tissue hypoxia causes villous capillary endothelial cell proliferation and capillary hypervascularity.

Altshuler [111] stated that the pathogenesis of chorangiosis is thought to be hypoxic stimulus which causes excessive villous capillary and connective tissue proliferation probably due to the induction of growth factors. This condition is usually associated with increased neonatal morbidity and mortality.

De La ossa et al [112] emphasized that chorangiosis should be considered as a placental sign of potential clinical significance because the interaction of maternal, placental and fetal factors may combine to produce this pathologic change.

Suzuki et al [113] has clarified a possible association of placental oxygenation status with the development of chorangiosis. They measured placental tissue oxygen index (TOI) values using near infrared spectroscopy (NRIS) before delivery and retrospectively compared them to the detection of placental chorangiosis, in a total of 47 pregnant women. Placental TOI values were significantly elevated in cases of chorangiosis. This indicates high oxygen saturation in the intervillous spaces because placental TOI values are expected to represent the oxygenation of maternal blood in the placental tissue.

Virupaxi et al [114] found localized fibrinoid necrosis, endothelial proliferation of arteries and hyalinization in the mosaicism of placenta probably leads to placental insufficiency and ultimately to foetal growth retardation. These
microscopic findings in the mosaicism of placenta are probably the aftermath of hypertension.

Tenney and Parker [115] were the first to recognize the syncytial knots in preeclampsia placenta which was widely referred to as tenny-parker changes. They found that syncytial knots were present on nearly all terminal villi in preeclamptic placenta, whereas they were only seen on 10% to 15% in normal placentas.

Myatt [116] observed increased syncytial knots and subtrophoblastic basement membrane thickness in the placenta of hypertensive pregnancy. Hypertension in pregnancy causes placental hypoxia leading to loss of large number of parenchymal cells, which causes appearance of syncytial knots and synthesis of fibrous tissue in their place. This fibrous tissue is synthesized by fibroblast of stroma, which are also responsible for subtrophoblastic basement membrane thickness.

Burton [117] studied placental villous development in hypoxia conditions and reported that the syncytial knots were increased in these cases. The syncytial knots formation results due to villous cytotrophoblast proliferation as a tissue response to hypoxia.

Nag et al [118] reported a significant increase in the cytotrophoblastic cellular proliferation and syncytial knot formation in the placental villi may also indicate a disturbance in the hormonal factors which may probably lead to altered morphometry of placenta resulting in pregnancy induced hypertension in the mother and low birth weight babies.

Genset [119] reported that stromal fibrosis and excessive syncytial knot formation are seen in generalized form as invariable results of overall reduction of foetal perfusion of the placenta.

Mallik et al [120] observed that if the syncytiotrophoblast suffers ischemic damage, the cytotrophoblast proliferaes in an attempt to replace the damaged tissue.
The degree of cytotrophoblastic hyperplasia is related to the extent of syncytial damage and thus serves as a rough quantitative index of the severity of ischemia.

Loukeris et al [121] provided normal reference data for the average percentage of syncytial knots for gestational ages ranging from 20 to 40 weeks. There was a significant positive correlation of gestational age with percentage of villi with syncytial knots. Term placentas (37-40 weeks) showed an average of 28% syncytial knots. A drop off to a mean of 22.5% was noted at 36 weeks; at 26 to 33 weeks, syncytial knots varied from 10.8% to 14.7%; between 20 to 25 weeks, syncytial knots ranged between 5.2% and 9.1%. These reference data can facilitate histologic assessment of normal placental maturation as well as evaluation of placental morphology in placental malperfusion.

Heazell et al [122] described an increased number of syncytial knots when tissue is cultured in hypoxia, hyperoxia or in the presence of ROS (reactive oxygen species). They suggested that the increased number of syncytial knots in placentae from pregnancies complicated by preeclampsia and fetal growth retardation can be replicated in vitro by ROS or hypoxia, supporting their involvement in the pathogenesis of these conditions.

Hamid Ansari et al [123] found that there was complete absence of vasculosyncytial membrane in placental villi of hypertensive patients resulting in foetal hypoxia, which in turn leads to perinatal morbidity and mortality.

Fox [124] found the deficient vasculosyncytial membrane in the placental villi of hypertensive pregnancies i.e present on less than 5% of the villi. This paucity of membranous area can be considered as a failure of trophoblast differentiation. He also noted that there was an inverse relationship between incidence of villous vasculosyncytial membranes and that of fetal hypoxia.

Devishankar et al [125] emphasized that in preeclampsia the hypoxic injury disrupts the syncytial architecture resulting in the increased density of syncytial knots and vasculosyncytial membrane thickness that consequently promotes the
release of soluble syncytial factors. The increased thickness of vasculosyncytial membrane causes impaired maintenance of feto-maternal exchange initiating the aponecrosis of syncytiotrophoblast as syncytial knots, subsequently culminating the systemic inflammatory response of the mother. These factors are suggested to pathologically active the maternal endothelium leading to maternal proteinuria and hypertension, the clinical hallmarks of preeclampsia.

Sargent et al [126] have proposed that when syncytial knots break off in increasing amounts from the placenta and are shed into the maternal circulation they may be the cause of the systemic endothelial activation that is seen in preeclampsia.

Neale et al [127] showed increased apoptosis of syncytiotrophoblasts may increase the amount of syncytiotrophoblast debris, syncytial knots, that leak into the maternal circulation and generate an exaggerated systemic endothelial activation.

Burton and Jones [128] suggests that turnover of syncytial nuclei takes place in the normal placenta, or that this occurs through an apoptotic-related process. Instead, they suggest that a proportion of syncytial nuclei are transcriptionally active, that epigenetic modifications underlie the changes in chromatin appearance, and that syncytial nuclei continue to accumulate until term. They have recognize that apoptotic changes can occur in pathologic pregnancies, but consider the deportation of trophoblast that has been linked to preeclampsia to be most likely of necrotic origin following ischemia injury.

Orozco and Jorgez [129] stated that syncytial knots are liberated into the maternal circulation from the villous surface as smaller syncytiotrophoblast fragments or microparticles. These syncytiotrophoblast microparticles (STBM), along with cell free fetal DNA (ffDNA), are defined in the maternal circulation and are abundant in preeclampsia.
2.4 STUDIES RELATED TO sFLT-1 EXPRESSION IN PREECLAMPTIC PLACENTA

Burrma et al [130] found that the syncytial knots are the principal site of expression of the antiangiogenic factors sFLT-1. In addition to that they reported significantly more placenta-derived syncytial aggregates in the autopsied lungs obtained from women died with preeclampsia, and these aggregates still contained the antiangiogenic factor sFLT-1 after their entrapment in the maternal lungs.

Augustine rajakumar et al [131] showed that placentas from preeclamptic women have more syncytial knots that are more heavily loaded with sFLT-1 protein compared with those from normal pregnancies. They concluded that detachment of syncytial knots from the placenta results in free, transcriptionally active syncytial aggregates that express sFLT-1 protein.

Clark et al [132] showed that placenta secretes sFLT-1, which would be expected to be a VEGF antagonist. They have found by in situ hybridization that the trophoblast contains the mRNA encoding a soluble version of the VEGF receptor known as sFLT-1, released into the maternal circulation.

Levine et al [133] reported alterations in the levels of sFLT-1 and free PLGF were greater in women with an earlier onset of preeclampsia and in women in whom preeclampsia was associated with a small-for-gestational age infant. Increased levels of sFLT-1 and reduced levels of PLGF predict the subsequent development of preeclampsia. The sFLT-1 level increased begins approximately 5 weeks before the onset of preeclampsia.

Hertig et al [134] confirmed in their study that the maternal serum sFLT1 concentration is markedly increased at delivery in women with preeclampsia and is measurably increased long before clinical onset (minimum of 6.5 weeks before onset).

Kendall et al [135] found soluble fms-like tyrosine kinase receptor mRNA, generated by alternative splicing of the same pre-mRNA used to produce
the full length membrane-spanning receptor, encodes the six N-terminal immunoglobulin-like extracellular ligand-binding domains.

Gilbert et al [136] found immunoreactive placental sFLT-1 was increased in RUPP (reduced uterine perfusion pressure) rats contrasted with the normal pregnant rats. The RUPP increases the expression of sFLT-1 and alters the balance of angiogenic factors in the maternal circulation.

Xu et al [137] reported that placental sFLT-1 production was higher in preeclampsia and antihypertensive drugs had no effect on placental production of sFLT-1 in vitro. They studied antihypertensive drugs clonidine (0.08-1.3 microg/ml), diazoxide (25-300 microg/ml), frusemide (60-1000 microg/ml) and hydralazine (6.3-100 microg/ml) have any effect on placental production of sFLT-1 and sEng in placentas from normal and preeclamptic pregnancies. Villous explants were cultured with increasing doses of antihypertensive drugs. Placental sFLT-1 and sEng production was examined using ELISA. Baseline sFLT-1 production was higher in placentas from women with preeclampsia than from normal pregnancy.

Ye et al [138] demonstrated significant correlation between the serum level of sFLT-1 and the expression of sFLT-1 mRNA or sFLT-1 in placenta of normal pregnancy group and preeclampsia group. Upregulation of sFLT-1 mRNA and excess expression of sFLT-1 in placenta may induce higher level of sFLT-1 in serum, which may be involved in the pathophysiological processes of preeclampsia.

Demir and Erbengi [139] observed strong VEGFR-1 immunolabeling on hofbauer cells during early pregnancy. They found hofbauer cells were numerous in the immature intermediate villi. Their distribution during the early days of pregnancy (between 22-48 days of pregnancy) were heterogeneous, and they were localized very near vascular pattern and close to ACC (angiogenic cord cells) in small clusters in the immature intermediate villi; these cells, which secrete VEGF moderately, expressed VEGFR-1 receptors strongly.
Roberts and Cooper [140] suggested that preeclampsia is a leading cause of maternal and fetal morbidity and mortality worldwide. It occurs in two phases: abnormal implantation of the placenta leads to impaired placental blood flow, which in turn induces the release of a critical placental substance into the maternal circulation.

Maynard et al [141] compared the gene expression profile in placental tissue from women with and without preeclampsia and identified soluble FLT-1 (sFLT-1), a vascular endothelial growth factor receptor, as a molecule that antagonizing the endothelial growth factor and or placental growth factor and eventually leading to clinical preeclampsia.

Richard et al [142] stated that, compared to women with a retrospective diagnosis of normal pregnancy (i.e without hypertension), preeclamptic women had increased serum sFLT-1 several weeks before the onset of clinical disease, suggesting that this protein might be used as a predictive marker for preeclampsia.

Chaiworaponga et al [143] showed plasma sVEGFR-1 concentration is elevated in preeclampsia prior to the clinical diagnosis of the disease. This elevation began 6-10 weeks prior to the clinical manifestations, and the increase was more pronounced at 2-5 weeks before the diagnosis, as well as at clinical presentation. Furthermore, in early-onset preeclampsia, plasma concentration of sVEGFR-1 is elevated earlier than the late onset disease.

Ahmad and Asif [144] suggested that removal of sVEGFR-1 by immunoprecipitation from preeclamptic CM (conditioned media) significantly restored angiogenesis further suggests that the elevated level of sVEGFR-1 in preeclampsia is likely to be responsible for the poorly developed feto-placental vasculature associated with this disorder. These findings provide potential therapeutic approaches for the prevention and treatment of preeclampsia and suggest that pharmacological intervention to inhibit sVEGFR-1 may be worthy of investigation.
Thandhani et al [145] demonstrated that sFLT-1 could be removed from the maternal circulation of preeclamptic women by apheresis safely, and that this therapy reduced both blood pressure and proteinuria, with a trend toward increased gestational duration.

2.5 STUDIES RELATED TO VEGF AND eNOS:

Leung et al [146] reported that vascular endothelial growth factor (VEGF) is a proangiogenic factor that promotes the proliferation, survival of endothelial cells and induces vascular permeability.

Sugimoto et al [147] found anti-VEGF therapies given to adult animals cause glomerular endothelial damage with proteinuria.

Eremina et al [148] found in humans, anti-angiogenesis cancer trails with anti-VEGF antibodies have led to proteinuria, hypertension and loss of glomerular endothelial fenestrae. Thus VEGF deficiency, whether induced by anti-VEGF antibodies or excess sFLT-1, is likely responsible for proteinuria and glomerular endotheliosis.

Lyall et al [149] demonstrated a reduction in villous placental vascular endothelial growth factor (VEGF) expression in placental villous tissue from pregnancies complicated by preeclampsia and intrauterine growth retardation.

Eremina et al [150] demonstrated in the mouse kidney that podocyte-selective knockout of VEGF in early postnatal life results in proteinuria, nephritic syndrome, endotheliosis, and eventually disappearance of endothelial cells from the glomerular tuft, recapitulating the classic renal lesion of preeclampsia.

Cooper et al [151] stated that expression of VEGF mRNA were significantly lower in the placenta of pregnancy complicated by preeclampsia compared with that of control. Such placenta exhibit deficient growth and differentiation of terminal villi and reduced fetal capillary branching and therefore reduced levels of VEGF could well account for these morphometric changes.
Kim et al [152] showed decreased expressions of VEGF and visfatin in the third trimester placental bed of pregnancies with preeclampsia compared with the normotensive controls. By this result they suggest that decreased expression of these angiogenic factors in placental bed may be associated with the pathogenesis of preeclampsia.

Sgambati et al [153] demonstrated that the level of VEGF mRNA expression were lower in the cases of preeclampsia with HELLP syndrome with respect to the control. This is probably related to haemodynamic changes that take place in these disorders, in order to attempt restoration of a normal uteroplacental flow.

Marini et al [154] stated that placentas from pregnancies with preeclampsia showed lowest VEGF mRNA expression levels compared with levels in the control group. This demonstrates a dysregulation of placental expression of VEGF family related to the degree of clinical severity of the hypertensive disorder.

Wang et al [155] reported that reduced vascular endothelial growth factor expression in preeclamptic placenta compared with the control may be responsible for the impaired vascular development which occurs in the preeclampsia.

Chen et al [156] observed decreased serum levels of vascular endothelial growth factor (VEGF) and reduced expression of vascular endothelial growth factor (VEGF) in placental tissues of preeclampsia pregnancies in rats.

Liu et al [157] showed that the reduced expression of VEGF in placenta of patients with pregnancy induced hypertension may be one of the important factors responsible for decreased placental vascular density and fetal intrauterine growth restriction.

Zhang et al [158] stated that VEGF is secreted mainly by syncytiotrophoblast in human placentae and the intensity of VEGF immunostaining in syncytiotrophoblast was significantly reduced in the pregnancy induced hypertension group compared with the normal group. This reduced VEGF may be
responsible for the impaired vascular development in pregnancy induced hypertension.

Cirpan et al [159] demonstrated vascular endothelial growth factor (VEGF) expression is significantly lower in placental bed biopsies of preeclampsia pregnancies.

Akercan et al [160] found that the vascular endothelial growth factor (VEGF) expression was significantly higher in placental biopsies of preeclampsia patients compared to that of controls.

Zhou et al [161] showed that VEGF was expressed in the syncytiotrophoblast and endothelial cells of vessels and capillaries in the placental tissue of normal and preeclampsia. Compared to normal the VEGF expression was decreased in preeclampsia. This VEGF deficit could lead to endothelium cell dysfunction, and the administration of VEGF could protect endothelium cells from injury. The lack of VEGF contributes to endothelial dysfunction, which may lead to the occurrence and development of preeclampsia.

Gilbert et al [162] demonstrated that chronic infusion of VEGF_{121} during late gestation restores glomerular filtration rate and endothelial function and reduces high blood pressure associated with placental ischemia. The VEGF_{121} may be a candidate molecule for management of preeclampsia and its related complications.

Li et al [163] reported the potential use of the VEGF family in the treatment of preeclampsia. They describe a preeclampsia model in pregnant rats induced by adenoviral overexpression of sFLT-1 (Adv-sFLT-1). Infection with Adv-sFLT-1 in rats resulted in hypertension and proteinuria. Histologically, the kidneys from these rats showed glomerular endotheliosis, reminiscent of the renal lesions associated with preeclampsia in pregnant women. Administration of recombinant VEGF-A_{121} resulted in a reduction in systolic blood pressure and proteinuria and an improvement in glomerular endotheliosis.
He et al [164] stated that VEGF have a direct vasodilatory effect on the systemic vasculature because infusion of VEGF leads to nitric oxide dependent vasorelaxation in the coronary arteries and other vessels in humans, likely through upregulation of nitric oxide and prostacyclin in vascular endothelial cells.

Yallampalli and Garfield [165] in their study produced an animal model of preeclampsia by infusion of an inhibitor of nitric oxide (NO) synthesis called L-NAME (L-nitroarginine methyl-ester), into pregnant rats, which produces hypertension, proteinuria and thrombocytopenia.

Kassab et al [166] found that chronic NO synthase inhibition in pregnant rats produces hypertension associated with peripheral and renal vasoconstriction, proteinuria, intrauterine growth restriction and increased fetal morbidity.

Davidge et al [167] stated that the nitric oxide (NO) production is elevated in normal pregnancy and these increments appear to play an important role in the vasodilatation that occurs in pregnancy.

Conrad et al [168] postulated that analogous to the endothelial cells, the syncytiotrophoblast cell layer that lines the intervillous blood space of the human placenta expresses endothelial nitric oxide synthase (eNOS).

Eis et al [169] found eNOS was expressed in the syncytiotrophoblast but not in the cytotrophoblast in the chorionic villi of mature placenta. They confirmed it by cell culture, in which nitic oxide synthase (NOS) was expressed with differentiation of cytotrophoblastic cells and formation of syncytium.

Myatt et al [170] demonstrated that the eNOS isoform is found in the endothelium of the umbilical, chorionic plate and stem villous vessels, but not in the terminal villous capillary endothelium of the normotensive placenta.

Ariel et al [171] found that eNOS immunoreactivity was most prominent in the apical portion of the syncytiotrophoblast in the first trimester placenta,
corresponding to the region with the most prominent histochemical reaction for NADPH diaphorase (indicating NOS activity).

Myatt et al [172] found eNOS reside in the endothelium of placental vessels such as umbilical arteries and veins, chorionic arteries and veins, but also in the layer of the syncytiotrophoblast.

Eis et al [173] observed the localization of eNOS in the syncytiotrophoblast layer and the endothelium of larger fetal vessels in the term human placenta.

Morris et al [174] reported lower endothelial nitric oxide synthase (eNOS) activity in gestations complicated by fetal growth retardation.

Kubes et al [175] stated that syncytiotrophoblast derived nitric oxide (NO) could potentially modulate the adhesion of maternal leukocytes to the syncytiotrophoblast, thereby preventing leukocyte migration through gaps in the syncytiotrophoblast cell layer and into the underlying villous core that contains tissue expressing paternally derived fetal HLA antigens.

Conrad and Vernier [176] reported that plasma levels, urinary excretion and metabolic production of cGMP are increased in gravid rats, and postulated that endogenous nitric oxide (NO) may mediate this change.

Forstermann et al [177] stated that the placenta would contribute to the production of nitric oxide (NO) during pregnancy because it contains fetal vessels with endothelium- a cell type known to possess NO synthase.

Myatt et al [178] stated that nitric oxide (NO) in the human placental villous serves to maintain basal tone as well as to attenuate the action of vasoconstrictor effects of thromboxane and endothelin in the feto-placental circulation.

Conard et al [179] identified increased biosynthesis of nitric oxide (NO) during pregnancy in rats, and proposed its involvement in maternal vasodilation and local immune modulation of normal gestation.
Myatt et al [180] showed expression of eNOS in placentas of pregnancies complicated by preeclampsia and/or intrauterine growth retardation (IUGR). Placentas from patients with preeclampsia with or without IUGR had a significantly more basal distribution of enos in syncytiotrophoblast. eNOS immunostaining was absent in terminal villous capillary and faint in stem villous vessel endothelium of normal placentas, but was intense in the endothelium of both of these types of vessels in the IUGR and preeclampsia groups, with significantly greater staining seen in stem vessels of patients with IUGR alone. This increased eNOS expression and hence increased NO production in the fetal-placental vasculature may be an adaptive response to the increased resistance and poor perfusion in these pathological pregnancies.

Ma et al [181] stated that inhibition of NO synthesis can cause imbalance of several vaso-regulatory factors, which may be responsible for the pathogenesis of pregnancy induced hypertension. They reported significantly decreased plasma NO (nitric oxide), PGI2 (prostaglandin I2) and Ang2 (angiotensin) while plasma ET (endothelin) and TXA2 (thromboxane) were increased significantly in rats given L-NAME (L-nitro arginine methyl ester) in pregnancy induced hypertension compared with controls.

Wang et al [182] demonstrated that infusion of L-NAME, an inhibitor of NOS, causes decreased serum NO level and signs similar to preeclampsia such as hypertension, proteinuria and intrauterine growth retardation. Nitroglycerine can reverse the lesions induced by the treatment of L-NAME. This may indicate that reduced level of NO may be a factor responsible for pregnancy induced hypertension.

Edwards et al [183] reported that long term nitric oxide synthase (NOS) blockade causes sustained hypertension, elevated levels of endothelin-1 and fetal growth restriction.