CHAPTER 5

DISCUSSION

Pregnancies complicated by preeclampsia are reflected in the placenta in a significant way both macroscopically and microscopically. In this study, the macroscopic changes observed in preeclamptic placenta compared to control were reduced placental weight, volume, thickness, diameter and number of cotyledons which may be due to insufficient blood supply to the placenta. These findings are in accordance with the findings of other studies in the literature [53-55].

The decreased placental volume and thickness in preeclampsia group observed in this study may be due to reduction in peripheral villi, fetal capillaries and intervillous space volume. The reduced villous tissue and insufficient blood supply may have resulted in small size of the placenta in preeclampsia. The significant reduction in diameter of placenta in preeclampsia group observed in this study may be due to the small size of the placenta. These data are supported by the findings of the Nobis and Das, Bhatia et al, Cibils, Fox, Das et al, Masodkar et al, who have studied the placental changes in hypertensive pregnancies [61-63, 72-74,186].

The placenta has the ability to control the growth of the fetus. In the present study the birth weight of the baby and APGAR score were reduced which may be due to inadequate blood supply to the placenta. The similar finding was reported by Damania et al that the placental weight and size were directly proportional to the birth weight of the babies. The low birth weight of the babies and its APGAR score in preeclampsia may be due to the altered arrangement of the intracotyledonous vasculature [56]. There is also evidence provided by Rath et al that fetal growth depends on placental weight, which is less with small for
gestational age infants. They also attributed that the marginal insertion of umbilical cord insertion hampers the growth of fetus in hypertensive pregnancies [58].

In this study the mean birth weight of baby and gestational age at delivery were lower in preeclamptic group compared to normal. This is similar to the study done by Barton et al, who have reported that the birth weight of the neonate was significantly lower in preeclampsia [65]. It is also homologous to the study of Xiong et al that the birth weights were significantly lower in mothers with preeclampsia who delivered at ≤ 37 weeks of gestational age [66]. Preeclampsia significantly increases the risk of preterm delivery/birth to reduce the maternal symptoms caused by this disorder.

In the present study the feto-placental ratio (fetal weight/placental weight) or placental ratio increased in preeclampsia pregnancies compared to normal. The placental ratio is higher when fetus growth is affected, because maturational changes are taking place throughout gestation within the placenta in order to increase the transfer capacities. This result corroborates with the findings of Verkauskiene et al that the placental ratio is high in preeclampsia. It is a strong indicator of impaired prenatal linear growth [71].

The umbilical cord length is one of the major cord morphological features in preeclamptic placenta. An abnormal umbilical cord length, either excessive or short, is a known risk factor for adverse prenatal outcome. A short umbilical cords, less than 35cm in length, were associated with pregnancy induced hypertension. The mean length of the umbilical cord observed in this study showed no significant difference between preeclampsia group with that of control. This finding is supported by a study conducted by Bhavina et al, who reported that there was no significant change in the length of umbilical cord in pregnancy induced hypertensive pregnancies [187].

Preeclampsia is more common in women with advanced maternal age as observed in this study. A women who is 35 years or older at the time of delivery has been defined as being of “advanced maternal age” (AMA). The similar finding was
reported by Lamminpaa et al that women of AMA were 1.5 times more likely to have preeclampsia compared to women less than 35 years of age. The mechanism behind this risk may be related to ageing of the uterine blood vessels [67]. An alternative explanation also exists in the literature that myometrial function deteriorates with age. Furthermore, women of AMA are also more likely to have chronic diseases, such as hypertensive disorders and diabetes mellitus, complicating their pregnancies [68, 70].

The gross reduction of the preeclamptic placenta impedes normal placentation and pathologically results in massive microscopic changes in the placenta. The histomorphometrical findings of the present study revealed that the number of villi, volume of villi and villi density were significantly increased in preeclampsia whereas the villous surface area, diameter of villi were decreased than those of controls. The increased villi density and decreased villi diameter in preeclampsia may be because of the continuous sprouting of the intermediate villi into terminal villi in order to compensate for the placental maldevelopment and dysfunction. These findings are in accordance with the findings of Egbor et al, Nakamura and Ahmed khairy makled et al, who had studied the histomorphometrical changes in the villi of preeclamptic placenta [81-83]. Although the number of villi increased, their diameter decreased because vasculature could not be established owing to unsustained normal villi size as noted in the normal placenta and is supported by the study done by Devishankar et al [80].

The mean number of villi count in preeclamptic placentas observed in this study was significantly higher than those in normotensive placentas. It may be because of an increase in the proliferation rate of the trophoblast in preeclampsia. This concept has been reported by Arnholdt et al that the reason behind this increased number of villi in hypertensive placentas could be that, these 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 deciduas [84].
In the present study the intervillous space volume was significantly reduced in relation to the total villous tissue volume of preeclamptic placenta compared to normal. There was proportionally more stem and less peripheral villous tissues in the placenta of the preeclampsia compared to the controls. The reduced intervillous space volume in preeclampsia group may be due to an abnormal pattern of maturation or arborization of the villous tree, characterized by significant decrease in the peripheral villous tissue (intermediate villi+terminal villi) and relative increase in stem villi. The similar finding was reported by Teasdale, that the intervillous space volume is reduced in preeclamptic placenta due to increased stem villous tissue [86]. It is reported by Kishwara et al that the volume of the intervillous space can also be reduced slightly by the deposition of fibrin [87].

In the present study the villous abnormalities observed in the preeclamptic placenta like increased stromal fibrosis, fibrinoid necrosis, obliteratorive endarteritis, hyalinized villi, thickening of trophoblastic basement membrane, cytotrophoblastic proliferation, syncytial knot formation, chorangiosis, CD68 cells and decreased vasculosyncytial membrane may be a response of the placenta due to the disturbances in blood flow. According to Boyd and Scott, these placental abnormalities can be seen not only in human toxemia, but also in animals with experimentally induced toxemia or with spontaneous toxemia. These histopathological findings in hypertensive placentas are due to the occlusion or narrowing of the uteroplacental vasculature which leads to the placental ischemia [100]. The similar finding was reported by Aparna Narasimha and Vasudeva, Majumdar et al, Soma et al, Lic et al, Fox, Troll Mann et al [101-106].

It is reported by Virupaxi et al, that the increased stromal fibrosis, fibrinoid necrosis, obliteratorive endarteritis and hyalinization of villi depict the mosaicism of placenta that are probably the aftermath of hypertension. Again the mosaicism of the placenta probably leads to placental insufficiency and ultimately to foetal growth retardation, thus creating a vicious cycle [114].

The marked thickening of the basement membrane observed in preeclampsia group indicates an altered trophoblastic activity which may be due to
increased secretion or decreased turnover of basal lamina molecules. The basement membrane thickening is the byproduct of cytotrophoblast cell hyperplasia as the basement membrane protein is secreted by these cells. This is in accordance with the findings of Mallik et al that the cytotrophoblastic proliferation and thickening of the trophoblastic membrane are often observed in the preeclampsia. They found that increased cytotrophoblastic proliferation and basement membrane thickening in preeclampsia leads to higher incidence of stillbirths, low birth weight babies and low APGAR score [120].

The above result is also supported by Fox, who reported that the cytotrophoblastic hyperplasia is a response to villous ischemia. If the syncytiotrophoblast suffers ischemic damage, the cytotrophoblast will proliferate in an attempt to replace the damaged tissue [196]. Thus the Cytotrophoblastic hyperplasia is a repair phenomenon and under ischemic conditions these cells are numerous and prominent mitotic figures can often be seen.

The thickening of trophoblastic basement membrane, cytotrophoblastic proliferation and increased syncytial knots observed in the placenta of preeclampsia may be due to placental hypoxia. The similar result obtained by Myatt, who studied the role of placenta in preeclampsia had reported that the placental hypoxia leads to loss of large numbers of parenchymal cells. It causes appearance of syncytial knots and synthesis of fibrous tissue in their place. This fibrous tissue is synthesized by fibroblast of stroma, which is also responsible for subtrophoblastic basement membrane thickness [116]. It is also supported by the study of Burton that the syncytial knots were increased in hypoxia [117]. A study by Collins et al has emphasized that increase in the number of syncytial knot formation, cytotrophoblastic cellular proliferation, stromal fibrosis and hyalinization of villi may be the result of reduction of foetal perfusion of the placenta [197].

Another study which also supports the findings of the present study is by Ifra Saeed et al, reported a significant increase in the cytotrophoblastic cellular proliferation and syncytial knot formation in the placental villi of preeclampsia. These changes indicate a disturbance in the hormonal factors which may probably
lead to altered morphometry of placenta resulting in pregnancy induced hypertension in the mother [198].

Chorangiosis is a vascular hyperplasia in the terminal chorionic villi. This characteristic feature is prominent in preeclampsia group of the present study. The vascular hyperplasia has been reported by Altshuler in long standing placental hypoperfusion or low grade tissue hypoxemia [110]. In the present study this may have occurred in preeclampsia because the placental tissue hypoxia might have caused villous capillary endothelial cell proliferation and capillary hypervascularity as a compensatory mechanism. A similar concept had been reported by Suzuki et al that incidence of chorangiosis is higher in women living in high altitudes, and thus a hypoxic stimulus may well lead to an excessive villous capillary and to connective tissue proliferative activity. In preeclampsia an increased quantity of blood vessels indicates that the placenta has properly adapted to lower levels of oxygenation [113]. It is also supported by a study of Altshuler that the pathogenesis of chorangiosis might be due to 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 [111].

The increased number of hofbauer cells in the preeclampsia group observed in the present study is analogous with the study done by Evsen et al that they have found 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 side of the placenta in patients with HELLP syndrome [107]. A both early and recent study present in literature also supports this finding [199-201]. It was reported that in preeclampsia, many cytokines are secreted from hofbauer cells that stimulate vasculogenesis and angiogenesis [139]. Seval et al showed a correlation between number of hofbauer cells in placental villous and number of vascular structures in preeclampsia [40]. Recent study showed that prostaglandin E2 (PGE2) and thromboxane (TXA2) were produced by hofbauer cells with PGE2 synthesis being predominant. Culturing
hofbauer cells in low oxygen showed a decreased production of PGE2 with TXA2 synthesis remaining unchanged [109].

The placental exchange between maternal and fetal components occurs in villi whose fetal vessels are in intimate contact with the covering syncytial membrane called vasculosyncytial membrane. In the present study the deficiency of vasculosyncytial membrane was found in the placenta of preeclampsia group compared to normal.

This paucity of vasculosyncytial membrane may be due to failure of trophoblast differentiation. This data is supported by the findings of the Hamid Ansari et al that the placentas from pregnancies complicated by preeclamptic toxemia have an unduly low proportion of villi with vasculosyncytial membrane. A low vasculosyncytial membrane count reflects the villous regression found in placentas from pregnancies complicated by preeclampsia [123]. The similar result was found in the studies of Fox that there was an inverse relationship between incidence of villous vasculosyncytial membranes and that of fetal hypoxia. Babies whose placentas had a low vasculosyncytial membrane count suffered from a high incidence of hypoxic complications [124]. In contrary Horkey found in fetal hypoxia there was an increased formation of these membranes in an attempt to increase the surface available for gas transfer [195]. Another homologous study had done by Devishankar et al. that, in preeclampsia the hypoxic injury to the placenta 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 [125].

In normal placentation, during the first and early second trimesters, the villous growth and arborization are regulated, which are necessary for fetal well-being [188,189]. The cytotrophoblast cells invade into the uterine spiral arteries and transform them from small-caliber resistance vessels into high-caliber capacitance
vessels capable of providing enhanced placental perfusion adequate for the growing fetus. For this transformation, a certain amount of hypoxia is needed to stimulate placental blood vessel formation. Until approximately 10 weeks of gestation, the embryo exists in a hypoxic environment with nutrients provided by the endometrial glands [190]. However, prolonged durations of hypoxia or oxidative stress leads to poor placental perfusion, which is the underlying pathogenesis of preeclampsia.

In preeclampsia, invasion of the uterine spiral arteries is limited to the proximal decidua, and 30–50% of the spiral arteries of the placental bed escape endovascular trophoblast remodeling. Persistence of muscular and elastic tissues of the media of spiral arteries, fail to dilate and remain responsive to vasomotor influences that lead to high resistance low flow choriodecidual circulation [191,192].

The reduction in the vascular dimensions is constantly accompanied by a significant impact on the lumen of the arteriole with changes in its muscular wall [193]. Thus, the average diameter of the blood vessels, which normally expands to 4 times their original size, is greatly decreased in preeclampsia. This was confirmed by this study through measuring the lumen and wall of the spiral arteries. The results showed statistically significant reduction in the blood vessel lumen and increased thickening of the wall in preeclampsia showing failure of trophoblast invasion which consequently leads to less blood supply to placenta that causes oxidative stress [89-91]. These findings are in agreement with those of Bokhari et al who has reported decreased luminal diameter of spiral arterioles in preeclamptic groups with mean diastolic blood pressure 108 and 123 mmHg [95]. Another similar study mentions this diameter to be reduced to 112µm with mean diastolic blood pressure of 110mmHg [92]. The study of Wolf et al also supports the present study by demonstrating a reduced luminal diameter in severe preeclampsia [194].

Hypertrophy of tunica media was another conspicuous feature observed in preeclamptic group of this study. An earlier study mentions hypertrophy of tunica media in severe preeclampsia with mean diastolic blood pressure 110mmHg [96-99]. This hypertrophy may be secondary to the development of hypertension and may act as a protective mechanism of the vessels against high pressure [93, 94].
The Programmed cell death or apoptosis plays an important role in cell homeostasis and tissue remodeling, particularly in placental development. Importantly, placental degeneration in preeclampsia may be due to unscheduled apoptosis of trophoblasts. The pregnancy associated remodeling of the spiral arteries is mediated by invasive cytotrophoblasts. However, if these trophoblasts are subjected to a high rate of apoptosis, this defective transformation of spiral arteries may result in local ischemia, thrombosis and infarction [128].

In the present study increased number of syncytial knots was observed in the preeclampsia group compared to control which may be due to apoptosis of trophoblast. The increased apoptosis of syncytiotrophoblasts may increase the amount of syncytiotrophoblast debris, the syncytial knots which leak into the maternal circulation and generate an exaggerated systemic endothelial activation in preeclampsia. This finding is supported by the study of Sargent et al and Neale et al, that when syncytial knots break off in increasing amounts from the placenta are shed into the maternal circulation. They may be the cause of the systemic endothelial activation that is seen in preeclampsia [126,127].

There is another study in accordance with the present study by Burton et al that the trophoblastic apoptosis in preeclampsia placentas causes increased syncytial knots formation in the peripheral zones. These zones are less oxygenated and are more prone for hypoxic changes. The state of hypoxia leads to the stimulation of apoptotic pathway, thereby increasing trophoblastic cell turnover process. The syncytial knotting represents a way of extrusion of unwanted aged nuclei [128]. Similarly Orozco and Jorgez in their study on preeclamptic placental release of microparticles into maternal circulation reported that these syncytial knots take either aponecrotic or necrotic route to reach the maternal circulation [129].

The syncytiotrophoblast can be affected by the altered rates of trophoblastic demise thereby reflecting the state of functioning of these cells in the placenta during implantation process. However an increase in the number of syncytial knots is a feature of normal pregnancy. Due to high degree of differentiation, the syncytiotrophoblast loses its power of division, so throughout
pregnancy, it depends upon cytotrophoblast. This continuous input is counterbalanced by continuous extrusion, by apoptosis of this material in the maternal circulation. This material is packed into membrane sealed structures called syncytial knots [198]. The study of Heazell et al supports the above findings that oxidative stress plays an important role in the pathogenesis of preeclampsia. They observed an increased number of syncytial knots in the placental tissue which is cultured in hypoxia, hyperoxia or in the presence of reactive oxygen species (ROS) [122].

The placenta of preeclampsia pregnancies produce soluble antiangiogenic factor which mediate endothelial dysfunction. The soluble fms like tyrosine kinase-1(sFLT-1) is an antiangiogenic protein that is overproduced by the placenta and induces systemic endothelial dysfunction in preeclampsia. In the present study it was observed that syncytiotrophoblast and syncytial knots express increased sFLT-1 in preeclampsia which may be associated with increased levels of maternal circulating sFLT-1, a finding that has been confirmed by several studies in the literature [132-134].

The syncytial knots express increased sFLT-1 in preeclampsia group compared to normal. This result of the present study is supported by the findings of Burrma et al that the syncytial knots are the principal site of expression of the antiangiogenic factor sFLT-1. They found syncytial aggregates in the autopsied lungs of women who died with preeclampsia. These syncytial aggregates contained the antiangiogenic factor sFLT-1 after their entrapment in the maternal lungs [130].

The present study also corroborates with the findings of Rajakumar et al who reported that the third trimester placentas spontaneously form living syncytial knots that detach from placental villi through fission. The detached syncytial knots form membrane bound multinuclear structures called syncytial aggregates. These syncytial aggregates has the ability to synthesis sFLT-1 protein from endogenous stores of mRNA. The increased number of active syncytial aggregates in preeclampsia, release sFLT-1 protein into the maternal circulation [131].
The current study also observed immunohistochemically that sFLT-1 expression was higher in Hofbauer cells of preeclamptic placenta compared to normal. This study also correlated the number of Hofbauer cells between normal and preeclampsia, which showed increased number of Hofbauer cells in preeclamptic placenta. Therefore in addition to syncytial knots, this study suggests that the Hofbauer cells also act as a source of sFLT-1 secretion. Moreover the locations of Hofbauer cells in the chorionic villi (near fetal capillaries) strongly suggest the participation of Hofbauer cells in the pathogenesis of this disorder. To our knowledge this may be the first study to correlate the number and increased expression of sFLT-1 in Hofbauer cells which may be one of the possible sources of increased sFLT-1 in preeclampsia.

In addition to demonstrating the excess sFLT-1 expression in the placental cells of preeclampsia, the present study also found the expression of VEGF and eNOS.

The VEGF is an angiogenic protein which was highly expressed in the early human placenta. According to immunohistochemical and in situ hybridization studies, villous trophoblast and Hofbauer cells are the main source of this cytokine. First trimester trophoblast and Hofbauer cells secrete VEGF in vitro. VEGF stabilizes endothelial cells in mature blood vessels and is particularly important in maintaining the health of the endothelium in the kidney, liver, and brain. VEGF induces nitric oxide and vasodilatory prostacyclins in endothelial cells and plays a role in decreasing vascular tone and blood pressure. Moreover, cancer patients receiving VEGF inhibitors therapeutically reported the signs of hypertension and proteinuria along with glomerular damage as adverse effects that resemble preeclampsia [148].

Studies on placental VEGF-A protein expression using immunohistochemistry have reported a decrease in VEGF-A immunostaining in preeclampsia and preeclampsia with HELLP syndrome compared with normotensive pregnancies which was analogous to the present study [149-151]. Such an abnormality of growth factor expression in the placenta will exhibit deficient growth
and differentiation of terminal villi and reduced fetal capillary branching. According to Liu et al, the reduced expression of VEGF in placenta of patients with pregnancy induced hypertension may be one of the important factors responsible for decreased placental vasculature density and fetal intrauterine growth restriction [157].

The VEGF is secreted mainly by syncytiotrophoblast in human placenta and the intensity of VEGF immunostaining in syncytiotrophoblast was significantly reduced in the preeclampsia group compared to normal. This reduced VEGF may be responsible for the impaired vascular development in preeclampsia. Therefore the reduced VEGF could lead to endothelial cell dysfunction, and the administration of VEGF could protect endothelial cells from injury. This finding is supported by the studies of Zhang et al and Zhou et al [158-161].

Various investigations on placental VEGF-A mRNA have shown that its level is reduced in preeclampsia placentas compared with normal placentas [159] while others have demonstrated an increase or no difference [160]. A recent study found placental VEGF mRNA to be higher in gestational hypertension, where hypertension occurs in the absence of proteinuria, but lower in preeclampsia with HELLP syndrome, while there was no difference in other preeclampsia placentas compared with normal placentas. It was proposed that the high VEGF-A expression in the gestational hypertension group may be a compensatory mechanism in an attempt to restore placental blood flow to normal. In preeclampsia and the HELLP syndrome, there is an attempt at compensation with only some components of the placenta being able to produce VEGF-A [153].

In the study of Eremina et al, the VEGF knockout mice showed the symptoms of proteinuria, nephritic syndrome, endotheliosis and eventual disappearance of endothelial cells from the glomerular tuft which is the classic renal lesion in preeclampsia [148]. Similarly Gilbert et al demonstrated that chronic infusion of VEGF121 during late gestation restores glomerular filtration rate and endothelial function and reduces high blood pressure associated with placental ischemia. The VEGF121 may be a molecule for management of preeclampsia and its related complications [162]. A 50% reduction of glomerular VEGF leads to
glomerular endotheliosis and proteinuria similar to that seen in preeclampsia [150]. A similar study conducted by Li et al, who had designed a preeclampsia model of pregnant rats by inducing adenoviral overexpression of sFLT-1 (Adv-sFLT-1). Injection 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 [163]. Collectively these data suggest that VEGF is important not only in blood pressure regulation but also in maintaining the glomerular filtration.

Recent study demonstrated increase of capillary formation after vitamin D3 treatment in endothelial colony-forming cells which could be mediated by the increased expression of VEGF that is known to stimulate endothelial cell migration and differentiation in vitro. There was a 1.74 fold increase of VEGF mRNA expression after treating endothelial colony forming cells with vitamin D3. Thereby vitamin D3 substitution in early pregnancy (i.e. in time of placental development) appears to reduce the risk of developing preeclampsia [205]. In another study it was reported that the phosphorylated form of α-tocopherol (αT), α-tocopheryl phosphate (αTP), increases the expression of VEGF. The stimulatory effect of αT on angiogenesis and vasculogenesis is potentiated by phosphorylation of αTP, which may act as a cofactor or active lipid mediator increasing VEGF expression. Increased VEGF expression and consequent enhanced angiogenesis and vasculogenesis induced by αTP may suggest the essential role of vitamin E on preeclampsia, ischemia/reperfusion injury [206,207].

Interestingly Belgore et al [204] showed that cigarette smoking is associated with a lower incidence of preeclampsia. Nicotine has been shown to have proangiogenic properties by inducing endogenous VEGF. Smoking has been shown to lower sFLT-1 levels in humans. Thus it was suggested that short term use of nicotine in cases of severe preeclampsia might be an effective treatment.
The eNOS is the vasodilatory factor that influences the vasculogenesis in placenta along with VEGF [202]. The NO biosynthesis increases during pregnancy and involves in maternal vasodilation and local immune modulation of normal gestation. The eNOS helps in the synthesis of NO in the placental villi which contribute to the regulation of vascular tone by counteracting the actions of vasoconstrictors [203]. The eNOS expression observed in this study was reduced in the placenta of preeclampsia group compared to normal. This finding was analogous with the existing literature that chronic nitric oxide synthase inhibition in pregnant rats produces hypertension associated with peripheral and renal vasoconstriction, proteinuria, intrauterine growth retardation and increased fetal morbidity [182].

It is important to note that eNOS gene expression in human placenta was found to increase with advancing gestation, which was found to be increased in placental syncytiootrophoblast with gestational age. It thus appears that there is enhanced biosynthetic demand for the production of NO in the growing human placenta [167]. The findings of the present study is supported by the study of Morris et al, who reported lower eNOS activity in gestations complicated by fetal growth retardation [174].

eNOS has been found to reside in the endothelium of placental vessels such as umbilical arteries and veins, chorionic arteries and veins [170]. It has been found that the syncytiotrophoblast cell layer that lines the intervillous blood space of the human placentas also express NO synthase [168]. Since it act as an endothelial layer that line the intervillous space. The syncytiotrophoblast derived NO may play a critical role in preventing platelet adhesion to the syncytiotrophoblast cell layer as well as inhibiting platelet aggregation within the intervillous space [172,173]. In preeclampsia, where the intervillous formation of thrombi is excessive, may conceivably result from deficient production of NO by the syncytiotrophoblast leading to increased aggregation of platelets. Evidence for exaggerated platelet activation and consumption has been reported in women with preeclampsia, although this abnormality may also reflect endothelial dysfunction in the maternal peripheral vasculature [177].
Syncytiotrophoblast derived 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. By this mechanism, as well as possibly by others, syncytiotrophoblast derived NO may contribute to the prevention of fetal semi-allograft rejection [175]. Myatt et al [180] found eNOS to 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. At term, the endothelium of all fetal blood vessels and the syncytiotrophoblasts were eNOS positive, while cytotrophoblasts had become negative. These data have been confirmed in this study by observing localization of eNOS in the syncytiotrophoblast layer and the endothelium of larger fetal vessels but not in the cytotrophoblast. This study also found increased expression of eNOS in hofbauer cells of preeclamptic placenta compared to normal which was previously unrecognized.

The NO is a potent vasodilator, blood flow through the fetal arterioles in the villous core of the stem and mature intermediate villi could be facilitated by NO formed in the overlying syncytiotrophoblast. In response to maternal hypoxemia or uteroplacental vasoconstriction, reduced oxygen availability in the intervillous space may limit NO production by the syncytiotrophoblast, thereby producing vasoconstriction of underlying fetal arterioles [181]. This finding was correlated in the present study that decreased expression of eNOS in syncytiotrophoblast results in reduced NO production which may lead to obliterative endarteritis in preeclampsia. In order to compensate this, hofbauer cells in the chorionic villi may produce increased eNOS to synthesis more NO to dilate the fetal blood vessels. Since the hofbauer cells are located close to the fetal blood vessels the increased expression of eNOS in hofbauer cells may play a compensatory role to overcome the insufficient blood flow in preeclamptic placenta.

The present study concludes that the expression of sFLT-1 was increased whereas VEGF and eNOS decreased in the placental cells of preeclampsia. The links visualized in the proposed work gives an idea about the molecular mechanism which occurs in the preeclamptic placenta.