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Angiogenic factors and histomorphological alterations encountered in preeclamptic placentae

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***Abstract*---**Background:Preeclampsia complicates 10% of pregnancies in the developed world. Alteration in angiogenic factors occurs in preeclampsia which leads to chronic hypoxic changes in placenta.Aim:To assess the histomorphological changes and expression of angiogenic factors in placenta of preeclamptic patients in comparison to normal placenta.Design: Cross sectional analytical study.Materials& Methods: This study was conducted from October 2013 to August 2015 and included 53 placenta from preeclamptic patients and 53 placenta from healthy pregnant women. Histomorphological changes were studied in the placenta. Expression of vascular endothelial growth factor (VEGF) and vascular endothelial growth factor receptor-1 (VEGFR-1) in the placental villi were evaluated using immunohistochemistry (IHC).Statistical analysis: Done using IBM-SPSS 21.0. 'p' value < 0.05 was taken as significant.Results: In our study, 92.5% of preeclamptic group were in the age group of 21 to 34 years and 62.3% of them had placental weight of less than 500 grams. The histomorphological findings observed in preeclamptic cases were increase in villous vascularity(62.3%), increase in syncytial knots(94.3%),

cytotrophoblastic proliferation(62.3%), Villous Stromal Fibrosis(45.3%) fibrinoid necrosis(50.9%) , diffuse Sub Chorionic Fibrin (3.8 %), diffuse infarction(37.7%) ,focal calcification(60.4%) and endarteritis obliterans(47.2%) with weak staining of VEGF and VEFGR-1 in 30.2 % and 43.4 % of cases respectively which significantly correlated with above features.Conclusion: We found that reduced expression of angiogenic factors in placenta of preeclamptic patients correlated with the impaired placental vascular adaptation. This would have resulted in reduced placental blood flow leading to the related histomorphological complications.

***Keywords*---**preeclampsia, placenta, angiogenic factors.

Introduction

Preeclampsia, characterized by new onset hypertension, with BP ≥ 140/90 mm of Hg on two occasions 4 hours apart and proteinuria occurring after 20 weeks of gestation[1]complicates 10% of pregnancies in the developed world.[2]In normal pregnancy, human placenta undergoes angiogenesis and pseudovasculogenesis. Alteration of this processin preeclampsia leads to chronic hypoxia. Morphological features in villi, like excessive syncytial knots, infarction and hypercapillarization are due to impaired uteroplacental blood flow. Placental expression of angiogenic factors; Vascular Endothelial Growth Factor (VEGF) & its receptors, Vascular Endothelial Growth Factor Receptors-1 &2 (VEGFR-1 &VEGFR-2),has shown to correlate with villous hyper maturity & severity of preeclampsia. However, some have also reported reduced expression of VEGF and VEGFR-1 in preeclampticpatients. Release of factors like soluble form of VEGFR-1 (sVEGFR-1/sFlt- 1) from diseased placenta into the maternal circulation is thought to be responsible for endothelial dysfunction & therefore the clinical manifestation of the disease.[3]The present study was conducted to assess the occurrence of various histomorphological changes and the expression of VEGF & VEGFR-1 in placenta of preeclamptic patientsand their correlation.

Materials and Methods

This was a cross sectional analytical study conducted in Departments of Pathology and Obstetrics and Gynecology, from October 2013 to August 2015. Placenta from 53 preeclamptic patients and 53 normal pregnant women were collected after getting informed written consent from the patients and controls.Inclusion criteria forstudy group was pregnant women with new onset hypertension with blood pressure ≥140/90 mm of Hg on two occasions 4 hours apart and proteinuria occurring after 20 weeks of gestation and for control groupwas pregnant age matched normotensive women with no co-morbidities. Exclusion criteria were pregnant women suffering from any concurrent disease like preexisting hypertension, gestational hypertension, eclampsiaand diabetes mellitus.

Patient's clinical details like age, parity, blood pressure, haematological and biochemical parameters like platelet count, LFT, RFT and fetal parameters like

mode of delivery, live or dead, term or preterm and baby weight were collected from the records from the Department of Obstetrics and Gynecology.

Placenta with umbilical cord and membranes was collected after delivery. In all cases, the membranes were removed from the placenta. The umbilical cord was cut at a distance of 5 centimetres from the site of attachment on the placenta. Placenta was assessed for gross features like average diameter & thickness (cm), weight (grams) in fresh state and kept for fixation in formalin for 24 hours. After fixation gross abnormalities like infarction, calcification and subchorionic fibrin deposits were assessed as:

Absent: When it is not significantly seen
+: When it is focally present
++: When it is seen diffusely / multiple

All these parameters were later confirmed microscopically.

Tissue sections were taken from the placenta, two sections from central and peripheral areas each. Additional sections were taken from grossly abnormal lesions. All the sections were stained with haematoxylin and eosin (H&E). Additional special stains were done for certain parameters. Masson Trichrome (MT) was done to highlight fibrosis in villi. MT will stain collagen blue. PAS stain was used to highlight fibrinoid necrosis which will be stained pink. All H&E stained slides were subjected to histopathological examination. Parameters like villous vascularity, syncytial knots, endarteritis obliterans (EAO), fibrinoid necrosis, cytotrophoblastic proliferation, villous stromal fibrosis were assessed.

Villous vascularity was assessed by counting number of capillaries per villus for 100 villi in each case. Normal average number of capillaries per villus is 3 to 5. We categorised vascularity into three groups as:

- Decreased - less than 3 capillaries per villus
- Normal - 3 to 5 capillaries per villus
- Increased - more than 5 capillaries per villus

Syncytial knots were assessed by counting the number of villi with syncytial knots for 100 villi in each case. It was categorised as less than or equal to 30 % and more than 30%.

Other parameters related to placental parenchyma like EAO, was assessed as whether they are absent or present. Cytotrophoblastic proliferation, villous stromal fibrosis and fibrinoid necrosis were assessed as normal and increased.

- Cytotrophoblastic proliferation –Increased: when in more than 20 % of villi
- Villous stromal fibrosis &Fibrinoid necrosis – Increased : when in more than 3 % of villi

All cases from study group and control group were subjected to immunohistochemistry using the biomarkers VEGF and VEGFR-1 by peroxidase method.Interpretation of markers expression were studied in the villous

trophoblast with scoring of staining intensity as score 0 - weak, score 1 – moderate and score 2 – strong.[4]

Statistical analysis was done using IBM-SPSS 21.0. The data on categorical variables related to maternal, fetal and histological parameters and angiogenicfactors were expressed as percentages. To compare categorical variables like staining intensity of angiogenic factors and histopathological changes between the study and control group Chi-square test was used. The data for continuous variables related to maternal and fetal were expressed as mean with SD. Correlation of histopathological parameters with birth weight and angiogenic factors expression was done and expressed by Pearson linear correlation coefficient. Five percentage level of significance and 'p' value < 0.05 was taken as significant.

Results

According to the predetermined inclusion and exclusion criteria, placenta from 53 preeclampsia cases and 53 normal controls were collected. The age range of preeclampsia group was 19 to 38 years with median of 25 years and mean of 25.90 ± 4.53 years. The control group was age matched with a range of 19 to 39 with a median of 24 years and mean of 24.86 ± 3.97 years. Majority of the subjects in both the groups were in the age range of 21 to 34 years. Out of 53 cases of preeclampsia, 38 cases (71.7%) were primipara and 15 cases (28.3%) were multipara. The Systolic BP ranged from 140 to 180 mm of Hg in preeclampsia and the Diastolic BP ranged from 80 to 120 mm of Hg. Preeclamptic patients with blood pressure more than or equal to 160/110 mmHg were considered to have severe preeclampsia. Mild preeclampsia cases were around 39(73.6%) and severe preeclampsia were 14(26.4%). Laboratory parameters included in 2013 ACOG criteria for assessment of severity of preeclampsia like platelet count; RFT (Serum creatinine) and LFT (Serum transaminases) were studied along with other parameters like proteinuria. In our study we collected data from preeclampsia and control groups based on dipstick method and categorized proteinuria as nil, trace and ≥ 1+and found that 42(79.2%) cases had trace proteinuria and 11(20.8%) cases had no proteinuria. None of the preeclampsia cases showed low platelet counts and mean platelet count2.06±0.54 in preeclampsia. Severity of preeclampsia based on liver transaminases and serum creatinineis depicted in (Graph 1) and (Graph 2).

The gestational age ranged from 24 to 41 weeks with mean of 36.39 ± 3.45 in preeclampsia and 38 to 42 weeks with mean of 39.27 ± 1.08 in controls. In preeclampsia group there were 23(43.4%) cases of preterm births whereas it was none in control. The statistical analysis for comparison of fetal outcome in relation to gestational age at birth between preeclampsia and control groups was significant ($p < 0.01$). Fetal birth weight was categorized as normal when it is more than or equal to 2.5 Kg, low birth weight(LBW) when it is 1.5 to 2.4 Kg, very low birth weight(VLBW) when it is 1 to 1.4 Kg and extremely low birth weight(ELBW) when it is less than 1 Kg. In preeclampsia group, 25(47.2%), 15(28.3%) and 1(1.9%) cases showed LBW, VLBW and ELBW respectively. In control group only 7(13.2%) cases showed LBW.The statistical analysis for comparison of fetal outcome in relation birth weight between preeclampsia and

control groups was significant ($p < 0.01$)Compared to the control group where there were no fetal deaths, the preeclampsia group had 5(9.4%) cases of fetal deaths. The statistical analysis for comparison of fetal deaths with live babies in preeclampsia and control groups was significant ($p = 0.02$)

We compared placental dimensions like placental diameter, thickness and placental weight (Table 1) and analysed placental weight comparison in both groups (Graph 3).The statistical analysis for comparison of placental weight in preeclampsia and control groups was significant ($p = 0.01$) Categorization ofhistomorphological features–subchorionic fibrin, infarction, calcification, villous vascularity, syncytial knots, endarteritis obliterans, cytotrophoblasticproliferation, villous stromal fibrosis and fibrinoid necrosiswas done asdescribed in the methodology (Figure :1,2,3,4&5). All these above mentioned features were compared between both groups and analysed(Table 2).In our study, other miscellaneous findings such as perivillous fibrin (13.2%), villitis (1.9%), intervillous hemorrhage (3.8%), intervillous thrombus (1.9%), chorioamnionitis (5.7%) andfunisitis (3.8%) were also seen(Figure:6&7). Correlation of fetal outcome (birth weight) with placental changes showed significance with placental weight, presence of infarction, calcification syncytialknots, cytotrophoblasticproliferation, villous stromal fibrosis and fibrinoid necrosis(Table 3). Expression of angiogenicfactors (Figure: 8), VEGF and VEGFR-1in preeclampsia and control groups was studied using immunohistochemicalmethods (Graph-4& Graph-5). The comparison of VEGF and VEGFR1 expression between normal and preeclampsia were statistically significant ($P < 0.01$). We also correlated the expression of angiogenic factors with varioushistomorphologicalchanges (Table-4).

Discussion

Numerous factors in the pathogenesis of preeclampsia have been proposed so far. Main factors associated with preeclampsia are defective placentation, placental ischemia, and endothelial cell dysfunction. [5, 6]The present study was undertaken to assess the histomorphological changes and angiogenic factor expression in preeclamptic placentas. Majority of cases, 49(92.5%) of preeclampsia and 51(96.2%) cases of controls were in the age group ranging from 21 to 34. Similar age group distribution was seen by *Doddamani et al*[7]and*Maly et al.*[8]

Predominant population in our study was primigravidae with 71.7% in preeclampsia and 67.9% in normal controls. Similar findings were observed by *Maynard et al*[9]and *Doddamani et al*[7]. It is not surprising considering that preeclampsia is seen mostly in primigravidae.Hypertension is the most important criteria for diagnosis of preeclampsia**.**Twenty six percentage of cases of preeclampsia had severe preeclampsia with a blood pressure of more than or equal to 160/110 mmHg.

In preeclampsia, there is hypervascularisation and vasoconstriction in liver and kidney. In liver, cell injury leads to alteration of cell membrane permeability and damage to the cells allowing intracellular enzymes to leak into the blood. In kidney, there will be alteration in renal tubular cell permeability and problem in filtration and reabsorption which leads to elevated urea and creatinine. [10] In the

present study,mean AST of 43.58±14.71 U/L, ALT of 23.9±9.30 U/Land serumcreatinine of 0.89±0.17 mg/dl was observed in preeclampsia.

In our study, preeclampsia cases had 43.4% of pretermbabies,22.6 % of normal birth weight babies, 47.2% of LBW babies, 28.3% of VLBW babies and 1.9% of ELBW babies and 9.4 % of still birth babies. In the study by *Akhlaq et al,*[11] preterm deliveries constituted about 76 % preeclampsia cases. Birth weight of our study was comparable with the study by *Doddamani et al*[7] Still birth in other studies are 20% in *Akhlaq et al,*[11] 20.8% in*Navbiret al*[12] and 17.4% in*Doddamani et al.*[7] The difference may be due to difference in the sample size.

Placental features like dimensions and weight are subjected to variation depending on the time of collection, whether assessed before or after fixation and duration of fixation. In our study, preeclampsia cases showed a mean placental diameter of15.93±3.25cm and thickness of 2.55±0.77cm which is comparable with the study by *Narashima et al,*[13]*Goswami et al*[14] and by *Al-Mamoriet al*[15].About 62.3% of placentas in preeclampsia cases weighed less than 500gms ,which was similar to the observation by *Tache et al*[2] where 66 % had placental weight of less than 10th percentile for gestational age.

We found that histomorphological alterations of preeclamptic placentas in our study were comparable with other studies (Table-5)We got statistical significance in the comparison of all above mentioned histomorphological alterations between normal and preeclampsia cases except for subchorionic fibrin.Infarction is seen more frequently in toxemia of pregnancy. It is due to thrombotic occlusion of uteroplacental vessels.[17]Preeclamptic patients have higher incidence of calcification. Calcification is an indicator of aging of the placenta.[18] It acts as a sign of premature aging in case of hypertensive diseases of pregnancy.[19]Vascularity of placental villi is difficult to assess as it has a tendency to collapse immediately after delivery. It also depends on mode of delivery, mode of cord clamping, time of fixation and composition of the fixative.[20]Our study results showed increased vascularity in preeclampsia which can be explained as when there is persistent hypoxia, there will be compensatory villous hypercapillarisation. Our result is concordant with *Resta et al*[21]study which showed hyper ramification of the capillary loops in terminal villi of preeclampticplacentas. In contrast, *Narashima et al,*[13]showed more of hypovascular villi in preeclampsia. This is discordant with our study results. According to them, preeclampsia is a condition of impaired remodeling of maternal spiral arterioles, which will reduce the overall blood flow to the placenta leading to decreased vascularity.Syncytial knots are normally present in the villi and increases with gestational age; therefore it is also an indicator of hyper maturity. It is increased in conditions like uteroplacental malformation. Exposure to hypoxia is an important inducing factor.[22] At term the cytotrophoblastic layer covering the villi becomes inconspicuous. Decreased oxygen levels can induce cytotrophoblasticproliferation.Stromal fibrosis is a feature of villous maturation in stem villi and it starts from the center in large stem villi, progressively replacing the loose reticular stroma of the stem and the intermediate villi. Towards term stroma of the intermediate villi is completely fibrosed. In contrast, stroma of the terminal villi will show very little collagen. Villous fibrosis is a marker of reduced fetoplacental perfusion.[23]

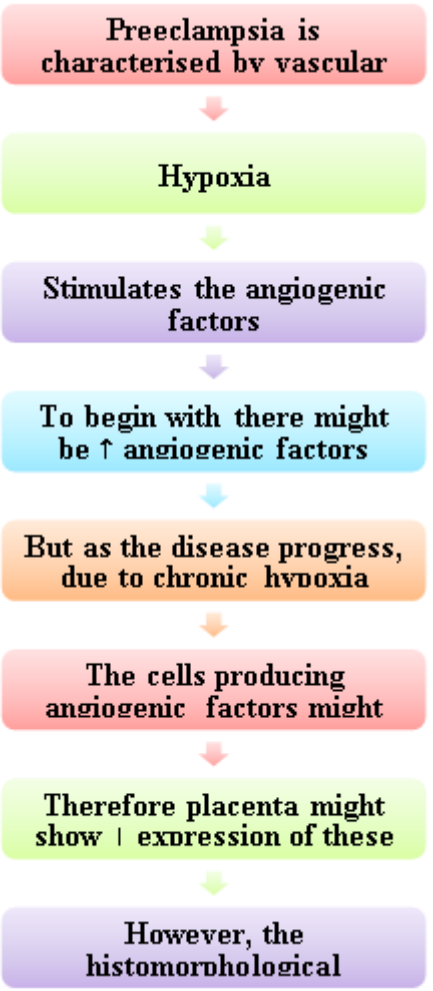
In our study we got significant positive correlation of birth weight with placental weight and significant negative correlation of birth weight with infarction, calcification, syncytial knots, cytotrophoblastic proliferation, villous stromal fibrosis and fibrinoid necrosis. Our result is comparable with that of *Navbir et al* who found significant correlation of histomorphological features like vasculosyncytial membrane, cytotrophoblastic proliferation and basement membrane with the fetal outcome. Birth weight measurement is a simple method to assess fetal outcome and it will show significant change for the changes in the maternal conditions like preeclampsia which will express its effect mainly on placenta.

A number of angiogenic factors are involved in development of vasculature of the placenta. Derangement of these angiogenic factors have been implicated in the pathogenesis of preeclampsia. VEGF and its receptors have been studied extensively in preeclampsia. VEGF plays an important role in angiogenesis. During normal pregnancy, VEGF induces cytotrophoblastic invasion and thereby remodelling of maternal spiral arteries takes place. According to studies the villous trophoblast secretes this cytokine. VEGF acts through different receptors amongst which VEGFR-1(also known as Flt-1) and VEGFR-2 (also known as KDR) have been studied in preeclampsia.[24]

In our study, in preeclampsia 30.2 % and 43.4% of cases showed of weak expression of VEGF and VEGFR-1 respectively and9.4 % and 5.7 % of cases showed strong expression of VEGF and VEGFR-1 respectively. This reduced expression in preeclampsia was concordant with studies by *Andraweera et al*[25] and *Zhouet al*[26] and discordant with studies by*Chung et al*,[27]*Helske et al*[24]and *Tacheet al*[2]which showed increased expression. It is not clear whether altered expression of VEGFR-1 is the cause or the result of the pathology of preeclampsia. Different studies have used different methods for demonstration of VEGFR-1. The expression of the mRNA and the protein need not always be the same. Expression of the Flt-1 in hypoxic/ischemic villi has been found to be more evident by in situ hybridization when compared to that observed by immunohistochemistry.[28] *Tache et al*[2] used a stain which could detect both Flt-1 and sFlt-1 by IHC in the placenta. Increased expression was seen in the syncytiotrophoblasts. Whether the increased staining was due to the sFlt-1 component is not clear. Increased sFlt-1 production is a universal finding which been implicated in causing endothelial dysfunction in preeclampsia. But in our study we used an IHC stain which can detect only VEGFR-1 and not the soluble form.

The discrepancies in the expression of these angiogenic factors could be due to different factors like genetic and environmental factors involved in the gene expression, varying sample size and the different methods used for studying the expression of these factors. In our study, we got significant negative correlation of VEGF expression with infarction, villous vascularity, syncytial knots, EAO and fibrinoid necrosis. We also got significant negative correlation of VEGFR-1 expression with villous vascularity and syncytial knots. So far there are not many studies on the correlation of angiogenic factors with histomorphological features in the placenta.

The following mechanism could be responsible for the altered expression of angiogenic factors and the histomrphological changes.



Though we got significant correlation in few of the histomorphological features, further studies with bigger sample size and as observed by*Kumazaki et al* [28]study, with other techniques like in situ hybridization might give us a better understanding of the pathogenetic mechanisms inpreeclampsia.

Conclusion

Preeclampsia is characterized by reduced expression of angiogenic factors in placenta which correlated with the impaired placental vascular adaptation. This would have resulted in reduced placental blood flow leading to the related histomorphological complications.

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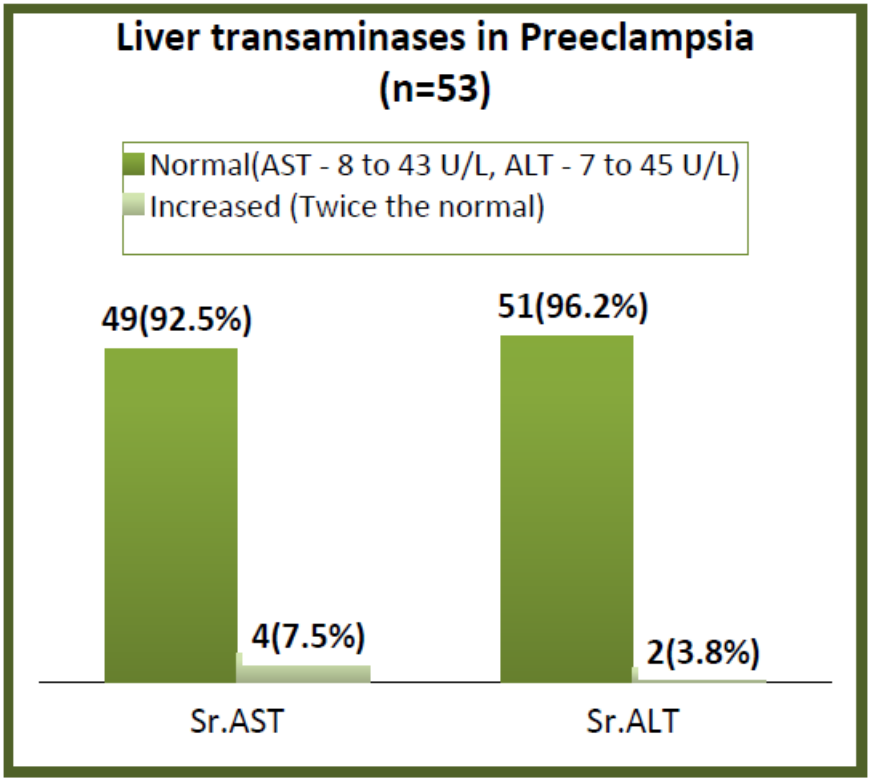
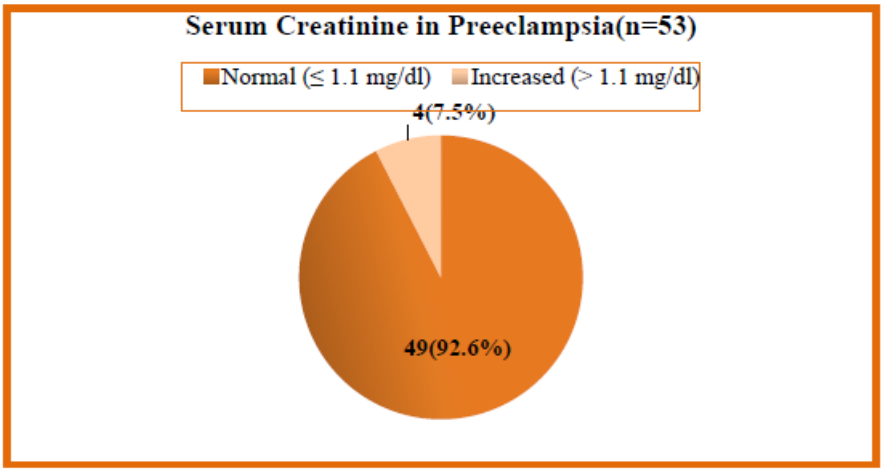
Graphs and Tables

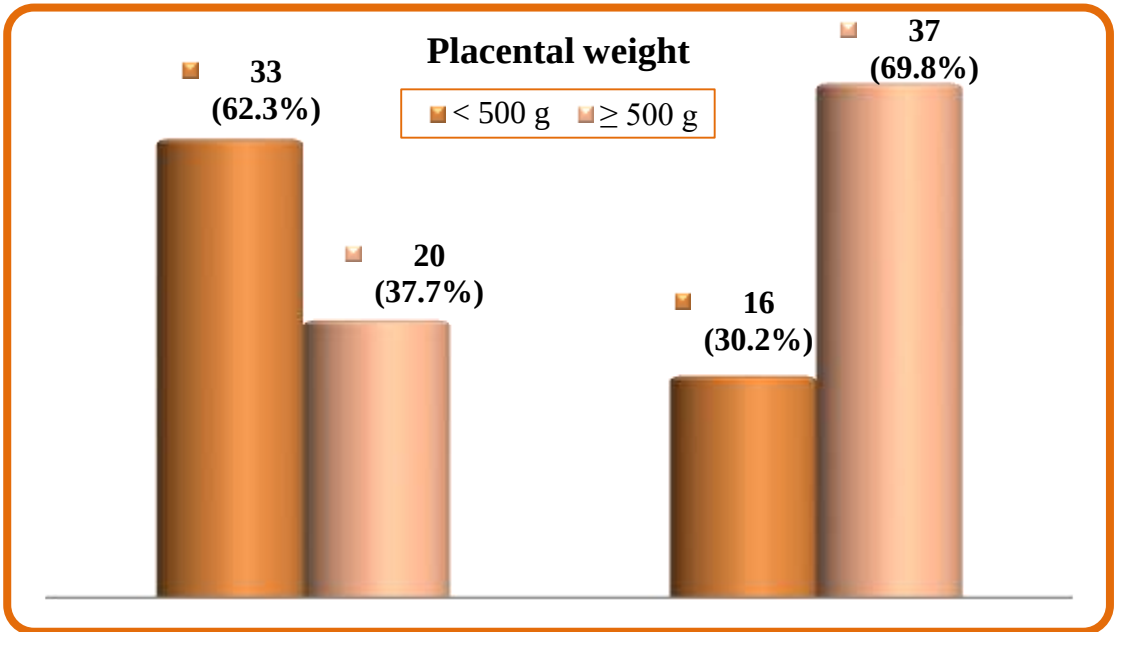
Graph 1: Severity of preeclampsia based on liver enzymes



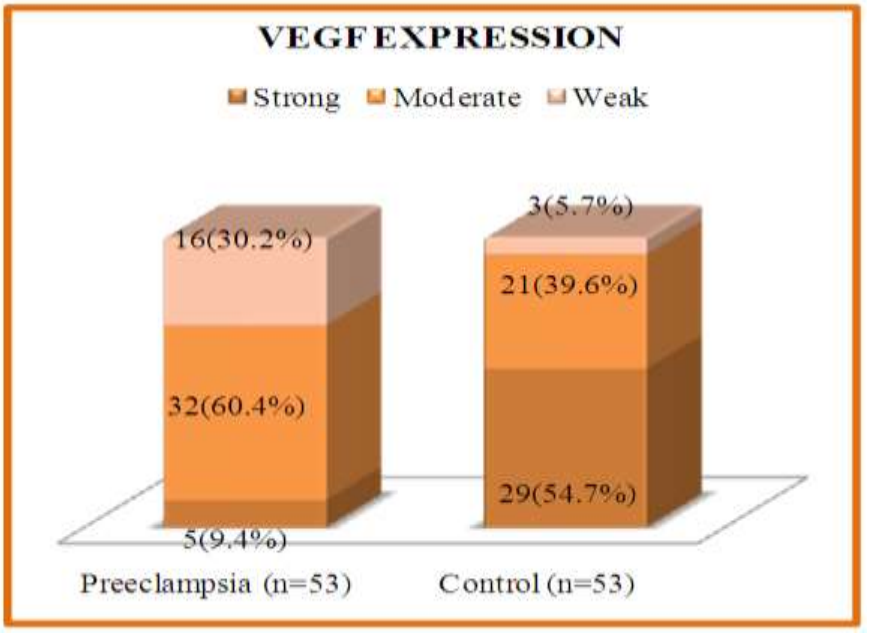
Graph 2: Severity of preeclampsia based on serum creatinine



Graph 3: Placental weight in preeclampsia and control groups



Graph 4: Comparison of VEGF expression in preeclampsia and control group



Graph 5: Comparison of VEGFR-1 expression in preeclampsia and control group

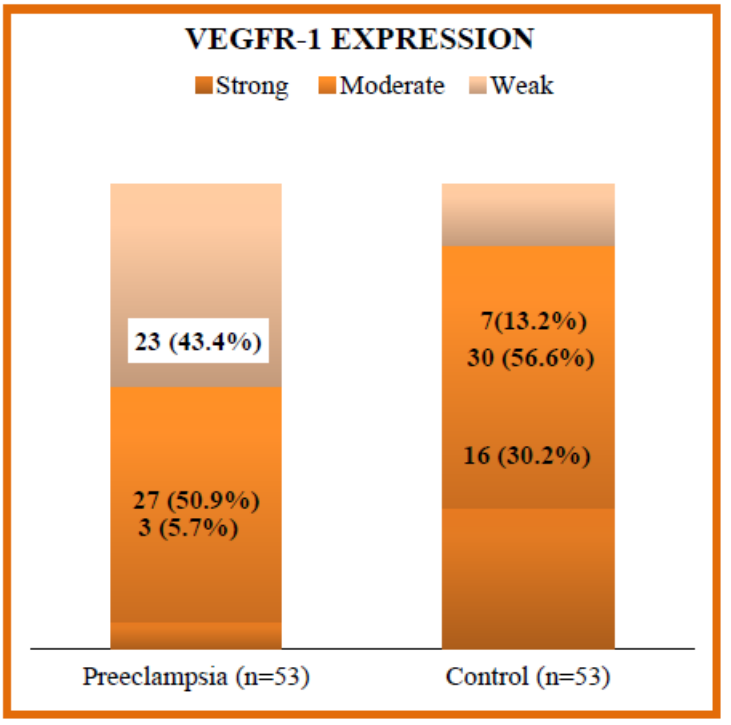


Table 1: Mean of placental dimensions

Groups	Mean±SD		
	Diameter(in cm)	Thickness(in cm)	Placental weight(in grams)
Preeclampsia	15.93±3.25	2.55±0.77	420.94±157.43
Control	17.14±1.51	2.06±0.51	532.07±128.63

Table 2: Histomorphological features comparison between preeclampsia and control

Histomorphological Features	Preeclampsia (n = 53) No. Of Cases (%)	Control (n = 53) No. Of Cases (%)	p Value
Subchorionic Fibrin			
Absent Focal Diffuse	45(84.9) 6(11.3) 2(3.8)	49(92.5) 4(7.5) 0(0)	0.27
Infarction			
Absent Focal Diffuse	30(56.6) 3(5.7) 20(37.7)	53(100) 0(0) 0(0)	< 0.01*
Calcification			
Absent	21(39.6)	43(81.1)	

Focal Diffuse	32(60.4) 0(0)	10(18.9) 0(0)	< 0.01*
Villous Vascularity			
Decreased Normal Increased	2(3.8) 18(34) 33(62.3)	0(0) 43(81.1) 10(18.9)	< 0.001*
Syncytial Knots			
≤ 30% > 30%	3(5.7) 50(94.3)	25(47.2) 28(52.8)	< 0.01*
Endarteritis Obliterans			
Absent Present	28(52.8) 25(47.2)	38(71.7) 15(28.3)	0.04*
Cytotrophoblastic Proliferation			
Normal Increased	20(37.7) 33(62.3)	42(79.2) 11(20.8)	< 0.01*
Villous Stromal Fibrosis			
Normal Increased	29(54.7) 24(45.3)	47(88.7) (11.3)	< 0.01*
Fibrinoid Necrosis			
Normal Increased	26(49.1) 27(50.9)	48(90.6) 5(9.4)	< 0.01*

*Significant p Value

Table 3 - Correlation Of Fetal Outcome (Birth Weight) With Placental Changes

Placental Changes	BIRTH WEIGHT	
	Correlation coefficient(r)	p Value
Placental weight	0.2729	0.0047*
Subchorionic fibrin	-0.1723	0.0773
Infarction	-0.5154	< 0.0001*
Calcification	-0.2094	0.0312*
Villous vascularity	-0.1862	0.0560
Syncytial knots	-0.2302	0.0176*
Endarteritis obliterans	0.01354	0.8904
Cytotrophoblastic proliferation	-0.3030	0.0016*
Villous stromal fibrosis	-0.4027	<0.0001*
Fibrinoid necrosis	-0.3078	0.0013*

*Significant p value (< 0.05)

Table 4 -Correlation Of Angiogenic Factor Expression With Histomorphological Features

Histomorphological features	VEGF		VEGFR-1	
	Correlation coefficient (r)	p Value	Correlation coefficient (r)	p Value
Subchorionic fibrin	-0.1040	0.2887	-0.0556	0.5710
Infarction	-0.2612	0.0068*	-0.1524	0.1188

Calcification	-0.1040	0.2887	-0.1332	0.1733
Villous vascularity	-0.2286	0.0184*	-0.2610	0.0069*
Syncytial knots	-0.2483	0.0103*	-0.2831	0.0033*
Endarteritis obliterans	-0.2433	0.0120*	-0.02459	0.8024
Cytotrophoblastic proliferation	-0.1444	0.1396	-0.1833	0.0600
Villous stromal fibrosis	-0.1283	0.1899	-0.1523	0.1191
Fibrinoid necrosis	-0.1936	0.0467*	-0.1737	0.0750

*Significant p value (< 0.05)

Table-5: Comparison of histomorphological alterations with other studies

Histomorphological Features	Our Study (%)	Narashima et al[13] (%)	Motwani etal[16] (%)	Navbir et al[12] (%)
Diffuse Subchorionic fibrin	3.8	7.4	-	-
Diffuse Infarction	37.7	40.7	43.33%	-
Focal Calcification	60.4	-	70	64.29
Increased Villous vascularity	62.3	7.4*	-	-
Syncytial Knots	94.3	100	-	90
Endarteritis Obliterans	47.2	77.77	-	-
Cytotrophoblastic proliferation	62.3	62.9	-	64.29
Villous stromal fibrosis	45.3	81.48	66.66	-
Fibrinoid necrosis	50.9	92.5	-	78.57

***Discordant results**

Figures

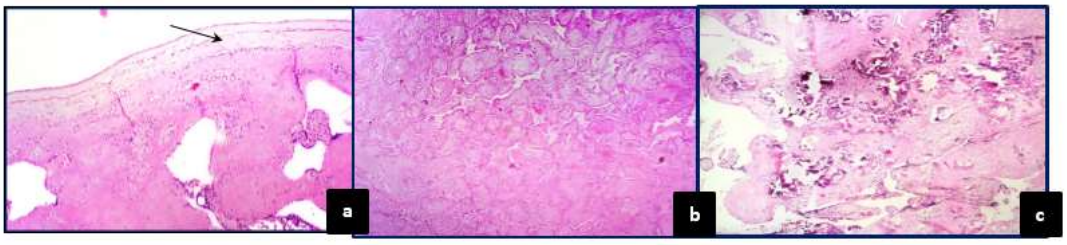


Figure 1: Photomicrograph of preeclamptic placenta showing (a)subchorionic fibrin deposition.Arrow depicts the chorionic layer; (b) Infarcted villi ;(c) Calcification (H&E,10 X).

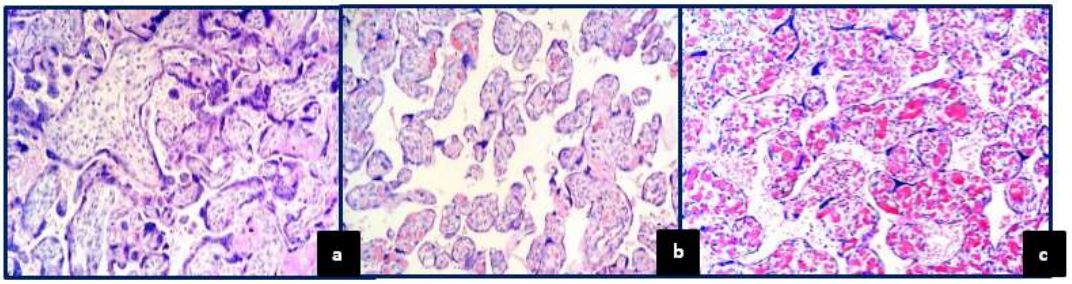


Figure 2: Photomicrograph showing villous vascularity.(a)Placental villi showing decreased vascularity;(b)normal vascularity;(c)increased vascularity (H&E, 20 X).

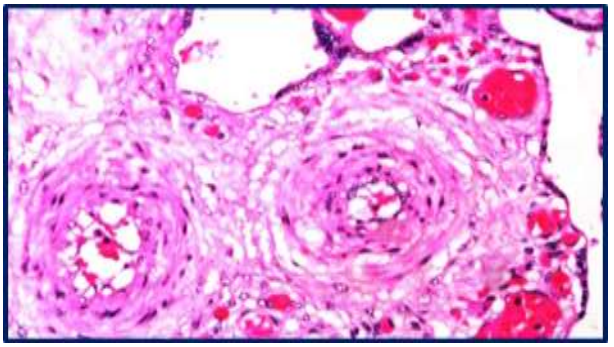


Figure 3: Endarteritis obliterans- Vessels showing narrowing of lumen andplumpendothelial cell lining in the placenta of a preeclamptic patient(H&E, 20X)

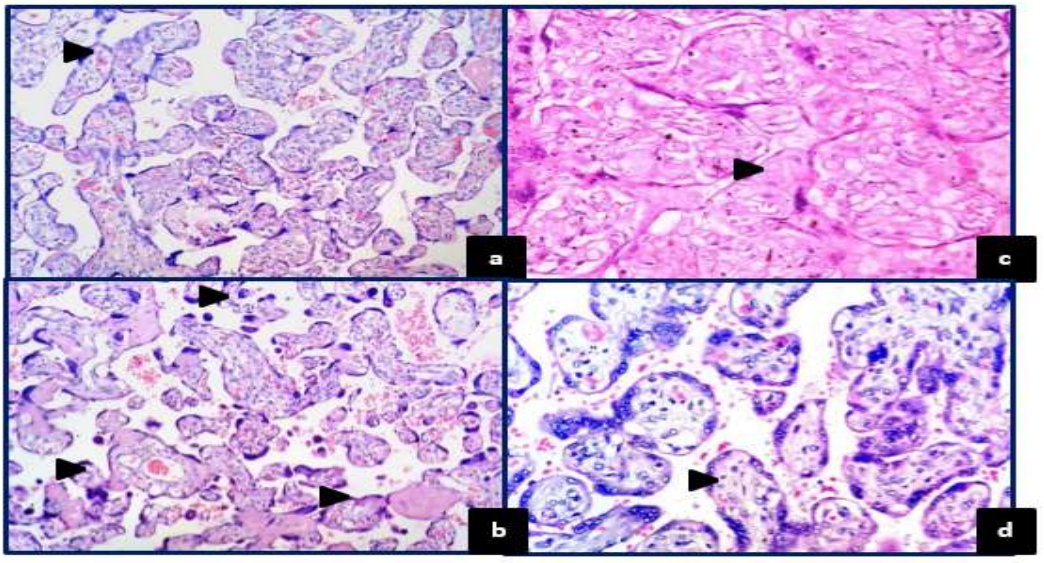


Figure 4: Photomicrograph showing normal(a) and increased(b) syncytial knots (arrow heads) (H&E,20X); (c)Normal villi showing flattened cytotrophoblastic lining(arrow head) (H&E, 40 X);(d) Cytotrophoblastic proliferation in the placenta of a preeclmapticpatient. There is multilayering of cytotrophoblastic lining(arrow head)(H&E, 40 X)

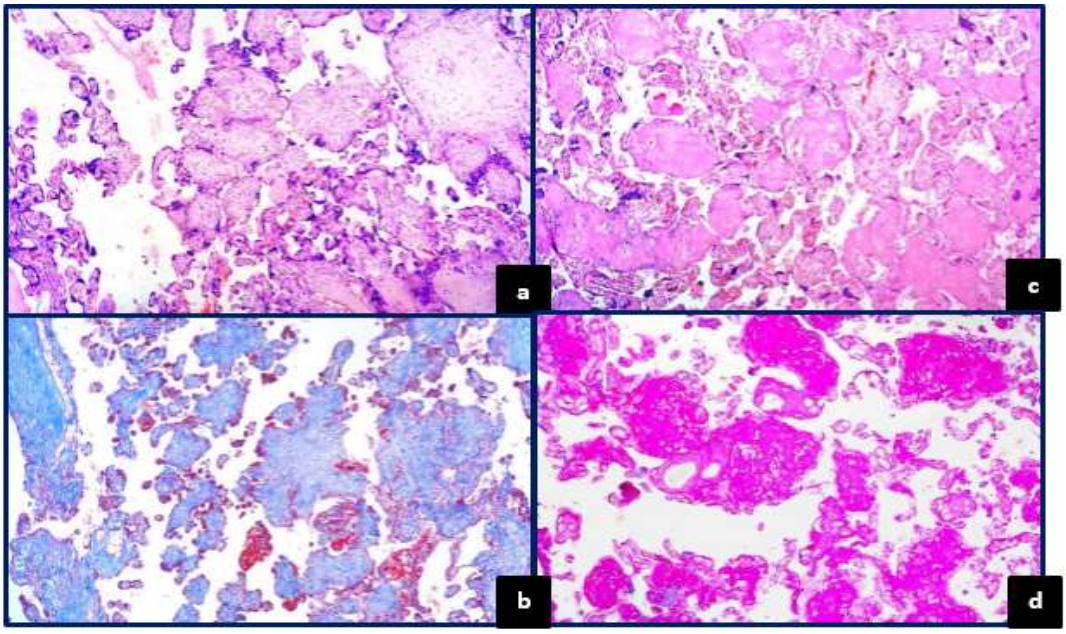


Figure 5: Photomicrograph showing (a)Villi with increased stromal fibrosis(H&E, 20X) ,(b)highlighted by Masson Trichrome stain (20 X); (c) Villi with increased fibrinoid necrosis (H&E, 20 X), (d) highlighted by PAS stain (20 X).

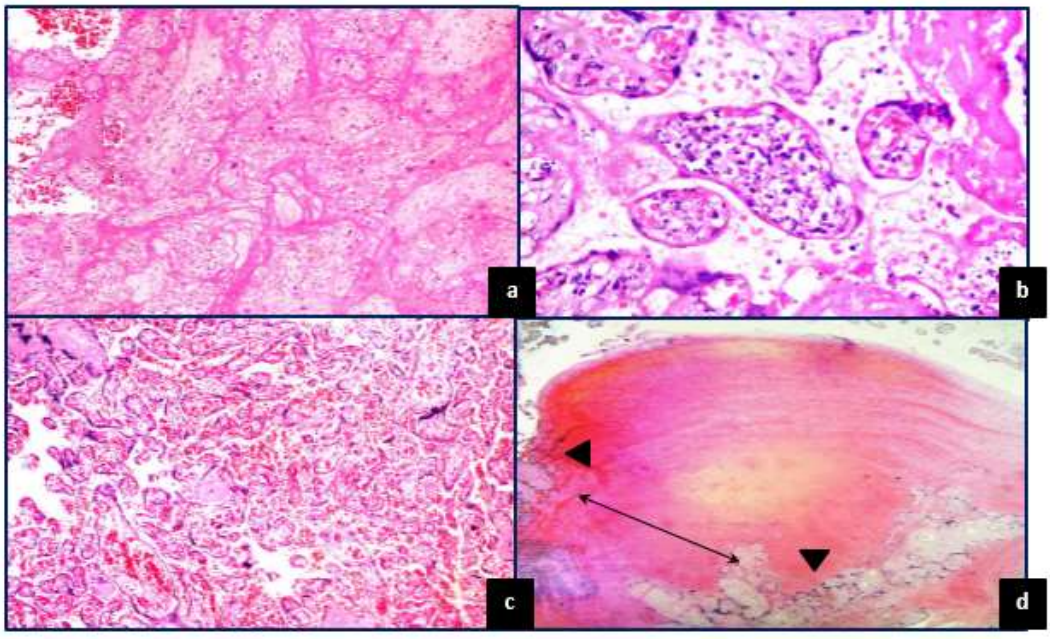


Figure 6 Photomicrograph showing preeclamptic placenta with (a)Perivillous fibrin deposition(H&E,20X); (b) Villi showing infiltration by neutrophils in acute villitis (H&E, 40 X); (c)Intervillous hemorrhage (H&E, 10 X); (d) Intervillous thrombus, with arrow heads showing villi and double arrow shows lines of Zahn (H&E, 10 X)

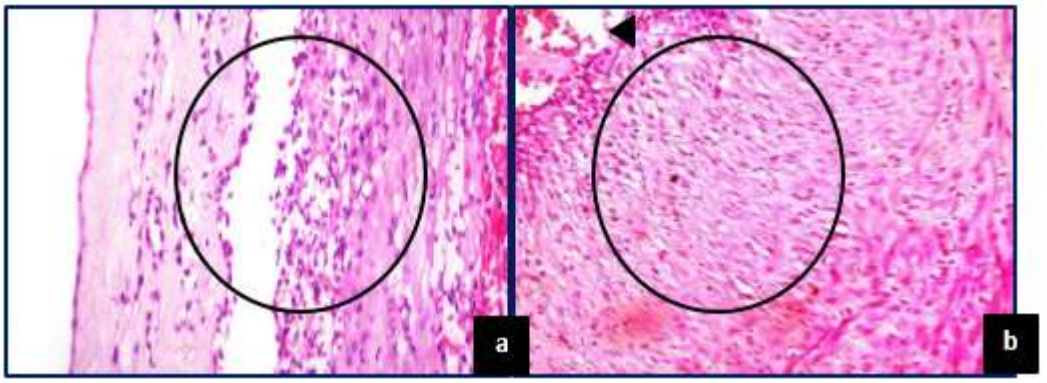


Figure 7: Photomicrograph showing (a)Acute chorioamnionitis- Membrane showing neutrophilic infiltration in both chorionic and amniontic layer (H&E, 10 X);(b) Funisitis- Wall of the umbilical artery showing neutrophilic infiltration. Arrow head showing lumen of the vessel (H&E, 40X)

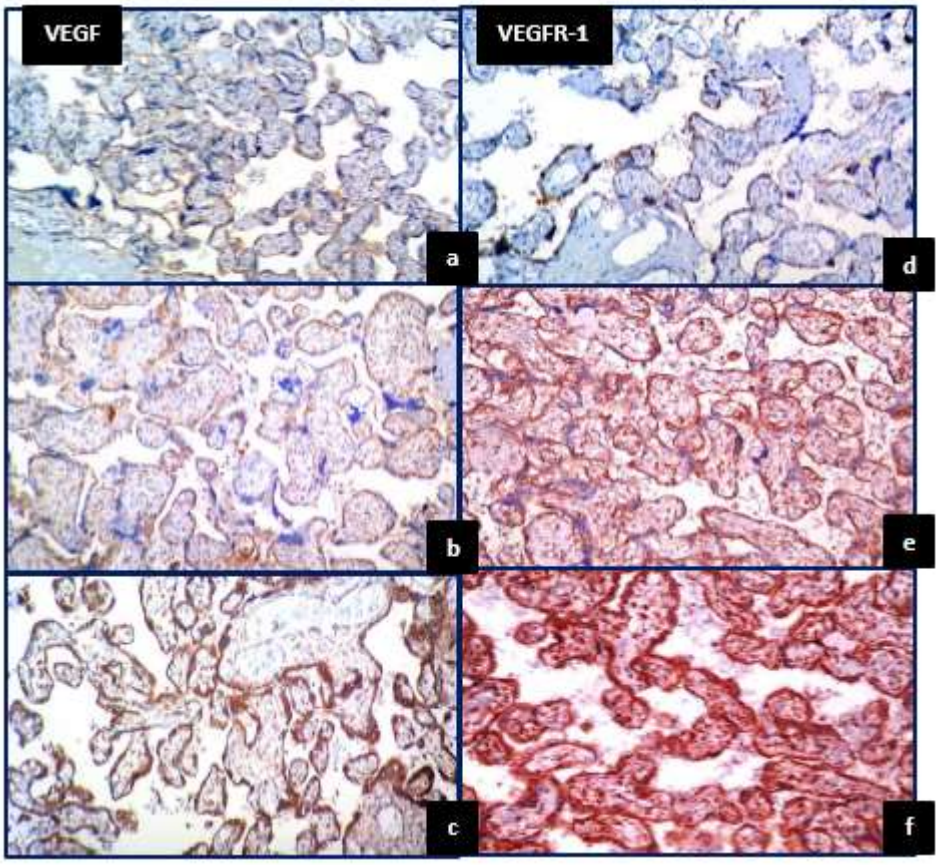


Figure 8: Photomicrograph showing placental villi with varied immunohistochemical(IHC) expression of VEGF and VEGFR-1; VEGF – (a)Weak, (b)Moderate, (c)Strong & VEGFR-1 – (d)Weak, (e)Moderate, (f)Strong (IHC,20X)