

Morphometric Analysis of Placental Terminal Villi, Capillaries, and Intervillous Space in Normal and Pre-Eclamptic Pregnancies

ABDULLAH KHILJI¹, SYED FAIZAN ALI RIZVI², SAMINA TARIQUE³, SABAHAT FATIMA⁴, AZHAR ALI SHAH⁵, QAMBER HAIDER KAZMI⁶, TABINDA UROOJ⁷

¹Associate Professor, Department of Anatomy, Khairpur Medical College, Khairpur mir's.

²Assistant Professor, Department of Anatomy, Ghulam Muhammad Mahar Medical College Sukkur.

³Lecturer, Department of Pathology, Ghulam Muhammad Mahar Medical College Sukkur.

⁴Assistant Professor, Obstetrics and Gynaecology Unit-II, Ghulam Muhammad Mahar Medical College Sukkur.

⁵Associate Professor, Department of Surgery, Ghulam Muhammad Mahar Medical College Sukkur.

⁶Assistant Professor, Department of Anatomy, United Medical and Dental College, Karachi.

⁷Assistant Professor, Department of Anatomy, Ziauddin Medical College Karachi.

Corresponding author: Syed Faizan Ali Rizvi, Email: drfaizvi@hotmail.com

ABSTRACT

Background: The placenta reflects the prenatal problems that are linked to high perinatal morbidity and mortality both macroscopically and microscopically. A pregnancy syndrome called pre-eclampsia has terrible consequences for both the mother and the unborn child. It may result in more frequent inductions of labor, fetal growth limitation, respiratory issues in newborns, increased admission rates to neonatal critical care units, and an elevated risk of perinatal or fetal death. To understand the relation of preeclampsia and fetal growth identification of its effects on various components of the placenta is mandatory hence the current study was designed to evaluate the Placental Terminal Villi, Capillaries, and Intervillous Space in Normal and Pre-eclamptic Pregnancies.

Methodology: It was a cross sectional study, carried out on 50 samples of placentas, out of which 25 were preeclamptic and 25 were normal. The placentas were recruited after cesarean session, processed as per guidelines and were analyzed for histopathological examination. The set inclusion criteria were Registered cases of Pre-eclampsia, Blood Pressure $\geq 140/90$ mmHg, Parity 0 – 4, Age of mother (25 – 35) Years., Gestational Age (36 – 42) Weeks, Mode of delivery Cesarean Sections. The set exclusion criteria were known hypertensive patients, and patient diagnosed with other chronic illnesses.

Results: Number of terminal villi, Transverse diameter of terminal villi (μm), Intervillous space (μm), and Transverse diameter of terminal villous capillaries decreased significantly ($p=0.001$) in preeclamptic group however, Number of terminal villous Capillaries were found to be increased in preeclamptic group ($p=0.001$).

Conclusion: Preeclampsia leads to more significant alterations in placental structure compared to normal pregnancies, emphasizing the importance of early detection and management of this condition to improve maternal and fetal outcomes.

Keywords: Pregnancy, preeclampsia, terminal villi, Intervillous space, terminal villous Capillaries

INTRODUCTION

The placenta reflects the prenatal problems that are linked to high perinatal morbidity and mortality both macroscopically and microscopically (1). To sustain pregnancy and encourage healthy fetal development, the placenta plays a crucial role (2). The fetus's respiratory, nutritive, excretory, endocrine, and immunological systems are all controlled by the placenta (3). Extraembryonic mesoderm give rise to umbilical cord that is covered by mesenchymal and epithelial stem cells. The umbilical epithelium contains two umbilical arteries, one umbilical vein and Wharton's jelly which serves as a protective cushion for the vessels (3, 4).

The human placenta's villous vasculature develops and the terminal villi are formed as a result of angiogenesis. Early in pregnancy, placental vascular development starts, and it continues throughout gestation. In the healthy development of the placenta, angiogenesis and vascular transformation are critical processes (5). A pregnancy syndrome called pre-eclampsia has terrible consequences for both the mother and the unborn child. It may result in more frequent inductions of labour, fetal growth limitation, respiratory issues in newborns, increased admission rates to neonatal critical care units, and an elevated risk of perinatal or fetal death (6).

Preeclampsia's etiopathogenesis is multifaceted, and the placenta has a significant impact on how the condition develops. Reduced uterine perfusion leads to placental ischemia and hypoxia, which play a key role in the pathogenesis of the condition. Secondary maternal consequences such as imbalance and endothelial dysfunction are brought on by placental ischemia (7, 8). To understand the relation of preeclampsia and fetal growth identification of its effects on various components of the placenta is mandatory hence the current study was designed to evaluate the Placental Terminal Villi, Capillaries, and Intervillous Space in Normal and Pre-eclamptic Pregnancies.

METHODOLOGY

It was a cross sectional study conducted in the Department of Anatomy, Basic Medical Science Institute, Jinnah Post Graduate Medical Centre Karachi, and the microscopic examination were carried out in the histology lab, Jinnah Medical and Dental College Karachi. In this investigation, a total of 50 full-term human placentas were used, 25 from normal (Group A) and 25 from pre-eclampsia (Group B). All the placentas were collected via caesarean section From the gynecology and obstetrics departments of the Jinnah Postgraduate Medical Center in Karachi. The set inclusion criteria was Registered cases of Pre-eclampsia, Blood Pressure $\geq 140/90$ mmHg, Parity 0 – 4, Age of mother (25 – 35) Years., Gestational Age (36 – 42) Weeks, Mode of delivery Cesarean Sections. The set exclusion criteria was known hypertensive patients, and patient diagnosed with other chronic illnesses.

Procedure: We collected placentae from registered cases in the Gynecology and Obstetrics units of Jinnah Postgraduate Medical Centre. The placentae were immediately placed in 10% formalin-filled jars with patient information marked on each jar. The placentae were then transferred to the Department of Anatomy at Jinnah Medical and Dental College Karachi and preserved in 10% formalin for 24-48 hours before histological sections were taken. Tissue pieces were processed for routine paraffin embedding by passing through alcohol, xylene, and paraffin infiltration. Paraffin blocks were prepared, tissue was placed in such a position covering all the area from chorionic plate to basal plate of placenta, then parallel sections of the tissues were obtained on the glass slides, 4 μm thick sections were cut on rotary microtome and allowed to float on hot water bath at 42°C. The floating sections were taken on glass slides from the water bath. Slides were kept on hot plate at 37°C for 24 hours for purpose of fixing the sections on the slides. Slides were numbered appropriately with lead pencil on frosted area.

The sections on the glass slides were stained using the routine Haematoxylin and Eosin (H and E) staining technique, allowing for observation of the general morphology of the tissue under a light microscope. A stage micrometer was used for calibration of ocular micrometer and the counting reticule. A stage micrometer with a scale of 10 mm divided into 100 parts was used for calibration. The ocular micrometer and counting reticule were calibrated at different magnifications (4X, 10X, and 40X) by comparing their divisions with those of the stage micrometer. The size of the counting reticule was also determined at each magnification. The results were used to calculate the size of objects viewed through the microscope. All the parameters, Intervillous space, number of villi, transverse diameter of terminal villi, number of terminal villous capillaries, transverse diameter of terminal villous capillaries were noted in H and E in both the groups. Statistical analysis was conducted using the independent sample t-test for quantitative variables Mean and standard deviation were reported for each group. Statistical significance was considered when the P-value was 0.05 or less. The analysis was performed using SPSS version 24.

RESULTS

Number of terminal villi: In 4 µm thick, H and E stained paraffin sections; number of terminal villi was counted in 10 random fields for each placenta in group A and group B.

The mean values of number of villi in control group A and pre-eclamptic group B were 100.16±0.72 and 64.28±1.51 respectively (table- 1).

Comparison was done between group A and group B, the data showed highly significant decrease (P< 0.001) in number of villi in group B as compared to group A (table- 1, photomicrograph- 1, 2, 3).

Transverse diameter of terminal villi: In 4 µm thick, H and E stained paraffin sections, transverse diameter of terminal villi of were measured with the help of ocular micrometer in 10 random fields for each placenta in control group A and group B.

The mean values of transverse diameter of terminal villi of placenta in control group A and pre-eclamptic group B were 61.38±0.79 µm and 43.48±0.90 µm respectively (table- 1).

Comparison was done between group A and group B, the data showed highly significant decrease (P< 0.001) in transverse diameter of terminal villi in group B as compared to group A (table- 3, photomicrograph- 4, 5).

Intervillous Space: 4 µm thick, H and E stained paraffin sections were observed for the measurement of intervillous space using ocular micrometer in 10 random fields for each placenta in control group A and group B.

The mean values of intervillous space in control group A and pre-eclamptic group B were 24.20±0.43 µm and 41.55±0.42 µm respectively (table- 3).

Comparison was done between group A and group B, the data showed highly significant increase (P<0.001) in Intervillous space in group B as compared to group A (table- 1, photomicrograph- 1, 2, 3).

Number of terminal villous capillaries: 4 µm thick, H and E stained paraffin sections were observed. Number of terminal villous capillaries was counted in 10 random fields for each placenta in control group A and group B.

The mean values of number of terminal villous capillaries in control group A and pre-eclamptic group B were 2.96±0.54 and 4.72±0.45 respectively (table- 2).

Comparison was done between group A and group B, the data showed highly significant increase (P<0.001) in number of capillaries in terminal villi in group B as compared to group A (table- 4, photomicrograph- 4, 5).

Transverse diameter of terminal villous capillaries: In 4 µm thick, H and E stained paraffin sections, transverse diameter of terminal villous capillaries were counted in 10 random fields for each placenta in group A and group B by using ocular micrometer.

The mean values of transverse diameter of terminal villous capillaries in control group A and pre-eclamptic group B were 18.77±0.60 µm and 8.90±0.43 µm respectively (table- 4).

Comparison was done between group A and group B, the data showed highly significant decrease (P<0.001) in transverse diameter of terminal villous capillaries in group B as compared to group A (table- 4, photomicrograph- 4, 5).

Table 1: Mean Values of Number of Terminal Villi, Transverse Diameter of Terminal Villi (µm) and intervillous space (µm) in Group A and Group B

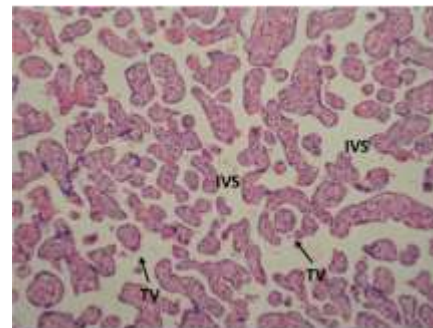
Parameters	Group A		Group B		p-value
	Mean	Standard Deviation	Mean	Standard Deviation	
Number of terminal villi	100.16	0.72	64.28	1.51	<0.001*
Transverse diameter of terminal villi (µm)	61.38	0.79	43.48	0.90	<0.001*
Intervillous space (µm)	24.20	0.43	41.55	0.42	<0.001*

*= Highly Significant

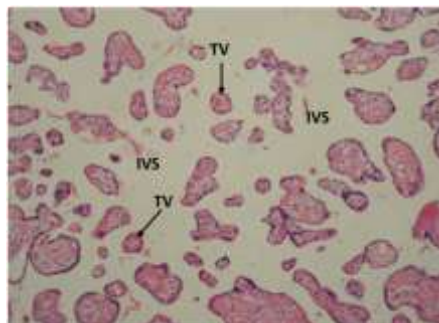
Table 2: Mean Values of Number of Terminal Villous Capillaries and Transverse Diameter of Terminal Villous Capillaries (µm) in Group A and Group B

Parameters	Group A		Group B		p-value
	Mean	Standard Deviation	Mean	Standard Deviation	
Number of terminal villous Capillaries	2.96	0.54	4.72	0.45	<0.001*
Transverse diameter of terminal villous Capillaries (µm)	18.74	0.60	8.90	0.43	<0.001*

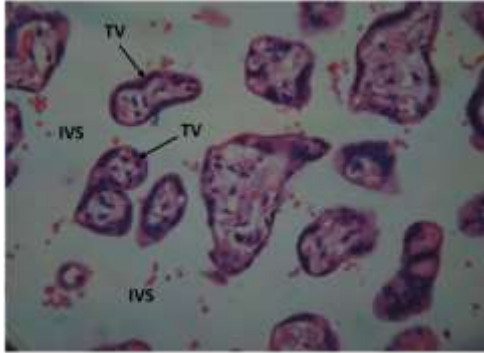
*= Highly Significant



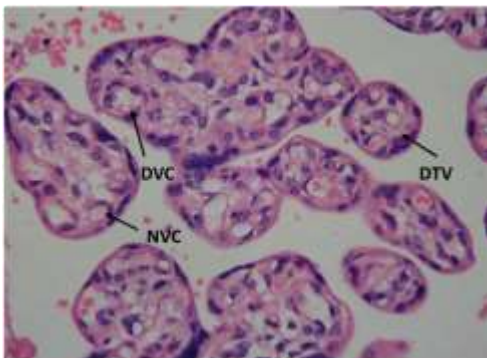
Photomicrograph: 1 A 4µm thick H&E stained paraffin section of full term normal human placenta from group-A showing terminal villi (TV) and intervillous space (IVS). x100



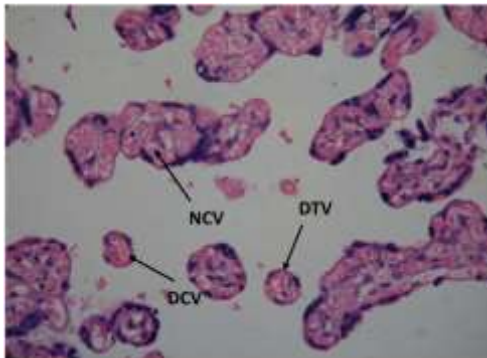
Photomicrograph: 2 A 4µm thick H&E stained paraffin section of full term pre-eclamptic human placenta from group-B showing decrease number of terminal villi (TV, black arrow) and wide intervillous space (IVS, black arrow). x100



Photomicrograph: 3 A 4µm thick H&E stained paraffin section of full term pre-eclamptic human placenta from group-B showing decreased number terminal villi (TV, black arrow) and wide intervillous space (IVS, black arrow). x400



Photomicrograph: 4 A 4µm thick H&E stained paraffin section of full term normal human placenta from group-A showing diameter of terminal villi (DTV, black arrow) and diameter of terminal villous capillaries (DVC, black arrow) and number of villous capillaries (NVC, black arrow). x400



Photomicrograph: 5 A 4µm thick H&E stained paraffin section of full term pre-eclamptic human placenta from group-B showing decreased diameter of terminal villi (DTV, black arrow) and decreased diameter of terminal villous capillaries (DVC, black arrow) and increase in number of villous capillaries (NVC, black arrow). x100

DISCUSSION

The placenta is a crucial organ for sustaining pregnancy and fostering healthy fetal growth. The terminal villous, which is in contact with maternal blood in the intervillous region, is the part of the placenta that performs its functions (9). The capillaries in the centers of these terminal villi contain fetal blood. The placental barrier keeps the blood of the mother and the fetus apart. The nutrition of the fetus is critically dependent on uteroplacental circulation. Pre-eclampsia is a syndrome that only occurs during pregnancy (6, 8). It is one of the main causes of maternal and fetal

morbidity and mortality worldwide, affecting 5-7% of pregnancies. The cause of pre-eclampsia is uncertain despite ongoing researches. Placental villous hypoxia and ischemia in pre-eclampsia are caused by insufficient trophoblast invasion of spiral arterioles (10). In our investigation, we found that group B (pre-eclamptic) had a much lower number of villi than group A (control) which is in parallel with the other reported findings who additionally noted a notable decrease in the number of villi in the pre-eclamptic group compared to the control group (11, 12).

Our findings were at odds with those of Saeed et al., who discovered a rise in the number of terminal villi in the pre-eclamptic group (13, 14). The intervillous space has increased as a result of the fewer villi. In our study, the pre-eclamptic group had considerably more intervillous space than the control group. This result was in accordance with the observation that pre-eclamptic women had much more intervillous space than control women (15). The results of our investigation differed from the studies who discovered that pre-eclampsia patients had significantly less intervillous space than the control group (16). In neither group did Kishwara et al. (2009) find any discernible differences in the intervillous space (17).

The transverse diameter of the terminal villi and the transverse diameter of the terminal villous capillaries were considerably smaller in group B pre-eclamptic placentae than in group A control placentae in the current study. As per documented data, the majority of the morphological alterations in the placenta in pre-eclampsia may be caused by decreased uteroplacental blood flow, which also accounts for the patho-physiological changes in internal components of the placenta (18, 19). Studies have reported that the transverse diameter of terminal villi and terminal villous capillaries were significantly smaller in the pre-eclamptic group than in the control group which is in parallel to current research (20, 21). According to Egbor et al. (2006), who showed no significant difference in the diameter decrease of both terminal villi and capillaries in both groups, the results of the present investigation were contrary to their findings (22).

As compared to group A control placentas, our study's findings revealed a significantly significant increase in the number of terminal villous capillaries in group B pre-eclamptic placentas. This was outlined by Mayhew et al. (2003) in their study, which found that pre-eclampsia causes increased angiogenesis, which the placenta uses to secure the flow of blood to the fetus. Endothelial cells are also implicated in the disease's adaption. Our results were consistent with those of Resta et al. (2006), who found that group B pre-eclamptic placentae had a much higher number of villous capillaries than did group A control placentae. Egbor et al. (2006) discovered no significant difference in the quantity of villous capillaries between the two groups, in contrast to this finding (22-24).

CONCLUSION

This study highlights the significant histological changes observed in the placentae of mothers with preeclampsia, indicating the multifocal nature of its origin. The findings suggest that preeclampsia leads to more significant alterations in placental structure compared to normal pregnancies, emphasizing the importance of early detection and management of this condition to improve maternal and fetal outcomes.

REFERENCES

1. Eastman AJ, Noble KN, Pensabene V, Aronoff DMJTJoM-F, Medicine N. Leveraging bioengineering to assess cellular functions and communication within human fetal membranes. 2022;35(14):2795-807.
2. Ortega MA, Fraile-Martínez O, García-Montero C, Sáez MA, Álvarez-Mon MA, Torres-Carranza D, et al. The pivotal role of the placenta in normal and pathological pregnancies: a focus on preeclampsia, fetal growth restriction, and maternal chronic venous disease. 2022;11(3):568.

3. Woods L, Perez-Garcia V, Hemberger MJ. Regulation of placental development and its impact on fetal growth—new insights from mouse models. 2018;9:570.
4. Tong M, Abrahams VM, Clinics G. Immunology of the Placenta. 2020;47(1):49-63.
5. Turco MY, Moffett AJD. Development of the human placenta. 2019;146(22):dev163428.
6. Magee LA, Nicolaides KH, Von Dadelszen PJ. Preeclampsia. 2022;386(19):1817-32.
7. Rana S, Lemoine E, Granger JP, Karumanchi SA. Preeclampsia: pathophysiology, challenges, and perspectives. 2019;124(7):1094-112.
8. Mayrink J, Costa M, Cecatti J. Preeclampsia in 2018: revisiting concepts, physiopathology, and prediction. 2018;2018.
9. Aplin JD, Myers JE, Timms K, Westwood MJ. Tracking placental development in health and disease. 2020;16(9):479-94.
10. Knöfler M, Haider S, Saleh L, Pollheimer J, Gamage TK, James JJC, et al. Human placenta and trophoblast development: key molecular mechanisms and model systems. 2019;76:3479-96.
11. Nizyaeva NV, Sukhacheva TV, Serov RA, Kulikova GV, Nagovitsyna MN, Kan NE, et al. Ultrastructural and immunohistochemical features of telocytes in placental villi in preeclampsia. 2018;8(1):1-15.
12. Huppertz B. The critical role of abnormal trophoblast development in the etiology of preeclampsia. 2018;19(10):771-80.
13. Saeed I, Iqbal I, Sarfaraz R, Qamar K, Butt SA, Shaukat SJ. Histomorphological Changes in Placentae of Pre-Eclamptic Mothers with Reference to Vasculosyncytial Membrane Thickness and Syncytial Knot Formation. 2012;16(1).
14. Iqbal I, Sarfaraz R, Qamar K, Butt SA, Shaukat SJ. Histomorphological Changes in Placentae of Pre-Eclamptic Mothers with Reference to Number of Villous Capillaries. 2014;18(1).
15. Donthi D, Malik P, Mohamed A, Kousar A, Subramanian RA, Manikyam UK. An objective histopathological scoring system for placental pathology in pre-eclampsia and eclampsia. 2020;12(10).
16. Mousa BA, Al Joborae SF. Study of placental shape and histopathological changes in pregnant ladies with preeclampsia. 2019;3(2).
17. Kishwari S, Nurunnabi ASM, Pal DR, Ara SJB. Study on the Histological Changes in the Parenchyma of the Placentae of Preeclamptic Women. 2009;24(2):63-6.
18. Ni G, Zhong J, Gao X, Wu R, Wang W, Wang X, et al. Three-dimensional morphological revealing of human placental villi with common obstetric complications via optical coherence tomography. 2023;8(1):e10372.
19. Kliewer MA, Bockoven CG, Reeder SB, Bagley AR, Fritsch MK. Ferumoxyl-enhanced magnetic resonance imaging with volume rendering: a new approach for the depiction of internal placental structure in vivo. 2023;131:104-10.
20. Copland I. Correlation of changes in morphology and TGF- β expression during human umbilical cord development 2000.
21. Waller E. Evidence for paternal imprinting of the Beta-3 adrenergic receptor gene and a variant association with reduced twinning rate in New Zealand merino sheep (*Ovis aries* L.): Lincoln University; 2017.
22. Egbor M, Ansari T, Morris N, Green C, Sibbons P. Maternal medicine: Morphometric placental villous and vascular abnormalities in early- and late-onset pre-eclampsia with and without fetal growth restriction. 2006;113(5):580-9.
23. Mayhew T, Ohadike C, Baker P, Crocker I, Mitchell C, Ong SJP. Stereological investigation of placental morphology in pregnancies complicated by pre-eclampsia with and without intrauterine growth restriction. 2003;24(2-3):219-26.
24. Resta L, Capobianco C, Marzullo A, Piscitelli D, Sanguedolce F, Schena F, et al. Confocal laser scanning microscope study of terminal villi vessels in normal term and pre-eclamptic placentas. 2006;27(6-7):735-9.