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The placenta in pre-eclampsia: association of histology with umbilical artery Doppler velocimetry

Abstract

Objectives: To study the association of umbilical artery Doppler velocimetry with histological changes of the placenta in pre-eclampsia.

Method: This was an observational study, conducted at two tertiary care centers in Sri Lanka between 2009 and 2010. A total of fifty placentae were studied; forty from women with pre-eclampsia and ten from normotensive women with uncomplicated pregnancies. Twenty pre-eclamptic patients with abnormal umbilical artery Doppler velocimetry were recruited in group 1. Group 2 included twenty gestational age-matched, pre-eclamptic patients with normal umbilical artery Doppler velocimetry. Ultra sound scan for umbilical artery Doppler was carried out to obtain the pulsatility index (PI) and resistance index (RI) approximately 24hours prior to delivery. Five randomly selected, haematoxylin and eosin-stained sections from the maternal surface of each placenta were examined under high power (magnification 40) of light microscopy. Hundred terminal villi in each slide were examined to identify the presence of four histological features; syncytial knots, proliferative villous cytotrophoblast cells, thickening of sub-trophoblastic basement membrane, and villous hypovascularity.

Results: All four histological features were present in a significantly higher proportion of placentae from pre-eclamptic patients ($P<0.05$) compared to normotensive placentae. The histological features of hypertensive placentae were not influenced by maternal age or parity but a statistically significant increase was noted with early onset hypertension (≤34weeks) compared to late onset hypertension (>34weeks). In hypertensive placentae, all four histological features were present in comparatively higher proportions in Group 1 (abnormal umbilical artery Doppler velocimetry) compared to Group 2 (normal umbilical artery Doppler velocimetry). But statistical significance was observed only in villous cytotrophoblast proliferation ($P=0.005$), and villous hypovascularity ($P=0.002$).

Conclusion: The placental histological changes are significantly increased in pre-eclampsia. Further deterioration of placental histology is associated with abnormal umbilical artery Doppler velocimetry.

Keywords: pre-eclampsia; placental histology; umbilical artery doppler velocimetry

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Abbreviations: PI, pulsatility index; RI, resistance index; PE, pre eclampsia; FGR, fetal growth restriction; SLE, systemic lupus erythematosus; US, ultra sound; SGA, small for gestational age; POG, period of gestation

Introduction

Hypertensive disorders are common during pregnancy requiring close antenatal care. Hypertension in pregnancy is defined as systolic blood pressure 140mmHg or diastolic blood pressure ≥90mmHg on two occasions at least 4 hours apart after 20weeks of gestation in a woman with a previously normal blood pressure. Pre-eclampsia (PE) is traditionally defined as new hypertension presenting after 20weeks of gestation with significant proteinuria (≥300mg/dl in 24-hour urine collection).[1] Worldwide, pre-eclampsia affects 5-10% of pregnancies and it is a leading cause of maternal morbidity, mortality, fetal growth restriction (FGR), and prematurity.[2] The aetiology of PE is still unknown, although an excessive maternal systemic inflammatory response and an imbalance between circulating angiogenic and anti-angiogenic factors have been described.[3] The placenta has long been recognized as the necessary component for the genesis of preeclampsia. A common pathological feature of preeclampsia is the failure of the maternal spiral arteries supplying the placenta to undergo the physiological adaptations of normal pregnancy to facilitate adequate placental perfusion.[4] This theory of placental hypo-perfusion is supported by clinical, pathological and experimental findings.[5]

The impaired placental perfusion precedes clinical manifestations of PE and it can be detected by Doppler ultrasound (US). Umbilical artery Doppler reflects downstream placental vascular resistance, correlated with the multisystem effects of placental deficiency. Recent meta analysis of randomized controlled trials suggests that incorporation of umbilical artery Doppler waveform analysis into management protocols for high risk pregnancies significantly decreases perinatal mortality.[6] Therefore, Doppler ultrasound findings have become an increasingly common tool for evaluating placental pathology in PE. A number of histopathological changes of the hypertensive placentae have been described; namely placental infarcts, increased syncytial knots, hypovascularity of the villi,

increased cytotrophoblastic proliferation, thickening of the sub-trophoblastic basement membrane, obliterated enlarged endothelial cells in the fetal capillaries and atherosis of the spiral arteries in the placental bed. The volume of the intervillous space and the terminal villi are also decreased in proportion to the degree of pre-eclampsia.[7,8] In this study, we assessed the histological features of the placentae in pre-eclampsia and evaluated the correlation between histological changes and umbilical artery Doppler velocimetry.

Materials and methods

This was an observational study carried out in two tertiary care centers in Colombo, Sri Lanka for a period of one year from 2009 to 2010. Women with a singleton gestation with pre-eclampsia were invited to participate, if they had fulfilled the inclusion and exclusion criteria to enroll in the study. Hypertension in pregnancy was defined as systolic BP ≥140mm Hg or diastolic BP ≥90mm Hg on two occasions at least 4hours apart after 20weeks of gestation in a woman with a previously normal blood pressure.[9] Proteinuria was assessed by urine dipstick method. A dipstick reading of +1 is considered as significant proteinuria where quantitative methods are not available.[9] We excluded pregnant women with gestational or pre-existing diabetes mellitus, connective tissue disorders (e.g. SLE), thrombophilic disorders, multiple pregnancies, placental tumours, and placental abruption. The confidence interval was set at 95% (α=0.05) and power of study at 80% (β=0.02) which gave the sample size of 37 by using the relevant formula. Based on a previous study, abnormal histological features were assumed to be present in 90% of placentae with abnormal umbilical artery Doppler and 60% of placentae with normal umbilical artery Doppler.[10] Ultra sound scan for umbilical artery Doppler was carried out to obtain the pulsatility index (PI) and resistance index (RI) in consented mothers 24hours prior to induction of labour or caesarean section. The Doppler US was performed by trans-abdominal examination with a GE-LOGIQ 3 PRO: AY-15CUI convex transducer. Umbilical artery measurements were taken in a free umbilical cord loop with insonation angle maintained below 60 degrees. Pulsatility index (PI) and resistance index (RI) were categorized as abnormal if the values were above the 95th percentile for the gestational age.[11]

Twenty patients with abnormal umbilical artery Doppler velocimetry were included in group 01 and twenty patients with normal umbilical artery Doppler velocimetry were recruited in group 02. The gestational age was matched between the two groups.

i. Group 1: Pre-eclampsia with abnormal umbilical artery Doppler velocimetry (N=20)

ii. Group 2: Pre-eclampsia with normal umbilical artery Doppler velocimetry (N=20)

As an index of normal histology, a conventional sample of ten placentae were collected from pregnant women with uncomplicated pregnancies whose gestational age was proportionately matched with the study group. The whole placenta was collected after the delivery and fixed in 10% buffered formalin for at least four weeks. Tissue sections were taken from the central and peripheral zones of the maternal surface of each placenta. Five random sections were selected from each placenta and stained with Haematoxylin and eosin. The stained slides were examined under high power (magnification 40) of light microscopy for histological features. In each slide, terminal villi were examined starting from one corner of the slide until 100 terminal villi had been encountered (Figure 1). Four histological features were analyzed in the terminal villi; syncytial knots, proliferative cytotrophoblast cells, sub-trophoblastic basement membrane thickening and villous hypovascularity. The proportions of terminal villi containing each of the four histological characteristics were calculated for each placenta. Syncytial knots in more than 30% of villi,[12] proliferative villous cytotrophoblast cells in more than 10% of villi,[13] basement membrane thickening in more than 20% of terminal villi and villous hypovascularity in more than 30% of terminal villi[14] were taken as abnormal histological findings. The birth weights of the babies were measured in both groups and were categorized as normal weight or small for gestational age (SGA). SGA was defined as birth weight below the 10th centile for the gestational age[15] Results were analyzed by relevant statistical formulae using SPSS-version17. Mann-Whitney U-test was applied to compare the histological features between normotensive and hypertensive placentae. The same test was applied to analyze the histological features in relation to maternal age, parity, and gestational age at the onset of hypertension. The X_2 test was used to analyze the association of histological features with umbilical artery Doppler velocimetry. Informed written consent was taken from the patients for collection of the placenta for histological examination. Ethical approval was taken from the Ethical Review Committee of Castle Street Hospital for Women, Colombo 08. Confidentiality of the subjects was maintained throughout the study.

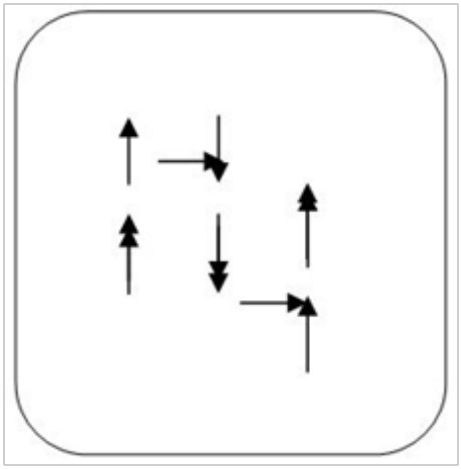

Figure 1 Technique of counting terminal villi on histology slide.

Results

A total of 40 participants were included in group 1 and group 2 each containing 20. The gestational age was matched between the two groups. The patient demographics; mean maternal age, parity, period of gestation (POG) at which hypertension was first detected and the POG at delivery were similar between the two groups (Table 1). We compared the histological features between the normotensive placentae and hypertensive placentae. All four histological characteristics; syncytial knots, cytotrophoblastic proliferation, sub-trophoblastic basement membrane thickening and villous hypovascularity were present in significantly higher proportions in hypertensive placentae compared to normotensive placentae (Table 2). Sub-analysis was carried out within the hypertensive group to study the association of histological features with maternal age, parity and gestational age at the onset of hypertension. Two age groups were defined for the analysis; age ≥35years and age <35years.

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The histological characteristics of hypertensive placentae were not significantly different between the two age groups. Similarly, there was no statistically significant difference in the histological features between primipara and multipara.

In contrast, the analysis of histological features in relation to the gestational age at the onset of hypertension revealed a significant difference. For the convenience of analysis, subjects were divided into two groups; onset of hypertension. For the convenience of analysis, subjects were divided into two groups; onset of hypertension ≤34weeks (early onset) and >34weeks (late onset) of gestation. With early onset hypertension, cytotrophoblastic proliferation, basement membrane thickening, and villous hypovascularity showed a statistically significant increase. Although syncytial knot counts also increased with early onset hypertension, the difference was not statistically significant (Table 3). Final statistical analysis was carried out to study the association of placental histology in pre-eclampsia with umbilical artery Doppler velocimetry. All four histological characteristics were observed in higher proportions in hypertensive placentae with abnormal umbilical artery Doppler velocimetry compared to normal Doppler velocimetry. Statistical significance was observed only in cytotrophoblastic proliferation and villous hypovascularity (Table 4). All the babies in group 1 were small for gestational age (SGA). In group 2, there were 13 babies (65%) who were small for gestational age.

Table 1 Patient demographics

	Group 1	Group 2	
	N=20	N=20	Significance
	Mean(SD)	Mean(SD)	
Age(years)	32.6(6.03)	31.25(3.86)	P=0.40, NS
POG at delivery(days)	249(11.62)	246(1.34)	P=0.35, NS
POG at which hypertension was first detected(weeks)	30.40(5.05)	29.25(9.072)	P=0.62, NS

SD, standard deviation; NS, not significant; POG, period of gestation

Table 2 Comparison of histological features between hypertensive and normotensive placentae

Histological characteristic	Number of terminal villi with a specific histological characteristic per 100 Villi		Significance
	Hypertensive placentae	Normotensive placentae	
	N=40	N=10	
	Mean(SD)	Mean(SD)	
Syncytial knots	46.87(11.25)	25.72(9.43)	P=0.00, S
Cytotrophoblastic proliferation	21.2(4.17)	9.67(3.24)	P=0.00, S
Basement membrane thickening	23.65(10.35)	12.47(5.78)	P=0.00, S
Villous hypovascularity	13.48(7.41)	6.23(3.12)	P=0.00, S

SD, standard deviation; S, significant

Table 3 Comparison of histological features of hypertensive placentae with gestational age at the onset of hypertension: After 34weeks versus Below 34weeks

Histological characteristic	Number of terminal Villi with a specific histological characteristic per 100 Villi		Significance
	After 34 W	Below 34 W	
	N=16	N=24	
	Mean(SD)	Mean(SD)	
Syncytial knots	43.19(18.44)	49.33(4.58)	P=0.12, NS
Cytotrophoblastic proliferation	18.25(5.62)	23.17(3.76)	P=0.00, S
Basement membrane thickening	14.88(9.85)	29.50(6.15)	P=0.00, S
Villous hypovascularity	11.04(5.33)	16.94(10.69)	P=0.04, S

SD, standard deviation; S, significant; NS, not significant; W, weeks

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Table 4 Comparison of histological features of hypertensive placentae with umbilical artery Doppler velocimetry

Histological Characteristic	Number of terminal villi with a specific histological characteristic per 100 Villi		Significance
	Group 1	Group 2	
	N=20	N=20	
	Mean(SD)	Mean(SD)	
Syncytial knots	49.00(9.55)	44.75(14.57)	P=0.28, NS
cytotrophoblastic proliferation	23.40(6.27)	19.00(2.17)	P=0.005, S
Basement membrane thickening	25.80(8.74)	21.50(12.02)	P=0.20, NS
Villous hypovascularity	17.80(11.11)	9.00(3.77)	P=0.002, S

SD, standard deviation; S, significant; NS, not significant

Discussion

Pre-eclampsia is a multisystem disorder unique to human pregnancy. It is known as "The Disease of Theories" as the exact course of events has not yet been elucidated. However, there is increasing evidence that pre-eclampsia is fundamentally related to poor trophoblast invasion of the myometrium which results in maternal spiral arteries being hampered in their normal physiological vasodilatation.[16] In normal pregnancy, the utreo-placental spiral arteries in the myometrium undergo structural modifications where the musculo-elastic tissue of the tunica media is replaced by invading trophoblastic cells surrounded by a thick layer of fibrinoid material. The affected vessels undergo progressive vasodilatation. This phenomenon is described as the "physiological changes of spiral arteries". These changes are responsible for the low vascular resistance of the placental bed and allow a large increase in blood flow to the intervillous space. In pre-eclampsia, the endovascular trophoblast does not invade the myometrium and physiological changes are confined to decidual segment. Therefore, the spiral arteries in the placental bed are less dilated than normal pregnancy and they remain responsive to vasomotor influences. The impaired intervillous blood flow results in inadequate perfusion and ischaemia of the placenta especially in the second half of pregnancy.[17] The histology of placenta is described mainly in relation to the terminal villi and its vasculature as they are the functional units of the placenta. The four histological features; syncytial knots, cytotrophoblastic proliferation, thickening of sub-trophoblastic basement membrane, and hypovascular villi were also observed in the placentae of normotensive women in varying degrees yet within normal limits. A statistically significant increase in the presence of all four histological features were observed in the hypertensive placentae. These prominent histological changes represent the structural adaptations for placental ischaemia which creates a hostile environment in hypertensive placentae.

For oxygen requirement, the syncytium depends on the maternal blood flow to the intervillous space through the utero-placental circulation. Reduced utero-placental blood flow in hypertension may result in hypoxic damage to the syncytium. The damaged syncytium stimulates syncytial nuclear proliferation leading to syncytial knot formation.[17] In an attempt to replace the degenerated syncytium, the cytotrophoblast cells undergo proliferation.[13] This phenomenon explains the existence of excess cytotrophoblast cells and syncytial knots in the terminal villi of hypertensive placentae. Thickened sub-trophoblastic basement membrane is presumed to be due to the proliferation of cytotrophoblastic cells, which secrete the basement membrane as a response to placental ischaemia.[18] But the presence of sub-trophoblastic basement membrane thickening in other conditions like diabetes mellitus and rhesus incompatibility raises the possibility that some other factors may also be involved in the pathogenesis in addition to ischaemia. A significant increase of collagen fibers in the villous stroma has been observed in hypertensive placentae.[14,19] This villous stromal fibrosis may collapse the fetal capillaries by a simple squeezing mechanism. Another observation in hypertensive placentae was obliteration of villous capillaries by endothelial thickening.[20] Both these factors may be responsible for the villous hypovascularity. The histological features in hypertensive placentae were not influenced by the maternal age or parity. We observed a statistically significant increase in cytotrophoblastic proliferation, basement membrane thickening and hypovascular villi with early onset hypertension.

Impaired placental perfusion caused by vascular abnormalities precedes clinical manifestations of PE and it can be detected by Doppler ultrasound. The umbilical artery Doppler reflects downstream placental vascular resistance and the multisystem effects of placental deficiency. Therefore, umbilical artery Doppler has been traditionally used for evaluation of the fetal status and prediction of adverse pregnancy outcome.[21] We assessed the umbilical artery Doppler in all the subjects. Both pulsatility index (PI) and resistance index (RI) of umbilical artery were measured by Doppler ultrasonography approximately twenty-four hours prior to delivery. The values above the 95th percentile for gestational age were classified as abnormal.[11] When histological features were analyzed in relation to the umbilical artery Doppler velocimetry, all four histological features showed a notable increase with abnormal umbilical artery Doppler. Proliferative cytotrophoblast cells and hypovascular villi showed a statistically significant increase. This observation suggests that progressive worsening of placental pathology in pre-eclampsia is responsible for the abnormalities observed in umbilical artery Doppler velocimetry. All the babies born to pre-eclamptic women with an abnormal umbilical artery Doppler (Group 1) were small for gestational age (SGA). In Group 2 (with normal umbilical artery Doppler velocimetry), there were thirteen SGA babies (65%).

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Some small for gestational age (SGA) babies fall into the category of fetal growth restriction (FGR) which is placenta-mediated. In this condition, the fetus fails to reach its potential growth due to placental insufficiency.[22] It is interesting to know whether placental insufficiency in pre-eclampsia differs histopathologicaly from that of FGR without pre-eclampsia. According to the available evidence, standard histological examinations do not appear to differentiate the abnormal placental findings between preeclampsia and FGR without preeclampsia.[23] The presence of similar placental histological features in two different clinical scenarios (pre-eclampsia vs FGR without pre-eclampsia) explains the multi-factorial nature of the origin of these conditions. In fact, there is a great debate going on whether placental inefficiency is a primary cause, contributory factor or a result of pre-eclampsia.[20]

In our study, placental histology was not analyzed in relation to the birth weight of the baby because of the small sample size and lack of a control group with FGR not complicated by pre-eclampsia. The study will be extended in future to address this issue.

Conclusion

Placental histology with regard to syncytial knots, cytotrophoblastic proliferation, sub-trophoblastic basement membrane thickening, and villous hypovascularity is significantly increased in pre-eclampsia compared to normotensive placentae. Progressive worsening of these placental histopathological features in pre-eclampsia is associated with the occurrence of abnormalities in umbilical artery Doppler velocimetry.

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Conflict of interest

The author declares no conflict of interest.

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