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Morphological and histological changes in placentas of preeclamptic patients

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Abstract

Preeclampsia is defined as a syndrome with onset of hypertension and proteinuria after 20th week of gestation in previously normotensive, non-proteinuric pregnant women. Placenta and its vascularity seem to be involved in preeclampsia.

In the present study a total of sixty placentas were collected from labour room of AIIMS and of theses thirty were from normotensive pregnancies and rest thirty were from preeclamptic patients. The placental tissue from each subject was fixed and subjected to paraffin embedding and H & E staining. The weight of placenta and baby's birth weight was recorded. The gross and histological examination of each placenta was done.

The normal term placenta showed normal morphology however occasional fibrinoid foci were seen externally in preeclamptic placentas. The placental and baby's birth weight was lower in preeclamptic patients than normal ($p<0.0001$). On light microscopic examination, a classic picture of normal placenta showed chorionic villi, extracytotrophoblastic matrix, connective tissue and blood vessels. However the histological picture of preeclamptic placenta showed increased branching of chorionic villi, syncytial knots, and increased vascularity of villi. The large number of intermediate villi were covered with cytotrophoblast

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and syncytiotrophoblasts which reduced the exchange surface area. In preeclampsia a state of hypoxia is known to be created which leads to increase in blood vessels and reduced fetoplacental circulation. These changes in turn may affect the growth and development of fetus.

Key Words: Preeclampsia, placenta, syncytiotrophoblast, cytotrophoblasts, fetal vessels.

Introduction

Preeclampsia is one of the major causes of perinatal and neonatal mortality and morbidity. It seems to affect the fetal growth and development (1,2). The exact etiology of preeclampsia is unknown, it has been reported to be multifactorial in nature that often involves placental or vascular susceptibility (3,4). The pathological abnormality of the placenta can contribute to the clinical understanding of premature delivery, fetal growth restriction and neonatal morbidity (5). It has been reported that placentas complicated by preeclampsia showed maldeveloped terminal villi, which led to impaired fetoplacental circulation (6). Placental vasculopathy has also been reported to be associated with decreased birth weight of babies (5). It has been reported that the histological changes in the placenta can lead to clinical manifestations, which could be cause/effect of preeclampsia. Thus it has been planned to study the morphological and histological changes of placenta in preeclamptic patients.

Materials and methods

Sixty pregnant women attending the antenatal OPD and labour room of the Department of Obstetrics and Gynecology, All India Institute of Medical Sciences, New Delhi, India, were selected for the study. The study was approved by the Institute Ethics Committee and informed consent was obtained from each patient. Of these thirty women had having pregnancy induced hypertension after twentieth week of gestation were included and were labeled preeclamptic. The pregnancies with chorioamnionitis, chronic hypertension, pregestational hypertension, renal disease, cardiac disease, active asthma, thyroid disease and preexisting seizures were excluded from the study. The rest thirty were normal pregnancies and served as control. Preeclampsia was defined according to the criteria of the

International Society for the Study of Hypertension in Pregnancy (ISSHP); systolic and diastolic blood pressures above 140mmHg and 90 mmHg respectively, in at least two consecutive measurements at least 6 hours apart occurring after twentieth week of gestation and accompanied by proteinuria (>300 mg per liter in a 24 hrs urine collection) and /or edema before commencing medication.

Hematoxylin and eosin staining

All placentas (control as well as preeclamptic) were collected immediately after delivery and examined grossly for any change in shape, size, color, and presence of any infarction, calcification, thrombus/ bleeding. The placental tissue was collected from central and peripheral part of each placenta. Each tissue was fixed in 10% buffered neutral formalin solution, and was processed for routine paraffin embedding. Cross sections (5-7μm thick) were cut, stained with hematoxylin and eosin (H and E) and visualized under light microscope for its histological features. The microphotographs were taken at different scale bars (50μm and 100μm).

Statistical analysis

The data was expressed in terms of the mean ± SD using student t test for independent samples. The chi square test was used for the analysis of differences of semi quantitative parameters.

Results

The systolic and diastolic pressure in control group was 112.5 ± 1.3mmHg (range between 100mmHg -124mmHg) and 71.6 ± 7.6mmHg (range between 60mmHg -84 mmHg) respectively whereas in preeclamptic group, the systolic and diastolic pressures were 139 ± 8.1mmHg (range 120mmHg - 150mmHg) and 98.4 ± 7.6mmHg (range 86mmHg-120 mmHg) respectively. Urinary protein was determined in 24-hour urine samples. The levels of protein and uric acid in the urine samples of control group were (0.6± 0.5 g/day) and (2.7± 1.2 mmol/L) respectively whereas these levels [5.1±1.8 g/day (range1.8g/day - 8.2g/ day) and 4.5± 1.3 mmol/L (range 1.5mmol/L - 7.9 mmol/L)] were raised in the urine samples of preeclamptic group. (Table 1)

The average maternal age was 28.2 ± 2.7 (yrs) in normal as compared to 29.9±3.4 (yrs) in preeclamptic patients while their gestational age was 38.8 ± 1.1 (wks) and 35.9± 2.6(wks) respectively (Table 1). The baby's birth weight was 2884.9 ± 241.3 gm (range 2480gms -3610 gm) of normal pregnant

Table 1 : Obstetrics and clinical characteristic of normal and preeclamptic patients.

Baseline characteristics	Mean ± SD		Difference in mean (95% C.I.)	p value
	Normal (n-30)	Preeclampsia (n-30)		
Demographic characteristics				
Maternal age (yrs)	28.2±2.7	29.9±3.4	1.7 (0.13, 3.33)	0.05
Gestational age (wks)	38.8±1.1	35.9±2.6	2.9 (1.91, 4.02)	0.0001
Baby's birth weight (gms)	2884.9±241.3	2446.5±95.3	422.4(327.56, 517.24)	0.0001
Placental weight (gms)	533±70.5	353±73.4	180(142.81, 217.18)	0.0001
Blood pressure				
Systolic BP (mmHg)	112.5±1.3	139 ± 8.1	26.8(22.80, 30.79)	0.0001
Diastolic BP (mmHg)	71.6±7.6	98.4 ± 7.6	26.73(22.80, 0.66)	0.0001
Clinical evaluation				
Protein (g/day)	0.6±0.5	5.1 ± 1.8	4.59(3.87, 5.30)	0.0001
Uric acid (mmol/l)	2.7±1.2	4.5 ± 1.3	1.82(1.15, 2.49)	0.0001

Student t test for independent samples. p< 0.05, statistically significant

women as compared to 2446.5 ± 95.3 gm (range 2270gms- 2640 gm) of preeclamptic patients at a statistically significant value of p<0.0001. The placental weight of 533 ± 70.5 gm (range 430gms-710 gm) vs. 353 ± 73.4gm (range 240gm- 490gm) was observed in normal pregnancy compared to preeclampsia (p<0.0001).

The maternal age was more than 28yrs of 9/30 (30%) and 17/30(56.6%) of normal and preeclamptic respectively while 21/30(70%) of normal patients and 13/30 (43.3%) of preeclamptic patients were less than 28yrs. The possibility of preeclampsia was increased with maternal age. The gestational age was less than 37 wks in 3/30 (10%) and 21/30 (70%) of normal and preeclamptic patients while gestational age was more than 37 weeks in 27/30(90%) and 9/30(30%) of normal and preeclamptic patients respectively. The baby's birth weight was less than 2629gms in 2/30(6.7%) and 28/30 (93.3%) and greater than 2629gms in

28/30(93.3%) and 2/30(6.7%) of normal and preeclamptic patients respectively. Similarly the placental weight was less than 459gms in 3/30(10%) and 28/30(93.3%) and greater than 459 gms in 27/30 (90%) and 2/30(6.7%) of normal and preeclamptic placenta respectively (Table2).

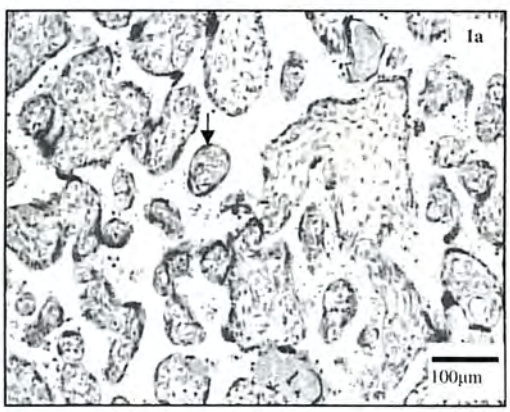
The Receiver operating characteristics curve (ROC) analysis of maternal age (yrs) and gestational age (wks) revealed a sensitivity of 56.6% and 90.0% and a specificity of 70.0% and 70%, at cutoff value of 28 and 37 respectively. The ideal cutoff value for birth weight of baby (gm) and placental weight (gm) to differentiate preeclamptic and normal patient was 2629gm and 459gm respectively, which demonstrated a sensitivity of 93.3% and 90.0% and a specificity of 93.3% and 93.3% respectively.

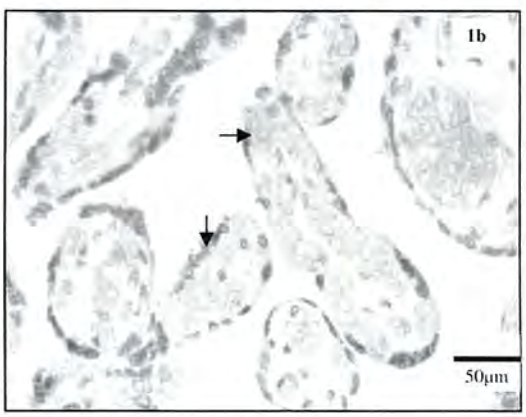
Table 2 : Percentage of maternal age, gestational age, baby's birth weight and placental weight in normal and preeclamptic patients.

Clinical characteristics	Number (%)		Difference in mean (95% C.I.)	p value
	Normal (n-30)	Preeclampsia (n-30)		
Maternal Age (yrs)				
≥ 28	9 (30.0)	17 (56.7)	3.05 (1.05, 8.84)	0.37
< 28	21 (70.0)	13 (43.3)		
Gestational age (wks)				
≤ 37	3 (10.0)	21 (70.0)	21 (5.05,87.37)	0.0001
>37	27 (90.0)	9 (30.0)		
Baby's birth weight (gms)				
≤ 2629	2 (6.7)	28 (93.3)	196 (25.77,1490.5)	0.0001
> 2629	28 (93.3)	2 (6.7)		
Placental weight (gms)				
≤ 459	3 (10.0)	28 (93.3)	126 (19.51,813.90)	0.0001
>459	27 (90.0)	2 (6.7)		

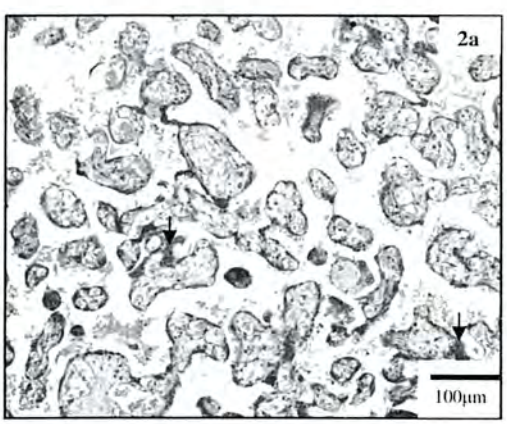
Chi square test. p< 0.05, statistically significant

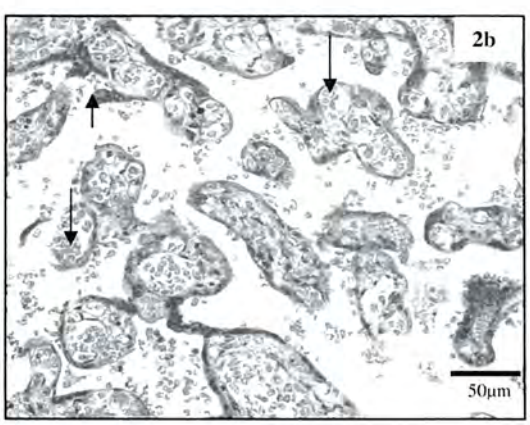
The normal mature placenta showed the normal maturation and branching pattern of stem chorionic villi from where short finger like villi branched. The terminal villi were relatively short and richly branched. On cross sections most of the terminal villi were of more or less uniform caliber except for some immature intermediate and stem villi (Fig. 1). There was minimal space between the villi. The stem villi had distinct layers of





Figures 1 : Photomicrographs show the normal histology of normal placenta. Arrows indicate the terminal villi, which are covered by a single layer of syncytiotrophoblast cells (Fig 1a at 100ì m and Fig 1b at 50 ì m scale bar).





Figures 2 : Photomicrographs show the scattered terminal villi. Small arrows indicate the terminal villi, which are covered by trophoblastic cell layers (cytotrophoblasts and syncytiotrophoblasts). There are excess of blood capillaries in the intermediate villi (shown by long arrows) (Fig 2a at 100ì m and Fig 2b at 50 ì m scale bar).

cytotrophoblasts and syncytiotrophoblasts. These villi were characterized by one or more arteries and veins or arterioles and venules having clearly visible muscular walls. The small villi were lined by a single layer of cytotrophoblast cells only. At places extravillous trophoblast was replaced by matrix type fibrinoid substance. Some syncytial knots consisting of pink homogeneous mass were also observed. The histology of preeclamptic placenta on the other hand showed slightly variable and disturbed pattern of villi. There was increased trophoblastic proliferation and increased villous capillary growth which changed the outer shape of terminal villi. The trophoblast directly covered the capillary bed as the connective tissue was reduced. The villi were of all sizes i.e. large, medium and small. The small villi were relatively less in number and were scattered with lot of space between them as compared with normal placenta (Fig. 2). The villi consisted of thick core of mesenchyme, rich blood vessels and were lined by cytotrophoblast and syncytiotrophoblast. Very few villi were covered with only a single layer of cytotrophoblast because of which the diffusion at the level of terminal villi was not very effective. Although due to preeclampsia there were too many blood vessels in stem and intermediate villi and these vessels also had thin walls unlike those of a normal placenta. However, the maternofetal exchange was probably not possible at these sites. There were small focal areas of calcification and fibrinoid material.

Discussion

Within the womb, fetal growth and development depends primarily upon inherited genetic potential, environmental factors and interaction between the two. For this the placenta plays an important role (7-9). The placenta must primarily initiate maternal adaptations to support the growing pregnancy and then successfully distribute substrates and waste across the uterine interface. The mother must adequately respond to placental demands without jeopardizing her own health. Disturbances at any of these steps, from implantation to maternal adaptation, may impact the uterine environment and trigger alterations in fetal growth and development.

In the present study, therefore the status of placenta was studied in terms of gross and histological features and compared with preeclamptic placentas and to see if placental environment had any effect on growing fetus. On gross examination of normal full term

placenta, most of them showed normal morphology such as shape which was either disc like, flat or round to oval, normal colour and had no obvious areas of focal fibrosis or necrosis. The preeclamptic placenta also did not show gross morphological abnormalities except small occasional fibrinoid foci which may be either a normal feature or due to hypoxia which is responsible for placental changes in women with preeclampsia, hypertension and anemia (10-15). Similar findings were also observed on experimental studies on long term effects of hypoxia on pregnant guinea pigs (16).

The average weight of normal placenta was 533±70.5gms whereas the weight of the preeclamptic placenta was 353± 73.4gms which was about 180gms lower than the normal placental weight ($p<0.0001$) (Table1). It has been reported that in preeclamptic placenta, the connective tissue is reduced and there is rich branching of capillaries which are covered by trophoblasts and there are less number of small villi which are mainly responsible for diffusion (16-18). These changes may contribute to reduced weight of preeclamptic placenta.

The histological changes observed in preeclamptic placenta in the present study have probably reduced the exchange surface available by terminal villi because they are mostly covered by both cytotrophoblast as well as syncytiotrophoblasts and also due to increased maternofetal diffusion distance between the terminal villi affecting the growth and development of the baby. In the present study when the weight of babies was taken, the sensitivity and specificity was 93%. Some of these changes of placenta correlate with IUGR as suggested by Vogel (19). However Macara (20) on the other hand, reported that other findings like paucity of villous trophoblast, reduction of cytotrophoblast proliferation, increased incidence of pyknotic nuclei and syncytial knotting and increased amounts of stromal collagen may also contribute to IUGR. These findings were in agreement with the experimental studies of Panigel and Myers (21). All these changes were probably due to decreased release of oxygen from the terminal villi into the fetoplacental circulation. Our findings did not completely correspond with the above authors. Although few of these changes were observed in preeclampsia which were reflected in the form of low birth weight of babies and low placental weight (Table1) ($p < 0.0001$) but the parameters for IUGR were not calculated in our study.

The clinical manifestations of preeclampsia are probably attributable to diffuse endothelial damage with capillary leak, hypercoagulability and release of vasoactive mediators leading to hypertension (22). Macara (20) noted that the terminal villi in IUGR placentas were smaller in diameter than those in normal placentas and had increased syncytiotrophoblast cell nuclei, reduced cytotrophoblast cell nuclei, thickened basal lamina and increased stromal deposition of collagen and laminin. Krebs (23) has shown that villous capillary loops in IUGR placentas were relatively sparse in number and significantly longer than those in normal placentas. These capillary abnormalities were found in association with elongation of the terminal villi, deposition of fibrin plaques of the trophoblast surface, and villous atrophy.

In a previous study, placentas of 82% of the patients with pregnancy induced hypertension (PIH) had one or more of these histological findings. Various authors (24) have reported that placental infarction was seen in 32.3% and maternal atherosis and fibrinoid medial necrosis was seen in 21.4% of these placentas of mothers with PIH. Examination of placentas with PIH should be based on a combination of a number of histological changes which included placental infarction, perivillous fibrin deposition and chronic villitis. Extensive perivillous fibrous deposition has also been associated with the decrease of blood flow in the intervillous space and has been frequently associated with the presence of placental infarction (25). Although all these findings collectively were not seen in preeclamptic placentas in the present study but there was nonavailabilty of enough diffusion surface due to thick walled less number of terminal villi with increased distance between them.

The investigation of the placenta is often limited to cases with severe or early disease-the variant of preeclampsia most closely associated with fetal growth restriction. Various authors (1) have reported that cases of mild preeclampsia at term are underrepresented and may show no histological, biological or molecular disturbance.

In the present study, there were histological differences in the villous size, its covering and vascularity in preeclamptic placentas. There were immature villi in preeclamptic placenta as compared to normal placentas which comprise the main exchange area of term placenta. Grossly we have not been able to see significant difference in preeclamptic patients except that these placentas were small in size and

had low birth weight babies. Theses histological changes along with other factors such as increased maternal age, decreased gestational age, low birth weight of babies and low placental weight may probably contribute to the physiopathology of preeclampsia.

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