Histomorphometry of Umbilical Cord and its Vessels in Pre-Eclampsia as Compared to Normal Pregnancies

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ABSTRACT

Aims: To study and compare the morphometric alteration in the umbilical cord obtained from thirty normal pregnancies (Group-I) and thirty pre-eclamptic pregnancies (Group-II).

Methods: Morphological changes in umbilical cords were examined on formalin fixed paraffin embedded section. Various parameters were measured by planimeter.

Results: In pre-eclamptic group total vessel area, total vein area, total luminal area of vein, and wall thickness of arteries were increased, whereas jelly area and wall thickness of vein were decreased as compared to normal group.

Conclusion: Increased umbilical arterial thickness, decreased umbilical vein wall thickness and increased luminal area of vein which could be a predictor of intrauterine growth retardation in term pre-eclamptic patients.

Keywords: Fetus, histomorphometry, pre-eclampsia, umbilical cord.

INTRODUCTION

Pre-eclampsia (PE) is one of the most common pregnancy associated pathological syndrome which is characterized by generalized edema, hypertension and proteinuria presenting after 20th week of gestation. It occurs in 5% of all pregnancies and is a major contributor to maternal and neonatal morbidity and mortality. Pre-eclampsia is one of the main causes of intrauterine growth restriction(IUGR). Occasionally PE and IUGR are also associated with chronic hypoxia of the growing fetus which in turn, might be responsible for pathological contractions in the vascular bed of cotyledons leading to an increased placental resistance.[1] In India, it is one of the major causes of maternal death and poor perinatal outcome. Prediction of PE is also very difficult in early pregnancy. Some epidemiological factors associated with pre-eclampsia include nulliparity, previous pre-eclampsia, family history, black race, obesity, diabetes mellitus, multi-fetal pregnancies, age of mother (< 18 years and >35years) and previous renal diseases. [2]

The umbilical cord forms connection between placenta and the fetus. The cross-sections of umbilical cord show a specific gross morphology of vein and arteries surrounded by Wharton's jelly. Umbilical vessels are not supplied by vasa vasorum and thus depend on their own oxygen supply making them more vulnerable to changes in hemodynamic condition. Literature is scarce with regard to alteration in morphometry of umbilical cord vessels due to pre-eclampsia in Indian population. Aim of this study was to compare the morphological alteration in umbilical cord vessels in normotensive and pre-eclamptic pregnancies on histological basis.

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METHODS

The study included 60 pregnant women who delivered at the labor room of Department of Gynecology and Obstetrics in Pt. B.D. Sharma University of Health Sciences, Rohtak. Subjects were divided into two groups. Group I consisted of umbilical cords obtained from normal pregnant women (n=30) with normal blood pressure (Systolic BP <140 mmHg and Diastolic BP <90mmHg), gestational age 37-40 weeks. Group II consisted of umbilical cord obtained from pre-eclamptic women. Women were diagnosed with pre-eclampsia if they had systolic BP ≥140mmHg, diastolic BP ≥90mmHg measured on two or more occasions at least 4 hrs apart after 20th week of gestation with proteinuria. Proteinuria was considered to be present when there was dipstick value of at least 1+ (≥300mg/dl) on two separate occasions at least 6 hr apart. None of the women were on Magnesium Sulphate. After delivery umbilical cords were cut five centimeter away from placental end and fixed in formaldehyde. Two blocks were made from each umbilical cord. After section cutting and mounting, slides were stained with hematoxylene and eosin stain. Systematic random samples of umbilical cord were taken for study. Stained slides were projected on a paper by a microprojector having thirty four times (34X) magnification. Various histomorphometric parameters of umbilical cord were measured by planimeter. The parameters measured for each umbilical cord included total cord and Wharton's jelly areas, total vessel and luminal areas and wall thickness. Wall thickness measurements express the whole thickness of vessel wall from endothelium to Wharton's jelly. All morphometric measurements were done in a blind fashion, without prior knowledge of clinical data.

Statistical analysis: Statistical analysis was performed using SPSS (SPSS 15 for windows, Chicago, IL, USA). The variables were tested for Kolmogoroy- Smimov test. The data obtained from the study was compiled and expressed as mean ± standard deviation. For paired variables having normal distribution, paired t test was used . A *p* value of <0.05 was taken as significant.

RESULTS

Demographic and clinical profile of control and pre-eclamptic groups are summarized in Table 1.

Table 1: Clinical characteristics of normal and pre-eclamptic pregnancies

S.N.	Parameters	Normal	Pre-eclamptic
1	Age	23.63±2.45	23.13±2.67
2	Parity Nulli/Multi	14/16	15/15
3	Systolic BP	117.60±9.80	155.86 ±15.51*
4	Diastolic BP	75.73± 6.90	105±11.28*
5	Period of gestation	38.8±0.886	38.46±1.56
6	Sex M/F	13/17	16/14
7	Weight of baby	2.76 ±0.35	2.50±0.59*
8	Mode of delivery FTVD/Caesarean	25/5	22/8
9	Apgar score	7±0.0	6.68±1.30

As expected from the inclusion and matching criteria, the patients' age was not significantly different, but blood pressure was significantly elevated in pre-eclamptic group. Birth weight of newborn babies was significantly lower in pre-eclamptic group.

Histological examination of the umbilical cord showed several distinct layers under the light microscope in the control group. On the surface, there was a well defined single layer of squamoid amniotic epithelium. Deep to this was a mucoid substance ie Wharton's jelly (Fig 1a).

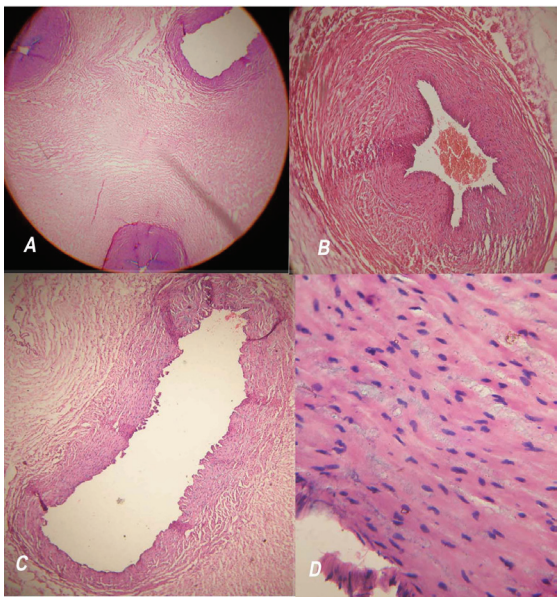

Fig 1. Photomicrographs of umbilical cord taken from normal pregnant woman (a) Normal umbilical cord with vessels (b) Umbilical artery(40x) (c) Umbilical vein(40x) (d) Higher magnification of umbilical vein wall (100x).

Embedded within Wharton's jelly are the umbilical vessels. Two arteries and a single umbilical vein were present in umbilical cord. The arteries possess no elastic lamina and have a double layered muscular wall having interlacing smooth muscle bundles. Vein has inner elastic lamina (Fig 1c).

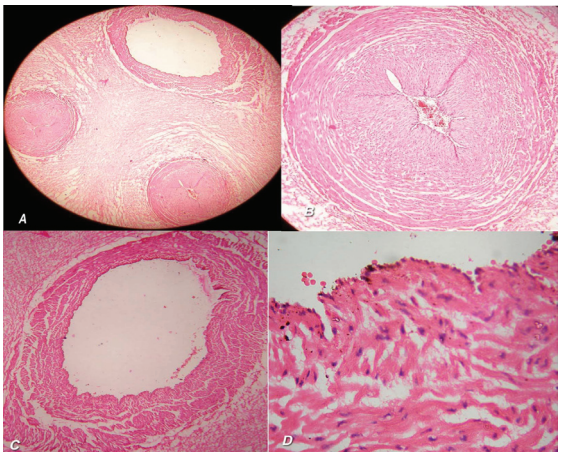

Fig 2. Photomicrographs of umbilical cord taken from pre-eclamptic woman (a) Normal umbilical cord with vessels (b) Umbilical artery (40x) (c) Umbilical vein (40x) (d) Higher magnification of umbilical vein wall (100x).

The umbilical vein has a larger diameter as compared to arteries (Table 2) and possesses a thin muscular coat consisting of a single layer of circular muscle coat. In transverse section mean cord area was 51.56 ± 2.34mm^2, jelly area was 42.27 ± 2.12mm^2, and total vessel area was 8.96 ± 0.82mm^2.

Umbilical cord vessels which were taken from pre-eclamptic group were hypoplastic (Fig 2), when examined under light microscope. In arteries, contraction of muscle cells occurred with wave like appearance of nucleus. Separation appeared between the muscle cells due to the increase in fluid between the cells, which was associated with edema. This edema in relation to connective tissue between the layers of muscle made it much easier to distinguish. Thickness of arteries was significantly increased. The vein lumen was seen to be significantly enlarged (7.10 ± 1.29mm^2 vs2.41 ± 0.66mm^2) whereas wall thickness of vein was significantly reduced (308.76 ± 25.47μm vs 385.73 ± 27.38μm). The endothelium, subendothelium and muscle layer of vein were observed to have completely joined each other. Hypoplasia could be clearly detected in this group. Jelly area was significantly decreased because of total vessel area which significantly increased. Hypoplasia was also seen in wharton's jelly.

DISCUSSION

The state of vascular system of mother and of the placenta is known to exert a great influence on intrauterine development of the fetus. Pre-eclampsia is the most common pathological syndrome associated with pregnancy which is known to affect the vascular system of mother as well as the fetus.

Four of the placenta in study group had anomalous umbilical cord insertion (marginal type) inserted. Age, gravidity, and parity between the two groups were matched. Birth weight was significantly decreased in pre-eclamptic babies, this represents intrauterine growth restriction**.** The smaller umbilical cord cross sectional area was found in pre-eclamptic group than normal group but this decrease in umbilical cord cross-sectional areas was not statistically significant ($p < 0.05$). These findings were consistent with the findings of Inan et al.[3] Lean umbilical cord on prenatal sonography possesses a risk that the fetus will be small for gestational age at delivery and will have distress during labor.[4]

The Wharton's jelly is a metabolically active tissue involved in fluid exchange between amniotic fluid and umbilical vessels. The lean umbilical cords are usually accompanied by torsion and fibrosis of Wharton's jelly and a thickening of vascular wall which obstructs the fetoplacental circulation lead to anoxia and fetal death.[5] A localized absence of Wharton's jelly in the involved area of the cord is believed to be the etiological factor in the constriction and subsequent torsion.[6] This reduction in Wharton's jelly area could be attributed to fetal starvation as a result of poor maternal nutrition. Umbilical cord anomalies, e.g. absence of Wharton's jelly, may result in antenatal fetal death.[7] Present histomorphometric study shows significant decrease ($p<0.01$) in Wharton's jelly area that is supported by biochemical studies. It is reported that accumulation of sulphated glycosaminoglycans (GAGs) in extracellular matrix of Wharton's jelly affect the biology of umbilical cord tissue. High concentrations of GAGs and proteoglycans surrounding the collagen fibers affect the solubility of this protein.[8] Such changes may make the collagen less soluble and jelly more compact and affect the umbilical cord's mechanical properties and macroscopic appearance.

Bertrand et al reported physiological properties in umbilical vessels.[9] In umbilical veins from pre-eclamptic mothers, the passive stretch-tension curve is shifted downward at passive tension higher than 6mN/mm. This suggests that high compliance at low stretch is not affected in umbilical veins from pre-eclamptic women and that these vessels are less compliant than the ones from women experiencing normal pregnancy at high stretch. In contrast to umbilical vein, passive stretch-tension curve of umbilical artery and chorionic vessels is shifted upward indicating increased compliance of these vessels. In a situation of increased placental resistance, an increase in intraluminal pressure in umbilical artery will tend to increase compliance to keep transmural pressure relatively constant. Conversely, the intraluminal pressure in umbilical vein will decrease, and compliance of vessel will diminish, again to keep transmural pressure constant.[9] As described by Dobrin[10], a vertical upward shift of stretch-tension curve implies an increase in strength or in the number of effective contractile units within vascular muscle cells.[9]

In present study, total umbilical vein area and luminal area were significantly increased ($p < 0.01$) whereas wall thickness of umbilical vein was significantly reduced

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($p<0.05$) as compared to normal group (Table 2).

These findings were suggestive of hypoplastic effect via various factors. Hypoplastic effect and vasoconstriction follow different mechanism or may follow each other [11] In contrast to our study, Inan et al demonstrated decrease in total vein area, wall thickness of vein and narrowing of lumen of vein in cases with pathological Doppler umbilical flow waveform.[3] Further study is needed to explain these findings. Umblical vein from pre-eclamptic women also showed break in endothelial layer and splitting of internal elastic lamina (fig 2c). Total umbilical artery area and luminal area were significantly not changed. However, wall thickness of umbilical arteries was significantly increased ($p<0.05$) (fig 2, Table 2). This thickness of arteries was 20% increased in pre-eclamptic group as compared to control group while Junek et al (2000) found 15% increased thickness of arterial wall.[12] This enlargement may be due to an increase in both tunica intima and tunica media thickness. The thickening of tunica intima has been attributed to migration of smooth muscle cells towards the endothelium, accompanied by splitting of internal elastic lamina.[12] This migration is supposed to be due to augmentation of sulphated glycosaminoglycons in umbilical cord arteries and reduced expression of elastin.[12] Thickness was also added by interstitial edema indicated by widening of intercellular spaces. These findings were consistent with the study of Bertrand et al.[9]

The current study and other experimental and clinical observation led us to hypothesize about the possible cascade of events occurring in pre-eclamptic fetuses before an altered umbilical artery hemodynamic state became manifested. A persistent reduction in umbilical blood flow velocity with increasing fetoplacental impedance might induce remodeling of umbilical cord and its vessels. Wharton's jelly area is decreased because of hypoplasia or decreased hydration. Further study is needed to evaluate these findings to see hypoplasia in jelly. As the pressure rises, the blood vessels are distended and vascular smooth muscle fibers surrounding the vessels contract. This is probably due in part, to intrinsic contractile response of smooth muscle to stretch. As further rise in pressure of vessel stimulate the increase in contractile unit that is smooth muscle fibers. Maintenance of a given wall tension as the pressure rises would regain a decrease in luminal radius and increase in wall thickness of umbilical arteries. Umbilical vein has lesser amount of smooth muscle fibers as compared to umbilical arteries. Umbilical arteries are more compliant than umbilical vein. Pressure rise in the lumen of umbilical arteries stretches the arterial wall and it tries to compensate for pressure by increase in the wall thickness or increase in the effective contractile units. But the umbilical vein compensates or increases the effective contractile units only up to certain limit of rise in pressure and with further rise in luminal pressure, it is unable to compensate and becomes dilated and thin walled.

In summary, pre-eclampsia associated changes in maternal hemodynamic conditions lead to fetal hypoxia, fetal hypertension and a discontinuous umbilical blood flow.[12] Increased vascular resistance of fetal aorta may act as chronic stimuli on the in umbilical vessel walls, leading to morphometric alteration as demonstrated in the present study.[13]We observed increased umbilical arterial thickness, decreased umbilical vein wall thickness and increased luminal area of vein which could be a predictor of intrauterine growth restriction in term pre-eclamptic patients. Clinically, these observations can be made through analysis of Doppler flow velocity waveform. The subclinical presence of vascular changes at term may also predict vascular diseases in early childhood like early onset of systemic arterial hypertension.[14]

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