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Original Article

Comparative histomophometric differences between umbilical cords from normal and pre-eclamptic pregnancies

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ABSTRACT

Introduction: To study and compare the histomorphometric parameters of umbilical cord and its vessels in normal and pre-eclamptic pregnancies.
Material and methods: One hundred umbilical cords, fifty each of normotensive and pre-eclamptic pregnancies were studied. Various histomorphometric parameters were measured with the help of vernier scale and ocular micrometer.
Results: In the present study, mean cord area was $43.45 \pm 9.26\ mm^2$, mean jelly area was $37.55 \pm 9.94\ mm^2$ and total vessels area was $5.90 \pm 1.91\ mm^2$ in control group. Whereas, in pre-eclamptic group, mean cord area was $34.85 \pm 11.78\ mm^2$, mean jelly area was $27.32 \pm 11.41\ mm^2$ and total vessels area was $7.51 \pm 3.25\ mm^2$. Mean wall thickness of the vein was $378.8 \pm 113.65\ \mu m$ and vein area was $2.38 \pm 1.01\ mm^2$ in control group. In pre-eclamptic group, mean wall thickness of vein was $307.2 \pm 107.15\ \mu m$ and vein area was $3.97 \pm 4.32\ mm^2$. Total cord area, jelly area and wall thickness of vein were significantly lower in pre-eclamptic group. Total vessels area and vein area were significantly higher in pre-eclamptic group.
Discussion: Umbilical cords in pre-eclampsia shows significant structural changes, including decrease in cord area, jelly area and umbilical arterial area, whereas increase in total vessels area and vein area. These differences are due to adaptation of the umbilical cord under the altered hemodynamic conditions in pre-eclampsia. Morphological modifications of the umbilical vessels directly influence the fetal blood stream, which impact upon fetal development. So, prenatal monitoring of the feto-placental circulation may reduce the postnatal complications in pre-eclamptic pregnancies.

1. Introduction

Umbilical cord is the only organ of the fetus that dies when life begins. It is the life-line connection between the fetus and mother for the supply of oxygen, nutrients and transfer of waste materials; and is necessary for the growth and development of the fetus.[1] Umbilical cord is mostly assessed for the impedance of the umbilical arteries to blood flow in the fetus with developmental abnormalities.[2] In view of this, abnormalities associated with umbilical cord would have adverse effects on the perinatal outcome.[3]

Pregnancy associated complications like hypertension or gestational diabetes are reflected in the umbilical cord and its vessels in a significant way both macroscopically and microscopically.[4,5] Pre-eclampsia is a common pregnancy associated pathological syndrome which is characterized by generalized edema, hypertension and proteinuria presenting after 20th week of gestation. It complicates about 6–20% of all pregnancies and is the major cause of maternal and neonatal death in developing countries. It also represents as the most important cause of intrauterine growth retardation, premature delivery, low birth weight, perinatal mortality. Prediction of preeclampsia is very difficult in early pregnancy. Some epidemiological factors associated with pre-eclampsia include nulliparity, previous pre-eclampsia, family history, obesity, diabetes mellitus, multi-fetal pregnancies, age of mother (<18 years and >35 years) and previous renal diseases.[6] Preeclampsia is associated with increased vascular resistance and decreased uteroplacental perfusion that results in fetal hypoxia and intrauterine growth retardation.[7,8]

Morphologically, cross section of umbilical cord comprises two umbilical arteries and one vein continuous with the vascular

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architecture of the placenta. Histologically, the umbilical cord in normal pregnancies shows well defined single layer of squamoid amniotic epithelium on the surface. Deep to epithelium, umbilical cord contains mucoid connective tissue known as Wharton's jelly within which umbilical vessels are embedded. The vessels are lined by a continuous single layer of flattened and elongated endothelial cells. Umbilical arteries do not possess an internal elastic lamina. The media of artery is thick, showing an inner layer of longitudinal smooth muscle cells (SMCs) and an outer layer of crossing spiraled SMCs. The vein has an internal elastic lamina and possesses a thinner muscular coat with single layer of circular SMCs. The umbilical arteries showed varying degrees of contraction, and often possess triangular, stellate or sickle shaped lumen. However, the vein was usually not so strongly contracted, and its lumen was comparatively patent. Umbilical vessels are not supplied by vasa vasorum and depend on their own oxygen supply; making them more vulnerable to hemodynamic changes.[9] Histological appearance of the umbilical cord vessels in pre-eclamptic group is nearly normal with varying degree of hypoplastic vessel wall. Edema due to the increase in fluid between the cells causes separation between the muscle cells. The morphological modifications of the umbilical cord in preeclampsia indicates some important postnatal and fetal hemodynamic deficiencies.[4,10]

The aim of the present study was to compare the morphological changes in umbilical cord and its vessels in normotensive and pre-eclamptic pregnancies on histological basis.

2. Material and methods

The study was carried out in the Department of Anatomy, Gandhi Medical College, Bhopal (M.P). One hundred umbilical cords were collected from pregnant women who delivered in Sultania Zanana Hospital associated to Department of Obstetrics and Gynaecology, G.M.C. Bhopal, after informed consent and due permission from institutional ethics committee. Women were diagnosed with preeclampsia if they had systolic BP $\geq$140 mmHg and diastolic BP $\geq$90 mmHg measured on two or more occasions at least 4 h apart after 20th week of gestation with proteinuria. Proteinuria was considered present when there was a urine dipstick value of at least 1+ (>30 mg/dl) on two separate occasions at least 6 h apart. On this basis, subjects were divided into two groups. Group I consisted of umbilical cords obtained from normal pregnant women (n = 50) with gestational age 37–40 weeks. Group II consisted of umbilical cords obtained from pre-eclamptic women (n = 50). Patients with essential hypertension, diabetes mellitus, anemia, renal disorders and other illness associated with pregnancy were excluded from study.

In all cases, segments of umbilical cord obtained at 2 cm distance from the placental side, of thickness of approximately 4–5 mm were routinely processed for paraffin embedding, sectioning and H & E staining. Two diameters from each histological section of umbilical cord were measured at right angle in x-axis and y-axis by using vernier scale on the stage of microscope. The diameters of blood vessels were measured by ocular micrometer in x-axis and y-axis [Figs. 1B, 2A]. Vessels wall thickness was measured by ocular micrometer which expresses the whole thickness of vessels wall, from the endothelium to the Wharton jelly.

Total cord area, Jelly area and total vessel area were calculated for each cord by the formulae given below:

(1) $A = \pi D_1 D_2/4$, where A is area, D_1 is diameter in x-axis and D_2 is diameter in y-axis.
(2) Total vessel area = (Total area of artery 1 + Total area of artery 2 + Total area of vein) in cross section.
(3) Jelly area = (Total umbilical cord area – Total vessels area) in cross section.

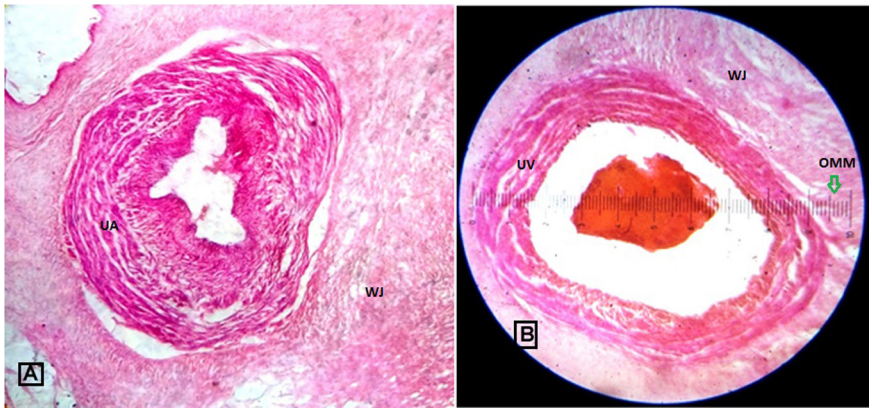

Fig. 1. Hematoxylin & eosin (H&E) staining of umbilical cord taken from normal pregnancy. (A) Umbilical artery (UA) embedded within Wharton's jelly (WJ, 100×). (B) Umbilical vein (UV) embedded within Wharton's jelly (WJ) with ocular micrometer (OMM, arrow, 100×).

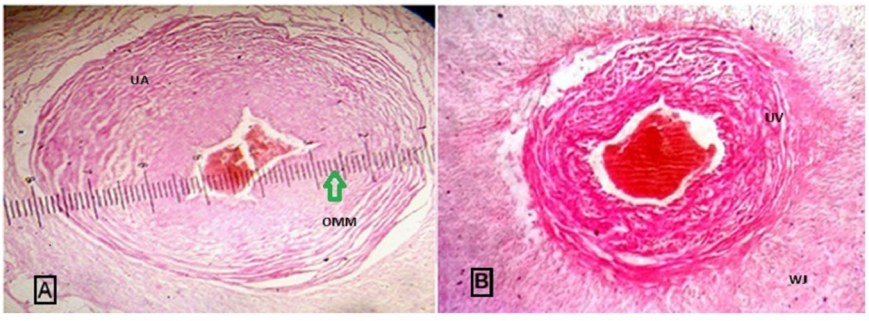

Fig. 2. Hematoxylin & eosin (H&E) staining of umbilical cord taken from pre-eclamptic pregnancy. (A) Umbilical artery (UA) with ocular micrometer (OMM, arrow, 100×). (B) Umbilical vein (UV) embedded within Wharton's jelly (WJ, 100×).

2.1. Standardizations of the methods

All measurements were taken by using 4× magnification of objective lens. The measurements which were taken from the vernier scale on the stage of microscope, shows the real size of sample, because it is not affected by the magnification of microscope.

The measurements which were taken by using ocular micrometer are the magnified value of objective lens. The reticle is mounted in the eyepiece, which is magnified by objective lens. Such as a real particle with a diameter of 1 mm at the microscope stage, when viewed with 4× objective lens will appear as 4 mm diameter on the reticle. Hence such magnifications have to be taken into account while calculating real measurements.

Sample 'Real Size' = Size of sample displayed on reticle, divided by 'Magnification of Objective lens'.

2.2. Statistical analysis

Statistical analysis of data was performed by using Statistical Package for Social Sciences (SPSS) version 15.0 (Chicago, IL). All results were expressed as mean values ± standard deviation. The significance of differences between group parameters was analyzed by Student's *t*-test and differences were considered significant if $p < 0.05$.

3. Results

Demographic and clinical parameters under study showed blood pressure was significantly higher, whereas gestational age and birth weight were significantly lower in pre-eclamptic group. In turn, appearance, pulse, grimace, activity and respiration rate (APGAR) of newborn in pre-eclamptic group were found to be significantly low [Table 1].

Morphometric analyses showed difference in cord parameters in two groups [Table 2, Figs. 1 and 2]. In control group, mean cord area was 43.45 ± 9.26 mm^2, mean jelly area was 37.55 ± 9.94 mm^2 and total vessels area was 5.90 ± 1.91 mm^2. Whereas, in pre-eclamptic group, mean cord area was 34.85 ± 11.78 mm^2, mean jelly area was 27.32 ± 11.41 mm^2 and total vessels area was 7.51 ± 3.25 mm^2. The mean wall thickness of the vein was 378.8 ± 113.65 μm and vein area was 2.38 ± 1.01 mm^2 in control group. Whereas, in pre-eclamptic group, mean wall thickness of vein was 307.2 ± 107.15 μm and vein area was 3.97 ± 4.32 mm^2. Total cord area, jelly area and wall thickness of vein were significantly lower in pre-eclamptic group. Total vessels area and vein area were significantly higher in pre-eclamptic group. There were no significant differences in total arterial area and mean wall thickness of artery [Table 2].

4. Discussion

Teenage pregnancy and nullipara are risk factors for pre-eclampsia. Women with preeclampsia has a shorter gestational period and delivered baby with lower birth weight.[11] In present study, gestational age and birth weight were significantly lower in pre-eclamptic group [Table 1], which is similar to the observation of earlier researchers.[4,10]

Histologically, two umbilical arteries and one vein embedded in Wharton's jelly with varying degree of lumen patency and muscular wall thickness in umbilical arteries whereas in vein lumen was patent. In pre-eclamptic cases separation of the muscle cells due to edema was observed which was similar to earlier observations[10] [Fig. 3A, B].

Wharton's jelly is a collagen-rich soft connective tissue that protects the umbilical blood vessels. It prevents disruption of flow in case of compression or bending caused by fetal movements or uterine contractions during labor.[12] The elastic and protective properties of Wharton's jelly are mainly due to the high proportion of the amount of collagen type III and of hyaluronic acid, the most abundant component of sulfated glycosaminoglycans contained in the umbilical cord.[13] It has been reported that the diameters and areas of umbilical cords changed during gestation, and these differences depended on the reduction of Wharton's jelly rather than the umbilical vessels themselves.[14]

Togni et al., found a strong correlation between the cross sectional areas of umbilical cord components and fetal anthropometric parameters.[15] Stricture of umbilical cords, characterized by a reduced amount of Wharton's jelly and a thickening of the vascular wall, has been described in unexplained intrauterine fetal

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

S. No.	Parameters	Normal ($n = 50$)	Pre-eclamptic ($n = 50$)	*p* value[a]
1.	Gestational age (weeks)*	38.04 ± 1.33	36.1 ± 0.93	0.0001[b]
2.	Systolic blood pressure (mmHg)*	118.28 ± 5.55	149.2 ± 10.72	0.0001[b]
3.	Diastolic blood pressure (mmHg)*	78.36 ± 5.26	105.16 ± 7.54	0.0001[b]
4.	Fetal weight (g)*	2633.6 ± 302.2	2435.2 ± 280.56	0.001[b]
5.	APGAR score*	8.1 ± 1.07	6.54 ± 0.93	0.001[b]

[a] *p*-Values were calculated by using Student's *t*-test. $p \leq 0.05$ was considered significant.
[b] Statistically significant.
* Parameters were expressed as mean value ± standard deviation.

Table 2
Comparison of histomorphometric parameters of umbilical cord in normal and pre-eclamptic pregnancies.

S. No.	Parameters*	Normal ($n = 50$)	Pre-eclamptic ($n = 50$)	*p* value[a]
1.	Total cord area (mm^2)	43.45 ± 9.26	34.85 ± 11.78	0.0001[b]
2.	Jelly area (mm^2)	37.55 ± 9.94	27.32 ± 11.41	0.0001[b]
3.	Total vessels area (mm^2)	5.90 ± 1.91	7.51 ± 3.25	0.046[b]
4.	Vein area (mm^2)	2.38 ± 1.01	3.97 ± 4.32	0.014[b]
5.	Wall thickness of vein (μm)	378.8 ± 113.65	307.2 ± 107.15	0.001[b]
6.	Total arterial area (mm^2)	3.52 ± 1.02	3.34 ± 1.00	0.38[c]
7.	Wall thickness of artery (μm)	486.0 ± 151.59	455.05 ± 107.16	0.086[c]

[a] *p*-Values were calculated by using Student's *t*-test. $p \leq 0.05$ was considered significant.
[b] Statistically significant.
[c] Statistically not significant.
* Parameters were expressed as mean value ± standard deviation.

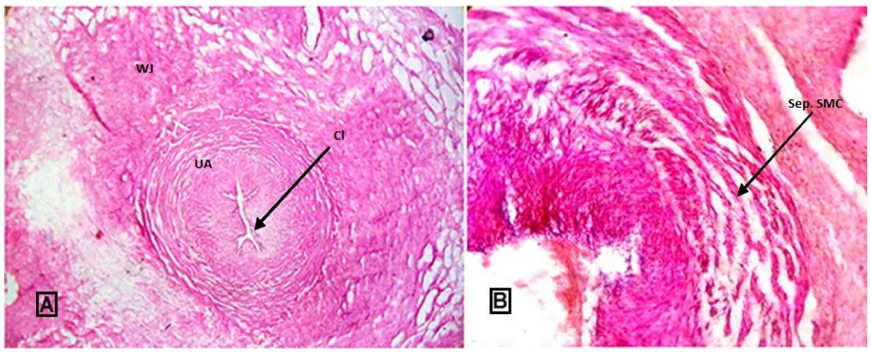

Fig. 3. Hematoxylin & eosin (H&E) staining of umbilical cord taken from pre-eclamptic pregnancy. (A) Umbilical artery (UA) with constriction of lumen (Cl, arrow, 100×). (B) Wall of the umbilical artery showing separation of smooth muscle cells (Sep. SMC, arrow, 400×).

deaths.[16] The lean umbilical cords are accompanied by torsion and fibrosis of Wharton's jelly and thickening of vascular wall which obstructs the feto-placental circulation, leading to anoxia and fetal death.[17] In present study, total cord area and jelly area were significantly decreased in pre-eclamptic group [Table 2]. These findings are consistent with those reported by earlier observers.[4,10]

Pre-eclampsia is characterized by an increased vascular resistance and modifications in the mechanical properties of blood vessels.[18] The vessels walls could react with these alterations to maintain their transmural pressure at an optimal level. In such situation an increase in intra-lumen pressure in the umbilical artery will tend to increase compliance to keep transmural pressure relatively constant. Conversely, intra-lumen pressure and compliance of the vein will decrease.[19] In present study, wall thickness of umbilical vein was significantly reduced [Table 2]. This finding is in-line with the study of Inan et al.,[10] whereas it differs with observation of Junek et al.[20]

Junek et al., demonstrated that umbilical arteries were thicker in the preeclamptic group than in uncomplicated pregnancies. These differences were observed in the tunica media and intima which were accepted as a result of the adaptation of the umbilical cord arteries under the altered hemodynamic conditions in preeclampsia.[20] In constrast to above, we observed that the thickness of arterial wall remained nearly constant, which is similar to findings reported by Inan et al.[10]

Inan et al., found that total arterial area was reduced in pre-eclampsia with normal and pathological Doppler flow velocity waveform.[10] We also observed that total arterial area was reduced in pre-eclamptic group as compared to control group, but this finding was statistically not significant [Table 2]. This finding suggests mainly a vasoconstrictive effect. The constricted lumen and contracted smaller smooth muscle cells were suggestive of a predominant hypoplastic mechanism. Both hypoplastic and vasoconstrictive effect may be different events or may follow each other. The first response to hypoxemia is vasoconstriction of the vessels. If the hypoxemia continues, it shall produce hypoplastic modifications; with immediate and late hemodynamic consequences.[21] Morphological modifications of the umbilical vessels directly influence the fetal blood stream, which has an impact upon fetal vascular system. The modification of the fetal vascular system may represent a main factor, for vascular affections of the future adult.[22] Assessment of maternal disease during pregnancy as well as intrinsic fetal sickness is facilitated by umbilical cord evaluation by sonography because of its close relation to fetal development.[11,23]

5. Conclusion

Umbilical cord and its vessels in pre-eclampsia show significant structural changes, including decreases in cord area, Wharton's jelly area, and vein wall thickness, whereas, total vessels area and vein area increases. These differences are due to adaptation of the umbilical cord under altered hemodynamic conditions in pre-eclampsia. A systematically prenatal monitoring of hemodynamics of the feto-placental circulation may reduce the incidence of intrauterine growth retardation and developmental defects in the new-born babies with mothers suffering from pre-eclampsia.

This study acts as a prelude to a cohort study, to assess long term pathological changes in infants born to pre-eclamptic mothers. These vascular abnormalities may be expressions of "early vascular aging" because the structural and mechanical properties of the large vessels can be permanently affected by altered hemodynamic stress early in life. The hemodynamic status of the fetus of pre-eclamptic mother is characterized by hypoxia or/and ischemia with an immediate and late impact upon fetal cerebral development. A good quantification of the morphological modifications of the umbilical cord in pre-eclampsia provides an informational support to the practitioner concerning the baby's neurological and cardiovascular future.

Conflicts of interest

The authors have none to declare.

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