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Original Article

Comparative Morphological Differences between Umbilical Cords from Chronic Hypertensive and Preeclamptic Pregnancies

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To compare morphological changes in the umbilical cords from chronic hypertensive and preeclamptic patients having normal or pathological umbilical artery Doppler ultrasonographic results. Umbilical cords from 34 normotensive, 31 chronic hypertensive and 70 preeclamptic women with normal and abnormal Doppler flow velocity waveforms (FVW) at 35-40 gestational weeks were studied. Morphological changes in the umbilical cords were examined on formalin-fixed, paraffin-embedded sections. The total umbilical cord area, total vessel area, and wall thickness of umbilical vessels were measured in systematic random samples using unbiased stereology methods. An ANOVA test was used for statistical analysis. In the chronic hypertensive and preeclamptic groups with normal Doppler FVW, the thickness of the umbilical cord vessels remained nearly constant, whereas both the total area and the lumen area were reduced. These changes correlate with the histopathological findings, suggesting a mainly vasoconstrictive effect. By contrast, analysis of the preeclamptic group with pathologic Doppler FVW showed a comparable reduction of all parameters of the umbilical cord. Histopathological findings were related to smaller, contracted smooth muscle cells of the vessel wall, which is suggestive of a predominant hypoplastic mechanism. As a result of reduced uteroplacental perfusion, fetal hypoxia and intrauterine growth retardation become unavoidable in preeclampsia. The histopathological changes in the umbilical cord between the chronic hypertensive and preeclamptic patients depend on the Doppler results. In conclusion, the umbilical artery Doppler FVW indices provide good values for predicting intrauterine growth retardation in preeclamptic patients.

Key words: umbilical cord, morphometry, hypertensive induced pregnancy

Hypertensive disorders in pregnancy are responsible for a significant amount of maternal and perinatal morbidity and mortality. The etiology of these disorders is still unknown. It complicates about 6-20% of all pregnancies. Preeclampsia and eclampsia constitute about 70% of these disorders, whereas chronic hypertension represents the remaining 30% of hypertensive disorders in pregnancy [1, 2].

Although preeclampsia is one of the major causes of maternal death, especially in developing countries, perinatal outcomes are also not favorable. Intrauterine growth retardation, premature delivery, low birth weight,

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fetal death and neonatal death due to premature birth are common complications. Predicting preeclampsia is difficult in early pregnancy, but some epidemiological risk factors are known, such as nulliparity, previous preeclampsia, family history, black race, obesity, diabetes mellitus, multi-fetal pregnancies, age of mother (<18 and >35), and previous renal disease [3].

Although the etiology of preeclampsia is still unknown, the placenta plays a crucial role in the development of the disease [4, 5]. Preeclampsia is associated with increased vascular resistance and decreased uteroplacental perfusion. According to the degree of decrease in uteroplacental perfusion, fetal hypoxia and intrauterine growth retardation can become unavoidable [6]. Many studies have demonstrated significant differences in the morphological structures of the placenta and umbilical cord vessels between normal and preeclamptic pregnant women [7–12]. Most of the women with preeclampsia show histological and biochemical evidence of poor placentation and ischemia. Bruch *et al.* reported that growth-retarded fetuses with or without umbilical artery Doppler abnormalities have a smaller umbilical cord cross-sectional area at delivery than do normal healthy fetuses [10]. Di Naro found that the diameters and areas of umbilical cords changed during gestation, and these differences depended on the reduction of Wharton jelly rather than the umbilical vessels themselves [11]. Junek *et al.* demonstrated that umbilical arteries were thicker in the preeclamptic group than in uncomplicated pregnancies. These differences were especially observed in the tunica intima and media. These differences were accepted as a result of the adaptation system of the umbilical cord arteries under the altered homodynamic conditions in preeclampsia [12].

It was observed that umbilical arterial vasoconstriction was induced by an excess of either endothelium or platelet-derived thromboxane A2, as described in IUGR [13]. A higher production of endothelin in IUGR was also described [14]. But the degree of defective placentation and placental ischemia may not adjust to the severity of preeclampsia. Therefore some other preexisting factors must also be present. These alterations in the placenta and umbilical cord vessels may develop as a result of a decrease in a vasodilator substance or an increase in vasoconstrictors due to a pathophysiologic event [15–18]. An abnormal endothelial hyperstimulation and dysfunction might be the main event, and preeclampsia can be an acute form of such a situation. Both preeclampsia and chronic hypertension include similar risk factors and biochemical alterations. Although the perinatal risk for women with chronic hypertension is less than for preeclamptic women, the histopathological differences in both groups may be important for the developing fetus. The aim of this study was to compare the morphological changes in the umbilical cord vessels in normotensive pregnancies, chronic hypertensive pregnancies and preeclamptic patients with and without pathologic umbilical artery Doppler ultrasound study results.

Materials and Methods

Subjects. The Ethics Committee of the Research Center of Aegean Social Security Hospital approved the protocol. Studies were performed on the umbilical cords of 135 newborns, delivered between 35–40 weeks of gestation. In all cases, 10 cm long sections of the umbilical cord were cut, beginning from their placental end, for morphometric study of the umbilical cord vessels. Patients were separated into 4 groups. There were no statistically significant differences in the mean ($\pm$ SD) age of the women among the groups. Patient characteristics are given in Table 1. For all patients, arterial umbilical flow velocity waveforms (FVW) from 24 h to 1 week before delivery were recorded with Toshiba 250 pulse-waved Doppler USG, using a 5 MHz abdominal transducer. Normal umbilical Doppler indices were defined as a systolic / diastolic value between 5% and 95% with respect to gestational age. Values out of this range were taken as abnormal.

Group 1: The control material was taken from newborns delivered by healthy mothers, aged 23–32, with normal blood pressure (systolic 100–135 mmHg, diastolic 60–85) and having normal umbilical Doppler FVW ($n=34$). Exclusion criteria included multiple pregnancies, essential hypertension, diabetes, chronic renal disease, platelet disorders, and epilepsy. The mean body weight of the newborns was $3{,}261.7 \pm 418.3$ grams.

Group 2: In this group, the umbilical cords were taken from newborns delivered by mothers, aged 24–33, with essential hypertension before the 20 th week of pregnancy and having normal umbilical Doppler FVW ($n=31$). The mean body weight of these newborns was $3{,}153.2 \pm 314.8$ grams.

Group 3: In this group, the umbilical cords were taken from newborns delivered by mothers, aged 22–32, with preeclampsia. Women were diagnosed with preeclampsia if they had blood arterial pressure as follows:

Table I Clinical characteristics of normal, chronic hypertensive and preeclamptic pregnancies

GROUP	GROUP 1	GROUP 2	GROUP 3A	GROUP 3B
	Control	Chronic HT with normal Doppler FVW	Preeclampsia with normal Doppler FVW	Preeclampsia with pathological Doppler FVW
	n = 34	N = 31	n = 32	n = 38
Age	27.7 ± 4.6	28.3 ± 4.4	27.6 ± 5.3	25.5 ± 3.8
Parity (Nullipar/multipar)	21/13	10/21	23/9	26/12
Gestational age (wk)	39.0 ± 0.5#	38.3 ± 1.0	37.3 ± 0.9§	36.4 ± 1.1*
Sistolic Blood Pressure (mmHg)	107.0 ± 10.8#	148.3 ± 11.8	149.8 ± 11.8	156.5 ± 12.7*
Diastolic Blood Pressure (mmHg)	69.4 ± 9.9#	95.9 ± 6.1	101.0 ± 11.4§	105.1 ± 10.7*
Edema	1.0 ± 0.6#	0.9 ± 0.9	2.2 ± 0.4§	2.2 ± 0.4*
Spontant delivery/ Cesarian section	25/9#	16/15	6/26§	7/31*
APGAR	8.3 ± 0.6#	8.0 ± 0.7	7.4 ± 0.8	6.8 ± 1.2*
Fetal weight (gm)	3261.7 ± 418.3	3153.2 ± 314.8	3015.6 ± 426.6	2109.2 ± 589.9*

#, $P < 0.05$ Group 1 *vs.* Group 2, 3A, 3B; §, $P < 0.05$ Group 3A *vs.* Group 2; *, $P < 0.05$ Group 3B *vs.* Group 3A, 2.

systolic > 140 mmHg, diastolic > 90 mmHg measured on 2 or more occasions at least 4 h apart after the 20 th week of gestation. Proteinuria was considered present when there was a urine dipstick value of at least 1 + (> 30 mg/dl) on 2 separate occasions at least 6 h apart. None of the women had an MgSO4 or betamethasone injection before the blood samples were drawn. This group was subdivided into 2 groups according to Doppler FVW results.

Group 3A: With normal umbilical artery Doppler FVW (n = 32). The mean body weight of these newborns was 3,015.6 ± 426.6 grams.

Group 3B (4): With abnormal umbilical artery Doppler FVW (n = 38). The mean body weight of these newborns was 2,109.2 ± 589.9 grams.

Methods. Each umbilical cord was immediately clamped at delivery. In all cases, 10 cm-long sections of umbilical cord were cut, beginning from their placental end, for morphometric study of the umbilical cord vessels. Five blocks of cord cross-sections were cut. A routine paraffin procedure was done. In brief, tissue samples were fixed in 10% formalin solution. They were dehydrated in a graded ethanol series, cleaned in xylene and embedded in paraffin. Sections were cut at 5 μm thickness, deparaffinised and hydrated. Serial sections of the umbilical cords were stained with hematoxylin and eosin (H.E). Systematic random samples of umbilical cord sections were identified under a microscope (× 40), and unbiased morphometric study was performed using an Olympus microscope. The view from the microscope was directly projected onto the computer screen. A systematic grid of crosses was randomly thrown onto the viewed object. The inter-cross spacing in the x and y direction is Δx and Δy units, respectively. This means that each cross has an associated area of a/p units^2. The number of crosses that hit the object multiplied by a/p is an unbiased estimate of the object's area [18]. The following parameters were measured for each umbilical cord: total cord and Wharton jelly areas, total vessel and lumen areas, and wall thickness. Wall-thickness measurements express the whole thickness of the vessel wall, from the endothelium to the Wharton jelly. All morphometric measurements were done in a blind fashion, without preexisting knowledge of the clinical data.

Statistical analysis. All results are expressed as mean values ± SEs. Statistical analysis of data and SEs was calculated for each parameter and estimated in each group. For computation we used the SPSS Advanced Statistical package. The data was analyzed by an ANOVA test, and differences were considered significant if $P < 0.05$.

Results

Demographic and clinical findings of the control group (Group 1), chronic hypertensive + pregnancy (Group 2), preeclamptic with normal Doppler (Group 3A) and preeclamptic with abnormal Doppler (Group 3B) are summarized in Table 1. As expected from the inclusion and matching criteria, the patients' ages were not significantly different, but other parameters such as blood pressure and edema were significantly elevated ($P < 0.05$) in the preeclamptic group. The method of delivery was usually cesarean section in Groups 3A and 3B. We also saw that the gestational age and birth weight were significantly lower in the preeclamptic group, especially in the pathologic Doppler flow patterns. Related with these findings, APGAR scores in Groups 2, 3A and 3B were found to be low. The results of the morphometric parameters of umbilical cords are given in Table 2.

Histological examination of the umbilical cord shows several distinct layers under the light microscope in the control group (Fig. 1A). On the surface is a well-defined single layer of squamoid amniotic epithelium. Deep in the epithelium that comprises the surface of the cord is the substance known as Wharton's jelly. Embedded within the Wharton's jelly are the umbilical vessels. The vasculature of the umbilical cord is composed of 2 arteries and a single vein. The arteries possess no elastic lamina and have a double-layered muscular wall (Fig. 1B). Each of these muscular layers is composed of a network of interlacing smooth muscle bundles. The vein has an inner elastic lamina (Fig. 1C). The umbilical vein, which generally has a larger diameter, possesses a thinner muscular coat consisting of a single layer of circular smooth muscle. In the control group, the mean cord area was 63.58 ± 2.00, the total area of the vein was 6.28 ± 0.44 and the wall thickness of the vein was 471.75 ± 33.27. The thicknesses of the arteries were similar to each other. The mean wall thickness of the arteries was 597.08 ± 18.02 and their total area was 2.97 ± 0.18.

When the umbilical cord vessels in Group 2 (chronic hypertensive + pregnancy) were examined under the light microscope, the histological appearance appeared to be close to normal (Fig. 2A). The endothelium and the subendothelium of the umbilical artery (Fig. 2B) and vein (Fig. 2C) were seen to be in their normal state. Although a few contractions in the nucleus could be seen, the smooth muscle cells generally had the appearance of being normal and of normal size (Fig. 2D). The intercellular gaps had an unnoticeable widening between them. The morphometric analyses of this group showed that the total

Table 2 Correlation's cord parameters

	GROUP 1	GROUP 2	GROUP 3A	GROUP 3B
UMBILICAL CORD PARAMETERS	Control	Chronic HT with normal Doppler FVW	Preeclampsia with normal Doppler FVW	Preeclampsia with pathological Doppler FVW
	n = 34	N = 31	n = 32	n = 38
Umbilical cord				
Total cord area	$63.58 \pm 2.00^{\#}$	54.09 ± 2.65	48.99 ± 3.18	41.84 ± 1.58*
Jelly area	$51.37 \pm 1.79^{\#}$	43.95 ± 2.43	$40.39 \pm 3.09^{\S}$	35.22 ± 1.77*
Total vessel area	$8.44 \pm 0.69^{\#}$	7.01 ± 0.29	$6.07 \pm 0.22^{\S}$	5.01 ± 0.19*
Total lumen area	$3.77 \pm 0.13^{\#}$	3.11 ± 0.28	2.58 ± 0.10	1.61 ± 0.11*
Vein				
Total area mm^2	$6.28 \pm 0.44^{\#}$	4.95 ± 0.09	$3.61 \pm 0.11^{\S}$	2.51 ± 0.22*
Lumen area mm^2	$2.97 \pm 0.29^{\#}$	2.47 ± 0.05	$1.96 \pm 0.21^{\S}$	1.10 ± 0.22*
Wall thickness μm	471.75 ± 33.27	459.58 ± 11.58	437.75 ± 10.79	398.58 ± 11.54*
Artery				
Total area mm^2	$2.97 \pm 0.18^{\#}$	2.59 ± 0.14	$2.49 \pm 0.12^{\S}$	2.06 ± 0.06*
Lumen area mm^2	0.40 ± 0.05	0.32 ± 0.04	0.31 ± 0.05	0.25 ± 0.06*
Wall thickness μm	597.08 ± 18.02	544.90 ± 17.40	547.58 ± 12.01	426.66 ± 19.22*

$^{\#}$, $P < 0.05$ Group 1 *vs.* Group 2, 3A, 3B; §, $P < 0.05$ Group 3A *vs.* Group 2; *, $P < 0.05$ Group 3B *vs.* Group 2, 3A, 1.

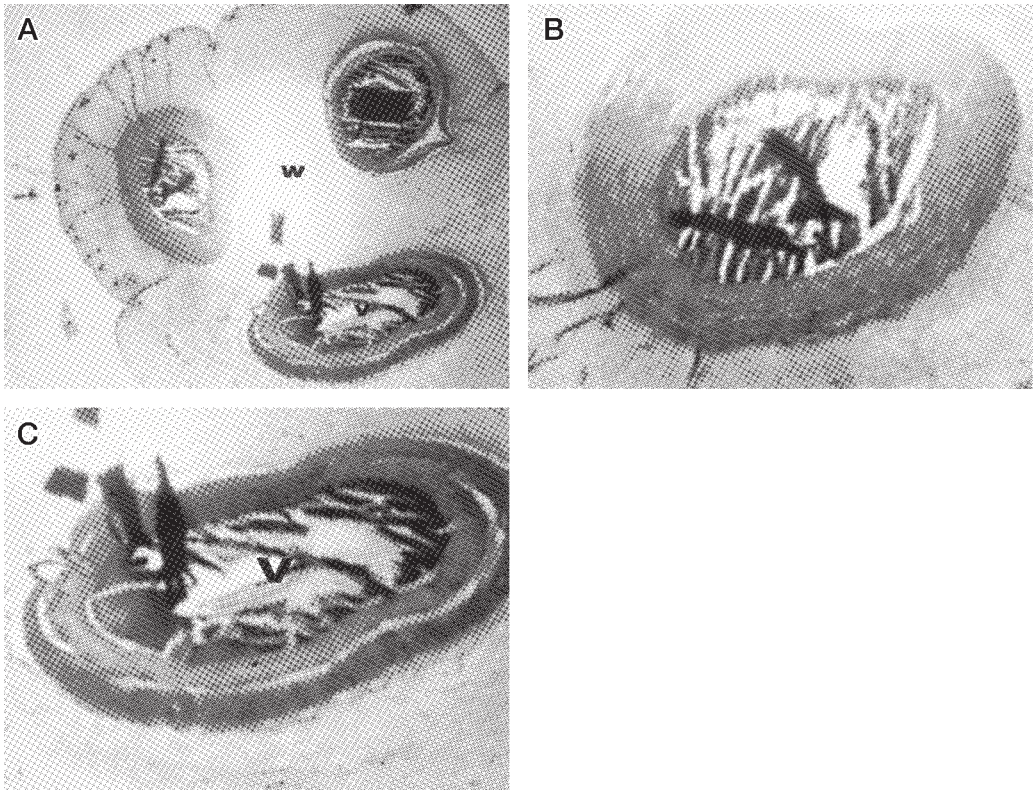

Fig. 1 Photomicrographs of umbilical cord taken from a newborn with a normotensive, healthy mother (Control group). Normal appearance of umbilical cord and vessels were seen. On the surface is a well-defined single layer of amniotic epithelium (a), embedded within the substance of Wharton's jelly (w) are the umbilical vessels. General view of umbilical cord at a magnification of × 10 (**A**); umbilical cord artery, × 40 (**B**); umbilical vein (V), × 40 (**C**). H.E.

cord and jelly area, and the total vessel area were significantly reduced (Fig. 2A). The wall thickness of the umbilical vessels was decreased in Group 2, but the difference with the control group was not statistically significant.

It was observed that in preeclamptic patients having normal Doppler FVW (Group 3A), a widening under the epithelium of the artery and between the muscle layers was present due to the edema (Fig. 3B). The contraction of the muscle cells occurred with a wave-like appearance of the nucleus. Separations appeared between the muscle cells due to the increase in fluid between the cells, which was associated with the edema. This edema in relation to the connective tissue between the layers of muscle made it much easier to distinguish between the layers. The vein lumen was seen to have narrowed due to the edema on the vein wall and to the vasoconstriction (Fig. 3C, 3D). Macroscopically, the cord thickness was significantly reduced in this group in comparison with the control and hypertensive groups. When Group 3A was compared with Group 2, a significant reduction of the total vessel area of the cord was observed. In contrast, no difference was observed in the wall thickness of the vessels.

The umbilical cord vessels, which were taken from preeclamptic patients who had an abnormal Doppler (Group 3B), seemed to be morphologically hypoplastic (Fig. 4). When examined under a light microscope, the diameters of the vessels were significantly reduced (Fig. 4A). The muscle area separated from the connective tissues, which led in turn to the substantial narrowing of the diameter of the lumen, which was especially noticeable in the arteries (Fig. 4B). This narrowing of the lumen resulted in the narrowing of the vein diameter (Fig. 4C). The muscle cells were seen to be hypoplastic and smaller

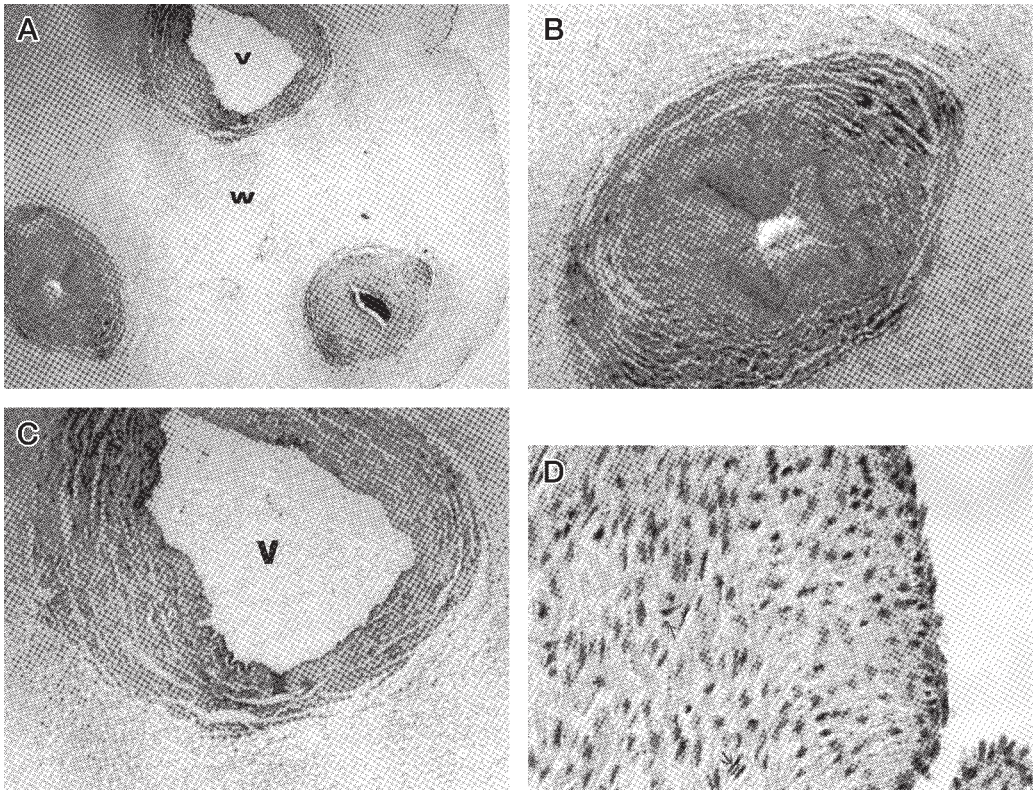

Fig. 2 Photomicrographs of umbilical cord taken from chronic hypertensive mother (Group 2). The endotel and subendotel seemed to look close to normal, the smooth muscle cells on the vessel walls were also seen to be on their normal state. Although it could be seen that a few vasocontrictions had a patched state in the nucleus (arrow). General view of umbilical cord at a magnification of × 10 (**A**); umbilical cord artery, × 40 (**B**); umbilical vein (V), × 40 (**C**); higher magnification of the vein wall, × 200 (**D**). H.E.

than their normal size (Fig. 4D). The contracted smooth muscle cells were seen to have separated their links from each other in some parts. The endothelium and subendothelium of the vessels and the inner layers of muscle were observed to have completely joined each other. With this observation, hypoplasy could be clearly detected. In this group, it was observed that all parameters of the umbilical cord were significantly reduced in comparison to the normal and hypertensive groups. A significant correlation was also observed between the thickness of a vessel wall and the pathologic Doppler values.

Discussion

The umbilical cord appears to play an important role in interactions between the mother and fetus during pregnancy. Pregnancies with growth retardation are associated with smaller placentas and thin umbilical cords [7–10]. In this study, the histopathological and morphometric differences associated with pregnancy-induced hypertension (preeclampsia) and chronic hypertension were observed. Abnormal umbilical cord arterial Doppler FVW was associated with the reduced umbilical cord diameter. It was also associated with both reduced total cord areas and reduced Wharton jelly areas.

Chronic hypertension is characterized by an increased vascular resistance and modifications in the mechanical properties of blood vessels [20]. Vessels contract via a variety of pharmacological agents including serotonin, potassium chloride, bradykinin, angiotensine, oxytosin and others [21, 22]. These properties have not been fully investigated in pregnancy-induced hypertension. But like chronic hypertension in preeclampsia, the inhibition of prostacycline synthesis, hypersensitivity to vasocon-

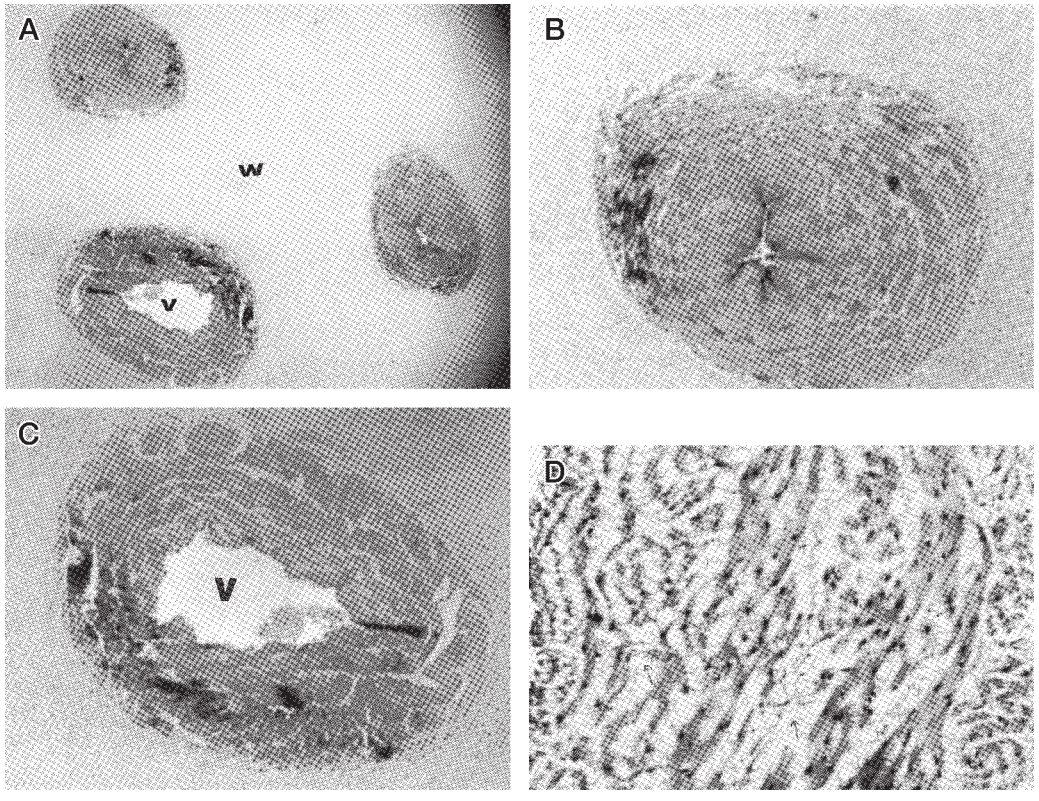

Fig. 3 Photomicrographs of umbilical cord taken from preeclamptic mother having normal Doppler FVW (Group 3A). A widening under the epithelium between the muscle layers and the contraction of the muscle cells was seen with a waved like appearance of the nucleus. Separations appeared in between the muscle cells and in between the layers of muscle (arrow). The vein lumen was seen to have narrowed. General view of umbilical cord at a magnification of × 10 (**A**); umbilical cord artery, × 40 (**B**); umbilical vein (V), × 40 (**C**); higher magnification of the vein wall, × 200 (**D**). H.E.

strictors and endothelial cell death were observed [23].

Dobrin reported that blood vessels exhibited characteristic changes during fetal development [24]. The widening of the media, an increased number and a thickening of elastic lamella, decreased cellularity and augmented collagen content characterize the morphologic development during this period. It was reported that the umbilical perfusion decreased in preeclampsia [25]. The vessel walls could react with the alterations, but their composition to maintain their transmural pressure at an optimal level would have to be sustained. In a situation of increased placental resistance, an increase in intralumen pressure in the umbilical artery will tend to increase compliance in order to keep transmural pressure relatively constant. Conversely, the intrauterine lumen pressure in the umbilical vein will decrease, and the compliance of the vessel will diminish, again to keep transmural pressure constant. Romanowicz *et al.* demonstrated that the insoluble elastin content decreased in the umbilical cord veins of newborns delivered by mothers with preeclampsia [26]. Reconstructing the umbilical cord vein wall may disturb fetal blood flow and affect the vascular system in adulthood [27].

Our morphometric results were in agreement with the results of previous studies in the control and preeclamptic groups [10–23]. Our results show that when Groups 2 and 3A were compared, the thickness of the umbilical cord vessels remained nearly constant, whereas both total areas and lumen areas were reduced with respect to the control group. These changes correlate with our histopathological findings, which included a widening under the epithelium, the contraction of the muscle cells, and

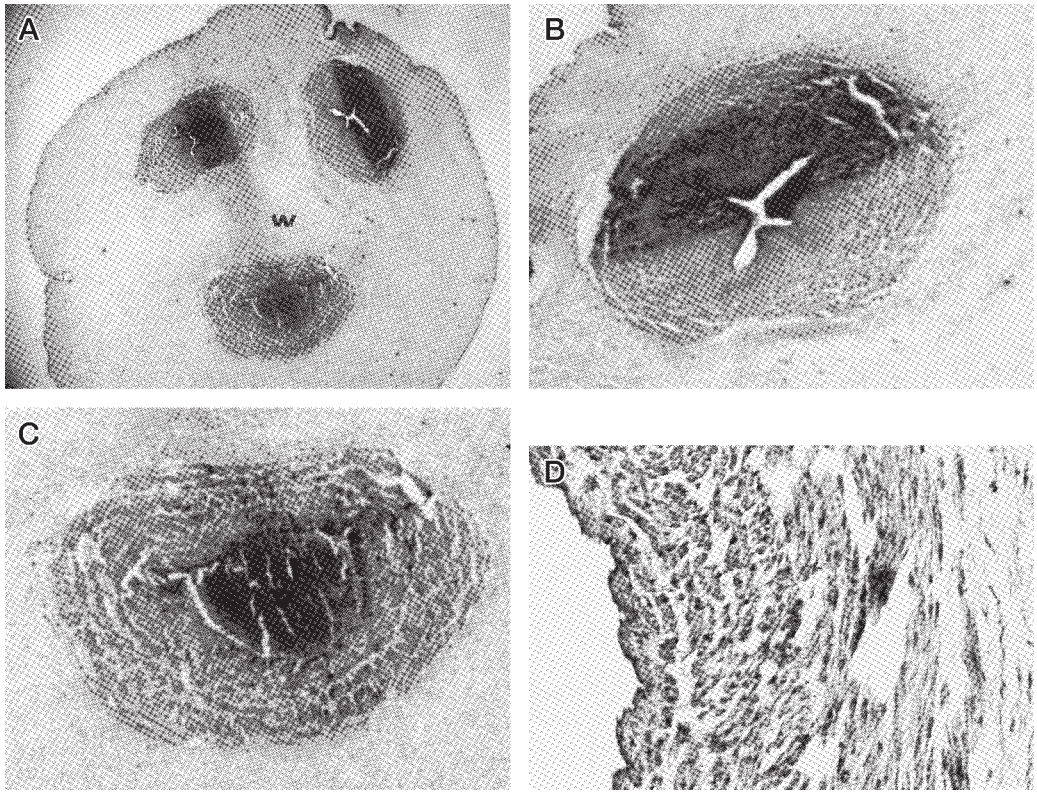

Fig. 4 Photomicrographs of umbilical cord taken from preeclamptic mother having abnormal FVW (Group 3B). The muscle area was seen to have separated from the connective tissues. The muscle cells were seen to be hypoplastic and smaller than their normal size. There was a significant decrease in the lumen area in this group, compared to the normal and chronic hypertensive group. General view of umbilical cord at a magnification of × 10 (**A**); umbilical cord artery, × 40 (**B**); umbilical vein (V), × 40 (**C**); higher magnification of the vein wall, × 200 (**D**). H.E.

separations between the muscle cells associated with the edema. These findings suggest a mainly vasoconstrictive effect. By contrast, a comparison of Group 3B to Groups 2 and 1 showed a comparable reduction of all parameters of the vessels. In Group 3B, our histopathological findings are related to the narrowing lumen of the vessels and the contracted smooth muscle cells that were smaller than their normal size. These findings are suggestive of a predominant hypoplastic mechanism. These 2 mechanisms, vasoconstriction and a hypoplastic effect, may be different events or may follow each other [10]. The first response to hypoxemia is vasoconstriction of the vessels. If hypoxemia continues, it may cause morphological changes such as hypoplasia.

Changes of the composition of Wharton jelly such as the glycosaminoglycans, water content, and extracellular matrix components were the main results of the reduction of the diameter of the umbilical cord [11, 26–27]. These changes might be responsible for the growth factors, which modify myofibroblast proliferation gene expression, protein biosynthesis and /or other processes.

Recent studies have suggested that Doppler waveform indices from the umbilical artery, fetal aorta and fetal middle cerebral arteries are useful in identifying IUGR and determining the risk of subsequent perinatal morbidity [28, 29]. An abnormal umbilical artery Doppler waveform is a strong predictor of adverse perinatal outcome in patients with preeclampsia. A correlation between the umbilical artery Doppler indices and adverse perinatal outcome was found in previous studies [24, 29]. The umbilical artery Doppler indices are related to placental vascular resistance. These earlier studies

showed the use of umbilical artery Doppler waveform indices in the prediction of abnormal neonatal morphometry. Doppler ultrasonography of the umbilical arteries is increasing in importance in the antenatal diagnosis of fetal well being. The umbilical vascular architecture is interesting not only from a morphologic point of view but also as a basis for functional interpretation. It is suggested that waveforms reflect placental impedance to blood flow, and that changes of flow patterns may be caused by histomorphologic alterations of the fetoplacental vessel tree. Abnormal Doppler systolic / diastolic ratios might reflect a pathologic fetal circulation resulting in intrauterine growth retardation, whereas normal values reflect a normal fetoplacental circulation associated with small fetal size [30-34].

In conclusion, the umbilical artery Doppler FVW indices provide good values for predicting intrauterine growth retardation in preeclamptic patients. We also observed that the umbilical vessel's wall thicknesses were reduced in the group of preeclamptic patients with pathological Doppler. It is not clear whether the morphological changes disturb the flow in the vessels or if a reduction of the flow causes the morphological changes. If a progressive increase in blood flow was a key factor contributing to the embryonic development of the vascular tree, an initial umbilical vasoconstriction in response to a hypoxic stress produced a reduction in the umbilical blood flow and in turn led to a less- developed arterial tree with an expected increase in total placental vascular resistance [10]. Chronic hypertension and preeclampsia may share similar pathophysiologic events. Further studies are necessary to elucidate the exact mechanisms.

References

1. Zuspan FB: New concepts in the understanding of hypertensive diseases during pregnancy. An overview. Clin Perinatol (1991) **18**, 653-659.
2. Eskenazi B, Fenster L and Sidney S: A multivariate analysis of risk factors for preeclampsia. JAMA (1991) **266**, 237-241.
3. Mittendorf R, Lain KY, Williams MA and Walker CK: Urinary tract infections and other risk factors for preeclampsia. J Reprod Med (1996) **41**, 491-496.
4. Piering WF, Garancis JG, Becker CG, Beres JA and Lemann J Jr: Preeclampsia related to a functioning extrauterine placenta: Report of a case and 25-year follow-up: Am J Kidney Dis (1993) **21**, 310-313.
5. Friedman SA, Taylor RN and Roberts JM: Pathophysiology of preeclampsia. Clin Perinatol (1991) **18**, 661-682.
6. Roberts JM and Redman CW: Pre-eclampsia: More than pregnancy-induced hypertension. Lancet (1993) **341**, 1447-1451.
7. Las Heras J, Baskerville JC, Harding PG and Haust MD: Morphometric studies of fetal placental stem arteries in hypertensive disorders ('toxaemia') of pregnancy. Placenta (1985) **6**, 217-227.
8. Kaufmann P, Luckhardt M and Leiser R: Three-dimensional presentation of the fetal vessel system in the human placenta. Res (1988) **3**, 113-137.
9. Johnstone FD, Ugaily-Thulesius L, Thulesius O and Nasrat AN: Umbilical artery reactivity and ultrastructural changes in pregnancy-induced hypertension and other complicated pregnancies. Clin Physiol (1987) **7**, 493-502.
10. Bruch JF, Sibony O, Benali K, Challier JC, Blot P and Nessmann C: Computerized microscope morphometry of umbilical vessels from pregnancies with intrauterine growth retardation and abnormal umbilical artery Doppler. Hum Pathol (1997) **28**, 1139-1145.
11. Di Naro E, Ghezzi F, Raio L, Franchi M and D'Addario V: Umbilical cord morphology and pregnancy outcome. Eur J Obstet Gynecol Reprod Biol (2001) **96**, 150-157.
12. Junek T, Baum O, Lauter H, Vetter K, Matejevic D and Graf R: Pre-eclampsia associated alterations of the elastic fibre system in umbilical cord vessels. Anat Embryol (2000) **201**, 291-303.
13. Templeton AG, Kingdom JC, Whittle MJ and Mc Grath JC: Contractile responses of the human umbilical artery from pregnancies complicated by intrauterine growth retardation. Placenta (1993) **14**, 563-570.
14. Hartikainen-Sorri A, Vuolteenaho O, Leppaluoto J and Ruskoaho H: Endothelin in umbilical artery vasospasm. Lancet (1991) **337**, 619.
15. Howard RB, Hosokawa T and Maguire MH: Hypoxia-induced fetoplacental vasoconstriction in perfused human placental cotyledons. Am J Obstet Gynecol (1987) **157**, 1261-1266.
16. Stuart MJ, Clark DA, Sunderji SG, Allen JB, Yambo T, Elrad H and Slott JH: Decrease prostacycline production: A characteristic of chronic placental insufficiency syndromes. Lancet (1981) **23**, 1126-1128.
17. Bodelsson G, Marsal K and Stjernquist M: Reduced contractile effect of endothelin-1 and noradrenalin in human umbilical artery from pregnancies with abnormal umbilical artery flow velocity waveforms. Early Hum Dev (1995) **42**, 15-28.
18. Roberts JM: Endothelial dysfunction in preeclampsia. Semin Reprod Endocrinol (1998) **16**, 5-15.
19. Howard CV and Reed MG: Unbiased Stereology. Three-Dimentional Measurement in Microscopy. 1st Ed, BIOS Scientific Publishers Ltd, Oxford, UK (1998) pp 28-37.
20. Meekins JW, Pijnenborg R, Hanssens M, McFadyen IR and van Asshe A: A study of placental bed spiral arteries and trophoblast invasion in normal and severe pre-eclamptic pregnancies. Br J Obstet Gynaecol (1994) **101**, 669-674.
21. Nasiell J, Nisell H, Blanck A, Lunel NO and Faxen M: Placental expression of endothelial constitutive nitric oxide synthase mRNA in pregnancy complicated by preeclampsia. Acta Obstet Gynecol Scand (1998) **77**, 492-496.
22. Khong TY, De Wolf F, Robertson WB and Brosens I: Inadequate maternal vascular response to placentation in pregnancies complicated by pre-eclampsia and by small-for-gestational age infants. Br J Obstet Gynaecol (1986) **93**, 1049-1059.
23. Bertrand C, Duperron L and St-Louis J: Umbilical and placental vessels: Modifications of their mechanical properties in preeclampsia. Am J Obstet Gynecol (1993) **168**, 1537-1546.
24. Dobrin PB: Mechanical properties of arterises. Physiol Rev (1978) **58**, 397-460.
25. Biagiotti R, Sgambati E and Brizzi E: Placental morphometry in pregnancies complicated by intrauterine growth retardation with absent or reversed end diastolic flow in the umbilical artery. Ital J Anat Embryol (1999) **104**, 201-207.

26. Romanowicz L and Sobolewski K: Extracellular matrix components of the wall of umbilical cord vein and their alterations in pre-eclampsia. J Perinat Med (2000) **28**, 140–146.
27. Romanowicz L, Bankowski E, Sobolewski K and Jaworski S: Activities of some glycosaminoglycan-degrading enzymes in Wharton's jelly and their alteration in EPH-gestosis (Pre-eclampsia). Biol Neonate (1999) **76**, 144–152.
28. Mitra SC, Seshan SV and Riachi LE: Placental vessel morphometry in growth retardation and increased resistance of the umbilical artery Doppler flow. J Matern Fetal Med (2000) **9**, 282–286.
29. Yoon BH, Lee CM and Kim SW: An abnormal umbilical artery waveform: A strong and independent predictor of adverse perinatal outcome in patients with preeclampsia. Am J Obstet Gynecol (1994) **171**, 713–721.
30. Bartha JL, Comino-Delgado R, Gonzalez-Mena C, Lopez I and Arrabal J: Umbilical blood flow and neonatal morphometry: A multivariate analysis. Eur J Obstet Gynecol Reprod Biol (1998) **79**, 27–33.
31. Atkinson MW, Maher JE, Owen J, Hauth JC, Goldenberg RL and Copper RL: The predictive value of umbilical artery Doppler studies for preeclampsia or fetal growth retardation in a preeclampsia prevention trial. Obstet Gynecol (1994) **83**, 609–612.
32. Berkowitz GS, Chitkara U, Rosenberg J, Cogswell C, Walker B, Lahman EA, Mehalek KE and Berkowitz RL: Sonographic estimation of fetal weight and Doppler analysis of umbilical artery velocimetry in the prediction of intrauterine growth retardation: A prospective study. Am J Obstet gynecol (1988) **158**, 1149–1153.
33. Chang TC, Robson SC, Spencer JA and Gallivan S: Identification of fetal growth retardation: Comparison of Doppler waveform indices and serial ultrasound measurements of abdominal circumference and fetal weight. Obstet Gynecol (1993) **82**, 230–236.
34. McCowan LM, Harding JE and Stewart AW: Umbilical artery Doppler studies in small for gestational age babies reflect disease severity. BJOG (2000) **107**, 916–925.