Original Article
https://doi.org/10.5115/acb.19.158
pISSN 2093-3665 eISSN 2093-3673
Anatomy & Cell Biology acb

Human umbilical cord and its vessels: a histomorphometric study in difference severity of hypertensive disorders of pregnancy

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Abstract: The umbilical cord (UC) is a platform for fetal nourishment and growth. The fetus, mother and placenta with UC form a triad, which contributes to pregnancy outcome. When pregnancy is complicated by a medical condition like hypertension, affects both maternal and fetal health. Being a fetal structure it can be used as a window to know the maternal dysfunctions and their impacts on fetal wellbeing. The present study is to explore the histomorphometric changes of the UC and its vessels involved in the development of hypertension during pregnancy. Sixty UCs were used and the following parameters, total UC area; total vessel area; jelly area; wall area, luminal area and wall thickness of umbilical arteries 1 and 2 and vein were studied using ImageJ software. From the results, the mean differences of above parameters of hypertensive UCs were found to be lesser than control and it was significantly higher in cases of severe preeclampsia ($P \leq 0.05$). From the present study, we conclude hypertensive cords and its vessels are associated with significant structural changes. Since it is a global health issue it is important to know the factors contributing it to diagnose and prevent.

Key words: Human umbilical cord, Umbilical vessels, Histomorphometry, Vasoconstriction, Hypertension

Received July 13, 2019; Revised August 17, 2019; Accepted September 9, 2019

Introduction

The umbilical cord is a bridge between the placenta and fetus that developed during the fifth week of intrauterine life and grows till the 28th week of gestation. It is an organ of pregnancy and fundamentally destined for nutritional exchange between the maternal and fetal circulation which is necessary for fetal growth and development [1, 2].

The umbilical cord contains two arteries and one vein suspended in the mucoid extracellular matrix, the Wharton's jelly. Umbilical vessels play a key role in maintaining and regulating the fetoplacental circulation [3]. Due to lack of vasa vasorum, the umbilical vessels depend on their own oxygen supply and thus making them more susceptible to hemodynamic alterations caused by maternal disorders like hypertension [4, 5].

Hypertension is the most common medical problem encountered during pregnancy complicating 6%–20% of all pregnancies, and responsible for 10%–15% of maternal deaths worldwide [6-8]. Hypertension is greatly responsible for maternal and perinatal mortality and morbidity [9, 10]. Women with hypertension are at increased risk of both maternal and fetal consequences such as cardiovascular and cerebrovascular diseases, disseminated intravascular coagulation, liver and kidney failure, HELLP syndrome, preterm delivery, fetal

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growth restriction, placental abruption, and even stillbirth and neonatal asphyxia [11, 12].

In hypertension, there is increased resistance to uteroplacental circulation due to an inadequate trophoblastic invasion of the maternal spiral arteries resulting in reduced blood flow across the placenta that leads to vascular insufficiency and placental dysfunction which adversely affects the growth of the fetus [13].

Extensive structural and functional changes of the cardiovascular system occur during pregnancy to fulfill the fetal demands. Thus any disease during pregnancy which affects both the mother and fetus has shows the great impact on the umbilical cord. Being the extension of the fetal cardiovascular system, it also exhibits the same stress and strain. To date, literature is scarce with regard to histomorphometric alterations of the umbilical cord and its vessels in hypertensive disorders of pregnancy in relation to the severity. Hence the present study was undertaken.

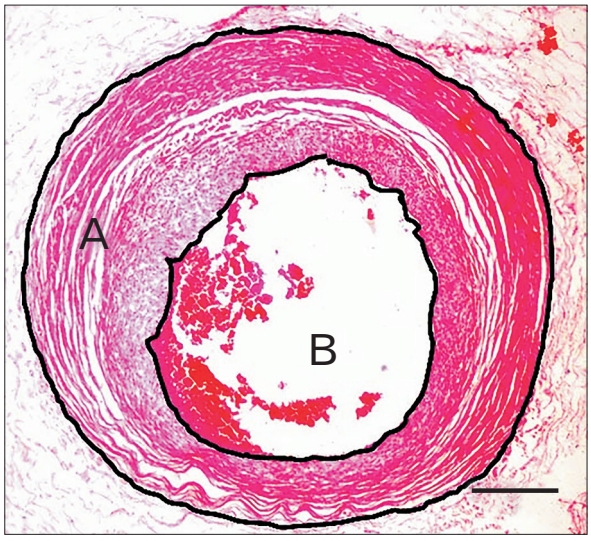

Fig. 1. Diagramatic representation of umbilical vessel (either artery or vein)-control stained with hematoxylin and eosin (H&E). A, vessel area; B, luminal area. Scale bar=7 μm.

Materials and Methods

For the present study, a total of 60 women who delivered at the Department of Obstetrics and Gynecology, Narayana General Hospital, Nellore, Andhra Pradesh, India were included. Written informed consent was obtained from each participant who took part in the study with proper institutional ethical committee approval. The criteria adopted for selection of cases were defined according to the National High Blood Pressure education program of USA (NHBPEP) followed by the American College of Obstetrics and Gynecologists [14, 15]. The study subjects were divided into four groups: control (G1), women with an uncomplicated pregnancy; gestational hypertension (G2), women with gestational hypertension with blood pressure 140/90 without proteinuria; preeclampsia mild (G3), women with mild preeclampsia with blood pressure 140/90 and proteinuria +1 dipstick in urine analysis; preeclampsia severe (G4), women with severe preeclampsia who had blood pressure 160/110 with proteinuria +2 dipstick in urine analysis. Immediately after delivery, the umbilical cords were collected and fixed in 10% formalin for 24–48 hours. One centimeter length cord was cut from the placental site and dehydrated in graded ethyl alcohol, cleaned in xylene and embedded in paraffin. The 4-μm-thick sections were made with Leica microtome and stained with hematoxylin and eosin.

The sections were photographed at ×0.75 by using a digital camera attached to the stereo-zoom microscope. The following parameters were performed with Image J software (Fig. 1). Wall area of artery-1 (WA-A1), wall area of artery-2 (WA-A2), wall area of the vein (WA-V), luminal area of artery-1 (LA-A1), luminal area of artery-2 (LA-A2), luminal area of the vein (LA-V), wall thickness of artery-1 (WT-A1), wall thickness of artery-2 (WT-A2), wall thickness of vein (WT-V), total vessel area (TVA), total umbilical cord area (TUCA), jelly area (JA).

Data from the current study were statistically analyzed with One-way analysis of variance (ANOVA) using SPSS version 16.0 (SPSS Inc., Chicago, IL, USA) to know the mean difference between four groups. Results of the study were represented as mean±SD, *P*-value of <0.05 was considered as statistically significant.

Results

The results of the histomorphometric parameters of the umbilical cord and its vessels between the control (G1) and hypertensive pregnancies (G2, G3, and G4) are summarized in Table 1.

In the present study, we observed that all the parameters, i.e., TUCA, TVA, JA, WA-A1, WA-A2 and WA-V, LA-A1, LA-A2 and LA-V, WT-A1, WT-A2, and WT-V were decreased in hypertensive cords (G2, G3, and G4) as compared with the control (G1). Above said all the parameters of hypertensive

https://doi.org/10.5115/acb.19.158

Table 1. Histomorphometrical parameters of the umbilical cord and its vessels in the control and hypertensive groups

Histomorphometric parameter	Mean±SD	*F*-value	*P*-value
Total umbilical cord area (mm^2)			
G1	70.246±22.710	5.266	0.001**
G2	61.840±20.445		
G3	58.689±13.830		
G4	42.945±18.983		
Total vessel area (mm^2)			
G1	10.504±5.309	5.725	0.002**
G2	7.799±3.071		
G3	6.841±1.917		
G4	5.581± 2.109		
Jelly area (mm^2)			
G1	59.742±18.363	4.546	0.006**
G2	54.042±18.810		
G3	51.847±13.804		
G4	37.363±17.765		
Wall area of artery-1 (mm^2)			
G1	2.024±0.727	3.536	0.020*
G2	1.522±0.949		
G3	1.495±0.397		
G4	1.161±0.749		
Wall thickness of artery-1 (mm)			
G1	1.550±0.779	3.509	0.021*
G2	0.457±0.545		
G3	0.351±0.467		
G4	0.312±0.385		
Luminal area of artery-1 (mm^2)			
G1	0.322±0.116	1.182	0.325
G2	0.242±0.151		
G3	0.238±0.063		
G4	0.185±0.119		
Wall area of artery-2 (mm^2)			
G1	2.671±1.241	2.781	0.049*
G2	2.517±1.535		
G3	2.202±0.671		
G4	1.623±0.542		
Wall thickness of artery-2 (mm)			
G1	1.285±1.007	2.654	0.057
G2	1.269±1.219		
G3	1.138±1.016		
G4	0.487±0.639		
Luminal area of artery-2 (mm^2)			
G1	0.425±0.198	2.171	0.102
G2	0.390±0.239		
G3	0.351±0.107		
G4	0.259±0.086		
Wall area of vein (mm^2)			
G1	5.809±4.731	3.646	0.018*
G2	3.760±1.585		
G3	3.145±1.861		
G4	2.797±1.243		
Wall thickness of vein (mm)			
G1	8.710±12.591	3.652	0.018*
G2	4.050±4.326		
G3	2.946±3.028		
G4	1.990±1.660		
Luminal area of vein (mm^2)			
G1	0.925±0.753	2.814	0.047*
G2	0.609±0.249		
G3	0.501±0.296		
G4	0.445 ±0.198		

G1, control; G2, gestational hypertension; G3, mild preeclampsa; G4, severe preeclampsia; *F*-value, *F* statistic value in ANOVA. **P*<0.05, ***P*<0.01.

cords were found to be statistically significant except LA-A1 and LA-A2, WT-A2, among which severe preeclampsia (G4) was found to be highly significant (Table 1, Figs. 2–4).

Discussion

Although hypertensive disorders of pregnancy are contributing a major role in maternal and fetal mortality and morbidity, the etiology of these disorders is still unknown. Many studies have shown that hypertensive related morphologic and or morphometric alterations of the umbilical cord are associated with adverse fetal outcomes. Due to structural alterations of umbilical cord in hypertensive pregnancies can contribute to placental ischemia, hypoperfusion and thus lead to decreased fetoplacental circulation. In this study, we have made an attempt to evaluate histomorphometric changes of the umbilical cord and its vessels between the control and with different severity of hypertensive pregnancies.

The TUCA was reduced in hypertensive cords when compared with control. This finding is similar to the previous histological and ultrasonographical findings [16-18]. The mean difference of TUCA was more in severe preeclampsia. Raio et al. demonstrated the reduced cross-sectional area of the umbilical cord is associated with intrauterine growth restriction. Preeclampsia is the most common pathology in pregnancy associated with intra-uterine growth retardation (IUGR) and preterm labor. These findings suggesting that the reduced total cord area in hypertensive pregnancies would be a predictor of IUGR and the lean umbilical cords are at increased risk of small for gestational age and fetal distress during labor [19, 20].

The development of the umbilical cord is dependent greatly on hemodynamic conditions such as the rate of blood flow, oxidative stress, and oxygen tension. In this study, we found the decreased TVA in hypertensive cords and the mean difference was more in severe preeclampsia. These findings are

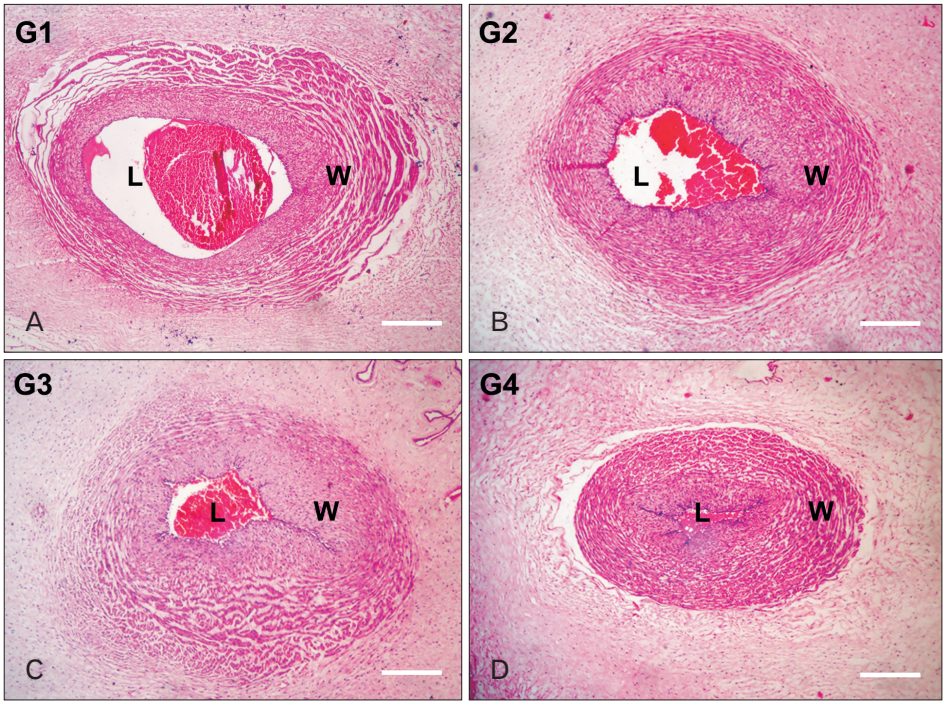

Fig. 2. Microscopic section of umbilical artery showing the wall (W) and luminal areas (L), stained with hematoxylin and eosin (H&E) under 4× magnification. (A) G1, control. (B) G2, gestational hypertension. (C) G3, mild preeclampsia. (D) G4, severe preeclampsia. Scale bars=29 μm (A–D).

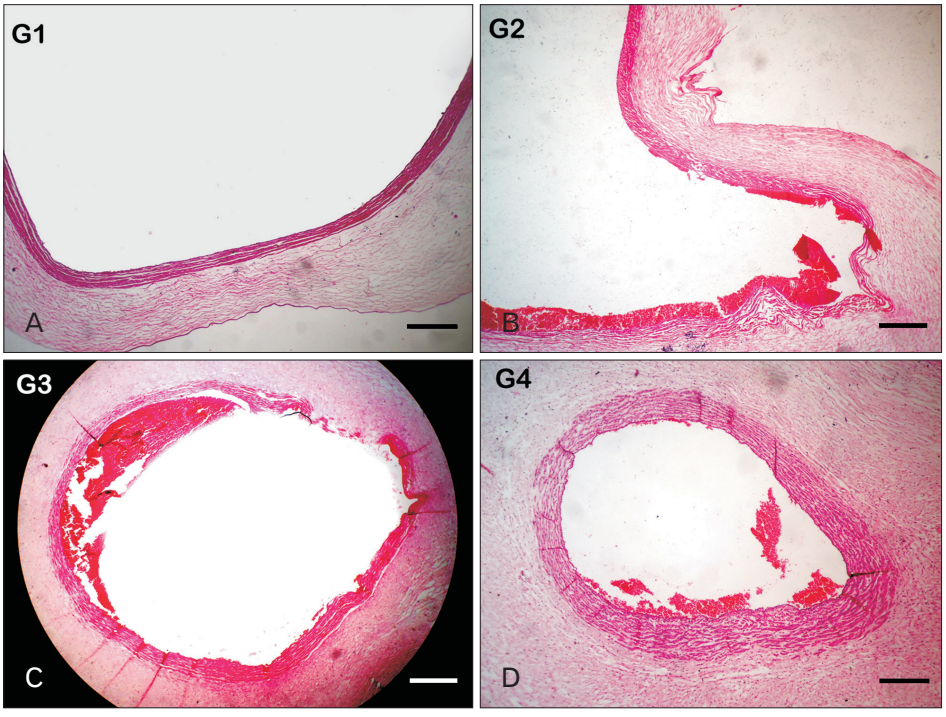

Fig. 3. Microscopic section of umbilical vein showing the wall and luminal areas, stained with H&E under ×4 magnification. (A) G1, control. (B) G2, gestational hypertension. (C) G3, mild unaccessible. (D) G4, severe preeclampsia. Scale bars=29 μm (A–D).

consistent with the findings of Bhavina et al. [21-23]. The decreased blood flow due to the inadequate trophoblastic invasion of the maternal spiral arteries in preeclampsia might be the reason for decreased size of umbilical vessels and thereby TVA [24, 25].

Ghezzi et al. [26] reported a high correlation between the umbilical cord cross-sectional area of Wharton's jelly and anthropometric parameters before the 32nd week of pregnancy Wharton's jelly is a protective mantle around the umbilical vessels. The most commonly represented glycosaminoglycans

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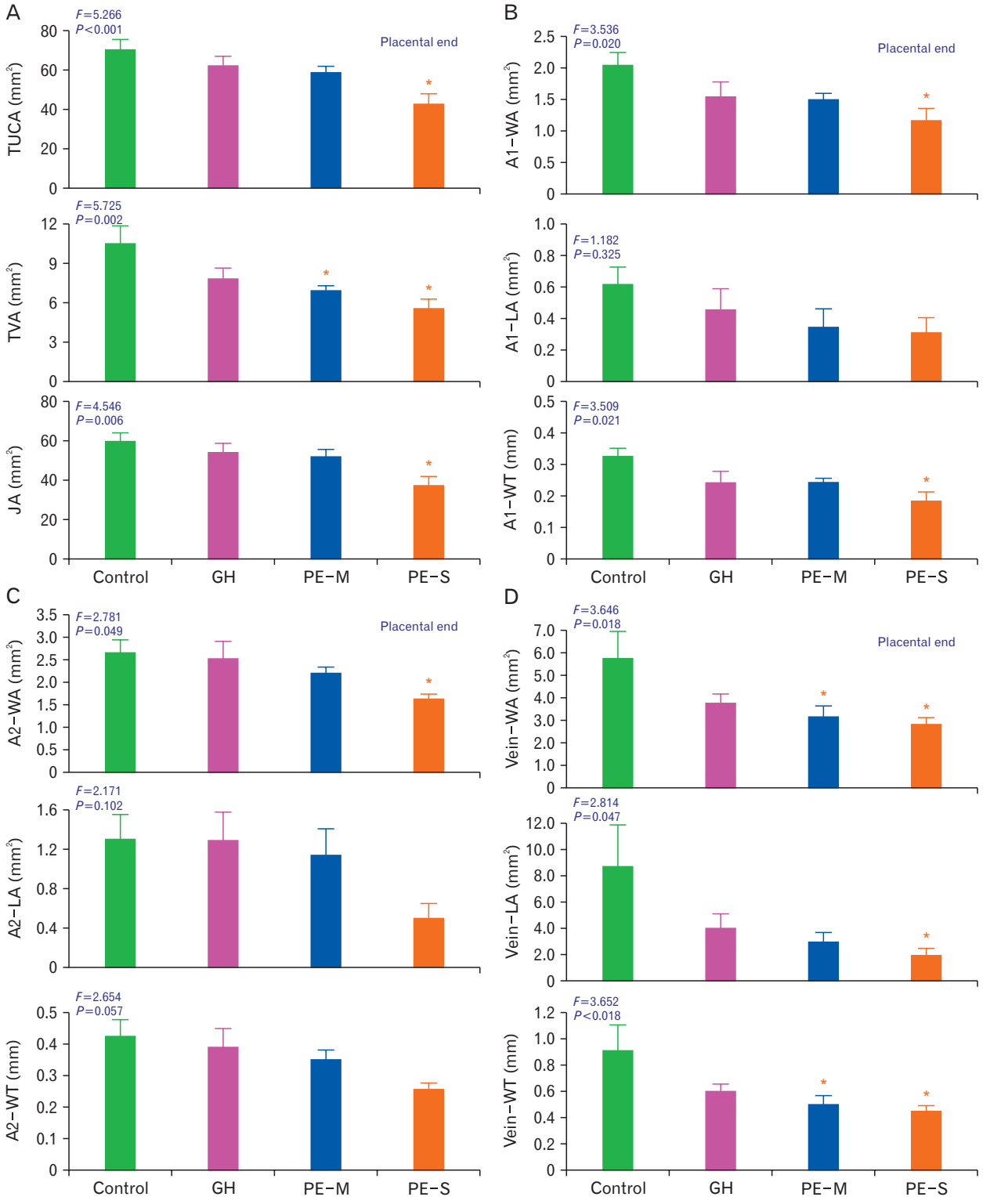

Fig. 4. Bar diagram showing the various parameters of umbilical cords from different groups. G1, control; G2/GH, gestational hypertension; G3/PE-M, mild preeclampsia; G4/PE-S, severe preeclampsia. (A) Comparison of jelly area (JA), total vessel area (TVA) and total umbilical cord area (TUCA). (B, C) Comparison of wall and luminal area of umbilical artery 1 and 2 with its wall thickness respectively. (D) Comparison of wall and luminal area of umbilical vein with its wall thickness. A1 and A2, umbilical artery 1 and 2; LA, luminal area; WA, wall area; WT, wall thickness. *Significantly different from control.

is hyaluronic acid. It is hydrophilic in nature absorbs water and electrolytes. It gives elasticity to the cord that resists external pressure influencing fetoplacental circulation through umbilical vessels [26, 27]. Bańkowski et al. [28] observed increased sulfated glycosaminoglycans in place of hyaluronic acid in hypertensive pregnancies and this suggests that reduced hydration. This may be the reason for these observed reductions of Wharton's jelly in hypertensive cords of the present study. These results correlate with Yasoob et al. [29] who observed the reduced Wharton's jelly in preeclampsia. In this study, we have observed both wall area and luminal area was reduced in mbilical veins while in umbilical arteries, vessel wall area, as well as luminal area was decreased in hypertensive cords in relation to the severity, but the luminal area did not show any statistical significance as mentioned by Inan et al. [18]. These findings are also similar to the findings of Bruch et al. [16] in intrauterine growth-retarded fetuses with or without umbilical artery Doppler. He suggested the reduce size of vessels may be due to vascular hyperplasia or vasoconstriction. He also stated that shortening of smooth muscles due to vasoconstriction those results in reduced vessel area as well as luminal area [16, 18]. Schönfelder et al. [30] reported the existence of nitric oxide synthase in umbilical vein smooth muscles and none in the artery. Preeclampsia is associated with loss of nitric oxide synthase expression and its significant reduction in mRNA. Decreased nitric oxide may therefore lead to the vasoconstriction of the umbilical vein and thus resulting in a smaller lumen in the hypertensive group and in the present study, the mean difference was increased with disease severity. In contrast to our study, Barnwal et al. [31] reported increased vein wall area and luminal area in preeclamptic pregnancies as compared to the normal group [30]. Junek et al. [32] observed thicker umbilical arteries in preeclamptic cords than in control. Whereas, in our study, we observed a decreased wall thickness of umbilical arteries and vein in hypertensive cords. This finding was statistically significant and consistent with findings of Inan et al. [18] who observed reduced wall thickness of umbilical vessels with pathological Doppler umbilical waveform. Burckhardt et al. [33] observed reduced elastin content in walls of umbilical arteries in IUGR fetuses and lower insulin-like growth factor 1 in umbilical cord blood, which regulates the synthesis of elastin and thus resulting in thinner and stiffer arteries [18, 33].

Conclusion

Hypertensive disorders of pregnancy are a global issue and its frequency is increasing in developing countries including India. It complicates 10%–15% of all pregnancies. The umbilical cord and fetal vasculature are derived from extra and intraembryonic mesoderm and thus share the common embryonic origin. Therefore, the umbilical vessels can be used as a model instead of nonaccessible fetal vessels to know the vascular health of the newborn. In this study hypertensive cords and vessels are associated with significant structural changes and these changes are increased with its progressive pathology. This work represents an advance in biomedical science because the understanding of these histomorphometrical changes, where the roots of such disorders seem to be, is required to plan for better treatment and thus prevents the maternal and fetal mortality and morbidity with lifelong impact.

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Author Contributions

Conceptualization: SC. Data acquisition: SC. Data analysis or interpretation: SC. Drafting of the manuscript: SC, SDV, VR. Critical revision of the manuscript: SC, SDV. Approval of the final version of the manuscript: all authors.

Conflicts of Interest

No potential conflict of interest relevant to this article was reported.

Acknowledgements

The authors would like to thank all the participants who involved in this study for their cooperation.

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