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Research Article

Histomorphometry of umbilical vessels of intrauterine growth restricted neonates

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ABSTRACT

Background: A complex and coordinated interaction between maternal, placental and fetal factors influences the normal fetal growth. Any disruption in this complex system can lead to intrauterine growth restriction (IUGR).The main objective of the study is to evaluate histomorphometric differences in umbilical vessels of appropriate for gestational age (AGA) and intrauterine growth restricted (IUGR) neonates and to observe histomorphometric differences existing in umbilical vessels between male and female sex.
Methods: A prospective observational study included 110 umbilical cords (UC) procured from KLES Dr. Prabhakar Kore Charitable Hospital and Medical Research Centre, Belagavi, India. Formalin fixed, paraffin embedded tissues were processed and stained with hematoxylin and eosin, and Masson's trichrome stain. Histomorphometric measurements of cross sectional areas (CSA) of umbilical vessels like total vessel area (mm^2), lumen area (mm^2) and vessel wall area (mm^2) were taken with 40X magnification with the help of Olympus microscope having digital image analyzer attachment. The relevant personal data of mother and neonate were recorded. Independent t test was used to compare means and chi-square test for categorical variables.
Results: Umbilical vessels of IUGR neonates had significantly reduced total vessel area, vessel wall area and lumen area compared to AGA neonates (except lumen area of umbilical arteries). Ratios of total vessel area to lumen area and vessel wall area to lumen area were significantly increased in human umbilical vein (HUV) of IUGR neonates. No significant difference in CSAs of umbilical vessels was observed between male and female sex in both groups.
Conclusions: IUGR is associated with significant structural changes in umbilical vessels of full term neonates. These changes are more obvious in HUV than human umbilical artery (HUA). It can be detected prenatally and used as an indicator of impending IUGR.

Keywords: Cross sectional areas, Histomorphometry, IUGR, Umbilical vessels, Umbilical vein

INTRODUCTION

A complex and coordinated interaction between maternal, placental and fetal factors influences the normal fetal growth. Any disruption in this complex system can lead to intrauterine growth restriction (IUGR).[1] Establishment of good utero-fetoplacental circulation is necessary for transfer of nutrients and oxygen between fetus and mother. Utero-placental insufficiency is most common cause for IUGR. Umbilical cord (UC) plays an important role in maintaining and regulating feto-placental circulation and thus in fetal nutrition and wellbeing.[2]

Any structural variations and pathological changes in UC have potential to lead to IUGR, adverse pregnancy outcome and even stillbirth.[3,4] Ultrasonography (USG) and histopathological studies on UC in IUGR babies showed smaller UC components compared to appropriate for gestational age (AGA) babies.[5,6]

IUGR is associated with not only an increased risk of perinatal morbidity and mortality but also may influence adult health.[1,7] The compensatory mechanism develops in fetus to adapt to the inadequate supply of nutrition. This may bring about long term changes in the morphology, physiology and metabolism of fetus.[8] Presently there are no effective preventive or therapeutic strategies to manage IUGR. Basic research is very essential to understand the altered morphology of fetal tissues causing IUGR.[1]

Unlike placenta, UC is completely fetal in origin and plays a major role in fetal development and yet, has not received much attention in clinical practice. Hence, we undertook this study to analyze structural differences in umbilical vessels of IUGR babies. Objective of this study was to evaluate histomorphometric differences in umbilical vessels of AGA and IUGR neonates and to observe histomorphometric differences existing in umbilical vessels between male and female sex.

METHODS

This prospective observational study included 110 UC procured from the KLES Dr. Prabhakar Kore Charitable Hospital and Medical Research Centre, Belagavi, India. The study was approved by the institutional human ethical committee. Control or AGA group included 55 UC samples from women with uneventful pregnancy having AGA fetus; defined as more than 10^{th} and less than 90^{th} percentile of the estimated fetal weight by USG (Mediscan sonocare software). Cases or IUGR group included 55 UC samples from IUGR pregnancies identified by USG using the same software by taking abdominal circumference less than 10^{th} percentile or expected fetal weight less than 10^{th} percentile for the gestational age (GA).[9]

Inclusion criteria singleton gestation, mother age between 18-35 years, GA between 37-40 weeks determined by last menstrual period (LMP) or 1^{st} trimester USG. Patients were excluded having multiple pregnancy, pre-gestational diabetes, gestational diabetes, heart diseases and unknown GA. Perinatal outcome like GA at delivery, birth weight, mode of delivery, sex of newborn and neonatal outcome were recorded in predesigned proforma. Preterm delivery often results in low birth weight babies and UC cord may have different size hence, excluded from the study. Informed written consent was obtained from eligible subjects who agreed to participate in the study.

5cm of UC tissues were collected immediately after the delivery, 5cm from the placental end and fixed in 10% formalin. Paraffin wax embedded blocks were processed for light microscopy. 3µm thick series of transverse sections were taken and stained with hematoxylin & eosin (H&E) and Masson's trichrome stain using standard histological techniques. Tangential and incomplete sections were excluded to avoid the measurement error. The slides were studied under Olympus microscope CX40 (Tokyo Japan) with digital camera DP21. The reliability of the instrument was analyzed by inter and intra-observers calibration by Kappa statistics. For each umbilical vessel cross sectional areas (CSA) like; total vessel area (mm^2), lumen area (mm^2) and vessel wall area (mm^2), was measured with 40X magnification. Ratios of total vessel area to lumen area and vessel wall area to lumen area were computed using respective areas. Microscopic image was projected on the screen. Area was measured using the polygonal area tool that displayed measurements automatically on the screen in square micrometer (μm^2). Vessel wall area was calculated by taking difference of luminal area from total vessel area.[10] Smaller artery was taken as human umbilical artery (HUA) -1 and larger umbilical artery was taken as HUA -2. Newborn's birth weight was recorded using digital baby weighing machine immediately after the delivery.

Statistical analysis

Before statistical analysis, the normality distribution was analyzed by Kolmogorv-Smimov test and parameters met normal assumption. The data were analyzed by parametric test like independent t test for numerical data and chi-square test for categorical data for comparison between two groups by using statistical software SPSS 21.0 version. The statistical significance was set at 5% level of significance ($p<0.05$).

RESULTS

Normally UC consists of two umbilical arteries and one umbilical vein. In this study, we observed single HUA in two UC of IUGR group and one UC of AGA group. True UC knots were not observed in both cases.

Table 1 shows a comparison of the clinical features of AGA and IUGR groups. The mean maternal age was similar in both groups. Incidence of pregnancy induced hypertension (PIH) and maternal anemia was noted to be higher in IUGR group. Pregnancy outcome results of control and case groups are given in Table 2. GA at birth and birth weight was significantly reduced in IUGR group. This higher birth rate of female babies (65%) and increased (15%) admission to neonatal intensive care unit (NICU) were significant in IUGR group. No significant difference was found in mode of delivery ($p=0.3830$) and stillbirth ($p=0.1540$)

Photomicrograph (Figure 1A and B) shows human umbilical vein (HUV) of AGA and IUGR neonate respectively. In AGA group, the tunica media of HUV (Figure 1A) is thin and composed of smooth muscles with large lumen. CSAs of umbilical vessels measurements in control and case group results are summarized in Table 3. The HUV (Figure 1B) had significantly smaller total vessel area (1.55±0.45 vs. 2.61±0.54) and vessel wall area (1.14±0.37 vs. 1.76±0.37) in IUGR group versus AGA group. The microscopic measurements of HUV lumen (Figure 1B) showed significantly (p=0.0001) smaller lumen area (0.42±0.27 vs. 0.85±0.44) in IUGR group versus AGA group.

Table 1: Clinical features in control and case group.

Clinical features	AGA group		IUGR group		p value
	No	%	No	%	
Maternal age year †	25	3.5	24	3.4	0.071
Consanguineous marriage	5	9	9	16	0.252
Gravidity					
Primigravida	21	38	32	58	0.036*
Multigravida	34	62	23	42	
Anemia	2	4	24	44	0.0001*
Pregnancy induced hypertension	0	0	14	25	0.0001*

*- significant,†- mean & standard deviation.

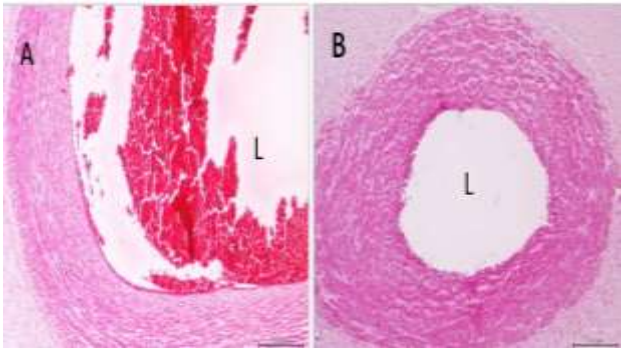

Figure 1A: HUV of AGA neonate with large total vessel area, vessel wall area and lumen area (L). Figure 1B: HUV of IUGR neonate showing reduced total vessel area, and vessel wall area and lumen area. H&E stain at 40X; bar=200µm.

Photomicrograph (Figure 2C and 2D) shows HUA of AGA and IUGR neonate respectively. HUA of control group (Figure 2C) has thick tunica media showing, inner longitudinal and outer layer of circularly arranged smooth muscle fibers. The two HUAs (Figure 2D) also had significantly (p<0.05) smaller total vessel area and vessel wall area in IUGR group compared to AGA group as shown in Table 3. No significant (p>0.05) difference was observed in luminal areas of both HUAs between two groups.

Table 2: Pregnancy outcome results of control and case group.

Outcome parameters	AGA group		IUGR group		p value
	No	%	No.	%	
Gestational age at delivery, weeks †	39.1	1.2	38.5	1.6	0.0250*
Birth weight, Kg †	3.1	0.40	2.2	0.27	0.0001*
Mode of delivery					
Normal	34	62	27	49	0.3830
Instrumental vaginal	1	2	2	4	
Caesarean	20	36	26	47	
Sex of baby					
Male	29	53	19	35	0.0350*
Female	26	47	36	65	
Neonatal outcome					
NICU admission	1	2	8	15	0.0150*
Stillbirth	0	0	2	4	0.1540

*- significant, † - mean & standard deviation, NICU - neonatal intensive care unit.

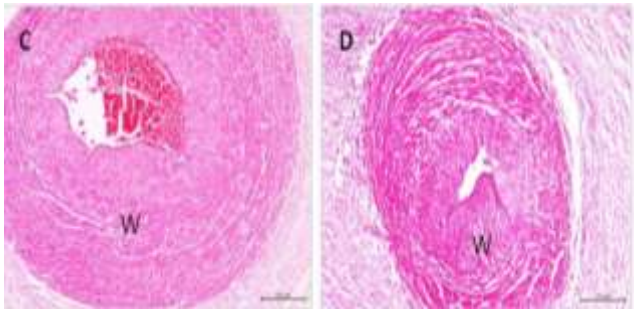

Figure 2C: HUA of AGA neonate with large total vessel area and vessel wall area (w). Figure 2D: HUA of IUGR neonates showing reduced total vessel area and vessel wall area (w). H&E stain at 40X; bar=200µm.

Ratio of total vessel area to lumen area (5.44±5.18 to 3.74±1.81, p=0.023) and vessel wall area to lumen area (4.44±5.18 to 2.74±1.81, p=0.023) were significantly increased in HUV of IUGR group compared to AGA group. In contrast, both ratios were increased in HUAs of IUGR but only HUA-2 increase was significant (p=0.018). No significant (p>0.05) differences in CSAs of umbilical vessels was observed between male and female in both groups.

Table 3: CSAs of umbilical vessels measurements in control and case group.

Vessels	Parameters	AGA group mean (SD)	IUGR group mean (SD)	t-value	p value
HUV	Total vessel area (mm^2)	2.61(0.54)	1.55(0.45)	11.077	0.0001*
	Lumen area (mm^2)	0.85(0.44)	0.42(0.27)	6.252	0.0001*
	Vessel wall area (mm^2)	1.76(0.37)	1.14(0.37)	8.844	0.0001*
	Total vessel area to Lumen area ratio	3.74(1.81)	5.44(5.18)	-2.298	0.0230*
	Vessel wall area to Lumen area ratio	2.74(1.81)	4.44(5.18)	-2.298	0.0230*
HUA-1	Total vessel area (mm^2)	1.60(0.42)	1.14(0.38)	5.971	0.0000*
	Lumen area (mm^2)	0.08(0.07)	0.05(0.08)	1.816	0.0720
	Vessel wall area (mm^2)	1.51(0.41)	1.08(0.36)	5.871	0.0001*
	Total vessel area to Lumen area ratio	38.16(47.48)	45.23(40.27)	-0.818	0.4150
	Vessel wall area to Lumen area ratio	37.16(47.48)	44.23(40.27)	-0.818	0.4150
HUA-2	Total vessel area (mm^2)	1.98(0.58)	1.33(0.46)	6.461	0.0001*
	Lumen area (mm^2)	0.12(0.13)	0.07(0.15)	1.692	0.0930
	Vessel wall area (mm^2)	1.89(0.50)	1.31(0.38)	6.738	0.0001*
	Total vessel area to Lumen area ratio	36.3(32.74)	61.20(68.33)	-2.403	0.0180*
	Vessel wall area to Lumen area ratio	35.3(32.74)	60.21(68.33)	-2.403	0.0180*

*:significant , SD - standard deviation, HUV – Human umbilical vein, HUA - Human umbilical artery.

DISCUSSION

The UC is the only channel between mother and fetus and its growth has profound effect on fetal growth leading to IUGR.[11] UC is formed by fetal tissue and the growth of UC is dependent upon genetic factors. At genomic level, paternal chromosomes are involved in regulation and development of fetal component of placenta, UC and membranes.[12] The genes involved are mainly vascular endothelial growth factor (VEGF) and placental growth factor (PIGF) from trophoblast which induce and control angiogenesis. IGF-1, leptin and insulin also influences the growth of UC and fetus.[12,13] In IUGR, western blot analysis of placenta shows increased levels of PIGF while VEGF is reduced.[14] Increased genetic insulin resistance, lower IGF-I and leptin level were associated with impaired fetal growth and abnormal vascular development during fetal life and may initiates adult vascular diseases.[1,15] This altered gene expression leads to structural modifications or hypo-development of feto-placental vasculature. Such intrauterine programming may lead to fetal growth disorders.[7,8]

We have observed that there is significant reduction in total vessel area in IUGR neonates in our study as was mentioned by Raio et al and Bruch et al Vessel wall area also showed significant reduction in IUGR group,[5,6] which is in agreement with Burkhardt et al.[10] They showed that structural alteration was because of reduced elastin positive area in HUA of IUGR group and biochemically UC blood contained lower IGF-1, which is regulator of elastin synthesis. This leads to increased arterial stiffness and smaller size. This was supported by myographic measurements of maximal tension and maximal force in HUA.

Bruch et al suggested two possible mechanisms for reduction in the size of the vessels: structurally it could be vascular hypoplasia or functionally it may be due to vasoconstriction.[6] He stated that shortening of smooth muscles could be because of vasoconstriction resulting in reduced total vessel area and luminal area. We have also observed that both areas are reduced in HUV of IUGR group. While in HUAs, only total vessel area was reduced while luminal area did not show significant change as in Bruch et al study.[6] We have analyzed the ratios of total area to lumen area and vessel wall area to lumen area. Both ratios were significantly increased in HUV of IUGR group but only HUA-2 showed significant increase. These findings suggest a vasoconstrictive effect mainly in HUV. Vasoconstriction and increased feto-placental impedance may be cause for altered hemodynamics especially in the HUV and may initiate structural changes in vasculature.[16] Nitric oxide (NO) is the main regulator of vascular tone and promotes angiogenesis. In IUGR, increased PIGF level and PIGF-2 in turn inhibited cell growth in endothelial cells of HUV. Endothelial NO dependent relaxation was reduced and it led to reduced internal diameter of HUV affected with IUGR. This explains poor angiogenisis in umbilical vessel of IUGR.[17,18]

Hemodynamic factors also play a role in structural remodeling of vessels. Continuous blood flow through the vessels regulates the vascular transwall pressure and maintains structural integrity of umbilical vessels.[19] Pathological flow velocity was associated with maldevelopment and reduction in number of terminal villi and may account for reduced feto-placental blood flow leading to hypoxia.[20] Electron microscope study of HUA showed degenerative changes of the endothelium exposed to low oxygen tension.[21] Researchers had

observed vascular hypoplasia in IUGR fetuses with pathological umbilical artery blood flow.[5] In vascular hypoplasia there is reduction in smooth muscles which leads to reduction of total vessel areas and luminal area along with reduction in vessel wall thickness.

Here we have observed statistically significant reduction in CSAs of umbilical vessels (except arterial lumen area) in IUGR compared to AGA full term neonates. Ratios of total vessel area to lumen area and vessel wall area to lumen area were increased. Reduction in lumen area was more obvious in HUV of IUGR neonates. HUV behaves like small caliber artery in terms of structural components and basic biochemical properties.[19,22] HUV conveys oxygen rich blood and regulates blood flow.[1] Therefore HUV structure and function influences fetal growth to large extent.

Research in basic science has profound influence on the practice of medicine. UC studies as an indicator of the health of developing fetus can be used as a predictor of fetal growth restriction. Presently UC examination is limited to counting number of vessels and umbilical artery flow velocity.[23] Findings of this study suggest that assessment of UC and its component especially CSA and blood flow of HUV by advanced USG in antenatal period may provide useful early information about pregnancy at risk and may help in averting poor perinatal outcome. Further prospective longitudinal and morphological studies are needed to elucidate the clinical importance of UC throughout the pregnancy.

CONCLUSION

IUGR is associated with significant structural changes in umbilical vessels of full term neonates. These changes are more obvious in HUV than human umbilical artery (HUA). It can be detected prenatally and used as an indicator of impending IUGR.

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