Image J Analysis of tunica media of Hyrtl's anastomosis in Pregnancy Induced Hypertension

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***Abstract*---Changes in the maternal hemodynamics often lead to conditions of fetal hypoxia, fetal hypertension and irregularity in the flow of blood in the umbilical arteries. Any differences in the blood flow is said to be equalized by Hyrtl's anastomosis, a common connection between the umbilical arteries near the cord insertion.**

Transverse histological sections followed by routine H&E stain and using Image J software, for the measurement of the muscle thickness and luminal diameter. For continuous variables mean values ±SD was considered and for non continuous variables was expressed as percentages.

On statistical analysis, P values were significant for the muscle thickness between the different groups (P≤0.001).

There was a significant difference in the tunica media of the umbilical arteries and the Hyrtl's anastomosis in both normotensive groups and pregnancy induced hypertension. In conclusion, the Hyrtl's anastomosis showed a considerable difference in the muscular layers as compared to the umbilical arteries.

Key words- umbilical arteries, umbilical cord, hyrtl's anastomosis , pregnancy induced hypertension, smooth muscle .

I. INTRODUCTION

The developing fetus is connected by a narrow tubular structure, the umbilical cord to the placenta. The umbilical cord contains the umbilical vessels surrounded by a gelatinous substance, the Wharton's jelly. Two umbilical arteries and a single umbilical vein lie inside the umbilical cord connecting the foetus's cardiovascular system with the placenta. These umbilical arteries are anastomosed with each other near the cord insertion of the placenta. This anastomosis is called the Hyrtl's anastomosis. This anastomosis is believed in equalizing the blood pressure in the umbilical arteries, supplying to the different territories of the placenta.

Various studies have registered Pregnancy Induced Hypertension as a complication of pregnancy.6-20% of all the pregnancies are affected by hypertensive disorders. 70% constitutes of preeclampsia and ecclampsia and 30% chronic hypertensive disorder in pregnancy [1].

Mitra associates a rise in the placental- uterine vascular resistance to pregnancy induced hypertension and also stating pregnancy induced hypertension as one of the important cause for low birth weight, perinatal mortality, premature birth, intrauterine growth limitations [2].

The morphological structure of the placenta and the cord vessels differences between normotensive and preeclamptic groups have been demonstrated in various studies by many authors [3-6].

Authors like Junek T et al. and Di Naro observed in normal pregnancies, the reduction in the thickness of the umbilical cords were mainly due to the reduction in Wharton's jelly [7,8].

Due to the changes in the arterial walls of the Preeclamptic gestation which maybe partly responsible for decreased flow of blood in the arteries,[9] ischemia and poor placentation [6] and depending on the rate of reduction in the uteroplacental perfusion , fetal hypoxia and intrauterine growth retardation can become unavoidable[10]. Several authors conducted the morphology of the umbilical cord and displayed the outcomes of pregnancies and its relationship with the thickness of the umbilical cord mostly after delivery.

Differences in the tunica media and intima, and the arteries being thicker than in normal pregnancies in pre-eclamptic groups have been demonstrated by Junek [7]. Author Ullberg et al. studied the histological section of the anastomosis and in comparisons with the umbilical arteries showed a thin layer of circular smooth muscle layer without any elastic tissues [11]. There is scarcity in the literature relating to morphometry of Hyrtl's anastomosis in Pregnancy Induced Hypertension and normotensive cases. The current study aimed to analyze and co-relate hyrtl's anastomosis with the morphometric parameters of the umbilical arteries in pregnancy induced hypertension and normal pregnancy using Image J software application. In the research studies

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the measurement of the thickness of layers is an important element and one's assessment in the thickness of the umbilical arteries and Hyrtl's anastomosis can reveal the differences in the tunica media contributing to the changes in the blood flow and thereby decrease in uteroplacental perfusion, leading to fetal hypoxia and intra uterine growth retardations.

II.METHODS AND MATERIALS

The study, obtained its approval from the Institution Ethics Committee (IEC No: SMIMS/IEC/2012-2) and the written consent of the patients. Patients with diabetes mellitus, chronic hypertension, platelet disorders were excluded.

The study sampled 100 umbilical arteries from pregnancy induced hypertension and 100 umbilical arteries from normal pregnancy.30 cases of Hyrtl's anastomosis of Pregnancy induced Hypertension and 30 cases of Hyrtl's anastomosis of normal pregnancy. The value of blood pressure taken into account for normotensive women; systolic were ranging between 100-135mmHg and diastolic 60-85mmHg and of Pregnancy Induced hypertension were systolic >140mmHg and diastolic 90mm Hg.

After delivery, the umbilical cords with the placentas were collected, keeping the whole length of the umbilical cord. The umbilical cords were then fixed in 10% formalin solution. Fixed umbilical cord were then dissected longitudinally, wharton's jelly removed and the umbilical arteries were traced all along the length of the cord insertion. The tissue were cleared and the anastomosis was then observed.

Umbilical arteries of about 2cm were cut from the placental end and only one artery from each placenta were taken. Samples of Hyrtl's anastomosis which were too small in length were difficult and almost impossible to take.

Tissues were dehydrated, cleared in xylene and embedded in paraffin and several blocks were prepared. Separated blocks for normal umbilical artery, for pregnancy induced hypertension for Hyrtl's anastomosis (normal and PIH) were made. Thin transverse sections of 5µm thickness were made by rotator microtome, deparaffinised and hydrated. Sections of the arteries and hyrtl's anastomosis (normal and PIH) were stained with haematoxylin and eosin. Sections were identified under the microscope (T1SM Phase Contrast Microscope) the microscope which was connected to the computer. The view from the microscope was captured by the camera with an eye piece of 10x and objective of 10x and projected to the computer screen. Measurements were taken using Image J software.

The parameters measured for each umbilical arteries and Hyrtl's anastomosis (normotensive and pregnancy induced hypertension) included wall thickness, luminal diameter. Wall thickness measurement expresses the smooth muscular layer only. The arteries in which the contours were well defined, visible and intact smooth muscle layers were only considered. Other alterations in the tunics were also observed.

III.STATISTICAL ANALYSIS

For continuous variables mean value ±SD was considered and for non continuous variables was expressed as percentages. One way analysis of variance (ANOVA) was considered in the analysis for continuous variable among two or more groups. $P \leq 0.05$ was considered to be significant. SPSS 16.0 was used for data analysis.

IV. RESULTS

Table 1: Showing the muscle thickness and luminal diameter between the normotensive umbilical artery and Pregnancy induced hypertensive umbilical artery.

SPECIMEN	MUSCLE THICKNESS	LUMEN DIAMETER
Normotensive Umbilical Artery		
Mean	0.33	0.44
standarddeviation	±0.12	±0.32
N	80	80
Median	0.29	0.34
PIH/case umbilical artery		
Mean	0.36	0.41
Standarddeviation	±0.105	±0.24
N	80	80
Median	0.35	0.36
F VALUE	4.19	0.49
P	0.042	0.484

1. Upon microscopic examination of muscle thickness and luminal diameter between 80 normotensive and 80 pregnancy – induced hypertensive umbilical arteries, we observed a significant increase in the muscle thickness layer in the pregnancy- induced hypertensive groups. (Table.1). In addition, we also noted that the luminal diameter in

both the groups were significantly not changed. (Table.1)

Table 2: Comparison between the normotensive umbilical artery and normotensive hyrtl's anastomosis.

SPECIMEN	MUSCLE THICKNESS	LUMEN DIAMETER
Normotensive Umbilical Artery		
Mean	0.322	0.45
standarddeviation	±0.12	±0.35
N	30	30
Median	0.29	0.35
Normal Hyrtl's Artery		
Mean	0.17	0.52
Standarddeviation	±0.91	±0.24
N	30	30
median	0.15	0.51
F - VALUE	29.966	0.718
P	≤0.001	0.40

2. As shown in Table 2, although muscle layers were found in hyrtl's anastomosis from umbilical cords of normotensive mother, the distribution of the muscle thickness varied between the umbilical arteries and hyrtl's anastomosis in the normotensive umbilical cords. (Fig.1 a, b) In the normal umbilical artery the muscular layers were about 20 to 25 and in few above 40 to 50 layers , in contrast the muscular layer in the hyrtl's anastomosis only 5 to 10 layers were found, a 1.3 fold difference. (Fig.1b) (Table:2) . Similarly, findings were observed in the luminal diameter in both groups and no significant difference was noted. (Table.2)
3. While comparing muscle thickness and luminal diameter in the umbilical arteries and Hyrtl's anastomosis of pregnancy-induced hypertensive mothers, we observed significant differences not only, in the muscular layer but also in the luminal diameter. (Fig. 2 c, d) (Table:3)

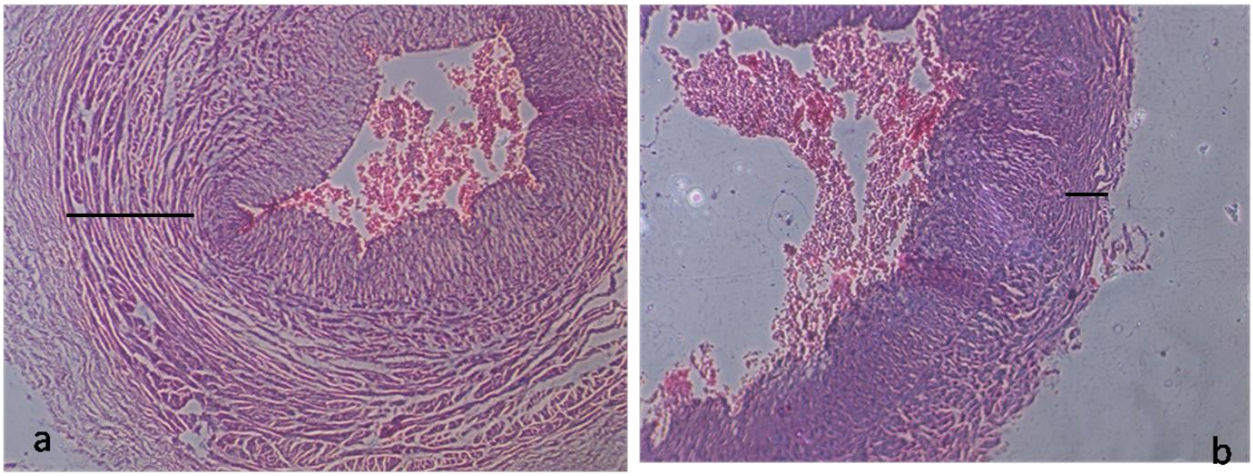

Fig 1 : a) Tranverse section of Normal umbilical artery b) Normal Hyrtl's anastomosis.

Table 3: Comparison between PIH umbilical artery and PIH Hyrtl's anastomosis.

SPECIMEN	MUSCLE THICKNESS (MM)	PIH LUMINAL DIAMETER
Normal Hyrtl		
Mean	0.17	0.22
N	30	30
std deviation	±0.091	±0.113
Median	0.14	0.197
PIH Hyrtl		
Mean	0.518	0.52
N	30	30
std deviation	±0.242	±0.29
Median	0.505	0.568
F-value	3.544	0.03
P	0.05	0.96

SPECIMEN	MUSCLE THICKNESS	LUMEN DIAMETER
PIH UmbilicalArtery		
Mean	0.35	0.38
standarddeviation	±0.97	±0.19
N	30	30
Median	0.35	0.36
PIH hyrtl's artery		
Mean	0.22	0.52
Standarddeviation	± 0.11	±0.29
N	30	30
median	0.19	0.56
F - VALUE	22.19	5.35
P	≤0.001	0.024

4. On overall comparison between the different categories a) normal umbilical arteries, b) normal Hyrtl's anastomosis, c) Pregnancy – induced hypertensive umbilical arteries, d) pregnancy –induced hypertensive Hyrtl's anastomosis, the muscular layer showed a significant difference between the groups, and the luminal diameter was not statistically significant.(Table:4).

Table 4: Comparisons between the normotensive umbilical artery, normotensive hyrtl's anastomosis ,PIH umbilical artery and PIH Hyrtl's anastomosis.

5. A significant difference of P=0.05 was observed in the muscular layer of hyrtl's anastomosis of pregnancy induced hypertensive and normotensive group.(Table : 5)

SPECIMEN	MUSCLE THICKNESS	LUMEN DIAMETER
Normotensive Umbilical artery		
Mean	0.322	0.45
standard deviation	±0.12	±0.35
N	30	30
Median	0.29	0.35
PIH UMBILICAL ARTERY		
Mean	0.35	0.38
Standard deviation	±0.97	±0.19
N	30	30
median	0.35	0.36
NORMAL HYRTL'S ARTERY		
Mean	0.17	0.52
Standard deviation	±0.91	±0.24
N	30	30
median	0.15	0.51
PIH HYRTL'S ARTERY		
Mean	0.22	0.52
Standard deviation	±0.11	±0.29
N	30	30
median	0.19	0.57
F –VALUE	18.83	1.89
P	≤0.001	0.136

Table 5: Showing the comparison between hyrtl's anastomosis of two groups

V. DISCUSSION

It has been demonstrated that the important causes of Intrauterine growth limitations, premature birth, low birth weight, perinatal mortality, the placental and vascular resistance are associated with pregnancy induced hypertension.[2]

Various studies in uterine growth limitation which are characterized by narrow cord and reduction in Wharton's jelly has been demonstrated by many

authors. The reduction in the vascular layer is mostly accompanied by significant structural disorder which have an impact upon the tunica intima, media and fibrillary structures. In case of pregnancy induced hypertension significant modification in the morphology was noted.

Often a reduction in the Wharton's jelly considered as a factor for the reduction in the diameter of the vessels mainly in the diameter of the umbilical cord [7, 12].

Several others were of the different opinion and reported diminished smooth muscle layer was due to the narrowing of the conjunctive tissue separating the muscular area [13,14].

Several authors reported changes in tunica media and intima of the umbilical arteries in preeclamptic groups.[7,13-16]

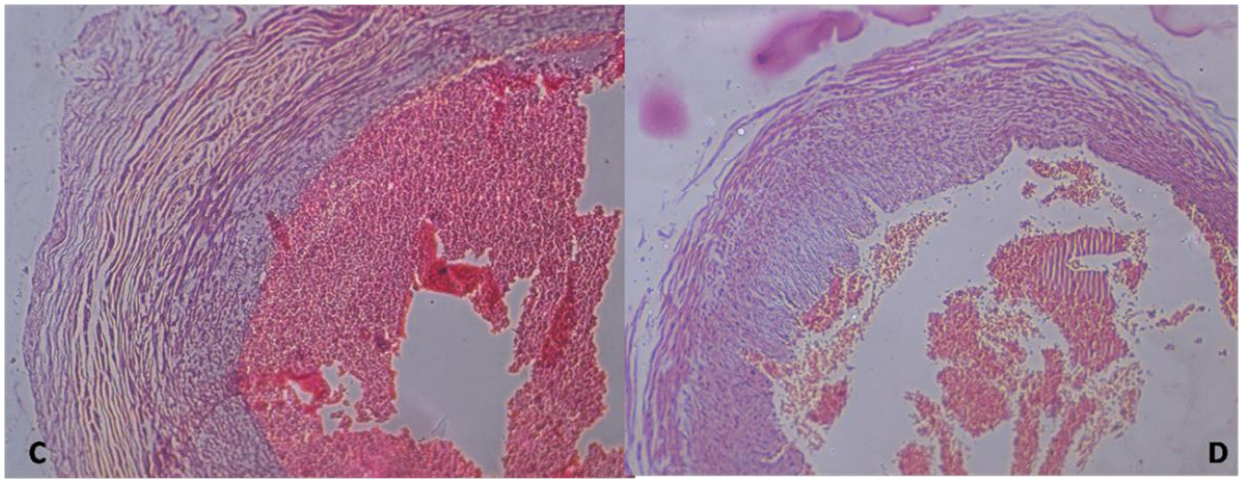

Fig 2 : c) Transverse section of Pregnancy induced hypertension umbilical artery
d) Pregnancy induced hypertensive Hyrtl's anastomosis

Similar to our study, Barnwal et al in their study reported a significant increase in the wall thickness of umbilical arteries ($p \leq 0.05$) and in pre-eclamptic the thickness of the arteries increasing by 20% as compared to the control groups [17].

Our study agreed to that of Barnwal were we observed an increase in the thickness of the muscular layer of the umbilical arteries of pregnancy –induced hypertension and also in the anastomosis of the pregnancy-induced hypertension when compared to the control.

Studies have been reported in the blood vessels having low calibre have differences in the blood flow and greater resistance in their vessel walls [18].

The increase in wall thickness in the umbilical arteries and decrease in luminal radius as explained by Barnwal might probably be due to the intrinsic contractile response of the smooth muscle to stretch and further increase in pressure of the blood vessel stimulates the increase in the smooth muscle fibers [17].

In our study might probably explain the thickness in umbilical arteries and the Hyrtl's anastomosis in pregnancy induced hypertension.

Several descriptions in the arrangement of the muscle fibers in the umbilical arteries as spiral or snail like are there but none for the Hyrtl's anastomosis [19, 20].

Others described the arrangement as nearly circular, smooth muscle layers arranged in 50-60 layers [2,22,23].

We observed a circular arrangement of the smooth cells in the umbilical arteries and except few areas where the layers were disrupted.

Results from our study was similar to Ulla Ullberg who studied a transverse histological section of hyrtl's anastomosis comparing to the connected arteries revealed a thin layer of circularly arranged smooth muscle cells without any elastic tissue [11].

Our study reports the absence of adventitia, vasa vasorum, Internal elastic lamina in the umbilical arteries as well as in hyrtl's anastomosis. These finding were in agreement with previous workers [22,23].

To conclude, umbilical arteries and Hyrtl's anastomosis in pregnancy- induced hypertension shows significant structural changes, including increase in wall thickness. Consequently, the analysis of these vessels maybe useful in detecting hemodynamic status of the foetus.

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REFERENCE

1. Zuspan, F.B, "New concepts in the understanding of hypertensive diseases during pregnancy. An overview" Clin Perinatol.1991, 18: 653-659.
2. Mitra C, Seshan SV, 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.
3. Las Heras J, Baskerville J C, Harding PG, et al, "Morphometric studies of fetal placental stem arteries in hypertensive disorders ('toxaemia') of pregnancy" Placenta.1985,6: 217-277.
4. Kaufmann P, Luckhardt M, Leiser R, " Three – dimensional presentation of the fetal vessel system in the human placenta" Res.1988,3: 113-137.
5. Johnstone FD, Ugaily-Thulesius L, Thulesius O, Nasrat AN, "Umbilical artery reactivity and ultrastructural changes in pregnancy induced hypertension and other complicated pregnancies" Clin Physiol.1987,7: 493-502.
6. Bruch J.F, Sibony O, Benali K, "Computerized microscope morphometry of umbilical vessels from pregnancies with intrauterine growth retardation and abnormal umbilical artery Doppler"Hum Pathol.1997, 28:1139-1145.
7. Junek T, Baum O, Lauter H, Vetter K, Matejevic D, Graf R, " Preeclampsia associated alterations of the elastic fibre system in umbilical cord vessels" Anat Embryol.2000,201: 291-303.
8. Di Naro, E Ghezzi, F Raio, L Franchi, M d'Addario V, " Umbilical cord morphology and pregnancy outcome" Eur J Obstet Gynecol Reprod Biol .2001, 96:150-157.
9. Bankowski E, Sobolewski K, Romanowicz L, Chyczewski L, Jaworski S, "Collagen and glycosaminoglycans of Wharton's jelly and there alterations in EPH-gestosis" Eur J Obstet Gynecol Reprod Biol .1996, 66: 109.
10. Roberts J M, Redman C W, " Pre-eclampsia; more than pregnancy induced hypertension" Lancet.1993, 341:1447-1451.
11. Ulla Ullberg, Gördan Lingman, Gunvor Ekman-Ordeber, Sandstedt B, " Hyrtl's anastomosis is normally developed in placentas from small for gestational age infants" Acta Obstet Gynecol Scand.2003,82: 716-721.
12. Romanovicz L, 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.
13. Dadak C, Ulrich W, Sinzinger H, "Morphological changes in the umbilical arteries ultrastructural study"Placenta.1984, 5: 419-426.
14. Cetin A, Kukner A, Ozturk F, "Ultrastructure of human umbilical vessels in pre-eclampsia" J Matern Fetal Neonatal Med.2002,12: 178-184.
15. Biagiotti R, Sgambati E, 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.
16. Morteza Tahmasebi and Reza Alighanbari, " Evaluation of umbilical cord thickness, cross-sectional area, and coiling index as predictor of pregnancy outcome" Indian J Radiol Imaging.2011, 21(3): 195-198.
17. Barnwal M, Rathi S K, Chhabra S, " Histomorphometry of umbilical cord and its vessels in Pre-eclampsia as compared to normal pregnancies" NJOG.2012, 7(1):28-31.
18. Predanci M, Perni SC, "Antenatal assessment of discordant umbilical arteries in singleton pregnancies" Croat Med J.2006, 47(5):701-708.
19. Herzog W.Die Rückbildung des Nabels und der Nabelgefässemit besonderer Berücksichtigung der Pathogenese der Nabelhernien. München,1892; . J.F .Lehmann.
20. Von Hayek, H Ztschr.f .Anat. U. Entwcklngsgesch .1935,.105: 15.
21. Mary Spivack, "The anatomic peculiarities of the human umbilical cord and their clinical significance" Am J Obst & Gyne. 1946, 52; 387-401.
22. Manuel V Blanco, Hilda R Vega, Rodolfo Giuliano, Daniel R Grana, Francisco Azzato, Jorge Lerman, Jose Milei, " Histomorphometry of Umbilical Cord Blood Vessels in Pre-eclampsia, The Journal of Clinical Hypertension . 2011, 13(1):30-34
23. Gebrane Younes J, Mink, H N, Orcel, L, " Ultrastructure of human umbilical vessels: a possible role in amniotic fluid formation" Placenta .1986,7: 173.

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