DIFFERENT ETIO-PATHOGENESIS IN INTRA UTERINE GROWTH RETARDATION IN DEVELOPING COUNTRY: HISTOMORPHOMETRIC EVIDENCE.

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ABSTRACT

The present study was conducted to compare histomorphometric parameters of umbilical cord and its vessels in intrauterine growth retarded fetuses and normally grown fetuses. The study was conducted on umbilical cords taken from thirty intrauterine growth retarded (IUGR) fetuses and thirty normally grown fetuses (NGA). Histomorphometric parameters were measured with the help of planimeter. In the present study it was found that in cross section the total umbilical cord area in IUGR group is 59.00+3.67 mm^2 and in the control group is 75.91+4.77mm^2 ($p<0.05$). Jelly area in IUGR group is 47.90 + 3.11 mm^2 and in control group is 60.26+ 4.55 mm2 ($p<0.05$). Total vessel area in control group is 15.45+1.73 mm^2 and in IUGR group is 10.95±1.20mm^2 ($p<0.05$). Total umbilical cord area, umbilical cord jelly area and total vessel area are significantly lower in the IUGR group but no significant difference was observed in total luminal area, total area of blood vessels and wall thickness of umbilical blood vessels. So in the study it was found that histomorphometric parameters of umbilical cord of IUGR fetuses are significantly lower than that of normally grown fetuses of similar gestational age.

KEY WORDS: IUGR, umbilical cord, umbilical artery, umbilical vein, Wharton's jelly.

INTRODUCTION

The birth weight of an infant is one of the most important determinant of its chances of survival, healthy growth and development. By International agreement low birth weight has been defined as a birth weight of less than 2.5 Kg (upto and including 2499 g); the measurement taken preferably within the first hour of life, before significant post natal weight loss has occurred[1]. Small for age (SGA) or small for date (SFD) babies are low birth weight babies whose birth weights are below 10th percentile for their gestational age[2]. It is estimated that from 3 to 10 percent of infants are growth restricted[3]. IUGR contributes specially to perinatal mortality and morbidity and an effective treatment of IUGR is not yet available[4].

The umbilical cord forms a vital link between fetus and mother. Considering its simple structure one may overlook its crucial function of modulating flood flow conveyance between the fetus and placenta. Umbilical cord characteristics such as cord length and thickness, number of vessels and their diameters, amount of Wharton's jelly, type of placental insertion, coiling and blood flow patterns are recognized contributors that may reveal increased risk for possible antenatal and perinatal complications.

It is important to study placentae and umbilical cords of IUGR babies to understand the pathophysiology of IUGR. Etiology of IUGR is multifactorial with causes related to mother, fetus and placenta. The different causes may be responsible for varying proportion of cases of IUGR in different populations and these causes may not be mutually exclusive. Thus a particular intervention may not have same effect in all populations. After extensive review of literature it was found that no study has been conducted in Indian population on histomorphometry of umbilical vessels in IUGR. This is first study conducted in Indian population in histomorphometry of umbilical vessels in IUGR.

MATERIAL AND METHODS:

Present study is a case-control study based on observations made on umbilical cords taken from subjects admitted to labour room in the department of Gynaecology and Obstetrics of Pt. B.D. Sharma, PGIMS, Rohtak. Sixty female subjects were separated into two groups. All subjects belonged to population of Haryana. Group I: the control group (n=30) with appropriate weight for gestational age;

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group II: IUGR (n=30). All the cases in group I and group II were full term vaginal delivery.

The criteria adopted for grouping of these cases were defined according to Usher and McLean5. Birth weight less than two standard deviations for gestation age was considered IUGR. Umbilical cords were taken only from subjects with gestational age of more than 36 weeks and less than 41 weeks to avoid confounding due to gestational age dependent modifications of morphometric parameters. Informed consent was taken from all participants. Patients with pregnancy induced hypertension, anaemia and other illnesses associated with pregnancy were excluded from study.

The placentae with umbilical cords were collected soon after delivery and were kept in ten percent formalin solution for 48 hours. Two pieces of umbilical cord for cross section were taken near placental insertion. After this pieces were routinely processed for paraffin embedding and sectioning. Sections from each block were then stained by haematoxylin and eosin. Morphometric analysis were performed after selection of best oriented (i.e. strictly transverse by visual observation) umbilical cord cross sections of two blocks to avoid tangential cross sections of cord and its vessels. Morphometric measurements were performed using a microprojector. Selected cross sections were projected at low power magnification on a paper sheet Outlines of following structures were drawn on a paper.

1. Circumference of umbilical cord
2. Outer circumference of vein
3. Inner circumference of vein
4. Outer circumference of artery-1
5. Inner circumference of artery-1
6. Outer circumference of artery-2
7. Inner circumference of artery-2

Length of these outlines was measured with the help of a planimeter. Three readings were measured and their mean was taken. Various parameters were calculated by the formulae given below:

1. $D=C/\pi$

Where C = Circumference and D is diameter.

2. $A = \pi D^2/4$.

Where A is area and D is diameter.

3. Thickness of vessel wall = (Outer diameter -Inner diameter)/2.

Sr. No.	Histomerphometric Parameter	Group-I Mean±SEM	Group-II Mean±SEM	P value
1	Total Umbilical Cord Area	75.91±4.77mm^2	59.00±3.67mm^2	<0.05
2	Jelly Area	60.26±4.55mm^2	47.90±3.11mm^2	<0.05
3	Total Vessel Area	15.45±1.73mm^2	10.95±1.20mm^2	<0.05
4	Total Vein Area	9.89±1.43 mm^2	6.37±1.04mm^2	>0.05
5	Total Luminal Area of Vein	7.46±1.40mm^2	5.10±1.12mm^2	>0.05
6	Wall Thickness of Vein	585.43±47.93µm	505.80±40.02µm	>0.05
7	Total Area of Artery-1	3.37±0.25 mm^2	2.82±0.29 mm^2	>0.05
8	Total Luminal Area of Artery-1	0.94±0.18 mm^2	0.93±0.24 mm^2	>0.05
9	Wall Thickness of Artery-1	1020.98±41.89µm	833.65±51.54µm	<0.01
10	Total area of Artery-2	2.39±0.24 mm^2	1.90±0.15 mm^2	>0.05
11	Total Luminal Area of Artery-2	0.67±0.19 mm^2	0.58±0.12 mm^2	>0.05
12	Wall thickness of artery-2	882.82±34.21µm	775.88±42.55µm	>0.05

Table-I : Comparison of Histomorphometric parameters in two groups.

Fig.1 - Artery 1 of umblical cord in control group

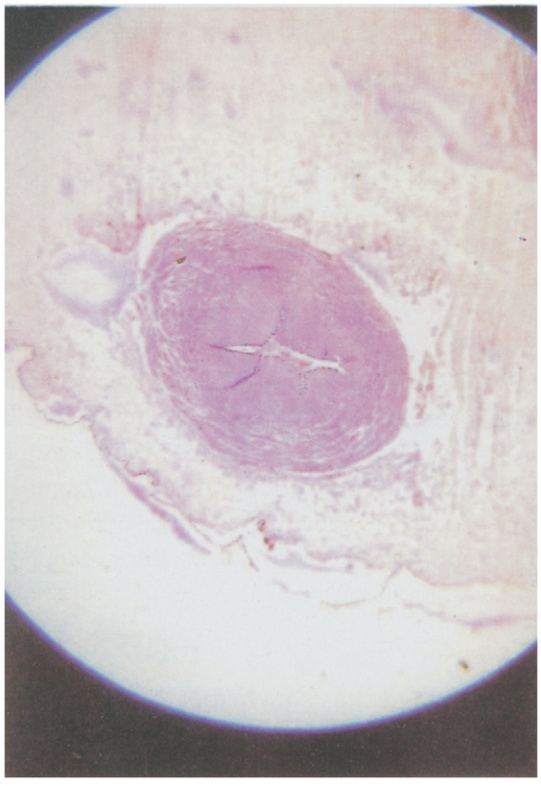

Fig.II - Artery 2 of umblical cord in control group

Fig.IV - Artery 1 of umblical cord in IUGR Group

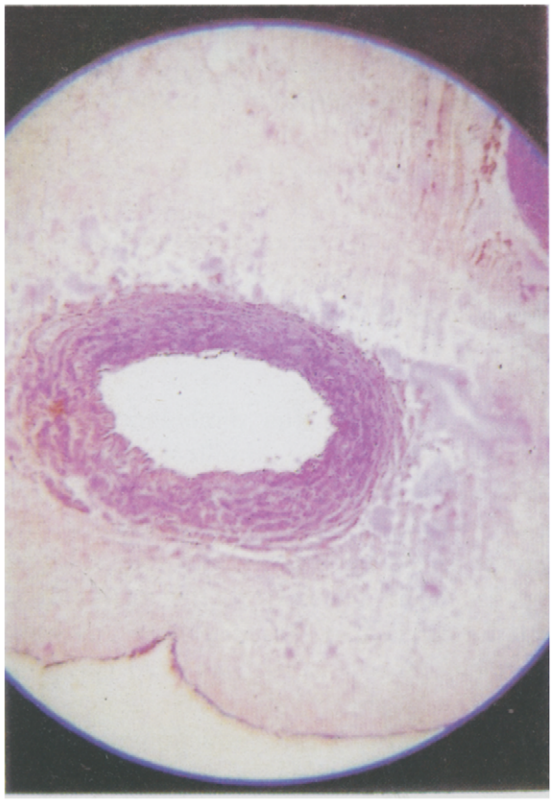

Fig.III - Vein of umblical cord in control group

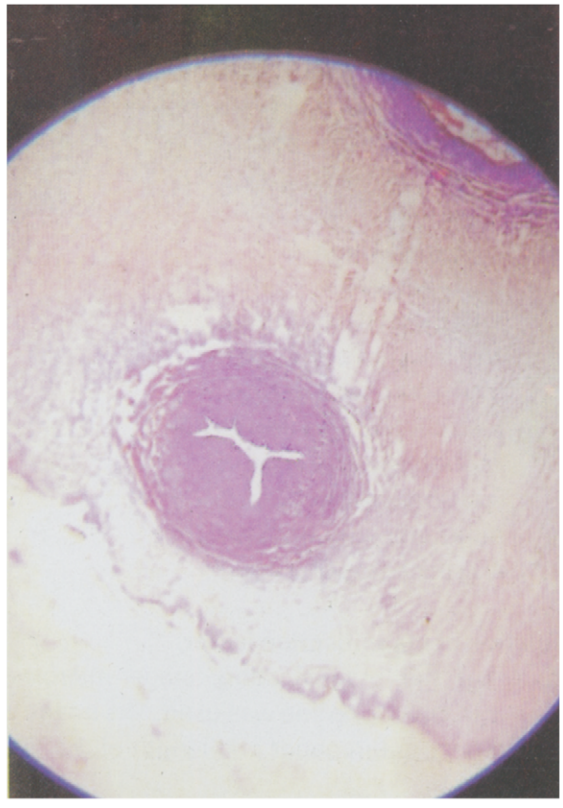

Fig.V - Artery 2 of umblical cord in IUGR group

Fig.VI - Vein of umblical cord in IUGR group

4. Jelly area = total umbilical cord area (Total area of artery 1 + Total area of artery 2 + Total area of vein) in cross section.
5. Total vessel area = (Total area of artery 1 + Total area of artery 2 + Total area of vein) in cross section.

From above data following parameters per section were calculated for each cord:

1. Total umbilical cord area
2. Jelly area
3. Total vessel area
4. Total vein area
5. Total luminal area of vein
6. Wall thickness of vein
7. Total area of artery-1
8. Total luminal area of artery-1
9. Wall thickness of artery-1
10. Total area of artery-2
11. Total luminal area of artery-2
12. Wall thickness of artery-2

Calculation of magnification factor:

To find out the magnification, stage micrometer marked with 2mm long line was used. The image was projected on a graph paper and length of the image was measured.

Length of stage micrometer scale = 2 mm

Length of projected image of stage micrometer scale = 68 mmData was statistically analyzed using student's t-test.

RESULTS:

Results of study are summarized in table I. We observed significant difference in some of cord parameters in two groups (Figures 1 to 6). Total umbilical cord area in IUGR group was 59.00 ± 3.67 mm2 and in control group was 75.91±4.77mm^2 ($p<0.05$). This reduction was related to both the Jelly Area and Total Vessel Area. Jelly area in control group was found to be 60.26 ± 4.55 mm^2 and in IUGR group 47.90±3.11mm^2 ($p<0.05$). Similarly Total Vessel Area in control group is 15.45±1.73mm^2 and in IUGR group is 10.95±1.20 mm^2 ($p<.05$). All other histomorphometric parameters were not found statistically significant.

DISCUSSION

This study has shown that the umbilical cord of IUGR fetuses have smaller total umbilical cord cross sectional area than those of appropriately grown fetuses. This finding is similar to the findings of previous ultrasonological and histological studies[6,7,8].

In western countries a number of anatomical studies have been conducted to investigate the umbilical cord structure in presence of fetal IUGR and hypertensive disorders during pregnancy[6,8]. It has been reported that umbilical artery Doppler parameters were characterized by a diminution of both the total vessel area and the Wharton jelly area in comparison with healthy fetuses6. These finding are in agreement with those reported by Inan et al8. In the present study we also found a significant diminution in total vessel area in addition to total umbilical cord area.

The Wharton's jelly area diminution leads to a macroscopically thin umbilical cord. At the microscopic level this is due to a decrease in extra cellular matrix components. Wharton's jelly is regarded as having a protective role towards the three vessels that are embedded in it[9]. It is likely that jelly reduction makes the cord more vulnerable to mechanical constrains such as cord compression and adversely affect flow of blood in umbilical vessels. A Wharton's jelly reduction has also been invoked as a possible cause of fetal death in the presence of single umbilical artery[10]. This reduction in Wharton's jelly area could be attributed to fetal starvation as a result of poor maternal nutrition.

Similar finding in umbilical cords of fetuses of pregnant women with hypertensive disorders when compared with healthy fetuses has been reported in another study recently[8].

Reduction of Total Umbilical Cord Area has been reported in IUGR group as compared to control group6. This may be due to the consequence of two distinct (but not mutually exclusive) mechanisms, Vasoconstriction or vascular hypoplasia. Vasoconstriction is linked to a shortening of muscle fibers in tunica media. It should give rise to a decrease of the luminal area and increase in mean wall thickness. In contrast, vascular hypoplasia is linked to a reduction of the muscle cell mass in the tunica media. It should give rise to a comparable reduction of both lumen area and mean vessel wall thickness. Interestingly the results in current study show that there is no significant reduction in total vein area of umbilical cord. This difference can be explained on the basis of different risk factor profile of IUGR in developing and developed countries[11,12]. Malnutrition of mother is most important risk factor for IUGR in developing countries whereas smoking is most important risk factor in developed countries. These different risk factors might be causing IUGR by different mechanisms.

It has been reported that proportion of lean umbilical cords (cross sectional area <10th percentile for gestational age) were significantly higher in IUGR fetuses as compared to AGA (Appropriate for Gestational Age) fetuses[13]. Present histological study supports these sonographic findings.

Reduction of blood flow in umbilical veins in IUGR has been observed in IUGR fetuses by in a study done in Italy[14]. Results of studies done in western countries can't be simply extrapolated to Indian population especially in a condition like IUGR where risk factor profile is quite different. Therefore such sonographic studies are required to be done in Indian populations also. Traditionally the prenatal assessment of umbilical cord is limited to the assessment of the number of vessels and to the evaluation of umbilical artery blood flow parameters. Morphological aspects of umbilical cord parameters have usually been studied by pathologists and retrospectively correlated with the perinatal outcome. The introduction of more sophisticated imaging techniques have offered the possibility to investigate the umbilical cord characteristic during fetal life from early to late gestation[15]. Nomograms of various umbilical cord components have been generated and allow the identification of lean or large umbilical cords, entities frequently associated with fetal growth abnormalities and diabetes[16].

Prenatal evaluation of umbilical cord is usually performed to assess the impedance of umbilical arteries to blood flow in fetuses with or at risk for growth and developmental abnormalities[17]. These studies have investigated sonographic morphologic and morphometric characteristics of the umbilical cord components in relation to fetal and maternal disease. None of these studies have been done in developing countries where problem of IUGR is largest and the etiology is different. It has been reported that lean umbilical cord on prenatal sonography poses a risk that the fetus will be small for gestational age at delivery and will have distress during labour[16]. Present study supports above study with histomorhpmetric evidence as the total cross sectional area in present study is significantly lower in IUGR group than in group with appropriately grown babies.

In other studies lean umbilical cords with reduced vein caliber and blood flow have been reported in intrauterine growth restricted fetuses with normal umbilical artery Doppler parameters[7,15]. The results of present study are different from these studies as no significant difference resulted in any of the histomorphometric parameters of vein including total area, total luminal area and wall thickness. It points to the need of sonographic studies on umbilical cord parameters in Indian population, as the prevention of low birth weight is a public health priority in many developing countries including India where condition is largely attributed to IUGR as compared to pre-maturity in developed countries.

CONCLUSION:

Present study has showed that histomorphometric parameters of umbilical cord and its vessels do not have same picture in India as in developed countries. Keeping this fact in view; further sonographic and pathological studies are required to be undertaken in India.The umbilical cord is crucially important link between fetus and mother, through very simple in structure while containing two arteries and one vein, it modulates blood flow conveyance between the fetus and placenta. Umbilical cord characteristics such as cord length and thickness, number of vessels and their diameters, amount of Wharton's jelly, type of placental insertion, coiling and blood flow patterns are recognized contributors that may

reveal increased risk for possible antenatal and perinatal complications. However during routine prenatal ultrasonography, only the number of umbilical cord vessels is recorded in majority of antenatal centres. Thereafter umbilical cord is inappropriately analyzed, except for umbilical artery blood flow patterns that are mainly used to further assess the well being of growth restricted fetuses. At present time, it appears prudent to exercise more detailed umbilical cord analysis including but not limited to vessel number, morphometric parameters of cord, during the fetal anatomical survey, because an abnormal presentation of these variables could be associated with adverse antenatal and prenatal events.

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