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Study of Placental Vascular Endothelial Growth Factor and Histopathology of Placenta in Healthy Mothers with Intrauterine Growth Restriction

Salil Digambar Barsode[1], Vivarna Gedala[2], Savita Mehendale[3]

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[1]Associate Professor [2]Junior Resident [3]Professor, Department of Obstetrics and Gynaecology, Bharati Vidyapeeth (Deemed to be University) Medical College, Dhankawadi, Pune, Maharashtra 411043, India.

Corresponding Author: Salil Digambar Barsode, Associate Professor, Department of Obstetrics and Gynaecology, Bharati Vidyapeeth (Deemed to be University) Medical College, Dhankawadi, Pune, Maharashtra 411043, India.

E-mail: barsodes@gmail.com

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Abstract

In developing countries like India the incidence of IUGR is accounting to 1/3rd live births every year.

Aim: To study histopathology and Vascular Endothelial Growth Factor levels of placenta of healthy mothers giving birth to Intrauterine growth restricted babies at term.

Materials and Methods: The study was conducted from August 2012 to August 2014 over a duration of two years. A total of 70 cases have been selected for present prospective observational study: 35 were study group mothers had delivered IUGR babies and 35 in control group with normal weight babies.

Results: The mean birth weight of babies at term from Group A was 2.297 kg and mean birth weight of babies from Group B was 2.796 kg. The difference in the mean birth weight of babies was statistically significant between the two groups (*p*-value <0.001). The average Ponderal index (PI) in Group A babies is 2.2 which is significantly low and in Group B babies it is 2.7 with statistical significance (*p*-value <0.001). Placental weight of babies born in study and control group had high statistical significance (*p*-value 0.000).

Conclusion: The structural abnormalities in the placentae of IUGR babies born at term is suggestive of placenta as the causative factor of IUGR. Co-relation of the VEGF levels with weights of the baby were estimated in both the groups and noticed that the VEGF levels were inversely related with the birth weight. In the IUGR babies the expression of VEGF in the placenta was found to be elevated than in the placenta of normal weight babies.

Keywords: Vascular Endothelial Growth Factor; Intrauterine growth restriction; Placental weight.

Introduction

Intrauterine Growth Restriction (IUGR) is defined as pathological restriction of fetal growth resulting in a fetus with birth weight below 10th percentile for that gestational age.[1]

It is the failure of the fetus to achieve his/her intrinsic growth potential, due to anatomical and or functional disorders and diseases in the feto–placental–maternal unit. IUGR is classified as: 1. Symmetric IUGR. 2. Asymmetric IUGR.

Symmetric IUGR: It is defined as Symmetrical IUGR if weight length and head circumference are proportionately low, which is usually indicative of a process originating early in pregnancy.[2]

Asymmetric IUGR: It is defined as Asymmetrical IUGR when brain sparing effect takes place and the head circumference is within normal limits. Intrauterine growth restriction (IUGR) is associated

with stillbirth, neonatal death, and perinatal morbidity as well as delayed effects including cerebral palsy (CP) and adult diseases.[3] IUGR may be caused by fetal, maternal or placental factors. These factors are usually multiple and overlapping. Many times, there may not be obvious maternal, fetal or placental factors responsible for IUGR. But there could be structural abnormalities demonstrated on histopathological examination of the placenta or there could be defective vasculogenesis or angiogenesis of the placenta.

Materials and Methods

The study was conducted from August 2012 to August 2014 over a duration of two years. Prospective observational study. Study was done in the Department of Obstetrics and Gynecology in collaboration with the Department of Pathology of a tertiary Medical College in Western Maharashtra. A total of 70 cases had been selected for present prospective observational study. Cases were divided into two groups: Group A — Study group, Group B — Control group.

Group A: 35 healthy mothers at term gestation (Gestational age 37 weeks or more) who have delivered IUGR babies (Birth weight <2.5 kg) were selected as study group.

Group B: 35 healthy mothers at term gestation who have delivered normal weight babies (Birth weight is 2.5 kg or more) were selected as control group.

Inclusion criteria: All healthy pregnant women who availed antenatal care at the hospital and were diagnosed to have IUGR and delivered at term gestation with babies having ponderal index less than 2.5 were included in study group. Healthy pregnant women who have attended the antenatal clinic and had delivered normal birth weight babies (above 2.5 kg) at term were included in the control Group for the present prospective study.

Exclusion criteria:

1. Mothers with low BMI.
2. Mothers with any medical complications such as chronic hypertension, severe anaemia, diabetes.
3. Women with high-risk pregnancies like preeclampsia, gestational diabetes, thyroid disorders, multifoetal pregnancies, oligohydramnios or polyhydramnios, anteparturm hemorrhage.
4. Preterm Deliveries.

Method of Collecting Data

During this study when the pregnant women were admitted in labor, detailed history of the patients were obtained. Thorough general, systemic and Obstetric examination was done. Events of labor were monitored. Immediately after delivery, baby was examined by neonatologist. Birth weight and length were noted. Apgar score after 1 minute and 5 minutes were noted. Thorough examination was done and ponderal index was calculated.

Ponderal index formula = Fetal weight in gm × 100/length in centimeter[3]

Babies having ponderal index less than 2.5 were categorized as IUGR babies and more than 2.5 as normal babies.

Informed written consent was obtained from the pregnant women who were willing to participate in the study.

The placentae of diagnozed cases of IUGR and control group were washed and weighed. Maternal and fetal surfaces of placentae were inspected, number of cotyledons, areas of any gross abnormality such as calcification, infarction, cord insertion site, number of umbilical cord vessels were noted. Then membranes were trimmed from the edges. Placenta was then cut into two halves: one half of it with cord is immersed in 10% formalin and sent to Department of Pathology for gross and histopathological examination and the other half of placenta was immersed in normal saline and was sent to for estimation of placental Vascular Endothelial Growth Factor (VEGF).

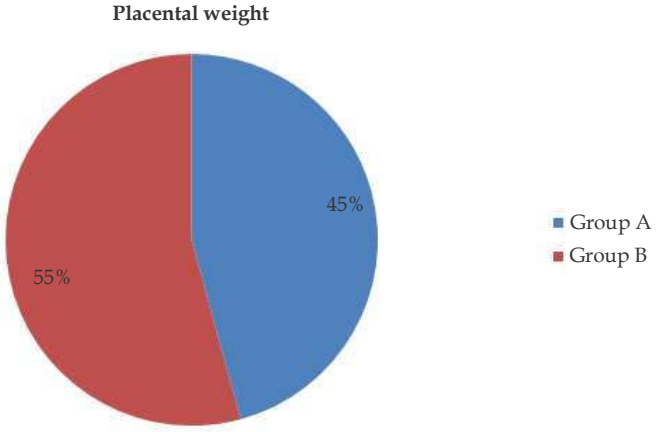
Results

Maximum cases in the study were between the ages of 25 and 27 years which were 12 in Group A and so was the case in Group B 14 cases. Distribution of maternal age did not have statistical significance (p-value >0.05). Distribution of parity did not differ significantly between the two groups (p-value >0.05 for both). The mean birth weight of babies at term from Group A was 2.297 kg and mean birth weight of babies from Group B was 2.796 kg. The difference in the mean birth weight of babies was statistically significant between the two groups (p-value <0.001) (Table 1). The mean placental weight from Group A was 417 gm and the mean placental weight from Group B was 498 gm and difference in the mean placental weight in the two groups was of statistical significance (p-value <0.000) (Graph 1). There is no

statistical significance in respect to mode of delivery and Apgar scores at 1 minute and 5 minutes in both the groups (Table 2). FTND (full-term normal delivery) was seen in both groups with 16 cases. FTVD (full-term vaginal delivery) was seen in 16 cases in Group A. LSCS was done in 2 cases in Group A and 3 cases in Group B respectively. VBAC in 1 Group A and 3 cases in Group B.

Table 1: Distribution of Mean Birth Weight Between Two Groups

Groups	Number	Mean birth weight (kg)	Standard deviation	F	*p*-value
Group A	35	2.2973	0.09325	83.876	0.001 (S)
Group B	35	2.7969	0.30894		



Graph 1: Mean placental weight in the two groups.

Table 2: Distribution of Mode of Delivery and Apgar Scores in the Two Groups

Mode of delivery	Group A	Group B	*p*-value
FTND (Full-term Normal Delivery)	16 (45.7)	13 (37.1)	
FTVD (Full-term Vaginal Delivery)	16 (45.7)	16 (45.7)	0.680 (NS)
LSCS	2 (5.7)	3 (8.6)	
VBAC	1 (2.9)	3 (8.6)	
APGAR score			
(1 min)	5 (14.3)	3 (8.6)	0.120 (NS)
>/= 7	29 (82.9)	26 (74.3)	
8	1 (2.9)	6 (17.1)	
9			
APGAR score			
(5 min)	1 (2.9)	0	0.547 (NS)
7	4 (11.4)	3 (8.6)	
8	30 (85.7)	32 (91.4)	
9			

The average Ponderal index (PI) in Group A babies is 2.2 which is significantly low and in Group B babies it is 2.7 with statistical significance (*p*-value <0.001). Incidence of NICU requirement did not differ significantly between two groups (*p*-value >0.05). Histopathological changes in placenta observed in Group A were ischemia in 10 cases, Dystrophic calcification in 9 cases accounting to statistical significance of (0.001) and the other changes like chorioamnionitis, chorangiosis, hyalinization were almost equal in both the groups. Normal histology was seen in only 5 cases among Group A subjects while 16 cases showed normal histology among Group B subjects (Table 3). Distribution of syncytial knots did not differ significantly between two groups (*p*-value >0.05) it was seen in 22 cases in Group A and 15 cases in Group B. The site of cord insertion among majority of Group A subjects was found to be eccentric (15/35) and among Group B subjects majority of them had central insertion of cord (23/35). It is statistically significant (*p*-value 0.006) (Table 4). Distribution of mean VEGF level is significantly higher in placentae of Group A compared to that of Group B subjects (*p*-value <0.05).

Table 3: Histopathological Changes in Two Groups

Hpe examination	Group A	Group B	Total	*p*-value
Chorioamnionitis	4	2	6	
Hyalinization	4	2	6	
Ishchemic changes	10	3	13	0.001
Normal histology	5	16	21	
Placental infarct	1	0	1	
Calcification	0	5	5	
Chorangiosis	1	1	2	
Dystrophic calcification	9	0	9	0.001(S)
Fibrinoid degeneration	4	2	6	
Fibrinoid necrosis	1	0	1	
Total	**35**	**35**	**70**	

Table 4: Umbilical Cord Insertion Site in the Two Groups

Cord insertion	Group A	Group B	Total	*p*-value
Central	9	23	32	
Eccentric	15	6	21	0.006 (S)
Marginal	9	6	15	
Vilamentous	2	0	2	
Total	**35**	**35**	**70**	

Mean VEGF level is elevated in Group A subjects with birth weight ranging from 2.1 to 2.3 kg than that of birth weight ranging between 2.3 and 2.5 kg. The mean VEGF level is seen elevated in Group A compared to Group B (*p*-value >0.05 for trend by ANOVA).

Discussion

IUGR is an important cause of perinatal morbidity and mortality. In developed countries, the incidence of IUGR is 3%, whereas in developing countries, it reaches 15–20%. It is one of the most commonly recognized abnormalities of the fetal condition and is a compounding factor in 26% or more of stillbirths.[4–6]

It is evident that both the groups had maximum number of women in the age group ranging from 22 to 27 years. This is statistically not significant.

A similar study carried out by Figen Barut[7] *et al.* in 2010 showed mean age of 27 yrs in Study Group and 29 yrs in Control Group similar to the results of this study. The present study did not show any co-relation between parity and IUGR. A study was carried out by Naoko Kozukil, Anne CC Lee *et al.* in 2013. It reported that majority of IUGR babies were born to women who were third para and above.[8]

In Group A mean birth weight was 2.297 kg and in Group B mean birth weight was 2.796 kg.

Group A had a mean placental weight of 417.7 ± 74.1 gm while Group B had 495.1 ± 93.3 gm and its mean placental weight had *p*-value (0.000) which was statistically highly significant. In the study conducted by Figen Barut *et al.* in 2010, the mean placental weight in study group was 404.6 ± 161.2 gm and the mean placental weight in the control group was 508.9 ± 404.6 gm which was highly statistically significant.

Nigam JS, Misra V *et al.* in 2014 conducted a case control histopathological study of placenta of low birth weight babies. They concluded that the mean age of mother in study group was 25.35 years and in control group was 24.75 years.[9]

Mean weight of fetus in the study group was 1.9 kg and that of control group was 2.8 kg.

Mean placental weight in the study group was 266.94 gm while it was 399.3 gm in the control group which was statistically significant.

Their results were similar with respect to maternal age, neonatal mean birth weight and placental weight to that of present prospective study. The average Ponderal index (PI) in Group A babies is 2.2 which is significantly low and in Group B babies it is 2.7 with statistical significance

(*p*-value <0.001). Low ponderal index could be used as a prognostic factor in predicting some morbidity in term neonates.[10] This index is independent of gender, race, birth order and to a certain extent, gestational age.

Placenta from IUGR pregnancies exhibit varying degrees of pathological lesions Salafia *et al.* 1995 had commented that it is not known to what extent placental function must be disrupted for IUGR to manifest.[11]

The fetus and the placenta share the same genetic makeup therefore are expected to have parallel growth potential.

Macpherson commented that one cannot assume that every adverse perinatal outcome is associated with an abnormal placenta or every abnormal placenta will have IUGR.[12] Total abnormalities in Group A were 30 in number while Group B showed only 19 abnormal placentae in our study. This difference was statistically significant. There was no statistical significance in the histopathological picture in placenta with respect to their birth weights in the two groups.

Syncytial knots were observed in 22 placentae in Group A and in 15 placentae in Group B. This difference was not of any statistical significance.

Mirchandani *et al.* reported syncytial knotting, observed that placentae were significantly smaller in premature IUGR babies. Syncytial knotting, trophoblastic basement membrane thickening, villous stromal fibrosis, fibrinoid necrosis, severe degree of intervillous fibrin deposition completely or partially filling up the intervillous spaces were noted by them in the placentae of IUGR fetuses.[13] Beebe *et al.* 1996 studied 1252 placentae of different categories, and concluded that histological evidence of placental ischemic changes and infarction were significantly increased in cases of IUGR as compared with normal babies. This corresponds to results of current study.[14] Marginal and velamentous insertion of cord are associated with IUGR.

Gediminas Meèëjus *et al.* (2005) in their study they had reported that cord insertion was eccentrical in 43%, marginal in 28%, central insertion 3%.[15]

In control group central insertion was 45%, eccentrical in 35%, marginal 16%, and velamentous was 4%. In group, Group A babies with birth weights ranging between 2100 and 2300 gm were 20 in number. They had mean VEGF value of 550.9 pg/ml. Group A babies with birth weights ranging between 2301 and 2500 gm were 19 in number. They had mean VEGF value of 509.7 pg/ml.

In Group B the average birth weight was 2.8 kg. Mean VEGF value of babies birth weights ranging between 2501 and 2800 gm was 450.6 pg/ml.

Figen Barut *et al.* in 2010 conducted a similar study aimed to investigate the role of placental angiogenesis in the development of intrauterine growth restriction (IUGR) by comparing the levels of expression of VEGF-A, b-FGF, and eNOS in normal-term pregnancy and IUGR placentae and they had concluded that it is necessary to explain the regulatory mechanism of placental vascular development in order to elucidate the pathogenesis of IUGR and the associated placental vascular insufficiency. The observed, increased expression of VEGF-A, b-FGF, and eNOS suggests that abnormal angiogenic activity, caused by insufficient uteroplacental perfusion, results in the pathophysiology of IUGR.

The difference between the mean birth weight of these babies showed statistical significance (*p*-value <0.001).

Figen barut *et al.* in 2010 also found in their study that the mean birth weight in normal pregnancy was 3041.5 ± 453.3 kg while the mean birth weight among the idiopathic IUGR group babies was 2023.9 ± 674.8 kg with statistical significance.

Mean birth weight of IUGR babies in this study is similar to their study but the mean birth weight from their control group was much higher than this prospective study.

At university of Otawa in 2014 a study was carried out in which the birth weight in IUGR group was of 2414 ± 457 gm and that of control group was 3082 ± 472 gm with statistical significance corresponding this prospective study. (*p*-value = <0.0001).[16]

The intrauterine existence of fetus is dependent on one vital organ "The Placenta".[17]

Conclusion

The structural abnormalities in the placentae of IUGR babies born at term is suggestive of placenta as the causative factor of IUGR. Abnormal placentation in early part of pregnancy characterized by shallow trophoblastic invasion results in malperfusion of the uteroplacental unit. Placental malperfusion leads to chronic ischemia, thus hypoxia of the placental tissue hampers the maternal-fetal exchange leading to IUGR. The binding of VEGF with VEGR 1 is essential for vasculogenesis in the

early pregnancy. Shallow trophoblastic invasion results in inadequate surface receptors (VEGR 1) for coupling. This results in elevated levels of VEGF levels in the IUGR placentae compared to the levels of VEGF in normal placentae.

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