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Role of Serum Levels of Inhibin A, Activin A and Leptin in Early Detection of Intrauterine Growth Restricted Pregnancy

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Abstract

Context: cross-sectional study. *Aims:* Estimation of the relationship of Serum levels of Inhibin A, Activin A and leptin in intrauterine growth retardation and appropriate for gestational age pregnancy *Settings and Design:*Cross-sectional study Total numbers of 100 pregnant women, were studied in department of Gynecology and Obstetrics in Postgraduate Institute of Medical Sciences and Research. Employee State Insurance Corporation, New Delhi. *Methods:* Hundred pregnant women, 50 with IUGR pregnancy were matched with 50 women with Appropriate for gestatational age (AGA) pregnancy. Inhibin A, activin A and leptin levels were measured in two samples. First sample was obtained by venous blood drawn from maternal peripheral circulation, which was collected before delivery, and second sample was taken from the umbilical vein after delivery. Data relating to delivery (e.g. mode, birth weight, signs of intrapartum fetal distress, and Apgar scores) were recorded. *Statistical Analysis Used:* Karl Pearson's correlation coefficient were calculated. Mann–Whitney U test was used for comparison between cases and controls.The data were entered into a multivariable linear regression model to allow a comparison between cases and controls subjects. Paired-Samples t-Test used to compare Ultrasound (USG) findings in cases and controls. The level of significance was set as p-value -£0.05. *Results:* Mean maternal inhibin A and activin A (0.57±1.50–0.08±0.04 ng/ml and 1.57±0.39–1.24±0.60 ng/ml respectively) and mean fetal inhibin A and activin A (1.304±2.92–0.13±0.06 and 1.43±0.58–1.31±0.36ng/ml respectively) concentrations were significantly higher in cases. However, a statistically significant correlation was not found between fetal leptin concentrations per kilogram of fetal weight and fetal activin A concentrations in cases (r=0.120; P>0.05). *Conclusions:* Inhibin A, activin A and leptin as serum markers in routine screening can detect IUGR

Keywords: Intrauterine Growth Restriction; Appropriate for Gestational Age; Inhibin A; Activin A; Leptin.

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Introduction

Intrauterine growth restriction (IUGR) is a composite condition for which definition has not reached a general agreement [1]. Normal fetal growth is dependent on several factors modulated by the fetus, the placenta and the mother. In IUGR pregnancies, cytotrophoblast invasion is limited with a restricted remodelling of spiral arteries, thus resulting in diminished uteroplacental perfusion [2]. The most common definition of IUGR is a birth weight lower than the 10th percentile for gestational age (GA) [1]. In the past years several molecules have been suggested as predictive markers of IUGR, including cytokines, neuropeptides, adhesion molecules, and glycoprotein's such like inhibin A and activin A [3]. However, limited data on maternal umbilical blood levels of inhibin, activin, and leptin exists. Hence, we designed a study to

determine maternal and umbilical blood levels in test group compared with controls.

Early detection of high-risk pregnancies for prevention of perinatal morbidity and mortality is a main goal of the obstetrician. IUGR is a frequent cause of perinatal morbidity as well as of impaired growth during childhood. IUGR can be lowered by timely detection and management of growth restricted fetuses. Estimation of the umbilical artery Doppler velocimetry gives information of the fetoplacental perfusion. Normally there is very minimal impedance against blood flowing through the umbilical arteries. As the placenta matures and the pregnancy advances, more tertiary villi is developed, which directly causes a rise in the end diastolic flow. Umbilical artery Doppler reflects downstream placental vascular resistance, which strongly correlates with IUGR [4]. Abnormalities in the umbilical artery waveforms are progressive with decrease, absent, and finally reversal of the diastolic flow. Reversed flow is associated with high incidence of perinatal and overall mortality and severe IUGR compared to absent end diastolic flow [5].

Inhibins and activins are members of the transforming growth factor b (TGFb) superfamily of growth and differentiation factors. Inhibins are heterodimers containing distantly related A and B (Bα or Bβ) subunits, while activins are â-subunit homodimers [6]. Leptin is produced by the human obesity gene. It controls appetite and body weight , and there are many reports that indicate the correlation of fetalleptin and intrauterine growth [7-11].

Human placenta and fetal membranes secrete considerable amounts of inhibin A and activin A in raising levels in maternal blood and umbilical blood, and their secretion alters in the presence of gestational diseases [12]. Consequently, their estimation may assume congruity with respect to a conjectural clinical application in the diagnosis, prevention, prognosis, and follow-up of different gestational pathologies [12]. Much of the incidence related to morbidity and mortality rates perinatally and in childhood is due to hypoxia and acidosis that was caused by abnormal placental function which leads to IUGR, whereas problems in later life are more likely to have underlying endocrine mechanisms perhaps because of abnormalities of placental transport of nutrients. Inspite of improving modalities of diagnosis and classification of the IUGR fetus , there is still limited knowledge of the underlying pathologic features of this condition. Current obstetric treatment of these fetuses is therefore limited to fetal surveillance and ultrasound (USG) colour Doppler with timing of delivery when necessary. The rationale for this research is for clinical application in the diagnosis of IUGR.

Subjects and Methods

The study was conducted in Department of Obstetrics and Gynaecology, Employee State Insurance Corporation, Post Graduate Institute of Medical Sciences and Research, Basaidarapur, New Delhi. Fifty women with a pregnancy with IUGR were included in the study. In all cases, gestational age was confirmed by ultrasound examination performed in the first trimester. Fetal growth restriction was diagnosed by antenatal estimation of fetal weight using ultrasound, and serial weight estimation was performed by evaluating fetal biparietal diameter, head circumference, abdominal circumference, and femur length. Fetal weight estimated and actual birth weights were below the 10^{th} percentile for gestational age in all cases. Multiple pregnancies or pregnancies with fetal chromosomal abnormalities and congenital malformations, severe anemia, severe malnutrition, severe respiratory disease, smoking, alcohol ingestion, thyroid disorders and cardiac disease were criteria of exclusion. The control group consisted of 50 women who had uncomplicated pregnancies, were delivered of infants of appropriate growth for their gestational age (AGA), and matched the study group with respect to pre pregnancy body mass index (BMI) and gestational week at delivery. The route of delivery was also taken into account while matching controls and study subjects to eliminate its effect on the studied variables.

Blood samples were collected at birth. The umbilical cord was clamped and 1 ml of umbilical arterial blood was collected into a heparinized syringe for automated blood gas determination (pH, PO2, and PCO2) immediately after delivery. Umbilical vein and maternal peripheral venous blood samples (5 ml) were also collected into plain vacutainer tubes for inhibinA, activin A and leptin assays. Samples were transported on ice and centrifuged at 3000 revolutions per minute and 4 °C for 10 min, and the plasma was stored at -20 °C until assay. Serum of activin A, inhibin A and leptin levels were measured by ELISA technique in two sittings.Standard curves were plotted for all the tests using multipoint calibration and values were calculated from the standard curve. Data relating to obstetric complications, delivery (mode, birth weight, signs of intrapartumfetal distress, and Apgar scores) were also recorded. Data are expressed as mean±S.D. data was obtained by

taking a detailed history regarding gravidity, obstetric history, demographic features was taken. Menstrual and obstetrics history was obtained, personal history including cigarette smoking, alcohol consumption, diet and history of any drug intake was taken. All the women were clinically evaluated including signs of IUGR (decrease fundal height, decrease abdominal circumference, serial weight measurement of pregnant women), including general, physical, systemic and antenatal examination. At the time of delivery clinical evaluation regarding recording of period of gestation at delivery, mode of delivery, birth weight of baby was recorded, Apgar score at 1 and 5 min of birth was also recorded.

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Statistical analysis of the data was performed with the software package SPSS for Windows 7.0 (Statistical Package for Social Sciences; SPSS Inc., Chicago, IL). Correlation analysis was performed using the Pearson correlation. Means between groups were compared using the t and Mann–Whitney U-tests. Multiple linear regression analysis was done to look for associations between different independent clinical parameters and elevated maternal or fetalinhibin A, activin A concentrations ($P<0.05$ was considered statistically significant).

Institutional Ethical Clearance and Informed consent was taken prior to study. The Study Period was approximately for 2 years, from October 2012 to March 2014.

Results

Demographic, obstetric, and neonatal data of the two groups are summarized in Table 1. There were no differences in mean maternal age and gestational duration at delivery between the groups. Mean birth weight was significantly lower in the study group than in the control group matched for gestational week at delivery. Mean BMI was significantly lower in the study group than in the control group. The modes of delivery were not significantly different in the two groups ($P>0.05$, x^2-test).

The mean maternal Serum of activinA, inhibin A and leptin levels of the two groups are shown in Table 2. Maternal serum inhibin A, activin A concentrations were increased significantly in cases compared with controls ($P<0.05$). Maternal serum Leptin concentrations was significantly decreased in cases compared with controls ($P<0.05$).The mean fetal Serum of activin A, inhibin A and leptin levels of the two groups are shown in Table 3. Fetal serum inhibin A, activin A concentrations were increased significantly in cases compared with controls ($P<0.05$). Fetal serum Leptin concentrations were significantly decreased in cases compared with controls ($P<0.05$).However, the leptin per kilogram of fetal weight (fetalleptin/kg) ratio in the study group

Table 1: Demographic, obstetric, and neonatal data for the groups

	IUGR (n=50)	Control (n=50)	P value
Maternal age, years[a]	25.6± 4.44	26.1±3.82	>0.05
Prepregnancy BMI[b]	20.7±2.05	24.5±2.37	<0.05
Gestational age, weeks[a]	37.5±2.03	39.3±1.16	>0.05
Birth weight, kgs[a]	1.95±0.32	3.07±0.38	<0.05
Cesarean delivery	12(24%)	10(20%)	>0.05
IOL[c]	14(28%)	12(24%)	>0.05
Apgar score	8.5±0.88	9.1±0.24	>0.05
Cord pH[a]	7.32±0.20	7.36±0.30	>0.05

[a]Mean±S.D.
[b] Body mass index.
[c] Induction of labor

Table 2: Umbilical vein and Maternal serum activinA, inhibin A, and leptin for the two studies groups

Umbilical vein serum activin A, inhibin A, and leptin for the two study groups.			
Umbilical vein	**[a] IUGR (n=50)**	**Control (n=50)**	***p-value**
Serum Activin A (ng/ml)	1.43±0.58	1.31±0.36	<0.05
Serum Inhibin A (ng/ml)	1.304±2.92	0.13±0.06	<0.05
Serum Leptin (ng/ml)	9.16± 8.37	17.90±16.66	<0.05

[a]Mean±S.D.,*Difference between the two studies groups, by Mann–Whitney U test

Table 3: Maternal serum activinA, inhibin A, and leptin for the two studies groups

Maternal serum activin A, inhibin A, and leptin for the two studies groups			
Maternal Serum	**[a] IUGR (n=50)**	**Control (n=50)**	***p-value**
Serum Activin A (ng/ml)	1.57±0.39	1.24±0.60	<0.05
Serum Inhibin A (ng/ml)	0.57±1.50	0.08±0.04	<0.05
Serum Leptin (ng/ml)	33.50±28.99	54.57± 29.83	<0.05

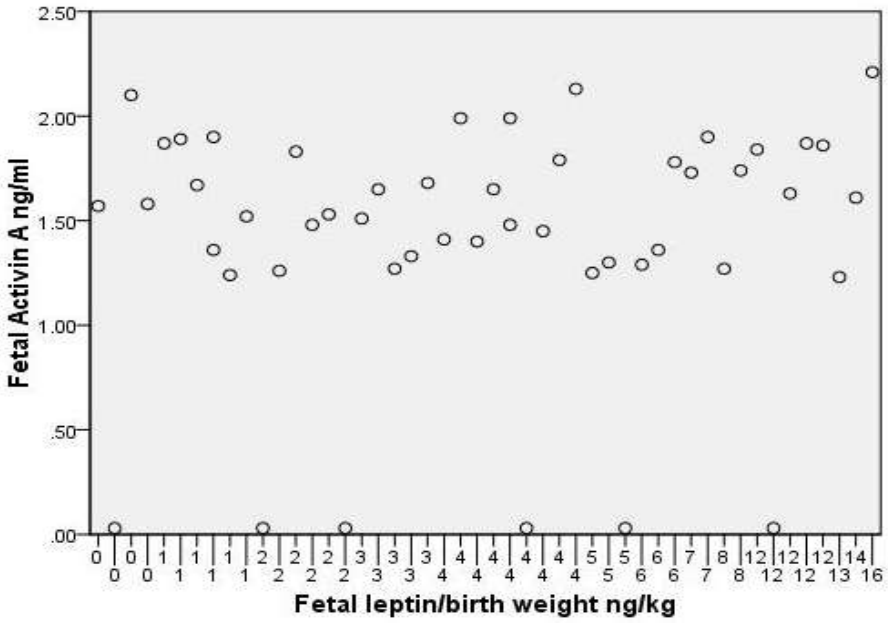

Fig. 1: Scatter of fetal activin A and leptin levels per kilogram fetal weight in pregnancies complicated with intrauterine growth restriction (r=0.120, P>0.05).

(4.74±4.05 ng/ml) was not significantly different from that in the control group (5.7654±5.48 ng/ml) (P>0.05, paired t-test).However, a statistically significant correlation was not found between fetalleptin concentrations per kilogram of fetal weight and fetalactivin A concentrations in pregnancies complicated with IUGR (r=0.120; P>0.05) [Figure 1]. Maternal and fetalactivin A, inhibin A and leptin levels were not affected by the mode of delivery (P>0.05, multiple linear regression analysis).

When all pregnancies were taken into account, there was no significant correlation between fetal and maternal activinA, inhibin A and leptin levels.

Discussion

In our study maternal serum inhibin A, activin A

concentrations were significantly increased in cases group compared with controls There were liner regression coefficients of 0.5250 and 0.4549 for activin A and inhibin A respectively, p value was 0.00056 and 0.0271 for activin A and inhibin A respectively. In our study umbilical vein serum inhibin A, activin A concentrations were significantly increased in cases group compared with controls. There were linear regression coefficients 0.6694 and 0.5147 for activin A and inhibin A respectively and the p value being <0.003 and 0.0003 for activin A and inhibin A respectively. Similar supportive data were reported by two studies [12,13]. Regarding Maternal serum levels of Leptin, difference between cases and controls, in our study maternal serum Leptin concentrations were significantly decreased in cases group compared with controls. There were liner regression coefficients of 0.8369 for Leptin and p value being 0.00048.

In present study umbilical vein serum Leptin concentrations were significantly decreased in cases group compared with controls. Linear regression coefficients was 0.9516 for Leptin in umbilical vein with p value being 0.00236. Similar supportive data were reported by ArslanM. et al.2004 who found mean fetal leptin concentration to be significantly lower in pregnancies complicated with IUGR [14] similarly in another study Mean fetal leptin level was found to be significantly lower in IUGR pregnancies as compared to control [15]. In 2009 Yildiz L et al. Found Leptin concentrations to be significantly lower in newborns with IUGR and in their mothers. In one study by Karowicz-Bilińska A. in 2004 it was found that in group of normal pregnancy leptin concentration was 5.347±1.098micrograms/l and in group of intrauterine growth restriction leptin concentration was 4.617±0.949 micrograms/l. The difference between both groups was statistically significant but the mean values in both groups were normal for non pregnant women [16]. Elevated serum activin A concentrations were recently reported in patients with preeclampsia plus intrauterine growth restriction, similar to those with preeclampsia only [17]. Conflicting results have also been reported from leptin studies that compare pregnancies complicated with IUGR and normal pregnancies. Shekhawat et al. [18] detected increased leptin levels in IUGR newborns compared with birth weight-matched AGA infants. In addition, in twin pairs, the smaller twin had a higher leptin level than the larger twin.

However, most studies in the literature have been in agreement that fetalleptin levels are lower in small for gestational age (SGA) newborns and in newborns with IUGR than in AGA newborns [11, 19–21]. Consistent with the result of present study, Cetin et al. [11] found lower leptin levels in newborns with IUGR compared with AGA newborns, but there were no differences between these groups regarding the fetalleptin/kg ratio. The same finding was reported by two more studies in IUGR newborns at term [22,23]. Previous studies have reported elevated maternal leptin levels in pre-eclampsia [23,24]. It has been demonstrated by Lea et al. that placental leptin is expressed by the syncytiotrophoblast in contact with maternal blood and in the vascular endothelial cells in contact with fetal blood [25]. Placental leptin may therefore be released into both the maternal and fetal compartments. Leptin release by vascular endothelial cells may act directly on the fetus. McCarty et al. found a positive correlation between maternal and fetalleptin levels for women with pre-eclampsia but not for healthy pregnant women [23]. It has been explained by a non communicating, two-compartment model of fetoplacentalleptin regulation in normal pregnancies and its possible disorganization in pre-eclampsia, resulting in communication between the two compartments. In our study we did not find any correlation between maternal and fetalleptin levels in either study or control groups. This finding also supports the two-compartment model for leptin regulation in pregnancy. Recent study in 2015 found mean cord leptin levels to be lower in growth restricted [25]. During the decade, the studies conducted on inhibin A, Activin A, and leptin suggested their possible involvement in the pathogenesis of IUGR [24]. Whether their altered secretion is the cause or simply reflects the placental problems is still far to be assessed; however, it has been assumed that the local changes in inhibin A, activin A, and leptin processing throughout gestation may be important not only in the paracrine control of the feto maternal communication required to maintain pregnancy, but also as specific markers of a derangement of that function. Indeed, the measurement of these proteins in maternal and fetal serum will offer new possibilities in the early diagnosis, prediction, monitoring and management of IUGR. It is not known why these analytes levels are change or whether they contribute to the cause of this disease. The present study strengthens the evidence of using inhibinA, activin A and leptin as serum markers in routine screening for early diagnosis which help in timely monitoring and management of IUGR which will still help in prevention of perinatal morbidity and mortality. But, large prospective studies are needed to further determine their role in clinical practice.

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