THE JOURNAL OF OBSTETRICS AND GYNECOLOGY OF INDIA

The Journal of Obstetrics and Gynecology of India (January–February 2013) 63(1):26–31
DOI 10.1007/s13224-012-0190-4

ORIGINAL ARTICLE

Umbilical Vein and Maternal Serum Inhibin A, Activin A, and Follistatin Concentrations in IUGR due to Placental Dysfunction Pregnancies

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Received: 1 June 2011 / Accepted: 23 April 2012 / Published online: 16 April 2013

Abstract
Objective The objective of this study were to (1) quantify the concentrations of inhibin A, activin A, and follistatin in maternal serum and umbilical vein (inhibin A, activin A) in IUGR due to placental dysfunction pregnancies and control group, (2) determine the concentration differences of these factors in maternal and umbilical vein serum in control and subject group, and (3) examine the relationship between fetal growth and placental function.
Method Sandwich ELISA was used to measure the concentrations in control ($n = 40$) and subject groups ($n = 30$).
Results Umbilical vein serum inhibin A, activin A concentrations were increased in subject group compared with controls (inhibin A regression coefficient, 0.7647, $P < 0.001$, activin A $P < 0.0005$). Maternal serum inhibin A, activin A were significantly increased in subject group compared with controls (inhibin A regression coefficient, 0.7614, $P < 0.001$, activin A $P < 0.0005$). Maternal serum activin: follistatin ratio was significantly increased in subject group compared with controls ($P < 0.0005$). Maternal serum inhibin A, activin A concentrations were more when compared to the umbilical vein inhibin A, activin A concentrations in subject group.
Conclusion The present study strengthens the evidence of using inhibin A, activin A, and follistatin as serum markers in routine screening for early detection of IUGR. But large prospective studies are needed to further define their role in clinical practice.

Keywords Inhibin A · Activin A · Follistatin · IUGR · Umbililical vein serum · Maternal serum

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Introduction

IUGR is a complex condition for which definition has not reached a consensus. From a pathological point of view, IUGR is characterized by a disrupted fetal growth and should not be confounded with low birth weight, which encompasses preterm infants with normal development, or even with small-for-gestational-age fetuses [1].

Normal fetal growth depends on several factors modulated by the fetus, the placenta and the mother. In IUGR pregnancies, cytotrophoblast invasion is restricted with a limited remodeling of spiral arteries, thus resulting in reduced uteroplacental perfusion [2]. The most common definition of IUGR is a birth weight lower than the 10th percentile when adjusted to gestational age (GA). 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 follistatin (FS) exists. Hence, we designed a study to determine maternal (inhibin, activin, and follistatin) and umbilical (inhibin, activin) blood levels in test group compared with control.

Identification of the pregnancies at risk for preventable perinatal morbidity and mortality is a primary goal of the obstetric care provider. IUGR is associated with significant morbidity. This can be lowered by timely identification and management of growth restricted fetuses. Assessment of the umbilical artery Doppler velocimetry provides information on the blood perfusion of the fetoplacental unit. Normally there is very little impedance against blood flowing through the umbilical arteries. As the placenta matures and the pregnancy advances, more tertiary villi is formed, which directly leads to an increase in the end diastolic flow. Umbilical artery Doppler reflects downstream placental vascular resistance, strongly correlated with IUGR and the multisystem effects of placental deficiency [4]. Abnormalities in the umbilical artery waveforms are progressive with reduction, loss, 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 β (TGFβ) superfamily of growth and differentiation factors. Inhibins are heterodimers containing distantly related α and β (β_A or β_B) subunits, while activins are β-subunit homodimers. FS is a structurally unrelated monomeric protein with several alternatively spliced molecular forms (designated FS288 and FS315) that irreversibly bind activin and neutralize activin's biological effects [6].

Human placenta and fetal membranes produce considerable amounts of inhibin A, activin A, and FS in increasing levels in maternal blood and umbilical blood, and their secretion changes in the presence of gestational diseases. Consequently, their measurement may assume relevance with respect to a putative clinical application in the diagnosis, prevention, prognosis, and follow-up of different gestational pathologies [7]. Much of the increased 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 [7]. Although detection and classification of the IUGR fetus is improving, there is still limited understanding of the underlying pathologic features of this condition. Current obstetric treatment of these fetuses is therefore limited to close observation and investigation with well-timed delivery when necessary. The rationale for this research is for clinical application in the diagnosis of IUGR.

Materials and Methods

Written informed consent was obtained from each pregnant woman and the permission of the local human investigation committee was granted for the study.

Subjects

The study population consisted of 30 women, age range between 25 and 37 years, BMI 23–30 kg/m^2.

Inclusion criteria were (1) singleton pregnancies, (2) clinically diagnosed IUGR, (3) confirmed subsequently on ultrasound when the fetal abdominal circumference was less then 2SD (standard deviation) from mean value, and (4) placental dysfunction was considered in pregnancies with umbilical artery Doppler S/D ratio $\geq$3 or those with absent diastolic or reversed diastolic flow, above the GA of 28 weeks.

Exclusion criteria were other causes of IUGR like (1) preeclampsia, (2) chronic hypertension, (3) chronic renal disease, (4) connective tissue disease, (5) diabetes with vascular lesion, (6) sickle cell anemia, (7) cardiac disease, (8) multiple gestations, (9) severe malnutrition, (10) smoking, (11) alcohol ingestion, (12) haemoglobinopathies, (13) infections, and (14) placenta previa. A detailed anomaly scan was performed on all fetuses and dysmorphic fetuses were also excluded from the study.

These women either attended the antenatal clinics at Department of Obstetrics and Gynecology, First Affiliated Hospital of Soochow University, Suzhou, China or were referred from the peripheral hospitals in view of IUGR over a period of 1 year (2009–2010). GA was determined from the first day of the last menstrual period and confirmed by ultrasound measurement of the fetal crown–rump length (CRL) in ongoing pregnancies.

Outcome data was collected including GA at birth, birth weight, apgar scores. Out of the total IUGR babies 99 % had live birth, 1 % were still born. Average GA at delivery was 34.2 weeks and 54 % of the IUGR babies were delivered by cesarean section. Average birth weight of these IUGR babies was 1900 g. Average S/D ratio ($n = 28$) was 3.82, ($n = 2$) fetuses had absent end diastolic velocity. The mean PI in the low end diastolic flow group was 1.345 and that in the absent end diastolic flow group was 3.14.

Controls

The control population was comprised of 40 women, age range between 26 and 33 years; BMI range between 20–24 kg/m^2.

Inclusion Criteria

(1) Singleton gestation, (2) accurate dating based on LMP and calculated GA by CRL measurement, and (3) having a term delivery with normal birth weight fetus.

Exclusion Criteria

All pathological conditions of pregnancy.

Study Design

Collection of Mother Blood

Five milliliters of blood sample was collected from each subject into a nonheparinized, serum separator tube (SST) containing clot activator and serum separator gel. The blood was allowed to clot. The samples were then centrifuged at 3,500 rpm for 10 min. The sera were separated and stored at 80 °C until assayed.

Collection of Umbilical Vein Blood

After delivery of the baby, the cord was clamped and cut, then 5 ml of blood sample was collected from the umbilical vein into the nonheparinized, SST tube before expulsion of the placenta. The blood was allowed to clot. The samples were then centrifuged at 3,500 rpm for 10 min. The sera were separated and stored at 80 °C until assayed.

Inhibin A, activin A, and FS were measured in duplicate using a commercial sandwich enzyme-linked immunosorbent assay (ELISA) kit (Diagnostic System Laboratories, suzhou, china) according to the manufacturers' protocol.

Statistical Analysis

Statistical analysis was performed with SPSS software package version 11.0 (SPSS, Chicago, IL, USA). Median and range (minimum–maximum) were used for maternal characteristic. Mann–Whitney *U* test was used for comparison between test and control group. To allow a comparison between inhibin A levels in IUGR and control subjects inhibin A and maternal age or maternal BMI was examined by linear regression. Log10-transformed data for inhibin A were entered into a multivariable linear regression model to allow a comparison between IUGR and control subjects that was adjusted for maternal BMI and maternal age results which are expressed as regression coefficients. Statistical significance was assumed with a value of $p < 0.05$ (Table 1).

Table 1 Demographics: clinical characteristics

Study group	Control subjects[a] ($n = 40$)	IUGR[a] ($n = 30$)	*P* value*
Maternal age (years)	30 (26, 34)	31.5 (25, 37)	0.123
Maternal BMI (kg/m^2)	22 (20, 24)	25 (23, 30)	<0.0005

[a] Data are median and interquartile range

* Differences between IUGR and control subjects, by Mann–Whitney *U* test

Results

Inhibin A, Activin A and A:F Ratio in Maternal Serum

Inhibin A was increased in IUGR pregnancies compared with control subjects, but the difference did not reach statistical significance ($P = 0.17$; Table 2). There was a positive linear correlation between inhibin A and maternal age (regression coefficient, 0.08; $P = 0.016$; Table 3) and a positive correlation between inhibin A and maternal BMI (coefficient, 0.04; $P = 0.086$; Table 3). Adjusted for both maternal age and maternal BMI, inhibin A was significantly increased in IUGR pregnancies compared with control subjects ($P < 0.001$; Table 3).

Activin A was significantly higher in the serum from IUGR pregnancies compared with control subjects ($P = 0.0005$; Table 2). The A:F ratio was significantly increased in IUGR pregnancies compared with control subjects ($P < 0.0005$) (Fig. 1).

Table 2 Maternal serum activin A, inhibin A, and activin A:follistatin ratio for the two study groups

Analyte	Control subjects ($n = 40$)	IUGR[a] ($n = 30$)	*P* value*
Activin A (ng/mL)	12.8 (4.67, 20.05)	17.6 (11.83, 31.28)	<0.0005
Inhibin A (ng/mL)	1.39 (1.1, 2.7)	1.55 (1.09, 2.81)	0.172
Follistatin (ng/mL)	4.8 (3.78, 5.93)	3.89 (3.28, 4.89)	<0.0005
Activin:follistatin ratio (ng/mL)	2.37 (0.82, 4.69)	4.66 (2.67, 9.28)	<0.0005

[a] Data are median and interquartile range

* Difference between IUGR and control subjects, by Mann–Whitney *U* test

Table 3 Differences between analyte in IUGR, compared with control subjects and adjusted for potential confounding factors

Analyte	Regression coefficient adjusted for maternal age	*P* value	Regression coefficient adjusted for BMI	*P* value	Regression coefficient adjusted for maternal age and BMI*	*P* value
Maternal serum inhibin A	0.08	0.016	0.04	0.086	0.75	<0.001

* Multivariable linear regression for categoric and continuous variables (growth restriction, maternal age, BMI)

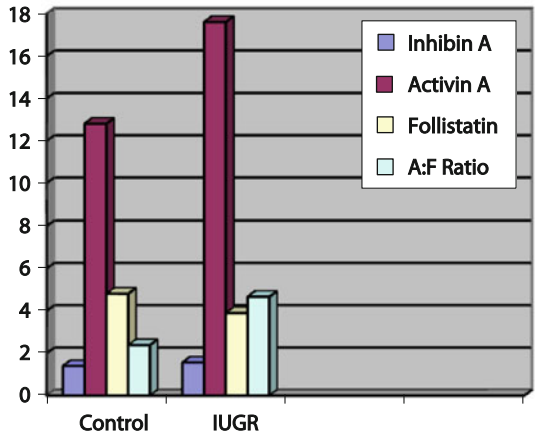

Fig. 1 Maternal serum activin A, inhibin A, follistatin, and activin A:follistatin ratio for the two study groups

Inhibin A, Activin A in Umbilical Vein Serum

Inhibin A was increased in IUGR pregnancies compared with control subjects, but the difference did not reach statistical significance ($P = 0.25$l; Table 4). There was a positive linear correlation between inhibin A and maternal age (coefficient, 0.07; $P = 0.019$; Table 5) and a positive correlation between inhibin A and maternal BMI (coefficient, 0.04; $P = 0.07$; Table 5). Adjusted for both maternal age and maternal BMI, inhibin A was significantly increased in test compared with control group ($P < 0.001$; Table 5) (Fig. 2).

Activin A was significantly higher in the serum from test compared with control group ($P = 0.0005$; Table 4).

Table 4 Umbilical vein serum activin A, inhibin A, for the two study groups

Analyte	Control subjects ($n = 40$)	IUGR[a] ($n = 30$)	*P* value*
Activin A (ng/ml)	0.41 (0.19, 0.57)	0.63 (0.33,1.27)	<0.0005
Inhibin A (ng/ml)	1.085 (0.85, 2.1)	1.195 (0.84,2.21)	0.255

[a] Data are median and interquartile range

* Difference between IUGR and control subjects, by Mann–Whitney U test

Maternal serum inhibin A concentrations in control group [range 1.10–2.70 ng/ml] was more than umbilical vein serum inhibin A concentration in control group [range 0.85–2.1 ng/ml]. Maternal serum activin A concentration in control group [range 4.67–20.05 ng/ml] was more than umbilical vein serum activin A concentration in control group [range 0.19–0.57 ng/ml]. Maternal serum inhibin A concentration in IUGR [range 1.09–2.81 ng/ml] was increased more than umbilical vein serum inhibin A concentration in test group [range 0.84–2.21]. Maternal serum activin A concentration in test [range 11.83–31.28 ng/ml] was increased more than umbilical vein serum activin A concentration in test pregnancies [range 0.33–1.27 ng/ml].

Discussion

This study represents a comprehensive analysis of maternal serum activin A, inhibin A, and FS concentrations and umbilical vein serum inhibin A and activin A concentrations in pregnancies that were complicated by IUGR secondary to placental dysfunction.

Maternal and umbilical vein serum inhibin A levels were raised in the test group compared with control group, but this only reached statistical significance after an adjustment was made for maternal age and maternal BMI. The finding of a positive correlation between maternal BMI and the levels of both analytes agrees with previous reports [8, 9]. We have been unable to find previous reports of studies of the relationship between maternal age and inhibin A. The relationship between maternal age and inhibin A concentrations suggest that it is important to adjust for maternal age in all human studies of inhibin A, where there can be marked variations between individual patients and within risk groups. Previous studies have reported an increase in levels of activin A [10] and inhibin A [11] in pregnancies with SGA fetuses, and also significant changes are confined to the subgroup of IUGR fetuses who are small because of placental dysfunction.

This study shows a significant increase in maternal serum and umbilical vein serum activin A in pregnancies that were complicated by IUGR secondary to placental

Table 5 Differences between analytes in IUGR, compared with control subjects and adjusted for potential confounding factors

Analyte	Regression coefficient adjusted for maternal age	*P* value	Regression coefficient adjusted for BMI	*P* value	Regression coefficient adjusted for maternal age and BMI[a]	*P* value
Umbilical vein serum inhibin A	0.077	0.019	0.046	0.073	0.75	<0.001

[a] Multivariable linear regression for categoric and continuous variables (growth restriction, maternal age, BMI)

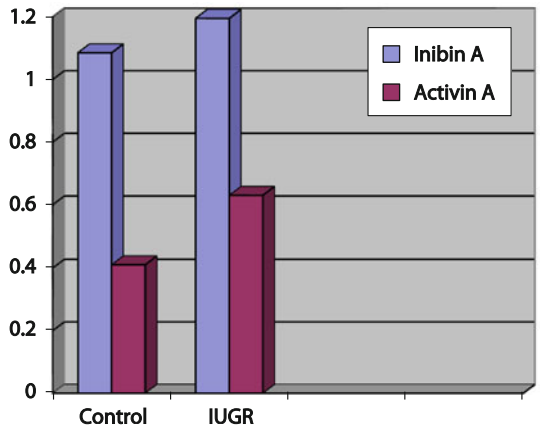

Fig. 2 Umbilical vein serum activin A and inhibin A for the two study groups

dysfunction when compared with control subjects. Elevated serum activin A concentrations were recently reported in patients with preeclampsia plus intrauterine growth restriction, similar to those with preeclampsia only [12].

Maternal serum FS did not increase in IUGR pregnancies, although the ratio of activin A to FS did suggests that there is more free activin A that is biologically active in the circulation in pregnancies affected by IUGR secondary to placental dysfunction compared with control subjects [10]. This study also showed that control group maternal serum inhibin A, activin A concentrations were more than the control group umbilical vein serum concentrations. This is comparable to the previous study done by Florio et al. [6].

Our study reported that IUGR group maternal serum Inhibin A, activin A levels were increased more than that of umbilical vein serum concentrations.

During the decade, the studies conducted on inhibin A, activin A, and FS suggested their possible involvement in the pathogenesis of IUGR. 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 FS processing throughout gestation may be important not only in the paracrine control of the fetomaternal communication required to maintain pregnancy, but also as specific marker 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, and monitoring of IUGR. It is not known why these analytes are increased or whether they contribute to the cause of this disease.

Activin A and inhibin A have also been raised in pregnancies that are complicated by preeclampsia [12, 13]. This is of interest because pregnancies that are affected by IUGR due to placental dysfunction, like those pregnancies that are affected by preeclampsia, are associated with abnormal trophoblastic invasion of the myometrium in the first half of pregnancy. It has been hypothesized that the increase in analytes in preeclampsia could be due to the abnormally functioning syncytiotrophoblast or the hyperplasic cytotrophoblast [2]. This could also be the case in IUGR.

In conclusion, inhibin A, activin A, and FS were increased in IUGR due to placental dysfunction, because during pregnancy the placenta exerts it's effects on fetal growth via metabolic and endocrine mechanisms. To achieve this, placenta exchanges a wide array of nutrients, endocrine signals, cytokines, and growth factors between the mother and the fetus. These exchanges modulate or program the fetal growth and development. In placental dysfunction there is decrease glucose and amino acid delivery to the fetus. This reduction in substrate availability leads to down-regulation of both the insulin and insulin-like growth factor 1, endocrine axis, and hepatic glucose metabolism. The result is glycogenolysis with a decrease in liver size redirection of gluconeogenic amino acid from endogenous protein breakdown and eventually, delayed longitudinal growth [10]. A reduction in fatty acid transfer decreases the availability of precursor molecules for a wide range of bioactive substances. These changes are important antecedents for the manifestation of IUGR [10]. When IUGR occurs, placenta takes part in the adaptive response to the adverse condition, and changes its hormonal secretary ability with the aim of modifying the adverse intrauterine environment. So inhibin A, activin A, and FS were increased in IUGR due to placental dysfunction pregnancies.

Our study reported that inhibin A, activin A, and FS influenced the fetal growth and development. The present study strengthens the evidence of using inhibin A, activin

A, and FS as serum markers in routine screening for early detection of IUGR. But, large prospective studies are needed to further define their role in clinical practice.

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