Placenta (2006), **27**, 432–437
doi:10.1016/j.placenta.2005.04.008

Umbilical Cord Serum Activin A Levels are Increased in Pre-eclampsia with Impaired Blood Flow in the Uteroplacental and Fetal Circulation

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Paper accepted 23 April 2005

The aims of the present study were to evaluate the umbilical cord serum activin A concentrations in complicated pregnancies and also to explore the relationship between activin A levels and blood flow velocity in fetal arteries. Umbilical cord blood samples were obtained postpartum after a full term uneventful gestation (control group, $n = 40$), and from pregnancies complicated by gestational diabetes ($n = 13$), preterm labour ($n = 18$), or pre-eclampsia ($n = 19$). Cord serum activin A levels were three-fold higher in pregnancies complicated by pre-eclampsia (1.17 ± 0.14 ng/ml, $p < 0.01$) than in the control group (0.43 ± 0.03 ng/ml), but were unaltered in the diabetes and preterm labour groups. The pre-eclampsia group had a marked increase of umbilical artery pulsatility index (PI) and also a decrease of middle cerebral artery PI ($p < 0.01$). Furthermore, activin A concentration correlated directly with the umbilical artery PI ($r = 0.540$, $p = 0.021$), with the length of stay in the Neonatal Intensive Care Unit ($r = 0.857$, $p < 0.001$) and also with cord blood pH ($r = -0.886$, $p < 0.001$). In conclusion, umbilical cord serum activin A levels are increased in the presence of pre-eclampsia and provide an indirect marker of impaired blood flow in the uteroplacental and fetal circulation.

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Keywords: Activin A; Fetus; Pre-eclampsia; Doppler

INTRODUCTION

Activin A is a dimeric glycoprotein composed by two βA subunits and belonging to the transforming growth factor-β superfamily of growth and differentiation factors [1]. Despite originally isolated from the ovarian follicular fluid [2], activin A is also expressed in a variety of extragonadal sources, where it exerts a number of different biological actions [1]. Intrauterine tissues (placenta, decidua, fetal membranes, embryo/fetus) represent important sites of activin A production throughout gestation [3], and from these sources activin A is secreted into maternal and fetal circulation, and amniotic fluid [3]. Furthermore, maternal and amniotic fluid concentrations, but not umbilical cord levels, significantly increase with advancing gestation, to reach the highest concentrations at term [3]. Previous studies have shown very high concentrations of activin A in the maternal serum of women with pre-eclampsia (PE) [3,4], but elevated activin A levels have been found also in pregnancies complicated by diabetes mellitus [5] and preterm labour [6].

PE is a multisystemic syndrome specific to pregnancy and is a leading cause of maternal and neonatal morbidity and mortality [7]. Although the pathophysiology of PE is not completely known, one important feature of the syndrome is the widespread vascular endothelial dysfunction due to an excessive systemic inflammatory response involving endothelial cells [8]. It is commonly accepted that PE is associated with an impairment of uteroplacental blood flow, possibly related to a deficient trophoblast invasion of spiral arteries, which then lack the physiological pregnancy-induced transformation into low-resistance vessels [7].

In pregnancies complicated by pre-eclampsia, the fetus may be exposed to variable degrees of hypoxemia, systemic inflammatory response and brain injury [9]. These conditions have been recently shown to increase activin A levels in fetal circulation [10,11]. Since most of the activin A present in the whole umbilical blood is of fetal origin [12], we hypothesized that the concentration of activin A in cord serum might reflect

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0143–4004/$–see front matter

the degree of uteroplacental blood flow resistance and/or fetal compromise due to pre-eclampsia, as fetal blood flow aberrations and hypoxemia are potential complications of this syndrome.

The present study aimed to evaluate activin A levels in umbilical cord serum samples collected from pregnancies complicated by PE, and to investigate whether they correlate with the disruption of the uteroplacental and fetal blood flow as measured through the umbilical artery and middle cerebral artery Doppler waveforms. In addition to a healthy control group, we compared PE with other conditions that rise maternal serum activin A levels and cause perinatal morbidity, namely, gestational diabetes and preterm labour.

MATERIALS AND METHODS

Patients and study design

In this controlled cross-sectional study, we evaluated 90 women with singleton pregnancies who received perinatal care at the academic hospitals of Siena and Catania, Italy. Written informed consent was obtained from each pregnant woman and the permission of the Local Human Investigation Committee was granted for the study.

The study population included a control group ($n = 40$) with uneventful, term gestation and delivery of a healthy infant; a group with gestational diabetes ($n = 13$) complicated by fetal macrossomia but without superimposed hypertensive disorders, preterm labour or infection; a group with preterm labour ($n = 18$) due to premature rupture of membranes, without evidence of maternal and/or fetal infection; and a group with PE ($n = 19$) not superimposed to chronic hypertension.

Gestational diabetes was diagnosed according to White's criteria [13]. All patients were treated with low calorie diet therapy, and no fetal malformation was detected in any of the newborns. Preterm labour was defined as labour starting before 37 complete gestational weeks. PE was defined as 300 mg or more of urinary protein excretion per 24 h and hypertension (blood pressure of 140/90 mmHg or higher), both first diagnosed after 20 weeks of gestation [14].

All pregnancies were dated by ultrasound with measurement of the biparietal diameter, head circumference, femur length, and abdominal circumference. All patients delivered by elective cesarean section under epidural anesthesia (in healthy women the cesarean section was performed for breech presentation or previous cesarean section) and their clinical characteristics are summarized in Table 1.

Umbilical cord blood samples were collected immediately after fetus delivery and cord clamping and before placental detachment. Mixed arterial/venous blood samples were allowed to clot and, after centrifugation for 10 min at 3000 rpm, the serum was harvested and stored at −20 °C until activin A assay. Cord blood pH was measured in a separate sample collected immediately after delivery.

Activin A assay

Serum activin A concentrations were measured using a specific two-site enzyme immunoassay purchased from Serotec (Oxford, Oxford, UK) as previously described [11]. All measurements were performed blinded to the patient's diagnoses and outcomes. The limit of detection for activin A was less than 100 pg/ml, and intra- and inter-assay CVs were 5.0% and 9.0%, respectively. The activin A assay has no detectable cross-reaction with inhibin A, inhibin B, follistatin, or activin B.

Doppler study

Umbilical artery and middle cerebral artery flow velocity waveforms were determined in the absence of fetal body or breathing movements by a single observer (F.M.S.) with a 3.5 MHz convex probe (Real Time Ultrasound Scan Equipment, Siemens Sonoline ELEGRA® Millenium Edition) before delivery, and the PI (peak systolic velocity − end

Table 1. Summary of clinical data

	Controls	Gestational diabetes	Preterm labour	Pre-eclampsia
N	40	13	18	19
Parity	1.05 ± 0.3	1.2 ± 0.1	1.2 ± 0.5	1.0 ± 0.3
Maternal age (y)	28.7 ± 1.2	27.9 ± 1.1	28.6 ± 2.0	28.3 ± 1.4
Weeks at delivery	39.3 ± 0.1	40.1 ± 0.2	32.4 ± 0.4*	31.4 ± 0.6*
Systolic BP (mmHg)	116.2 ± 1.4	115.3 ± 2.6	115.5 ± 2.3	166.3 ± 4.1*
Diastolic BP (mmHg)	67.1 ± 1.3	65.0 ± 1.7	64.1 ± 1.4	117.3 ± 3.3*
Birth weight (g)	3344 ± 66	4311 ± 56*	1902 ± 116*	1331 ± 133*
Centile	55	97	60	20
Umbilical artery PI (*z* score)	−0.11 ± 0.10	−0.47 ± 0.13	−0.14 ± 0.09	2.38 ± 0.09*
Middle cerebral artery PI (*z* score)	0.29 ± 0.12	0.33 ± 0.17	0.07 ± 0.24	−2.37 ± 0.10*
Apgar <7 at 1 min (*n*)	0	1	5#	14#
Apgar <7 at 5 min (*n*)	0	0	0	6#
Stay in the NICU (days)	4.8 ± 0.2	5.9 ± 0.3	17.1 ± 1.7*	83.1 ± 11.4*

BP: blood pressure; PI: pulsatility index; NICU: neonatal intensive care unit. *$P < 0.01$ vs. control group (Newman−Keuls test), #$P < 0.01$ vs. control group (Fisher's exact test).