# Umbilical cord activin A concentration in pregnancies complicated by mild preeclampsia and relationship between activin A levels and Doppler

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*Abstract*

***Objective.*** *To evaluate the significance of umbilical cord activin A concentration in preeclampsia and also explore the relationship between activin A levels and Doppler findings.* ***Methods.*** *The study population was constituted 40 pregnancies complicated with term mild preeclampsia and control group constituted 40 pregnancies with uneventful, term gestation. Umbilical cord blood samples were collected immediately after fetus delivery. Umbilical artery and middle cerebral artery flow velocity waveform were determined just before delivery.* ***Results.*** *Activin A levels were high in preeclamptic group (p<0,0001). Marked increase of umbilical artery S/D rate and pulsatility index (PI) (p<0,0001) and also decrease of middle cerebral artery PI (p<0,05) were detected in preeclamptic group. No significant changes in middle cerebral artery S/D rate were observed between two groups (p>0.05). There were no correlation between Doppler examination and activin A level.* ***Conclusion.*** *Umbilical cord activin A levels reflected directly fetoplacental unit resistance in preeclampsia.*

***Keywords:*** *activin A, preeclampsia, doppler, fetoplacental*

## Introduction

A multisystemic syndrome, preeclampsia is specific to human pregnancy. It can have a major impact both on perinatal and on maternal morbidity and prevalence are 5-7% of all pregnancies[1]. Although the exact pathogenesis of preeclampsia still remains to be unraveled and is most likely multifactorial, it is increasingly clear that systemic inflammatory response leading to generalized endothelial cell dysfunction and inadequate trophoblast invasion of spiral arteries contribute to the spectrum of the disease[2,3].

Endothelial cell dysfunction is a leading cause of deterioration in uteroplacental blood flow. Doppler ultrasound examination of the uteroplacental circulation has confirmed that increased impedance to flow of the uterine arteries is associated with an increased risk of fetal hypoxia[4]. It is suggested that umbilical activin A measurement represents neonatal oxygenation and hypoxia.

Activin A is a homodimeric glycoprotein hormone that belongs to the transforming growth factor β super family[5]. Activin A is produced by many tissues, including the fetus, placenta, decidua, and fetal membranes. The placenta has been postulated to be the principal source of activin A, which is detected in the circulation during pregnancy. Having been produced, activin A is secreted into amniotic fluid, maternal and fetal circulation[6]. Its biologic functions are diverse and, in pregnancy, include the regulation of trophoblast differentiation, placental steroidogenesis, prostaglandin production and angiogenesis[7]. In normal pregnancy, circulating activin A concentrations have been found to increase throughout gestation, peaking in the third trimester[8,9]. The adoptions of placenta to the hypoxia, hypertension, fetal and placental malformation contribute to the over expression of activin A[10,11]. So measurement of activin A may be helpful for early diagnoses of fetal hypoxia, hypertension, fetal and placental malformation.

Found elevated in pregnancies complicated with preeclampsia[10], activin A can be used as a predictor of preeclampsia. Some of the authors suggest that activin A levels can be found 10 times higher in preeclampsia than non-complicated pregnancies. In a low-risk population, it is reported that serum activin A concentrations at 15 to 19 weeks could discriminate preeclampsia with a sensitivity of 41%, a specificity of 89%, which gives a posttest probability of preeclampsia of 16%. At 21 to 25 weeks, the sensitivity was 59%, and the specificity was 87%.

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Similar sensitivity (60%) and specificity (90%) for activin A to predict preeclampsia in women who were at a low risk was reported. Activin A appears to be more predictive of early onset preeclampsia by detecting almost 90% of these women at 21 to 25 weeks of gestation, with a likelihood ratio of 11.4. If mothers were accepted as a cut of value for activin A, the sensitivity would be 61%, and the specificity would be 89%. We have hypothesized that activin A level may reflect fetal hypoxia or umbilical artery blood flow resistance[11,12].

There is a strong relationship between Doppler findings and preeclampsia. Today doppler imaging is used in routine management of preeclampsia in many clinics[11]. Middle cerebral artery and umbilical artery are the most common vessels used for Doppler examination[13]. If a pregnancy is complicated with preeclampsia, clinician may detect increased S/D, RI and PI indexes of umbilical artery and decreased S/D, RI and PI indexes of MCA[14]. We are of the opinion that these doppler findings and activin A levels may be used in management of preeclampsia.

The aim of the present study was to both evaluate the significance of umbilical artery serum activin A concentration in pregnancies complicated by mild preeclampsia and explore the relationship between activin A levels and blood flow velocity in fetal arteries.

## Methods

The study was approved by the institutional ethics committee and all participants signed an informed consent form regarding both CS and anesthetic technique. This study was conducted in Obstetrics and Gynecology Department of Gulhane Military Medical Academy.

A total of 80 pregnant women with previous caesarean between 37$^{th}$ and 40$^{th}$ weeks of gestation admitted to our department were investigated. About 40 pregnant women with uncomplicated healthy singleton pregnancies constituted our control group and 40 singleton mild preeclamptic women constituted our study group. Women who reported histories of preeclampsia were not eligible to be controls. Patients with multiple pregnancies, with chronic renal and vascular disease or previous thromboembolic complications were excluded and women taking anticoagulant therapy or having preeclampsia superimposed on chronic hypertensions were not included in the study.

Diagnosis of preeclampsia was done according to the criteria agreed by the National High Blood Pressure Education Program Working Group of National Institutes of Health (NIH) in 2000[15]. Preeclampsia was defined as blood pressure (BP) of at least 140/90 mmHg after 20 weeks gestation on at least two occasions 6 hours apart with proteinuria more than 0.3 g per 24 hours and edema <1+ after bed rest and gestational trophoblastic disease or multiple pregnancies were not evaluated. Blood was measured with a calibrated aneroid manometer in the supine position after a five-minute-rest. Gestational age was calculated according to last menstrual period and confirmed by ultrasonographic fetal biometrical measurements including biparietal diameter, head circumference, abdominal circumference, and femur length. All patients delivered with an elective caesarean section under epidural analgesia and were followed until discharged from hospital. Anesthetic and obstetric procedures were all standardized maneuvers, and all newborns were attended at the time of delivery by a pediatrician. Pediatricians who assigned the APGAR scores were blinded to the patients' diagnose. Birth weight, APGAR score, perinatal complication and gestational week were recorded.

**Measurement of activin A:** After double-clamping, arterial+venous cord blood was obtained from approximately 10-20 cm length of the umbilical cord. Samples were centrifuged for 10 minutes at 3000 rpm then those samples were stored at -20°C until assayed. Activin A was measured by ELISA (Synergy HT, USA) using commercial kits (OBI Aktivin A ELISA-Oxford Bio-Innovation UK).

## Doppler Imaging

Siemens Antares Sonoline colored Doppler ultrasound machine with a Doppler unit and a 3.5 MHz convex linear probe was used for ultrasonographic examination. All sonographic examinations were performed by a single doctor. Sonographic examination began with routine obstetric evaluation by B-Mode imaging. For avoiding supine hypotension secondary to aortacaval compression, sonographic examinations were performed in a semi-recumbent position with the head and chest slightly elevated. The number of fetus, fetal heart activity, localization and maturation of the placenta, amniotic fluid volume, fetal presentation, fetal anatomic structures and biophysical profile and fetal biometric measurements were evaluated. After this routine obstetric evaluation, Color Doppler Ultrasonogarphy was started.

The recording was performed during periods of fetal apnea, because of a potential effect of fetal breathing movements on waveform variability. Once waveforms of good quality were collected and analyzed on average, 3 separate readings were performed. The combination of a vein, two arteries and structure of cord was investigated during the UA measurements. All measurements were carried out from any of the two arteries and performed from the free portion of umbilical cord. For measurements of the middle cerebral artery doppler index, an axial view of the fetal head was obtained at the level of the cerebral peduncles. Color Doppler was used to visualize the circle of Willis. The Doppler sample volume was placed within 1 cm of the origin of the middle cerebral artery that was easily identified as a major branch

running in anterolateral direction from the circle of Willis towards the lateral edge of the orbit. Doppler indices were calculated by the software provided by the Doppler device. Doppler index measurements, gestational age, birth weight, APGAR score and perinatal complications were recorded separately for each pregnancy.

## Statistics

Kolmogorov-Smirnov test was used to check the data normality. According to these results, either two-sided independent samples t test or Mann-Whitney U test was used to compare the differences between groups. Pearson's correlation analysis was used to identify the associations between variables.

*Table 1* Demographic evaluations of both groups

| Parameters | Control Group | Preeclampsia Group | P value |
|---|---|---|---|
| N | 40 | 40 | |
| Age(mean±SD) | 27.1±4.9 | 25.9±4.6 | 0.266* |
| Parity(mean±SD) | 0.52±0.5 | 0.57±0.6 | 0.828** |
| Gestational week (mean±SD) | 38.4±0.8 | 38.2±0.8 | 0.179** |
| Birth Weight (mean±SD) | 3385.2±349 | 3153±411 | 0.008* |
| APGAR score (mean±SD) 1st minute | 8.4±0.5 | 8.0±0.5 | 0.007** |
| APGAR score (mean±SD) 5th minute | 10.0±0.0 | 9.7±0.4 | 0.003** |
| NICU acceptance(n,%) | 0(0) | 0(0) | 1* |

*Table 2* Evaluation of both groups for Doppler imaging and cord activin A value

| Parameters | Control Group | Preeclampsia Group | P value |
|---|---|---|---|
| N | 40 | 40 | |
| UA S/D (mean±SD) | 2.21±0.29 | 2.86±0.62 | <0.0001* |
| UA PI(mean±SD) | 0.80±0.12 | 0.99±0.24 | <0.0001* |
| MCA S/D(mean±SD) | 3.79±0.72 | 4.08±1.30 | 0.222* |
| MCA PI(mean±SD) | 1.44±0.17 | 1.32±0.28 | 0.017* |
| Cord aktivin A(mean±SD) | 0.40±0.19 | 0.91±0.38 | <0.0001** |

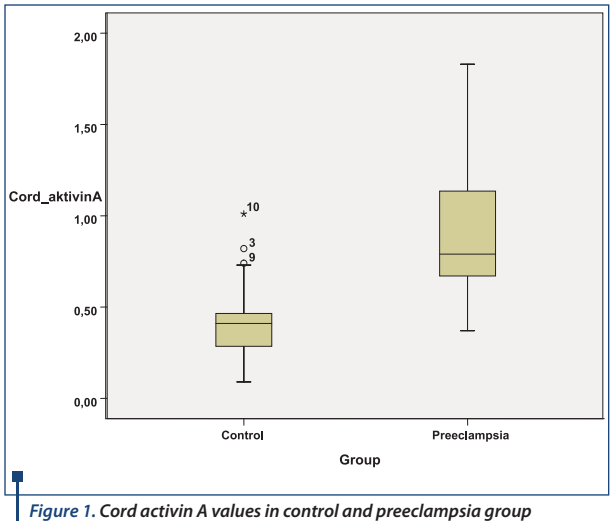


*Figure 1. Cord activin A values in control and preeclampsia group*

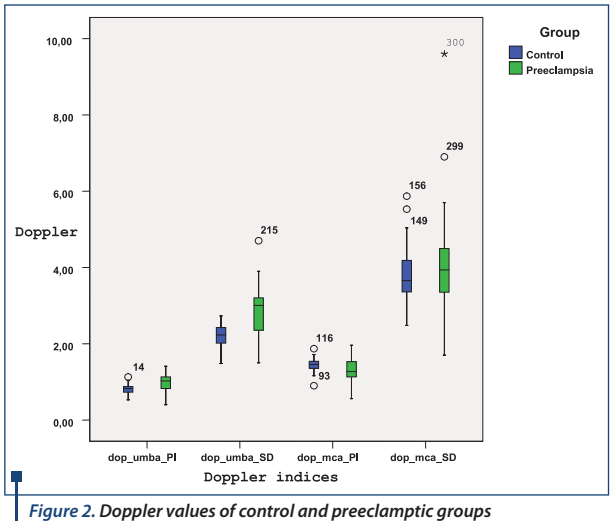


*Figure 2. Doppler values of control and preeclamptic groups*

Values are expressed as mean ± standard deviation. Analyses were performed using SPSS 16.0 software. $p<0.05$ was considered statistically significant.

## Results

There were no statistically significant differences for age, gestational week and parity. Birth weight, APGAR scores in 1st and 5th minutes were significantly high in control group (Table 1).

Umbilical artery S/D and PI values were statistically significantly high in preeclamptic group. Additionally, cord activin A and MCA PI values were statistically significantly high in preeclamptic group as well. There was no statistically significant difference between both groups for MCA S/D value (Table 2 and Figure 1).

Mean value of umbilical artery S/D was 2.86±0.62 in preeclamptic group and it was 0.99±0.24 in control group. Mean value of MCA S/D was 4.08±1.30 in preeclamptic group and it was 3.79±0.72 in control group. Mean MCA PI value was 1.32±0.28 in preectamptic group and it was 1.44±0.17 in control group (Figure 2).

Correlations between activin A and UA S/D, UA PI, MCA S/D, MCA PI illustrated r:-0.053, r:0.129, r:-0.012, r:0.082, respectively ($p>0.05$) and there was no correlation between serum activin A and Doppler results.

## Discussion

Preeclampsia represents a disorder which is responsible for an important procent of the maternal and perinatal morbidity and mortality in general, though it afects only 2% of the total pregnancies. In all cases of preeclampsia, only one quarter (0.5% of pregnacies) represents the severe forms or those with an early onset; these forms present in fact, a great influence on the morbidity and mortality rates[16].

Activin A is a homodimeric glycoprotein hormone that belongs to the transforming growth factor β super family[5]. Although maternal serum and fetal activin A levels increase throughout gestation, umbilical cord levels of activin A remain constant. Different level of activin A between maternal serum and umbilical cord emphasizes that activin A is produced by many tissues which include the fetus, placenta, decidua, and fetal membranes. The placenta has been accepted to be the principal source of activin A, which is detected in the circulation during pregnancy. After activin A is produced by trophoblast, it is secreted into amniotic fluid, maternal and fetal circulation[16].

Strikingly, the source of activin A is not only trophobasts. A multitude of *in vitro* data exist, which highlights a number of potential cell types capable of synthesizing and secreting activin A following inflammatory stimuli, including monocytes, macrophages, bone marrow and the vascular endothelium. Especially under inflammatory stimuli is obviously an important and strictly regulated process. However, the identification of cells responsive to inflammatory stimuli and releasing activin A into the circulation are problematic due to the wide cellular distribution of activin A[17]. As blood vessels comprise approximately 2% of body mass, one potentially large source of activin A is vascular endothelium[18].

Some conflicting results reported about usefulness of activin A in preeclampsia prediction. Blackburn et al have concluded that in women who are at high risk of the development of preeclampsia, serum activin A levels are not elevated with preeclampsia in which Activin A is not a useful predictor of preeclampsia[19]. However Bersinger et al concluded that activin A can be used in prediction of preeclampsia[20]. This situation can be interpreted that activin A measurement from maternal serum may reflect feto-placental unit partially, which leads to conflicting results. Direct measurement of activin A from umbilical vessels may be an accurate indicator of feto-placental unit.

Activin A was significantly elevated in preeclamptic group in this study. This observation is potentially interesting as this may reflect an underlying hypoxic condition in the placenta - a feature proposed to occur in preeclampsia[21].

This condition of hypoxia may on the one hand arise from inadequate modification of the maternal spiral arteries by the invading cytotrophoblast[21] or on the other hand it may occur as hypothesized via preeclampsia is an inflammatory disease[22]. Regardless of which event occurs, it is possible that activin A may act as a signal to both improve the oxygen supply to the fetus under these conditions of placental oxidative stress and ensure an adequate supply of oxygen to the fetal tissues.

Florio et al examined activin A levels and Doppler examination of 19 preeclamptic and 40 healthy pregnancies. Similar to our study, activin A levels were three times higher in preeclamptic group (1.17 ± 0.14 ng/ml vs 0.43 ± 0.03 ng/ml ) and they concluded that the group of fetuses whose gestation was complicated by PE had a marked increase of umbilical artery PI and also a decrease of middle cerebral artery PI in comparison to the control group[23]. Although we did not detect any correlation between activin A level and Doppler measurement, they concluded that there were significant and positive correlation between umbilical PI value and activin A level.

It is difficult to establish relationship between activin A level and Doppler findings. Fetal activin A production may be related to the effect of vasoactive peptides on vascular tonus. Endotelin-1 lead to increase placental activin A secretion[24]. Regardless of the source of activin A in umbilical cord, the role of such an increased secretion in PE was taken into account. Activin A, placenta and the fetus with vasoactive factors involved in the regulation of placental and fetal vascular tone. Activin A may play a role in the mechanism of vasorelaxation by triggering nitric oxide formation[25].

Experimental animal studies have reported intracerebroventricular injection of recombinant human activin A due to reduced neuronal loss after hypoxic-ischemic injury[26]. In our study there was no newborn whose APGAR score was under 7 at first and fifth minute in PE group. Thus, we consider the hypothesis that activin A is released in response to impaired blood flow in the uteroplacental and fetal circulation and favors brain adaptation to intrauterine hypoxemia.

## Conclusions

In conclusion, there is limited marker that can be used to determine uteroplacental and fetoplacental blood flow in pregnancies complicated by PE. There is no objective criterion for the effect of uteroplacental and fetoplacental blood flow impairment on fetus. Activin A levels are increased in umbilical cord circulation in the presence of PE with biophysical signs of impaired blood flow in the uteroplacental and fetal circulation. Additionally, there were no correlations between doppler imaging and activin A level. Therefore, not only activin A but also Doppler imaging may be a useful tool in the management of PE. There is a need for further, larger scale, prospective and homogenous studies. ■

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