Journal of Experimental and Clinical Medicine

Clinical Research

doi: 10.5835/jecm.omu.30.02.007

Comparison of maternal serum and umbilical cord concentrations of nitric oxide and asymmetric-dimethyl-arginine in pre-eclamptic and uncomplicated pregnancies

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ARTICLE INFO

Article History
Received 02 / 12 / 2012
Accepted 04 / 05 / 2013

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Keywords:
Asymmetric dimethyl arginine
Cord blood
Maternal serum
Nitric oxide
Pre-eclampsia

ABSTRACT

The aim of this study was to compare maternal serum and umbilical cord levels of asymmetric-dimethyl-arginine (ADMA) and nitric oxide (NO) in pre-eclamptic and non-pre-eclamptic women and the possible effects of ADMA and NO on fetal outcome. Mean umbilical cord and maternal serum NO and ADMA levels of 29 women with pre-eclampsia (PE) and 24 randomly selected healthy pregnant women were compared. Maternal venous blood samples were collected before delivery and umbilical cord venous blood samples were obtained after delivery. Birth weight, apgar score, cord blood pH, duration of stay in the neonatal intensive care unit (NICU), and maternal and neonatal complications were recorded. Umbilical cord ADMA and maternal NO levels were significantly higher in women with pre-eclampsia compared to women without pre-eclampsia. There were no significant differences between means of maternal serum ADMA levels and cord blood NO levels in women with and without pre-eclampsia. Maternal and cord serum NO and ADMA levels in the pre-eclampsia group did not show a significant correlation with cord blood pH, umbilical artery pulsatility index (PI). In conclusion, umbilical cord ADMA and maternal NO levels were significantly higher in women with pre-eclampsia compared to women without pre-eclampsia.

J. Exp. Clin. Med., 2013; 30:125-128

This work was supported by The Research Fund of Istanbul University, Project Number: T-450/25062004.

1. Introduction

Pre-eclampsia (PE) is characterized by the onset of high blood pressure and proteinuria after the 20th weeks of gestation. It occurs in about 3-5% of all pregnancies and results in substantial maternal and neonatal morbidity and mortality (Cunningham et al., 2001; Sibai et al., 2005).

Altough the pathogenesis of pre-eclampsia is unclear, researchers have suggested that an association exists among impaired angiogenesis, changes in local oxygen tension (Ahmad and Ahmed, 2004), endothelial dysfunction (Pinheiro et al., 2013) and immunological alterations in the early placental microenvironment (Laresgoiti-Servitje et al., 2010). Endothelial nitric oxide (NO) is important in regulation of blood flow and vasomotor tone through inhibiting smooth muscle contraction (Myatt and Webster, 2009). It is well known that endothelial NO could increase blood volume, enhance cardiac output and decrease blood pressure. Consequently, it is obviously important for maintenance of the maternal systemic vasodilatation and reduction of vascular reactivity during normal pregnancy. Recent studies suggested that reduced endothelial NO formation was associated with hypertensive disorders of pregnancy, especially in PE (Savvidou et al., 2003; Garcia et al., 2007). Endothelial dysfunction results in a decrease of substances such as the NO and nitric oxide synthase (NOS) which normally function as vasodilators and regulators of vascular tone. Asymmetric-dimethyl-arginine (ADMA) competes with L-arginine for NOS. Inhibition of NOS by ADMA decreases the synthesis of NO and interferes with other related vasodilator mechanisms (Holden et al., 1998; Maeda et al., 2003; Savvidou et al., 2003).

The aim of this study was to compare maternal serum and umbilical cord levels of ADMA and NO in pre-eclamptic and non-pre-eclamptic women and the possible effects of ADMA and NO on fetal outcome.

2. Materials and methods

The study included 29 pre-eclamptic and 24 randomly selected healthy pregnant women. The study was approved by the ethics committee of Department of Obstetrics and Gynecology, School of Cerrahpaşa Medical Faculty, and informed consent was obtained from every patient. The diagnosis of pre-eclampsia was made based on Working Group Report on High Blood Pressure (BP) in pregnancy (Maeda et al., 2003). Hypertension was defined as sustained BP readings of ≥140/90 mmHg (with reading taking place ≥ 6h apart). Proteinuria was defined as urine protein concentration ≥ 300 mg/L per 24 h urine collection (or >1+ a urine dipstick) and absence of urinary tract infection. Normal pregnancy was defined as a pregnancy with maternal blood pressure below 140/90 mmHg, absence of proteinuria or other medical and obstetrical complications. Exclusion criteria were multiple pregnancies, gestational diabetes mellitus, smoking, chronic hypertension, diabetes mellitus, polyhydroamnios, inflammation, prior renal diseases.

Vital signs were checked in every 6 hours; weight measurements were performed every day; ultrasonographic and doppler examinations (uterine and umbilical artery, fetal aorta, middle cerebral artery (MCA) and ductus venosus pulsatility index (PI), resistance index (RI), systolic-diastolic ratios (S/D) measurements, and 24 hour protein measurements were performed every week in all patients. Diagnosis of growth restriction was based on the evidence of fetal growth delay, with an estimated sonographic fetal weight below the 10th percentile for gestational age.

Maternal venous blood samples were collected before delivery and umbilical cord venous blood samples were obtained after delivery. Serum samples were stored at -80 ^{0}C until assayed for determination of NO and ADMA. Blood gas analysis from umblical artery was performed immediately. Birth weight, apgar score, cord blood pH, duration of stay in the neonatal intensive care unit (NICU), and maternal and neonatal complications were recorded.

Doppler velocimetry measurements were performed with 3.5-5 MHz convex transabdominal probe (with color Doppler velocimetry) GE 3000 ultrasonraphy. Uterine artery Doppler measurements were done with the probe suited at bilateral inferior lateral quadrants angulating to the uterine artery crossing at external iliac artery. The PI, RI, S/D measurements, and presence of diastolic notch were recorded after three wave forms were noticed. Umbilical artery measurements were performed at the site of placental insertion, MCA were done at the Wills poligone from the proximal segment of MCA at the axial crossection of fetal head after observation of three wave forms.

Assay of NO

NO was determined as the concentration of nitrate plus nitrite (NOx) in the plasma. Nitrate was reduced to nitrite by nitrate reductase, the sample was deproteinized with $ZnSO_4$, and the concentration of nitrite was measured spectrophotometrically at 430 nm using the Griess reaction with a commercial kit (Roche, Germany, cat.no:1 756 281). The intra-and interassay coefficients of variation for NO were 4.8% and 5.2%.

Assay of ADMA

Serum endogenous ADMA level measurements were done with Enzyme-Linked ImmunoSorbent Assay (ELISA) method following the recommendations of producer (Cardio Vasics-California).

Serum urea, uric acid, albumin, total protein were determined by routine laboratory methods using the Hitachi 704 auto analyzer (Boehringer Mannheim, Tokyo, Japan).

Statistical analysis

Data are presented as the mean ± standard deviation (SD). The statistical analysis was made with Statistical Package for Social Sciences (SPSS 17.0, Inc, Chigago,Ilinois) using student's t test for parametric variables and Kruskal-Wallis for non parametric variables. P value of less than 0.05 was considered significant.

3. Results

Twenty nine preeclamptic women and 24 normotensive controls were included in the study. The demographic characteristics of the study group are shown in Table 1. Pregnancy and fetal outcomes of the study group are shown in Table 2. The variation of mean systolic and diastolic blood pressure, birth weight and intra uterine growth restriction (IUGR) rate between the groups were significant. Difference between mean gestational age at birth was not significant between the normal and preeclamptic pregnancies (p=0.65).

There were 7 IUGR cases in the preeclamptic group (24%) and none in the control group. Five newborns died in the preeclamptic group between day 1 and day 8 postpartum due to respiratory distress syndrome and sepsis. All were under 1000 g of weight.

Table 1. The demographic characteristics of the study group

	Normal pregnant (n: 24)	Pre-eclamptic pregnant (n: 29)	P
Maternal age (years)	27.6 ± 3.7	28.9 ± 6.5	>0.05
Weight (kg)	70.3 ± 14.3	71.7 ± 12.6	>0.05
Gravida	2.18 ± 1.63	2.06 ± 1.20	>0.05
Parite	0.78 ± 1.50	0.77 ± 1.08	>0.05
Diastolic blood pressure (mmHg)	69.29 ± 6.16	95.86 ± 5.01	0.001
Systolic blood pressure (mmHg)	107.7 ± 7.2	148.5 ± 5.1	0.001

Statistically significant: p<0.05.

Table 2. Pregnancy and fetal outcomes of the study group

	Normal pregnant (n: 24)	Pre-eclamptic pregnant (n: 29)	p
Gestational age at sampling (weeks)	34.14 ± 3.48	31.72 ± 3.48	0.65
Birth weight (g)	2374.29 ± 815.7	1587.93 ± 641.7	<0.001
IUGR (%)	0	7 (24%)	0.001
Apgar score	6.79 ± 1.42	5.38 ± 2.13	0.031
Duration of NICU (day)	1.21 ± 0.14	8.55 ± 1.98	<0.05

IUGR, intra uterine growth restriction

Statistically significant: p<0.05.

Apgar scores were significantly lower in the preeclamptic group (p=0.031). The mean duration of stay in the NICU in the study and control group was 8.55±1.98 days and 1.21±0.14 days,respectively (p<0.05).

Cord serum ADMA values were significantly higher in normotensive cases (p: 0.027). However there was no statisti-

cally significant difference in maternal serum ADMA values between the normal and pre-eclamptic women (p: 0.468). Maternal serum NO levels were higher in patients with pre-eclampsia (p=0.021). Cord serum NO levels did not differ significantly between normal and pre-eclamptic women (p= 0.10) (Table 3).

Table 3. NO and ADMA levels of maternal serum and umbilical cord

	Normal pregnant (n: 24)	Pre-eclamptic pregnant (n: 29)	P
Maternal serum			
ADMA (µmol/L)	0.42 ± 0.25	0.53 ± 0.35	>0.05
NO (µmol/L)	23.7 ± 4.1	26.8 ± 4.1	0.021
Cord blood			
ADMA(µmol/L)	0.43 ± 0.24	0.68 ± 0.36	0.027
NO (µmol/L)	22.6 ± 3.9	24.8 ± 3.6	>0.05

ADMA, Asymmetric Dimethyl Arginine; NO, nitric oxide
Statistically significant: p<0.05

Maternal and cord serum NO and ADMA levels in the pre-eclampsia group did not show a significant correlation with cord blood pH, umbilical artery pH.

4. Discussion

Systemic endothelial dysfunction due to ineffective trophoblastic invasion is the most reasonable theory in preeclampsia (Roberts et al., 1989).

NO is a potent local vasodepressor synthesized by endothelial NOS (eNOS) that inhibits the aggregation of platelets and leukocytes (Moncada et al., 1991). It is secreted from maternal endothelium and placental trophoblasts (Myatt et., 1993). Vascular tone might be regulated by NO as it helps to decrease the effects of vasopressors in the placental bed (Gude et al., 1990; Myatt et al., 1991). Deficiency of NO has been claimed in the pathophsyiology of preeclampsia (Seligman et al., 1994). Although in vitro studies have attributed the role of NO and NOS in pre-eclampsia, in vivo studies have given conflicting results. Pre-eclamptic patiens were found to have increased (Mutlu-Türkoglu et al., 1999), unchanged (Egerman et al., 1999) or decreased (Shaamash et al., 2001) nitrate levels.

ADMA competitively inhibits NOS with L-arginine and decreases the levels of NO of which itself is a vasodepressor. Holden et al. (1998) have shown a positive correlation between ADMA levels and increasing gestational age. They found higher ADMA levels in non-pregnant women than normotensive pregnants and concluded that vascular adaptation in pregnancy was linked to deacreased ADMA levels. They also found higher ADMA levels in in third-trimester pre-eclamptic patients than normotensive third-trimester subjects.

Savvidou et. al (2003) compared ADMA levels and flow dependent brachial artery dilatation of groups with or without a uterine artery notch at 20-24 weeks gestation. Flow dependent brachial artery dilatation as an indicator of endothelial function was poorest in preeclamptic patients with high ADMA levels at later stages of pregnancy.

In the present study, we detected increased serum NO levels in preeclamptic group compared to controls. This finding is in correlation with the studies that previously proposed increased NO levels as a result of insufficient compensatory mechanisms to supress vasoconstriction (Shaamash et al., 2001). The subclinic renal dysfunction may cause insufficient clearence of NO metabolites namely nitrate and nitrite. None of the previous studies focusing on NO and ADMA have compared renal dysfunction parameters between preeclamptic patients and control groups. Although, ADMA levels in maternal serum was higher in preeclamptic women, it did not reach statistical significance. Among the studies investigating ADMA and preeclampsia, only Meada et al. (2003) from Coulombia found indifferent levels of ADMA between preeclamptic and control groups. The other four studies in which ADMA was detected to be higher in preeclampsia came from Western Europe, US, Sweden and Japan (Holden et al., 1998; Savvidou et al., 2003; Maeda et al., 2003). Indifferent levels of ADMA in both groups in our study reminds us the possible ethnic differences and their effects on the physiopathology of preeclampsia. The low number of cases in our control group may be another reason for our findings. Higher number of patients may reveal statistically different levels. Another striking finding of our study is that NO levels of fetal serums of both groups did not reveal statistical differences. Previous studies also reported conflicting data concerning NO levels in fetal plasma.

There are studies that have shown decreased or unchanged NO levels in preeclamptic women (Sladek et al., 1997; Rudherford et al., 1995). In the present study, for the first time we have studied fetal ADMA levels in preeclampsia. We detected higher fetal plasma ADMA levels compared to controls. In their study, Meada et al. (2003) linked high fetal ADMA levels to relative hypoxic medium in the normotensive group. This relative hypoxic medium results in the degradation and methylation of proteins and arginine-linked proteins were are detected as ADMA. So the increased finding of ADMA in preeclamptic fetal serum may be a result of this hypoxic milieu.

It seems reasonable to suggest that increased fetal ADMA levels suppresses fetal NO levels that should normally rise in a compensatory fashion. The findings of normal levels of maternal ADMA and increased levels of maternal NO levels in preeclampsia compared to controls may be explained as ADMA in maternal serum was insufficient to suppress NO levels. It has to be noticed that decreased NO levels should not be expected in every pathology that ADMA increase. ADMA is not just a simple NOS inhibitor but in certain conditions may result in production of metabolites like peroxynitrates that contributes to the physiopathology of preeclampsia. But nevertheless, the absence of correlation of neither fetal nor maternal ADMA levels with the maternal and fetal syndrome parameters in our study weakens this theory.

Elevated ADMA levels in preeclampsia are detected before clinical symptoms develop; hence these findings suggest that ADMA may play a role in the pathogenesis of preeclampsia (Slaghekke et al., 2006).

We conclude that absence of significant relationship between maternal/fetal NO or ADMA and fetal prognostic parameters may imply that the increase in maternal NO was only a insufficient compensatory increase or this increase may result from subclinic renal dysfunction. Increase in fetal ADMA may be a result of methylation of degradeted

protein fragments. We believe that the insignificant increase in maternal ADMA levels in our patients was possibly due to ethnical differences in the pathogenesis of pre-eclampsia. Small number of cases was present in our study. Further randomized controlled studies with higher number of cases are needed.

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