Perinatal risk factors affecting the maternal and fetal asymmetric dimethylarginine levels

Mustafa Kul[1], Erkan Demirkaya[2], Osman Metin İpçioğlu[3], Rahmi Sinan Karadeniz[4]
Turan Tunç[2], Sebahattin Vurucu[2], Fahri Gürkan Yeşil[5], Hasan Öztin[5], Erdinç Çakır[6]

Departments of [1]Pediatrics, and [3]Biochemistry and Clinical Biochemistry, Gülhane Military Medical Academy Haydarpaşa Training Hospital, İstanbul, and Department of [2]Pediatrics, and [6]Emergency Medicine Laboratory of Biochemistry, [5]Gülhane Military Medical Academy, and [4]Department of Obstetrics and Gynecology, Ministry of Health Etlik Maternity and Women's Health Teaching and Research Hospital, Ankara, Turkey

SUMMARY: Kul M, Demirkaya E, İpçioğlu OM, Karadeniz RS, Tunç T, Vurucu S, Yeşil FG, Öztin H, Çakır E. Perinatal risk factors affecting the maternal and fetal asymmetric dimethylarginine levels. Turk J Pediatr 2009; 51: 141-145.

The aim of this study was to investigate the relationship between maternal risk factors, neonatal demographic features and asymmetric dimethylarginine (ADMA) levels in a randomly selected group of pregnancies during delivery.

The subjects were categorized into five groups as having: no maternal risk factor, maternal hypertension, gestational diabetes, maternal smoking history, and meconium staining. Blood samples were taken from the mothers before delivery and from the umbilical vein after delivery.

Mean ADMA levels were significantly lower in the cord blood when compared with maternal levels in all groups. Mean ADMA level of neonates in the meconium staining group was found to be significantly higher than in the other groups ($p<0.001$). Maternal age, delivery type, parity and sex did not show any effect on cord blood ADMA levels.

Overall, umbilical vein ADMA levels are modulated independent of several maternal features and risk factors. Although these factors are interrelated and it is difficult to interpret the relevant data separately, the most significant factor affecting umbilical vein ADMA levels seems to be perinatal hypoxia as in the case of meconium staining.

Key words: asymmetric dimethylarginine, cord blood, hypoxia, meconium, perinatal risk factors.

Pregnancy causes dramatic changes in the maternal systemic vascular function by increasing the cardiac output and decreasing the peripheral resistance, blood pressure and vascular reactivity to infused vasoconstrictors[1]. These changes ensure adequate delivery of oxygen and nutrients to the fetus. It has been shown that several factors -either of maternal or neonatal origin- affect the nitric oxide (NO) metabolism and play a role in the pathogenesis of the following serious disorders: hypoxic ischemic encephalopathy, persistent pulmonary hypertension, necrotizing enterocolitis and chronic lung disease[2-6].

Characteristic changes, i.e. increased cardiac output and decreased systemic resistance, also ensue in the fetal circulation[7]. To explain the mechanisms underlying these aforementioned adaptations during pregnancy, several vasodilator mediators including prostaglandins have been implicated. Yet, there is good evidence that NO -a potent vasodilator produced by the maternal endothelium- plays an important role[8]. NO, synthesized by NO synthase (NOS), is known to be not only a key regulator of the maternal systemic vascular resistance but also an endogenous dilator of the fetal vessels in the human placenta[9-11]. NOS is partially regulated by negative feedback from NO, but there are also other important endogenous factors in humans, like the competitive inhibitor asymmetric dimethylarginine (ADMA)[12]. Like NO, ADMA is synthesized almost everywhere and the amounts released are sufficient to inhibit NO production[13].

Data pertaining to perinatal ADMA metabolism is quite limited. Elevated ADMA levels during pregnancy have been reported as an important pathophysiological factor in the development of preeclampsia[14,15]. Although in normal pregnancy, ADMA levels were found decreased in the maternal circulation and increased in the umbilical cord blood[16], the effects of maternal factors or fetal problems during delivery on the ADMA levels are still not fully understood. Vida et al.[17] and Tsukahara et al.[18] investigated the plasma ADMA levels during the perinatal period and showed that ADMA might have an important role both in the control of the fetoplacental circulation and the neonate's postnatal circulatory adaptations. However, the effect of perinatal risk factors on ADMA levels have not been reported; therefore, the aim of this study was to investigate the relationship between maternal risk factors, neonatal demographic features and ADMA levels in a randomly selected group of pregnancies during delivery.

Material and Methods

Study groups and design

One hundred and eight term pregnant women in the first stage of labor at Ankara Etlik Maternity and Women's Health Teaching and Research Hospital and their babies after delivery were recruited. Maternal risk factors (hypertension, diabetes mellitus, smoking) and demographics (age, body weight, height), and data concerning the neonate (duration of pregnancy, type of delivery, sex, birth weight, presence of meconium) were recorded. Maternal hypertension was defined according to the Working Group (2000) criteria as high blood pressure ≥140/90 mmHg after the 20th week of gestation[19]. Gestational diabetes mellitus was defined as any degree of glucose intolerance with onset or first recognition during pregnancy[20]. Mothers who smoked more than five cigarettes per day during the whole pregnancy were eligible for this study.

Those neonates who required intense resuscitation were not included in the study. The subjects (neonates-mothers) were then categorized into five groups as follows: Group 1: (n:51) no maternal risk factor, Group 2: (n:14) presence of maternal hypertension, Group 3: (n:11) presence of gestational diabetes mellitus, Group 4: (n:19) presence of maternal smoking history, and Group 5: (n:13) presence of meconium staining. The groups were strictly arranged such that each group (except Group 1) had only one risk factor.

Blood samples were taken from the mothers before delivery and from the umbilical vein after delivery. They were collected into tubes without any additives and were then centrifuged (4000 rpm/10 min). Serum samples were kept at -80°C until ADMA measurements.

The study was approved by the local ethical committee of Gülhane Military Medical Academy. Informed consent was obtained from all subjects.

ADMA determination

Measurement of blood ADMA levels was accomplished by high-performance liquid chromatography (HPLC), using the method described by Chen et al.[21]. In brief, 20 mg of 5-sulfosalicylic acid (5-SSA) was added to 1 ml of serum, and the mixture was left in an ice bath for 10 min. The precipitated protein was removed by centrifugation at 2000 g for 10 min. Ten microliters of the supernatant, which was filtered through a 0.2-μm filter, was mixed with 100 μl of derivatization reagent (prepared by dissolving 10 mg of o-phthaldialdehyde in 0.5 ml of methanol and adding 2 ml of 0.4 M borate buffer [pH 10.0] and 30 μl of 2-mercaptoethanol) and then injected into the chromatographic system. Separation of ADMA was achieved with a 150x4-mm-I.D. Nova-pak C18 column with a particle size of 5 μm (Waters, Millipore; Milford, MA, USA) using 50 mM sodium acetate (pH 6.8), methanol, and tetrahydrofuran as mobile phase (A, 82:17:1; B, 22:77:1) at a flow rate of 1.0 ml/min. The areas of peaks detected by fluorescent detector (excitation, 338 nm; emission, 425 nm) were used for quantification. The variability of the method was <7%, and the detection limit of the assay was 0.1 mM.

Statistical analysis

Data were analyzed by using SPSS 10.0. Two group comparisons were done by Student's t test and multiple group comparisons were done by ANOVA and post-hoc Bonferroni tests. Statistical significance was set at $p<0.05$.

Results

Demographics of the subjects in each group are summarized in Table I. Sixty-two percent of the newborns were born via vaginal delivery and 62.0% of the newborns were male. Hypertension was present in 13.0%, diabetes in 10.2%, smoking in 17.6% and meconium staining in 12.0% of the subjects.

Mean maternal ADMA levels were significantly lower than those of the cord blood in all groups. Concerning Groups 1-4, mean ADMA levels of both the mothers and newborns were found to be indifferent among groups (all p values >0.05). Mean ADMA level of neonates in Group 5 -but not that of the mothers'- was found to be significantly higher than the other groups (p<0.001). In Group 1, maternal age (below or above 30 years), delivery type, parity and sex did not show any effect on cord blood ADMA levels (Table II).

Discussion

In this study, we have shown that (i) maternal risk factors including hypertension, gestational diabetes and smoking did not seem to affect ADMA levels of either the maternal or the cord samples; (ii) meconium aspiration during delivery might lead to elevated ADMA levels in the fetal circulation, and (iii) fetal ADMA levels were significantly higher than the maternal ADMA levels.

Several studies have demonstrated that NO played a role in the maternal vascular adaptation to pregnancy, in postnatal adaptation of the neonate and also in various neonatal disorders[2-6,22]. Therefore, maternal or fetal factors that may affect the perinatal NO metabolism are quite important for the neonate. It is known that, similar to NO, ADMA is synthesized in the vascular endothelium and it decreases the synthesis of NO by competitively inhibiting

Table I. Demographic Features and ADMA Level Comparisons Among Groups (Mean±SD)

	Group 1 (n=51)	Group 2 (n=14)	Group 3 (n=11)	Group 4 (n=19)	Group 5 (n=13)
Maternal age (year)	26.87±5.32	29.28±4.89	32.77±5.21	28.21±4.74	26.16±2.12
Maternal weight (kg)	74.13±9.91	89.72±15.86	99.57±21.91	79.61±6.09	82.20±3.35
Gestational week	38.68±1.49	35.85±3.88	38.30±1.25	38.31±1.79	39.76±0.92
Birth weight (g)	3362±603	2697±1083	3300±1202	3322±474	3380±366
*Apgar scores					
1 min	8.7 (7-10)	8.6 (7-10)	8.5 (7-10)	8.5 (7-10)	8.2 (6-9)
5 min	9.5 (8-10)	9.7 (8-10)	9.7 (8-10)	9.8 (9-10)	9.3 (6-10)
ADMA (µmmol/L)					
Maternal	0.66±0.30	0.62±0.21	0.61±0.19	0.56±0.16	0.63±0.08
Cord blood	0.87±0.33	0.88±0.25	0.87±0.24	0.88±0.21	1.50±0.65
	p=0.002	p=0.008	p=0.011	p<0.001	p=0.001

ADMA: Asymmetric dimethylarginine.
P values refer to comparisons between maternal and cord blood ADMA levels in each group.
*Values are given as mean (min-max).

Table II. Evaluation of the Effects of Maternal and Fetal Features on Cord Blood ADMA Levels in Group 1

	n	ADMA (µmmol/L) (Mean±SD)	P value
Female	24	0.88±0.39	p=0.79
Male	27	0.85±0.28	
Vaginal delivery	22	0.91±0.40	p=0.50
Cesarean section	29	0.84±0.27	
Maternal age >30	16	0.89±0.33	p=0.79
Maternal age <30	35	0.86±0.35	
Primipara	19	0.87±0.34	p=0.90
Multipara	32	0.88±0.34	

ADMA: Asymmetric dimethylarginine.

the endothelial NOS[23,24]. In this study, we evaluated the effects of perinatal factors -both concerning the mother and the neonate- on the plasma ADMA levels.

Evidence from experimental and clinical studies strongly suggests that NO synthesis is responsible for the maternal vascular adaptation to pregnancy[8,9]. The low maternal ADMA levels in our group were consistent with the earlier findings[17]. Several studies have suggested that NO also plays an important role in maintaining low vascular tone in fetoplacental vessels. In sheep, for example, the fetal plasma nitrate concentration is 2.5 times higher than that of the mother[10]. In addition, NOS is expressed in the human placental syncytiotrophoblasts and in fetoplacental and umbilical vascular endothelium, where basal production of NO contributes to low fetoplacental vascular resistance[8,22].

We have observed that umbilical vein ADMA levels were higher than maternal venous samples. This significant elevation could be a result of enhanced ADMA formation from high protein breakdown or reduced ADMA elimination. Arginine N-methyltransferase-1 is essential for ADMA formation from protein L-arginine residues and is vital for post-implantation mouse development[22], but its activity during intrauterine development has not yet been described. ADMA is metabolized by dimethylarginine dimethyl aminohydrolase (DDAH) to citrulline[23] or excreted directly into urine[23-25]. Interestingly, DDAH activity was shown to increase after birth, with a peak already after one day[26]. Thus, this decreased DDAH activity during intrauterine growth may lead to high venous cord blood ADMA levels in newborns.

Although ADMA levels of neonates with meconium aspiration were found to be higher than the other groups, the presence of maternal diabetes, hypertension or smoking history did not seem to have any effect on the umbilical vein ADMA levels. Meconium aspiration is one of the important indicators of acute or chronic fetal hypoxia. As is well known, in chronic fetal hypoxia, the blood flow to the periphery (kidneys, spleen, skin, muscle, liver) is decreased, shifting the circulation towards the vital organs[27]. ADMA is metabolized by DDAH or excreted directly into urine[24]. Decreased blood flow to the kidneys during hypoxia might have contributed to the impaired renal elimination of ADMA and thus might have caused increased plasma ADMA levels. Moreover, hypoxia might also have inhibited the DDAH enzyme activity and accordingly might have had a similar effect on the ADMA levels. Nevertheless, this observation is in line with the finding that in newborn piglets exposed to hypobaric hypoxia, lung DDAH activity was markedly suppressed, which would result in impaired ADMA metabolism, higher tissue ADMA levels, more efficient NOS inhibition and apparent reduction in NO production[26]. The rise in ADMA levels in meconium-stained newborns, therefore, interferes with pulmonary vascular adaptation and contributes to the development of persistent pulmonary hypertension.

In conclusion, umbilical vein ADMA levels are modulated independent of several maternal features and risk factors. Although these factors are interrelated and it is difficult to interpret their effects separately, the most significant factor with respect to umbilical vein ADMA levels seems to be perinatal hypoxia, as in the case of meconium staining. Future clinical and experimental studies that will uncover the role of ADMA in perinatal adaptation and its relation to perinatal disorders are warranted.

REFERENCES

1. Rosenfeld CR. Mechanisms regulating angiotensin II responsiveness by the uteroplacental circulation. Am J Physiol 2001; 281: 1025-1040.
2. Richir MC, Siroen MP, van Elburg RM, et al. Low plasma concentrations of arginine and asymmetric dimethylarginine in premature infants with necrotizing enterocolitis. Br J Nutr 2007; 97: 906-911.
3. Ergun O, Ergun G, Oktem G, et al. Enteral resveratrol supplementation attenuates intestinal epithelial inducible nitric oxide synthase activity and mucosal damage in experimental necrotizing enterocolitis. J Pediatr Surg 2007; 42: 1687-1694.
4. Maxwell AJ. Mechanisms of dysfunction of the nitric oxide pathway in vascular diseases. Nitric Oxide 2002; 6: 101-124.
5. Davis JM. Role of oxidant injury in the pathogenesis of neonatal lung disease. Acta Paediatr Suppl 2002; 91: 23-25.
6. Shi Y, Pan F, Li H, et al. Role of carbon monoxide and nitric oxide in newborn infants with postasphyxial hypoxic-ischemic encephalopathy. Pediatrics 2000; 106: 1447-1451.
7. Yoshimura T, Magness RR, Rosenfeld CR. Angiotensin II and α-agonist. I. Responses of ovine fetoplacental vasculature. Am J Physiol 1990; 259: 464-472.
8. Williams DJ, Vallance PJ, Nield GH, Spencer JA, Imms FJ. Nitric oxide-mediated vasodilation in human pregnancy. Am J Physiol 1997; 272: 748-752.

9. Sladek SM, Magnes RR, Conrad KP. Nitric oxide and pregnancy. Am J Physiol 1997; 272: 441-463.
10. Young D, Lang U, Greenberg SG, et al. Elevation of nitrate levels in pregnant ewes and their fetuses. Am J Obstet Gynecol 1996; 174: 573-577.
11. Böger RH, Bode-Böger SM. Asymmetric dimethylarginine, derangements of the endothelial nitric oxide synthase pathway, and cardiovascular diseases. Semin Thromb Hemost 2000; 26: 539-545.
12. Moncada S, Higgs A. The L-arginine-nitric oxide pathway. N Engl J Med 1993; 329: 2002-2012.
13. MacAllister RJ, Fickling SA, Whitley GS, et al. Metabolism of methylarginines by human vasculature; implications for the regulation of nitric oxide synthesis. Br J Pharmacol 1994; 112: 43-48.
14. Savvidou MD, Hingorani AD, Tsikas D, et al. Endothelial dysfunction and raised plasma concentrations of asymmetric dimethylarginine in pregnant women who subsequently develop pre-eclampsia. Lancet 2003; 361: 1511-1517.
15. Speer PD, Powers RW, Frank MP, et al. Elevated asymmetric dimethylarginine concentrations precede clinical preeclampsia, but not pregnancies with small-for-gestational age infants. Am J Obstet Gynecol 2008; 198: 112-117.
16. Maeda T, Yoshimura T, Okamura H. Asymmetric dimethylarginine, an endogenous inhibitor of nitric oxide synthase, in maternal and fetal circulation. J Soc Gynecol Invest 2003; 10: 2-4.
17. Vida G, Sulyok E, Ertl T, et al. Plasma asymmetric dimethylarginine concentration during the perinatal period. Neonatology 2007; 92: 8-13.
18. Tsukahara H, Ohta N, Tokuriki S, et al. Determination of asymmetric dimethylarginine, an endogenous nitric oxide synthase inhibitor, in umbilical blood. Metabolism 2008; 57: 215-220.
19. Report of the National High Blood Pressure Education Program Working Group on High Blood Pressure in Pregnancy. Am J Obstet Gynecol 2000; 183: 1-22.
20. American Diabetes Association. Diagnosis and classification of diabetes mellitus. Diabetes Care 2007; Suppl 1: 42-47.
21. Chen BM, Xia LW, Zhao RQ. Determination of NG, NG –dimethylarginine in human plasma by high-performance liquid chromatography. J Chromatogr B 1997; 2: 467-471.
22. Myatt L, Eis AL, Brockman DE, et al. Endothelial nitric oxide synthase in placental villous tissue from normal, pre-eclamptic and intrauterine growth restricted pregnancies. Hum Reprod 1997; 12: 167-172.
23. Leiper JM. The DDAH-ADMA-NOS pathway. Ther Drug Monit 2005; 27: 744-746.
24. Teerlink T. ADMA metabolism and clearance. Vasc Med 2005; 10 (Suppl): 73-81.
25. Pawlak MR, Scherer CA, Chen J, Roshon MJ, Ruley HE. Arginine N-methyltransferase 1 is required for early postimplantation mouse development, but cells deficient in the enzyme are viable. Mol Cell Biol 2000; 20: 4859-4869.
26. Arrigoni FI, Vallance P, Haworth SG, et al. Metabolism of asymmetric dimethylarginine is regulated in the lung developmentally and with pulmonary hypertension induced by hypobaric hypoxia. Circulation 2003; 107: 1195-1201.
27. Richardson BS, Bocking AD. Metabolic and circulatory adaptations to chronic hypoxia in the fetus. Comp Biochem Physiol A Mol Integr Physiol 1998; 119: 717-723.