Perinatal risk factors affecting the maternal and fetal asymmetric dimethylarginine levels

Mustafa Kul¹, Erkan Demirkaya², Osman Metin İpçioğlu³, Rahmi Sinan Karadeniz⁴
Turan Tunç², Sebahattin Vurucu², Fahri Gürkan Yeşil⁵, Hasan Öztin⁵, Erdinç Çakır⁶

Departments of ¹Pediatrics, and ³Biochemistry and Clinical Biochemistry, Gülhane Military Medical Academy Haydarpaşa Training Hospital, Istanbul, and Department of ³Pediatrics, and ⁶Emergency Medicine Laboratory of Biochemistry, ³Gülhane Military Medical Academy, and ⁴Department of Obstetrics and Gynecology, Ministry of Health Etilk Maternity and Women’s Health Teaching and Research Hospital, Ankara, Turkey


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 vasoconstrictors¹. 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 disease²-⁶.

Characteristic changes, i.e. increased cardiac output and decreased systemic resistance, also ensue in the fetal circulation⁷. 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 role⁸. 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 placenta⁹-¹¹. 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 production¹³.
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\textsuperscript{14,15}. Although in normal pregnancy, ADMA levels were found decreased in the maternal circulation and increased in the umbilical cord blood\textsuperscript{16}, the effects of maternal factors or fetal problems during delivery on the ADMA levels are still not fully understood. Vida et al.\textsuperscript{17} and Tsukahara et al.\textsuperscript{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 20\textsuperscript{th} week of gestation\textsuperscript{19}. Gestational diabetes mellitus was defined as any degree of glucose intolerance with onset or first recognition during pregnancy\textsuperscript{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.\textsuperscript{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 20,000 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-phthalaldialdehyde 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 150 x 4-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. 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)

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

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

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

ADMA: Asymmetric dimethylarginine.
hypoxia might have contributed to the impaired 

Evidence from experimental and clinical studies strongly suggests that NO synthesis is responsible for the maternal vascular adaptation to pregnancy\textsuperscript{8,9}. The low maternal ADMA levels in our group were consistent with the earlier findings\textsuperscript{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\textsuperscript{10}. In addition, NOS is expressed in the human placent al syncytiotrophoblasts and in fetoplacental and umbilical vascular endothelium, where basal production of NO contributes to low fetoplacental vascular resistance\textsuperscript{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\textsuperscript{22}, but its activity during intrauterine development has not yet been described. ADMA is metabolized by dimethylarginine dimethyl aminohydrolase (DDAH) to citrulline\textsuperscript{23} or excreted directly into urine\textsuperscript{23-25}. Interestingly, DDAH activity was shown to increase after birth, with a peak already after one day\textsuperscript{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\textsuperscript{27}. ADMA is metabolized by DDAH or excreted directly into urine\textsuperscript{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\textsuperscript{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**


