Asymmetric Dimethylarginine (ADMA) level in Serum of Preeclamptic patients

Faisal Gh. Al-Rubaye* **MBChB, MSc, PhD**

Summary:

Background: Preeclampsia, the *de novo* occurrence of hypertension and proteinuria after the 20th week of gestation, continues to exert an inordinate toll on mothers and children alike.

The idea that asymmetric dimethylarginine (ADMA) accumulation may be a cardiovascular risk factor in patients with preeclampsia was advanced in 2003. Furthermore, High ADMA levels have been associated with alterations in the regulation of cerebral blood flow and neural function, with insulin resistance, thyroid dysfunction, and alterations in bone homeostasis, fertility, and erectile function.

Subject and methods: the present study is a cross-sectional case-control study includes measurement of s.ADMA in 60 patients with preeclampsia. The results were compared with 60 apparently healthy pregnant women (as controls).

Results: showed a significant increase in serum ADMA in the preeclamptics as compared with the controls this was accompanied by a significant reduction of this parameter with advancing gestational age in normal pregnancy.

Conclusion: preeclamptics (in different gestational age groups) experienced vasospasm induced by inhibition of nitric oxide (which consider the natural vasodilator) when compared with healthy pregnant women matched with their age and gestational age; this was supported by the significant high level of s. ADMA, the endogenous inhibitor of NO .

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Introduction:

The term preeclampsia refers to the new onset of hypertension (140/90 mmHg) and proteinuria after 20 week of gestation in previously normotensive, nonproteinuric women 1. The first indication of a potential role for Nitric oxide (NO) came from the evaluation of guanosine 3',5'-cyclic monophosphate (cGMP), an important second messenger of NO. Plasma concentration, urinary excretion, and metabolic production of cGMP are increased in rat pregnancy and pseudopregnancy 2,3 as well as in human gestation 4,5 Urinary excretion of nitrate and nitrite, stable metabolites of NO, are increased in pregnant and pseudopregnant rats that consume a diet low in nitrite and nitrate corresponding to the rise in cGMP excretion 3. These data suggested that endogenous NO production is increased in gravid rats, as well as in human gestation 6. Supportive evidence for NO deficiency and in preeclampsia has been obtained from the reduced uterine perfusion in rat model 7. Also, women with high resistance placental circulation at risk of preeclampsia 8. Endothelial dysfunction is the common final pathway leading to clinical signs of preeclampsia. Asymmetric dimethylarginine (ADMA) is an endogenous inhibitor of nitric oxide synthase (NOS) and induces endothelial dysfunction by reversibly inhibiting nitric oxide production from L-arginine 8.

**Department of Chemistry & Biochemistry, College of Medicine, Al-Nahrain University.*

Subjects & Methods:

A-Subjects**:** The present study is a cross-sectional, case-control study conducted on 60 patients with preeclampsia (PE) attending the Obstetric Consultant-Clinic, Antenatal Clinic, and Labor Ward at Al-Kadhimiya Teaching Hospital, for re-evaluation of newly diagnosed PE, or for delivery. The diagnosis of PE was based on clinical criteria that were hypertension (absolute BP of 140/90 mmHg twice over 4 hr without prior comparison) and proteinuria (21.5 mg of urinary protein per µmol creatinine). The exclusion criteria used for cases and controls were gestational or chronic hypertension, diabetes mellitus, renal disease, multifetal gestation, intrauterine fetal death, and pregnancy less than 20 weeks of gestation. Depending on the gestational age, the 60 patients were divided into two groups:

1. Preeclamptics in the second trimester **(G1):** They were 30 with age range from 16 to 36 years (mean age $\pm$ SD = 25.1 $\pm$ 5.4 year) and gestational age range from 20 to 28 weeks (mean gestational age $\pm$ SD = 24.3 $\pm$ 1.6 week).

2. Preeclamptics in the third trimester **(G2):** They were 30 with age range from 18 to 38 year (mean age $\pm$ SD = 26.2 $\pm$ 6.7 year), and gestational age range from 29 to 40 weeks (mean gestational age $\pm$ SD = 36.5 $\pm$ 1.5 week). The study also included another 60 apparently healthy pregnant women attending the Antenatal clinic, and Labor Ward at Al-Kadhimiya Teaching Hospital, for re-evaluation of their pregnancy, or for delivery. They were considered as normal controls. They were divided

into two groups according to their gestational age:
1-.Normal pregnant women in the second trimester **(G3):** They were 30 with age range from 15 to 38 years (mean age ± SD = 26.6 + 5.6 year) , and gestational age range from 20 to 28 weeks (mean gestational age ± SD = 24.7 ± 1.9 week).
2-Normal pregnants during the third trimester **(G4):** They were 30 with age range from 18 to 35 year (mean age ± SD = 25.2 ± 6.5 year) and gestational age range from 29 to 40 weeks (mean gestational age ± SD = 37.6 ± 2.3 week). B. Blood samples: Ten milliliters of random venous blood were withdrawn from each patient and control, in supine position, without application of tourniquet. Samples were transferred into clean new plane tube, left at room temperature for 15 minutes for clotting, centrifuged, and the separated serum was transferred into Eppendrof tube and was used for measurement of ADMA. The tubes were stored at -20^{o} C until analysis, which was done within one month after collection. C-Methods: Serum ADMA levels were measured by reversed phase high performance liquid chromatography (HPLC) after pre-column derivetization with ortho-phthalaldehyde (OPA) as described previously by Zeigler et al. 1992 9. D. Statistical analysis: Statistical analysis was done using Excel system version 2003 and includes descriptive statistics (mean and standard deviation) and inferential statistics (*t-test*) to test the significancy of mean difference. When P-value was less than 0.05, the difference is considered statistically significant, and the difference is considered highly significant when P-value was less than 0.001.

Results:
Serum ADMA: Serum ADMA level was significantly higher in preeclamptics (G1 &G2) compared with normal pregnants (G3 & G4) [P < 0.001 for both]. Moreover, serum ADMA was significantly higher in third trimester groups G2 compared with G1 [P < 0.001]. Also, a significant difference was found between healthy pregnants G3 & G4, but serum ADMA was significantly higher in second trimester group G3 compared with G4 [P < 0.05] as in Table 1.

Table (1): The mean serum asymmetric dimethyl arginine (ADMA) in different preeclamptic and control groups (presented as mean ± SD).

Variable	G1	G2	G3	G4
s.ADMA (µmol/L)	0.9 ± 0.002*	1 ± 0.002*	0.7 ± 0.003**	0.6 ± 0.002

(G1):Preeclamptics in the second trimester.
(G2): Preeclamptics in the third trimester.
(G3):Control pregnants in the second trimester.
(G4):Control pregnants during the third trimester.
*** p < 0.001**
**** p<0.05**

Discussion:
In this study we have observed that the concentration of serum ADMA is significantly higher (all p<0.001) in all preeclamptic women compared to women with normal pregnancy, and even when compared with preeclamptic in the second trimester. Several studies reported elevated serum ADMA in women with preeclampsia: 10,11,8. Concerning studies in middle-east region, only one study 12 done in Syrian population yields a results similar to our results. In Iraq, there is no comparable data regarding ADMA and preeclampsia; but there is only one study of ADMA in chronic renal failure done by 13 who demonstrate a raised ADMA level in patients with chronic renal failure. One important difference and limitation of our study compared to other studies like the study by 8 is that we lack Doppler data on the majority of our subjects, and therefore we cannot make a direct comparison of these studies. Elevated circulating ADMA concentrations may be of pathophysiological importance in pregnancies complicated with preeclampsia. Lower ADMA levels in pregnant women compared to non-pregnant controls suggest that ADMA has a role in vascular dilatation and blood pressure changes; these findings suggest that ADMA has a role in the pathogenesis of preeclampsia 14. ADMA levels seem to fall during normal pregnancy but are increased in women with preeclampsia. Recently, it has been shown that ADMA levels are increased even before the development of pre-eclampsia, suggesting that ADMA might provide a novel risk marker for the early detection of women at high risk. It is not known where the ADMA originates, but ADMA is produced by the fetus and is present in large amounts in fetal plasma and urine. Furthermore, the placenta expresses high levels of dimethylarginine dimethylaminohydrolase-2 (DDAH-2) (ADMA-degrading enzyme) raising the possibility that failure of the placenta to clear the ADMA produced by the fetus is important. In women with high ADMA levels early in pregnancy, there was a clear relationship between the ADMA level and endothelial dysfunction, but this was only seen in the women who subsequently developed pre-eclampsia.85 This may suggest that a raised ADMA alone is insufficient, but that some individuals are particularly susceptible to the effects of raised ADMA and they are at most risk of developing complications 15. Endothelial-dependent vascular dysfunction is a central pathophysiological feature of preeclampsia. Endothelial dysfunction could be the result of impaired nitric oxide synthesis. Increasing evidence suggests that elevated concentrations of ADMA act as an endogenous inhibitor of NOS to contribute to endothelial-dependent vascular dysfunction in diabetes, hypertension, hypercholesterolemia, and obesity16. In addition to preventing NO production by competitive inhibition of NOS, ADMA may promote the uncoupling of NOS enzymatic activity

and convert NOS to a superoxide generator. This uncoupling activity of ADMA is proposed to further contribute to vascular dysfunction and may play a role in the pathophysiology of preeclampsia by serving as a source of vascular oxidative stress17. Recently, There has been an increased interest in the role of angiogenic growth factors (VEGF and PIGF) and their soluble receptors (sFIt1, antiangiogenic) in the pathophysiology of preeclampsia.16 ADMA is another important molecule capable of affecting angiogenesis in pregnancy and preeclampsia. Specifically, angiogenesis is negatively affected by high ADMA concentrations 6, 17, and the angiogenic activity of growth factors such as vascular endothelial growth factor (VEGF), placental growth factor (PIGF), and basic fibroblast growth factor (bFGF) are mediated through a nitric oxide dependent mechanism that is inhibited by ADMA. 18, 19 ADMA is also reported to decrease the expression of VEGF in cultured endothelial cells, and therefore may affect not only growth factor activity but its production. 20 Therefore, it is interesting to speculate that there may be interactions between pro-angiogenic growth factors (VEGF and PIGF) and antiangiogenic factors such as ADMA as well as sFIt1 in early pregnancy that could influence the risk of preeclampsia 18.

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