STUDY OF PLASMA NITRIC OXIDE, PLASMA MALONDIALDEHYDE AND SERUM URIC ACID CONCENTRATION IN NORMAL AND PREECLAMPTIC PREGNANT WOMEN

Tun Nay Oo[1], Myat Thandar[2], Yi Yi Myint[2], Zaw Lin Thein[2]

[1]*Physiology Department, University of Medicine Magway*
[2]*Physiology Department, University of Medicine (1), Yangon*

ABSTRACTS

Endothelial cell dysfunction and oxidative stress have been implicated in the pathophysiology of preeclampsia. Endothelial function and oxidative stress could be assessed by measuring the blood malondialdehyde, nitric oxide and uric acid levels. The aim of the study is to study plasma nitric oxide, plasma malondialdehyde and serum uric acid levels in preeclamptic pregnant women. A cross-sectional, comparative study was done. Samples were obtained from 35 normotensive and 35 preeclamptic pregnant women in their third trimester. Plasma nitric oxide, plasma MDA and serum uric acid levels were determined. Mean plasma nitric oxide, plasma MDA and serum uric acid levels were significantly higher in the preeclamptic than in normotensive pregnant women ($p<0.05$; $p<0.001$ and $p<0.001$ respectively). There was a significant positive correlation between plasma nitric oxide level and plasma MDA level, between plasma nitric oxide level and serum uric acid level, and between serum uric acid and plasma MDA levels in the preeclamptic pregnant women ($r = 0.38$, $p < 0.05$; $r = 0.35$, $p < 0.05$; $r = 0.31$, $p < 0.05$ respectively). No significant correlations between the above variables were observed in the normotensive pregnancy group. These findings reaffirmed that endothelial cell dysfunction and oxidative stress are involved in the pathophysiology of preeclampsia.

INTRODUCTION

Preeclampsia, a complex multisystem disorder that occurs during pregnancy, is associated with the highest maternal and fetal morbidity and mortality related to pregnancy complications[1].

Despite its morbidity and mortality, the etiology and pathogenesis of preeclampsia remain poorly understood. However, pathophysiological features of preeclampsia such as increased sensitivity to pressors, activation of the coagulation cascade, increased vascular permeability and endothelial changes in many organ systems suggest that generalized endothelial cell damage and dysfunction are major features[2].

Although the mechanisms underlying endothelial dysfunction may be multifactorial, there is a growing body of evidence that increased production of reactive oxygen species (ROS)

may contribute considerably to this phenomenon[3]. Deleterious effects of free radicals include initiation of lipid peroxidation, oxidative damage of biomolecules, and cellular dysfunction, and it is proposed that these may initiate maternal vascular endothelial dysfunction1. Malondialdehyde (MDA), a major metabolite of lipid peroxidation, can be used as an indicator of oxidative stress[2].

Nitric oxide (NO) is synthesized from L-arginine by endothelial nitric oxide synthase (eNOS) and it is an endothelial factor responsible for the relaxation of vascular smooth muscle cells. NO seems to be a major contributor to these physiologic adaptive changes of normal pregnancy such as an increase in heart rate, cardiac output, blood volume and a decrease in arterial pressure[5]. In animal models, the inhibition of NO production leads to findings similar to those reported in human preeclampsia, including hypertension, proteinuria and fetal growth restriction, suggesting that diminished production of NO by endothelial cells could account for the vascular physiology of preeclampsia[6].

Elevated concentration of uric acid in preeclampsia is not simply a marker of disease severity but rather contributes directly to the pathogenesis of the disorder[7]. Uric acid levels have been positively correlated with markers of endothelial damage[8] and elevated uric acid concentration could participate in reduced production of NO[9].

Although increased oxidative stress and endothelial dysfunction could explain many signs of preeclampsia, studies on the role of nitric oxide metabolites have yielded conflicting results. Circulating plasma levels of nitric oxide metabolites in preeclamptic patients have been reported as decreased[1], unchanged[10], and increased[11], [12]compared with normotensive pregnancies. Factors like diet, medications, and urinary excretion[13], source of the sample like serum, plasma or urine, disease severity and assay methods[14] can affect serum nitrate concentrations. The involvement oxidative stress and endothelial dysfunction in pathophysiology of preeclampsia still need to be elucidated.

Thus, in the present study, plasma nitric oxide metabolites (nitrites and nitrates), plasma malondialdehyde and serum uric acid levels were measured in preeclampsia and normal pregnancy to further understand the pathophysiology of preeclampsia.

MATERIALS AND METHODS

A cross sectional, comparative study was done. Seventy normal and preeclamptic pregnant women in the third trimester aged between 18 to 40 years were recruited. Each group consisted of 35 women. Preeclampsia was diagnosed by obstetrician. The subjects were recruited from the Obstetrics and Gynaecological units of Central Women's Hospital and Defence Services Obstetrics Gynaecology and Children's Hospital. Those who smoked and had complications such as diabetes mellitus, renal disease, cardiovascular disease and primary hypertension were excluded. Before blood sample collection, pregnant women were interviewed and thorough clinical history was taken. Informed consent was taken after explanation about the experiments.

About eight ml of blood was taken from the antecubital veins of each normal pregnant and preeclamptic pregnant woman after overnight fasting. Blood samples were collected in two separate test tubes. Six ml of blood was placed into the tube containing ethylenediaminetetraacetic acid (EDTA) for nitric oxide and MDA assay. Two ml of blood was placed into another test tube for serum uric acid assay.

All samples were collected with code numbers and analyzed batch by batch. The plasma for nitric oxide was stored at -80°C until analysis. Plasma MDA and serum uric acid level were determined on the same day of sample collection. Plasma nitric oxide level was determined by colorimetric method using Griess reagents (Nitrate/Nitrite Colorimetric Assay Kit- catalog no: 780001, Cayman Chemical Company, USA). Plasma MDA level was determined by spectrophotometric method using thiobarbituric acid[15]. Serum uric acid level was determined by simplified alkaline phosphotungstate method[16].

RESULTS

The plasma nitric oxide level (mean ± SD) of normal pregnant women was 22.45 ± 6.70 µmol/L whereas that of preeclamptic pregnant women was 26.50 ± 9.43 µmol/L. The plasma nitric oxide level of preeclamptic pregnant women was significantly higher than that of normal pregnant women ($p < 0.05$) (Figure 1).

The plasma MDA level (mean ± SD) of normal pregnant women was 1.72 ± 0.15 µmol/L whereas that of preeclamptic pregnant women was 2.54 ± 0.28 µmol/L. The plasma MDA level of preeclamptic pregnant women was significantly higher than that of normal pregnant women ($p < 0.001$)(Figure 2).

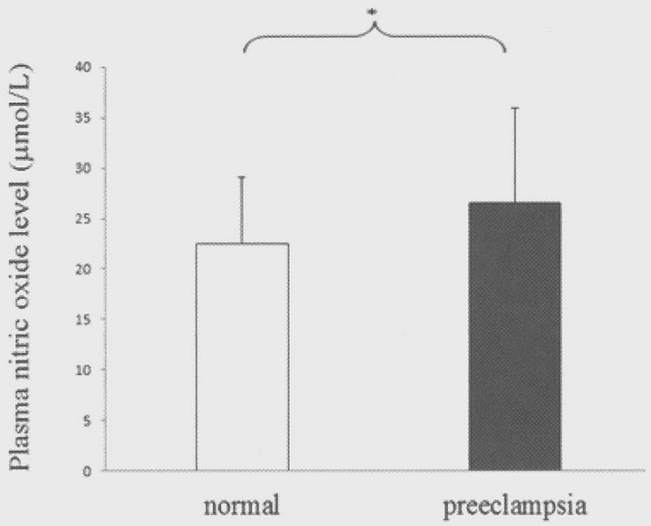

Figure1. Plasma nitric oxide level in normal pregnant and preeclamptic pregnant women
* indicates significant difference ($p < 0.05$) between normal and preeclamptic subjects

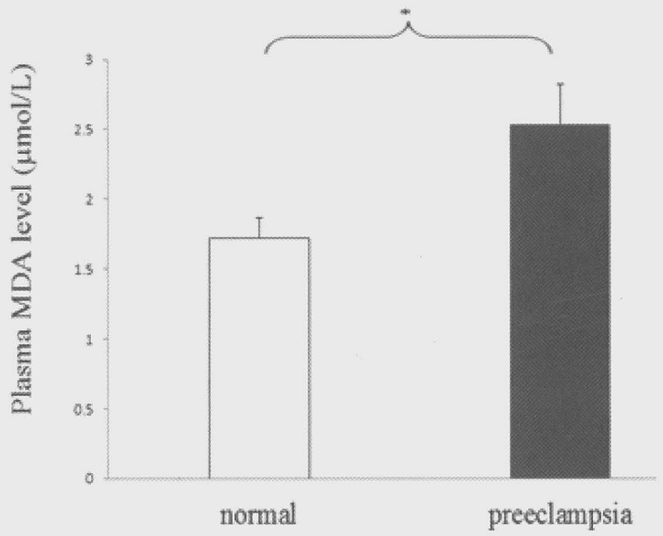

Figure 2. Plasma MDA level in normal pregnant and preeclamptic pregnant women
* indicates significant difference ($p < 0.001$) between normal and preeclamptic subjects

The serum uric acid level (mean ± SD) of normal pregnant women was 4.11 ± 0.51 mg/dL whereas that of preeclamptic pregnant women was 5.92 ± 1.32 mg/dL. The serum uric acid level of preeclamptic pregnant women was significantly higher than that of normal pregnant women ($p < 0.001$) (Figure 3).

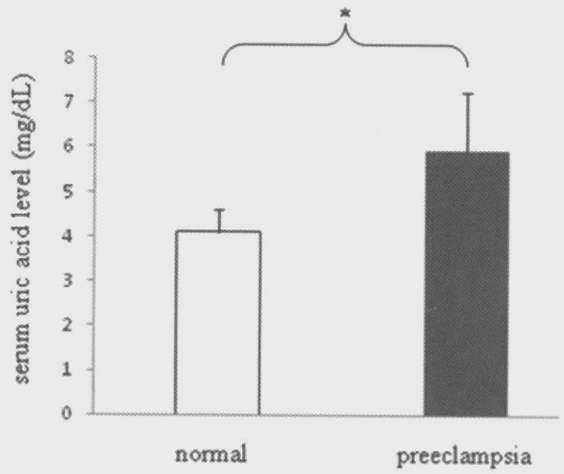

Figure 3. Serum uric acid level in normal pregnant and preeclamptic pregnant women

* indicates significant difference ($p < 0.001$) between normal and preeclamptic subjects

There was a significant positive correlation between plasma nitric oxide level and plasma MDA level in preeclamptic pregnant women ($r = 0.38$, $p < 0.05$) (Figure 4).

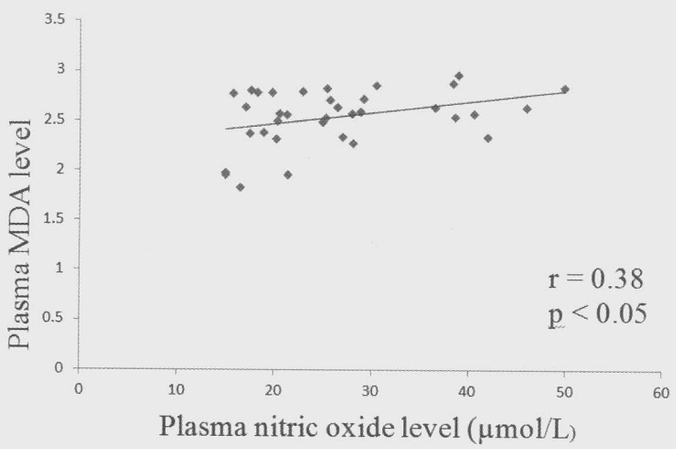

Figure 4. Correlation between Plasma Nitric Oxide and Plasma MDA levels in preeclamptic women

Similarly, there was a significant positive correlation between plasma nitric oxide level and serum uric acid level in preeclamptic pregnant women ($r = 0.35$, $p < 0.05$) (Figure 5).

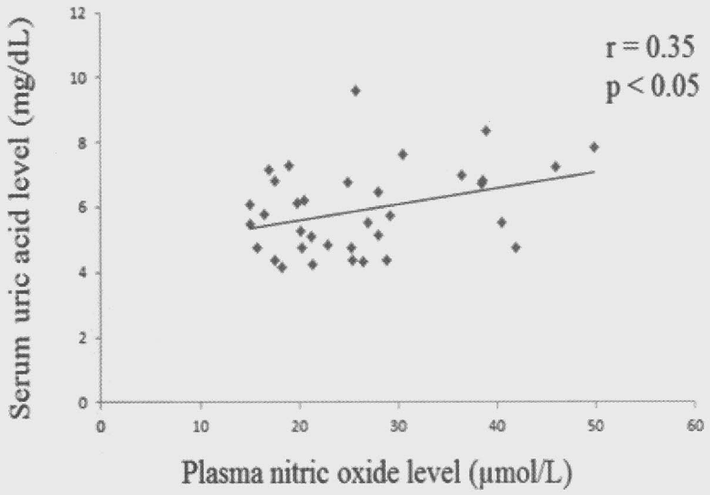

Figure 5. Correlation between plasma nitric oxide and serum uric acid levels in preeclamptic women

Also, a significant positive correlation between serum uric acid and plasma MDA levels was found in preeclamptic pregnant women ($r = 0.31$, $p < 0.05$) (Figure 6).

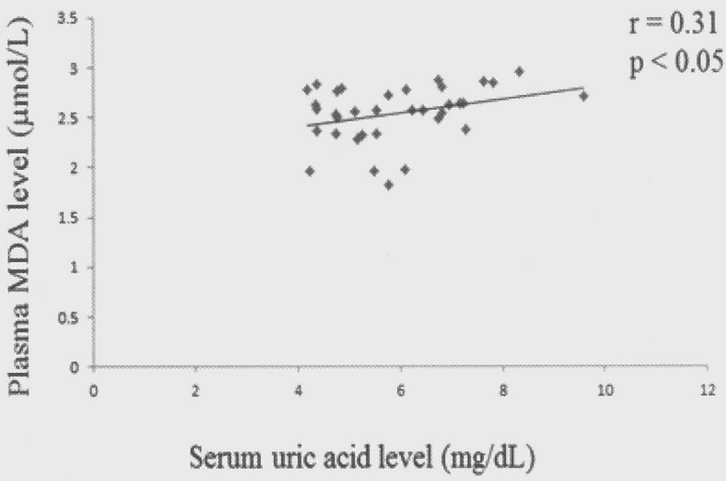

Figure 5. Correlation between plasma nitric oxide and serum uric acid levels in preeclamptic women

However, there were no correlations between the above variables in normotensive pregnancy group.

DISCUSSION

Etiology of preeclampsia is widely studied and role of oxidative stress in impaired endothelial cell function is still a subject of interest. Endothelial cell injury in preeclampsia could be caused by free radical-induced uncontrolled lipid peroxidation[17]. The present study was undertaken to evaluate plasma nitric oxide, plasma malondialdehyde and serum uric acid levels in third trimester pregnant women with or without preeclampsia.

In the present study, mean plasma MDA level was significantly higher in women with preeclampsia than that of normal pregnancy. A number of reports indicated that MDA levels were elevated in women with preeclampsia relative to normal pregnancy[18, 19, 20]. The findings in the present study provide further evidence for uncontrolled lipid peroxidation or increased oxidative stress as an important factor in the pathogenesis of preeclampsia.

In the present study, mean plasma nitric oxide level was significantly higher in preeclampsia than in normal pregnancy. Discrepancies between the present study and many studies were also noted[2] and Bernardi et al (2008) found a significant decrease in the nitric oxide level in preeclamptic women[21]. Diejomaoh et al (2004) and Silver et al (1999) found no significant difference in circulating nitric oxide levels between preeclampsia and normotensive control[10, 14]. Plasma nitric oxide concentration might be related to dietary factor. The alimentary supply of nitric oxide can be up to three times as high as the total body nitrite and nitrate porduction[11]. Lack of dietary control in some studies might explain the differences in these findings. In the present study, the blood was collected early in the morning after at least 8 hours of fasting in order to avoid the influence of diet.

A decrease in NO level was not observed in preeclampsia in the present study. But it cannot be interpreted that the bioavailability of nitirc oxide was not reduced. A reduced availability of NO may not be due to reduced nitric oxide synthesis but rather inactivation of NO by superoxide anions, which result from increased oxidative stress[22]. NO reacts with superoxide anion to generate the powerful oxidant peroxynitrite[23, 24]. Peroxynitrite is cytotoxic and causes oxidative damage to proteins, lipids, DNA[24], initiates lipid peroxidation[25], and increases platelet activation[26]. An increase in nitric oxide production and the concomitant increase in oxidative stress might also affect endothelial

function through peroxynitrite. In the present study, increased NO and MDA levels were found in preeclmapsia. NO levels were positively correlated with plasma MDA levels. Concomitant increase in NO and MDA levels could be an indirect evidence of increased peroxynitrite formation.

Excessive NO production could be compensation for the vasoconstriction and the higher platelet aggregation, which are the typical signs of preeclampsia[27,28]. Nobunaga, Tokugawa and Hasimoto (1996) reported that the plasma concentrations of nitric oxide in the patients with preeclampsia were higher than those in the normotensive pregnant women[29]. However, pregnant patients with underlying essential hypertension had significantly lower plasma concentrations. In addition, Bartha et al (1999) found that serum nitric oxide levels were higher in preeclamptic women, lower in chronic hypertensive women and similar in women with gestational hypertension in comparison to the control group[27]. Armas-Padilla et al (2007) found that serum NO levels were significantly reduced in patients with essential hypertension as compared with normotensive individuals[30]. On the other hand, increased endothelial NO production and increased NOS activity were found after exposure to plasma from the preeclampsia[31]. They noted that this was due to greater gene expression of the constitutive endothelial nitric oxide synthase enzyme, which could be up-regulated by a factor present in plasma from women with preeclampsia. Thus increased nitric oxide levels might be specific to preeclampsia and not simply the counter-regulatory response to high blood pressure.

Circulation levels of nitrite and nitrate might also be affected by elimination. Preeclamptic renal changes could affect plasma nitrite and nitrate levels[11]. Cameron et al (1993) reported that urinary nitric oxide excretion did not differ significantly between preeclamptic and normotensive pregnant women[32]. In the present study, serum uric acid levels were positively correlated with plasma nitric oxide levels in preeclampsia. Reduced renal clearance could be responsible for increased plasma nitric oxide level.

In the present study, gestational age was significantly higher in controls than in preeclamptic women (36.6 ± 2.5 vs. 34.3 ± 2.9 weeks). That was unlikely to affect nitrite and nitrate data, because plasma levels of nitrite and nitrate were reported to be independent of duration of gestation in normotensive subjects during the last trimester of pregnancy[11].

In the present study, patients taking antihypertensive drugs were not excluded. But in a study of Ranta et al (1999), preeclamptic women were given antihypertensive medication or betamethasone, but neither drug caused any changes in plasma nitrite and nitrate values[11].

In the present study, mean serum uric acid level was significantly higher in the preeclamptic women than in the normal pregnant women. Serum uric acid level was positively correlated with plasma MDA level in preeclamptic patients. Correlation of plasma MDA and serum uric acid levels may reflect the fact that the activity of xanthine oxidase, which yields uric acid and superoxide anion, is increased as a result of placental ischemia and the resultant free radicals might cause lipid peroxidation[33, 34]. The positive correlation might also suggest the relationship between the MDA level and severity of preeclampsia.

In the present study, serum uric acid level was positively correlated with plasma nitric oxide level and increased nitric oxide level might also indicate the disease severity or renal dysfunction.

CONCLUSION

Nitric oxide, malondialdehyde and uric acid level were higher in the preeclamptic women, and significant correlations between these variables were found only in the preeclamptic subjects. These findings reaffirmed that endothelial cell dysfunction and oxidative stress are involved in the pathophysiology of preeclampsia.

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