The Myanmar Health Sciences Research Journal, Vol. 21, No. 3, 2009

Plasma malondialdehyde, vitamin C and vitamin E levels of normal pregnant and preeclamptic pregnant women in West Yangon General Hospital

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This study was conducted to evaluate the plasma levels of vitamin C, vitamin E, and malondialdehyde (MDA) in 48 normal pregnant women and 35 preeclamptic Myanmar pregnant women between the ages of 18-40 years and at the gestational ages of ≥32 weeks. Plasma levels of vitamin C, vitamin E and MDA were measured by spectrophotometric methods. In normal pregnancy, plasma vitamin C level was 1.25±0.13 mg/dl, plasma vitamin E level was 1.88±0.47 mg/dl and plasma MDA level was 2.02±0.25 μmol/l. In preeclampsia, plasma vitamin C level was 0.73±0.11 mg/dl, plasma vitamin E level was 1.33±0.39 mg/dl and plasma MDA level was 3.20 ± 0.30 μmol/l. Plasma levels of antioxidant vitamins C and E were significantly lower in preeclamptic pregnancy than in normal pregnancy. Plasma MDA level was significantly higher in preeclamptic pregnancy than that of normal pregnant women. There were significant negative correlations between plasma MDA level and plasma levels of vitamin C and vitamin E in normotensive as well as in preeclamptic pregnant women. A significant correlation was also detected between diastolic blood pressure and plasma levels of vitamin C, vitamin E and MDA in normal pregnancy and in preeclampsia. In conclusion, plasma MDA level was increased and antioxidant vitamins C and E were decreased in preeclampsia.

INTRODUCTION

Pregnancy is a stressful condition in which many physiological and metabolic functions are altered to a considerable extent. Pre-eclampsia is one of the most serious complications of pregnancy and despite extensive research its etiology and pathogenesis remain obscure.

Normal pregnancy itself is an oxidative event as it has been demonstrated that circulating levels of lipid peroxides increase significantly during the gestation when compared to that of non-pregnancy [1]. It indicates that peroxidation reactions are enhanced during pregnancy. The major source of oxidative stress during gestation appears to occur in the placenta. Generation of free radicals increases during pregnancy and placental mitochondria are the major sources of reactive oxygen species. Reactive oxygen species attack the phospholipids of cell membranes and react with poly-unsaturated fatty acids, occurring lipid peroxidation, which results in primary lipid peroxidation products such as lipid hydroperoxides and secondary lipid peroxidation products such as MDA. Lipid hydroperoxides bind to lipoproteins and are carried to distant sites where the hydroperoxides can propagate lipid peroxidation.

The destructive process of lipid peroxidation may contribute to the development of vascular endothelial cell dysfunction. The elevated plasma concentration of MDA may precede the development of preeclampsia. Lipid peroxidation products are toxic to cells and membranes, but they are normally

controlled by the antioxidant scavenging system. Uncontrolled peroxidation may cause disruption of membrane lipids and other cell components, and then lipid peroxides are involved in endothelial cell injury, vasoconstriction, and imbalance between thromboxane and prostacyclin in preeclampsia.

In addition to the enzymic mechanism of the free radical removal, essential nutrients that can scavenge free radicals, such as vitamin C and E, constitute a strong line of defense in retarding free radical induced cellular damage. Antioxidant systems serve to protect them against the harmful oxidative reactions. However, chronic excessive generation of free radicals will deplete antioxidant vitamins.

Several studies have reported that there is an imbalance between lipid peroxides and antioxidants in preeclampsia and suggested that oxidative stress may contribute to the damage of endothelium which plays a role in the pathophysiologic mechanism of the disease [2-5].

The present study is carried out to determine plasma levels of MDA, vitamin C and vitamin E in normal pregnant and pre-eclamptic pregnant women. The outcome of the research may be useful in the determination of the need to give antioxidant vitamins in normal pregnancy as well as in preeclampsia.

Objectives

- To determine the plasma ascorbic acid level of normal pregnant and pre-eclamptic pregnant women in West Yangon General Hospital (WYGH)
- To determine the plasma tocopherol level of normal pregnant and pre-eclamptic pregnant women in WYGH
- To evaluate the plasma MDA level of normal pregnant and preeclamptic pregnant women in WYGH
- To compare the above biochemical parameters in preeclampsia with normal pregnancy

MATERIALS AND METHODS

A hospital-based study was performed on 48 normal pregnant women and 35 pre-eclamptic pregnant women presenting at WYGH antenatal clinic. They were interviewed and thorough clinical history was taken after their informed consents had been obtained. Their blood and urine samples were collected before starting any anti-hypertensive medication. All biochemical parameters; plasma vitamin C, vitamin E, and MDA levels were determined at the Research Laboratory of Biochemistry Department, University of Medicine I, Yangon, on the same day of sample collection. All samples were collected with code numbers and analyzed batch by batch in duplicate. Plasma vitamin C level was determined by simple colorimetric method for ascorbic acid [6]. Plasma vitamin E level was determined by spectrophotometric method [7]. Plasma MDA level was determined by spectrophotometric method using thiobarbituric acid [8].

Operational definition of preeclampsia is as followed: hypertension of at least 140/90 mmHg recorded on two separate occasions at least 4 hours apart and in the presence of at least 300 mg protein in a 24-hour collection of urine (1+ proteinuria), arising de novo after the 20^{th} week of gestation in a previously normotensive woman and resolving completely by the 6^{th} postpartum week [9].

Inclusion criteria for the present study were age between 18-40 years and 28-40 weeks gestational period at blood and urine sampling. Exclusion criteria were women in labour, multiple pregnancy, smoker, those taking antioxidant vitamins, and any concurrent medical diseases.

Data analysis was done by using the Statistical Package for Social Sciences (SPSS) software version 13. Standard statistical methods were applied for the calculation of mean, standard deviation and standard error. Student's "t" test (unpaired) was applied to calculate the significance of

difference between the means on 95% confidence interval of each parameter. Evaluation was done at the probability level of less than 0.05. Pearson's correlation coefficient (r) was calculated to assess the relationship between the variables.

RESULTS

Basic parameters

Both normal pregnant women and preeclamptic pregnant women had gravida 1 to 3. Systolic and diastolic blood pressures were significantly high in preeclamptic pregnant women (Table 1).

Table 1. Clinical parameters of normal pregnant and preeclamptic pregnant women

	Normal pregnant women[a] (n = 48)	Preeclamptic pregnant women[a] (n = 35)	Level of signi-ficance[b] (p)
Age (years)	28.02±6.05	28.31±5.28	0.819
Gestational age at blood sampling (weeks)	35.58±1.76	35.57±1.48	0.974
Systolic blood pressure (mmHg)	117.71±4.25	144.57±6.11	<0.001*
Diastolic blood pressure (mmHg)	77.71±4.25	94.29±5.02	<0.001*

a=The data shown are as mean ± standard deviation values.

b=The p values refer to the difference from the normal pregnant group.

*=denotes statistically significant according to Student's t-test.

Plasma vitamin C, vitamin E, and MDA levels

Plasma vitamin C, vitamin E, and MDA levels of normal pregnant and preeclamptic pregnant women are shown in Table 2. Mean plasma levels of vitamin C and vitamin E were significantly lower in pre-eclamptic pregnant women as compared to normal pregnant women. Mean plasma MDA level was significantly higher in pre-eclamptic pregnant women than that in normal pregnant women.

Correlation between plasma MDA level and plasma levels of vitamin C and vitamin E

There was a significant negative correlation between plasma MDA level and plasma vitamin C level in normotensive pregnant women ($r=-0.921$, $p<0.001$) and in pre-eclamptic patients ($r=-0.957$, $p<0.001$). There was a significant negative correlation between plasma MDA level and plasma vitamin E level in normotensive pregnant women ($r=-0.845$, $p<0.001$) and in pre-eclamptic patients ($r= -0.875$, $p<0.001$).

Table 2. Plasma vitamin C, vitamin E and MDA levels of normal pregnant and preeclamptic pregnant women

	Normal pregnant women[a] (n = 48)	Preeclamptic pregnant women[a] (n = 35)	Level of significance[b] (p)
Plasma vitamin C level (mg/dl)	1.25±0.13	0.73±0.11	0.001*
Plasma vitamin E level (mg/dl)	1.88±0.47	1.33±0.39	0.001*
Plasma MDA level (µmol/l)	2.02±0.25	3.20±0.30	0.001*

a=The data shown are as mean ± standard deviation values.

b=P values refer to the difference from the normal pregnant group.

*=Statistically significant according to student's t test

Correlation between plasma level of MDA, vitamin C and vitamin E with blood pressure

Plasma MDA level was found to be significantly and positively correlated with diastolic blood pressure in normal pregnant women as well as in preeclamptic pregnant women ($p<0.01$ and $p<0.05$, respectively).

Plasma vitamin C level was found to be significantly and negatively correlated with diastolic blood pressure in normal pregnant women as well as in preeclamptic pregnant women ($p<0.01$ and $p<0.05$, respectively).

Plasma vitamin E level was found to be significantly and negatively correlated with diastolic blood pressure in normal pregnant women as well as in preeclamptic pregnant women ($p<0.01$ and $p<0.05$, respectively). Plasma vitamin E level was also significantly and negatively correlated with systolic blood pressure in normal pregnant women ($p<0.05$).

DISCUSSION

Free radicals by their unstable and transient nature are difficult to measure directly. Their tendency to cause lipid peroxidation has been used as an indirect measure. Marker of lipid peroxidation (MDA) was found to be increased in preeclamptic pregnancy. A number of reports also indicated that plasma level of MDA, lipid peroxidation product, was elevated in women with preeclampsia relative to normal pregnancy, and it has been suggested that lipid peroxidation may play a role in the etiology of the disease [10-13].

Plasma vitamin C level of preeclamptic pregnant women was significantly lower than that of normal pregnant women and it might be due to increased utilization of vitamin C to scavenge free radicals and also to regenerate other antioxidants in preeclampsia.

Plasma vitamin E level was also significantly lower in women with preeclampsia than that in normal pregnancy. Decreased plasma vitamin E level in preeclampsia may be due to increased consumption of the vitamin during lipid peroxidation. Another possibility is decreased absorption of vitamin E from the gut as a result of vasoconstriction in preeclampsia.

Antioxidant nutrients counteract the free radical disturbances and thereby protect cell membranes against free radical mediated lipid peroxidation. As a water-soluble antioxidant, ascorbic acid traps most of the free radicals present in the aqueous phase of the plasma and functions as a first-line defense mechanism against free oxygen radicals. When the capacity of ascorbic acid is exceeded, free radicals can then diffuse to the cell membranes. Vitamin E is highly lipid-soluble and is distributed in the lipid layer of the membrane and acts as a free radical scavenger in preventing lipid peroxidation in the membrane of cells, mitochondria and cell organelles where lipid peroxidation occurs. Preeclampsia is thus associated with increased utilization of antioxidant vitamins.

The increase in oxidative stress was further supported by the observation of a negative correlation between lipid peroxidation product (MDA) with antioxidants (vitamin E and vitamin C).

An increase in diastolic blood pressure correlates positively with plasma MDA level and also correlates negatively with plasma vitamin C and vitamin E levels indicating that the severity of hypertension in pre-eclampsia was correlated with the extent of lipid peroxidation.

The findings of the present study are in agreement with the study including 30 preeclamptic pregnant women and 30 normotensive pregnant women in India [14]. Mean plasma vitamin C level was 1.06±0.41mg/dl in normal pregnancy and 0.81±0.1mg/dl in preeclampsia. Mean plasma vitamin E level was 1.12±0.05 mg/dl in normal pregnancy and 0.5±0.04 mg/dl in preeclampsia. Both plasma vitamin C and vitamin E levels were significantly decreased in preeclampsia as compared to controls ($p<0.05$).

In another study, assessment of plasma vitamin C and MDA levels in 20 normal pregnant and 38 preeclamptic pregnant women was carried out [4]. Plasma vitamin C level was significantly lower and plasma MDA level was significantly higher in preeclamptic pregnancy compared with normal pregnancy. The present study also agreed well with that result.

The results of the present study are also in agreement with a study in Japanese women [15]. Serum MDA and vitamin E levels were measured in 18 preeclamptic and 25 nomotensive pregnant women in Ondokuz Mayis University Hospital. The investigators concluded that there was an imbalance between lipid peroxidation and serum vitamin E level in preeclampsia and that increased lipid peroxidation was well correlated with the increase in systolic and diastolic blood pressure measurements.

The results of the present study suggested that vascular endothelial cell dysfunction in preeclampsia may be caused by uncontrolled lipid peroxidation which overwhelms the protective mechanisms of the antioxidants. In other words, the decrease in antioxidant vitamin levels observed in the present study indirectly supports the concept that free radical mediated lipid peroxidation and related antioxidant consumption may be involved in the pathophysiologic mechanisms of preeclampsia. The concept of increased utilization of vitamin C and vitamin E in preeclamptic pregnant women raises the possibility of a potential protective role for antioxidant vitamins for preeclampsia.

ACKNOWLEDGEMENT

The authors acknowledge with sincere gratitude to Dr. Htun Naing Oo, Director-General, Department of Medical Research (Central Myanmar), for his kind support. The authors would like to express their gratitude to Professor Htay Htay for her invaluable advice and Dr. Pe Win, Medical Superintendent of West Yangon General Hospital for granting permission to carry out the study in this hospital. The authors also greatly appreciate the help provided by the House Surgeons.

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