Serum selenium level and lipid peroxidation in normal and pre-eclamptic myanmar pregnant women

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Abstract

Preeclampsia is a major complication of pregnancy that is associated with high maternal and prenatal morbidity and mortality. Preeclampsia is characterized by abnormal placentation and maternal systemic response. This process depends on an imbalance of the oxidant-antioxidant system which normally operates effectively during gestation. Therefore, the present study was aimed to determine the serum selenium (Se) and malondialdehyde (MDA) in normal and pre-eclamptic pregnant women and non-pregnant women. Samples were obtained from 30 normal non-pregnant women, 30 healthy pregnant women at four weekly intervals from 24 weeks till 36 weeks and 30 pre-eclamptic pregnant women in their third trimester. The serum Se was determined by using Atomic Absorption Spectrophotometer and MDA by Thiobarbituric acid test reaction. Serum MDA was significantly higher ($P < 0.01$) and serum Se was significantly lower ($P < 0.01$) in pre-eclamptic pregnant women when compared with normal pregnant and non-pregnant women. Significant negative correlation ($P < 0.01$) between MDA and Se level was found in normal pregnancy. In conclusion, increased MDA indicated that increased oxidative stress could be one of the possible factors of lipid peroxidative damage to vascular endothelial cells in pre-eclampsia. It was suggested that selenium might be utilized in the lipid peroxidation process in pre-eclampsia.

Introduction

Normal pregnancy itself is oxidative event. It is evident by the presence of signs of oxidative stress during normal gestation, such as increased circulating lipid peroxides, increased leukocyte activation and increased circulating Melonylaldehyde (MDA)[1]. During the pregnancy, lipid peroxidation process appears to occur in placenta as shown by interaction of reactive oxygen species (ROS) with unsaturated lipids producing isoprostanes, lipid hydroperoxides (primary) and malonylaldehyde (MDA) (secondary). Therefore, MDA is measured in the blood as an index of lipid peroxidation[2]. Pre-eclampsia (PE) is a multi-system disorder and one of the important life-threatening problems during pregnancy.

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Clinically, it is characterized by maternal hypertension, proteinurea and edema. It can occur at any time from late 2nd trimester to the 3rd trimester of gestation[3]. Though an exact underlying pathophysiological mechanism of PE remained unsolved, placental under perfusion is thought to cause increased oxidative stress which overwhelms the antioxidant protective mechanisms, resulting in an imbalance of oxidant-antioxidant system and the clinical entity of PE[4].

An essential trace mineral Selenium (Se)[5], is present in the human body as selenocysteine (R-SeH) which is an integral component of selenoprotein (GPx)[6]. Glutathione peroxidase is a selenium dependent anti-oxidant enzyme (selenoprotein) which removes H_2O_2 and damaging lipid and phospholipid hydroperoxides generated in vivo by free radicals and oxygen derived species[7]. Thus, GPx acts as one of the anti-oxidant enzymes in the body. Se, an important component of anti-oxidant enzyme GPx, is required for cytosolic optimal activity of the enzyme[8] and therefore Se acts as anti-oxidant. Thus, conditions associated with increased oxidative stress are expected to be influenced by plasma Se[9]. Some studies reported lower Se levels in PE compared with healthy pregnant and non-pregnant women[10,11]. However, Rayman, 2003 found no association between Se status and risk for development of PE[12]. As the relationship between lipid peroxidation status and selenium dependent anti-oxidant enzyme activity in normal and PE pregnant women is still controversial, the aim of the present study was to evaculate lipid peroxidation status and selenium dependent glutathione peroxidase (anti-oxidant system) in Myanmar pre-eclamptic and normal pregnancy.

Materials and Methods

There were three study groups (pre-eclmaptic pregnant, normal healthy pregnant and non-pregnant women) (n = 30 for each group; age 18-35). The subjects were recruited from South Okkalapa Women and Children Hospital. Exclusion criteria for normal healthy pregnant were: no concurrent medical and surgical problems before or during this pregnancy such as hypertension, diabetes mellitus, heart diseases or renal diseases and no history of hypertension in previous pregnancy/pregnancies. For pre-eclmaptic (PE) pregnant group, PE was diagnosed clinically by the obstetrician and gynaecologist (OG) and identified by the presence of blood pressure of at least 140/90 mmHg or a rise of 30 mmHg in systolic or 15 mmHg in diastolic pressure and proteinuria in random urine specimen. Blood samples were taken before starting anti-hypertensive medication. As preliminary investigations, written informed consent was first taken. Demographic and clinical data, i.e. personal identification, previous medical drug, family history, previous obstetric history and general and obstetric examination were also taken. Blood pressure measurement and urine analysis for protein were also done. Blood samples were taken during two weeks after menstruation in group - 1 to ensure non-pregnant state, 24 weeks to term in group - 2, 32 to

34 weeks of the gestational period in group - 3. After centrifugation and serum separation, the blood samples were stored at - 20^{0}C until analysis. Serum MDA level was determined by Thiobarbituric acid test reaction[13]. Serum Se level was measured by Graphite fumace technique using Atomic Absorption Spectrophotometer[14].

Statistical Analysis

The computer based statistical package of statistical product and service solution (SPSS) version 11.5 was used for data handling and analysis. Results were reported as means±SD. Differences between pre-eclamptic patient and the control groups were compared with one way analysis of variance (ANOVA). For the comparison during pregnancy at various gestational periods, two ways ANOVA test was used. Correlation studies were calculated by using Pearson correlation test. Differences were considered significant when $P < 0.05$.

Approval by the University Academic Board

This research was done in 2006 for the degree of M.Med.Sc (Physiology). The study was approved by the Academic Board, University of Medicine 2, Yangon. The research procedure was done at the Obstetrics and Gynaecological unit of the South Okkalapa Women and Children Hospital, Yangon. All women gave written informed consent and signed in the consent form to participate in this study.

Results

The characteristics of the study groups were shown in Table 1. Serum MDA and Se concentrations of 30 normal pregnant women were measured at four weekly intervals from 24 weeks till to term. Mean serum MDA and Se concentrations at 24 weeks were (1.03 ± 0.20) μmol/L and (114.47 ± 23.99) μg/L, at 28 weeks were (1.26 ± 0.22 μmol/L) and (110.46 ± 23.49 μg/L), at 32 weeks were (1.46 ± 0.21 μmol/L) and (103.35 ± 22.93 μg/L), at 36 weeks were (1.64 ± 0.15 μmol/L) and (97.71 ± 20.82 μg/L) and at onset of labour pain (1.69 ± 0.09 μmol/L) and (98.41 ± 14.48 μg/L) respectively (Table 2).

Mean serum MDA concentration of normal healthy pregnant women significantly increased from 24 weeks gestation to 36 weeks ($P < 0.001$). Mean serum MDA levels at 36 weeks and at the onset of labour pain were more or less similar and maximum level was found at 36 weeks of gestation (Table 2).

Mean serum Se concentration of normal pregnant women significantly decreased from 24 weeks gestation to 36 weeks ($P < 0.001$). Mean serum Se concentration at 36 weeks and at onset of labour pain was more or less similar and minimum level was found at 36 weeks of gestation (Table 2).

Table 1. Characteristics of the non-pregnant, apparently healthy normal pregnant and pre-eclamptic (PE) pregnant women

Parameter	Non-pregnant (n = 30)	Normal Pregnant (n = 30)	PE Pregnant (n = 30)
Age (years)	18 - 35	18 - 35	18 - 35
Gestational period at blood sampling (weeks)	-	24 - term	32 - 34 weeks
Systoloic blood pressure (mmHg)	104 ± 9.50	104 ± 7.59	160.83 ± 6.17
Diastolic blood pressure (mmHg)	69 ± 2.48	70.83 ± 5.43	98.33 ± 2.40
Gravida	-	1 - 3	1 - 3
Proteinuria	-	-	+

Data are presented as mean ± SD.

Table 2. Changes in mean serum MDA and mean Se concentrations in normal pregnant women at various gestational periods

Parameters n = 30	24 weeks	28 weeks	32 weeks	36 weeks	Onset of labour pain
MDA (μmol/L)	1.03±0.20	1.26±0.22***	1.46±0.21***	1.64±0.15***	1.69±0.09***
Se (μg/L)	114.47±23.99	110.46±23.49***	103.35±22.93***	97.71±20.82***	98.41±14.48***

Data are presented as mean ± SD.

*** indicates - $P < 0.001$

Mean Serum MDA and Se in non-pregnant, apparently normal pregnancy and pre-eclamptic cases were shown in figure 1 and 2 respectively. The mean serum MDA and Se levels of 30 non-pregnant women were (0.71 ± 0.12 mmol/L) and (123.44 ± 18.32 mg/L), of 30 normal pregnant women at 32-34 weeks were (1.46 ± 0.21 mmol/L) and (103.36 ± 22.93 mg/L) and of 30 pre-eclamptic patients of same gestational age were (2.33 ± 0.23 mmol/L) and (78.51 ± 8.50 mg/L) respectively.

Mean serum MDA level was significantly higher in pre-eclamptic pregnancy and normal pregnancy group at 32-34 weeks of gestation compared with non-pregnant group ($P < 0.001$). Additionally, mean serum Se level was significantly lower in pre-eclamptic pregnancy and normal pregnancy group at 32-34 weeks of gestation compared with non-pregnant group ($P < 0.001$). The mean serum MDA level was significantly higher ($P < 0.001$) and mean serum Se was significantly lower ($P < 0.001$) in pre-eclamptic pregnant women compared with normal pregnant women of same gestational period.

There was a significant negative correlation ($r = -0.89$, $P < 0.001$) between MDA

and Se level in normal pregnancy at gestational age 32-34 weeks (Figure 3). However, there was no correlation between MDA and Se level in preeclamptic pregnancy (r = -0.10). Similarly, there was no correlation between MDA and Se level in control non-pregnant group (r = -0.16) (data not shown).

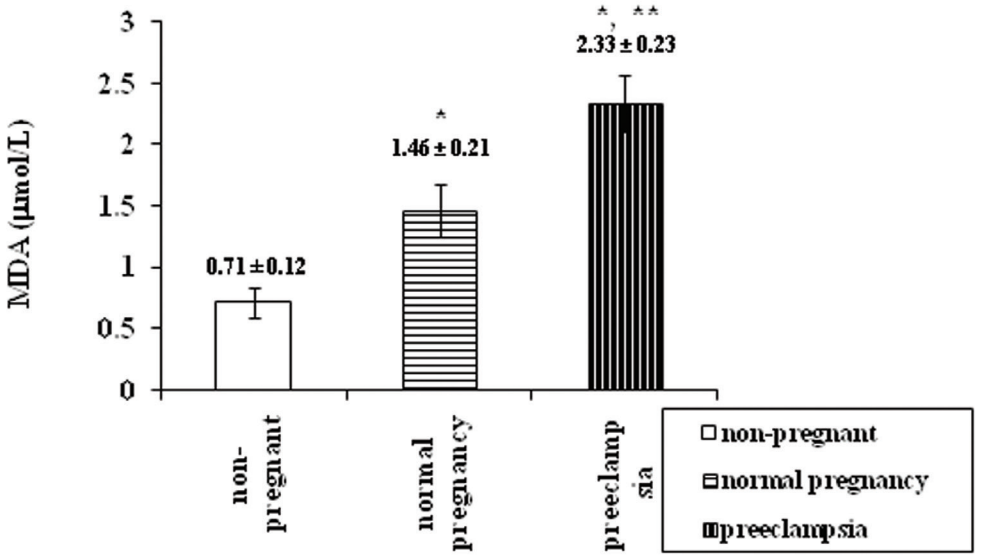

Figure 1. Mean Serum MDA level in non-pregnant women, normal pregnancy at 32 to 34 weeks and pre-eclamptic pregnancy of same gestational period
P* < 0.001 compared with non-pregnant group
P < 0.001 compared with normal pregnancy**

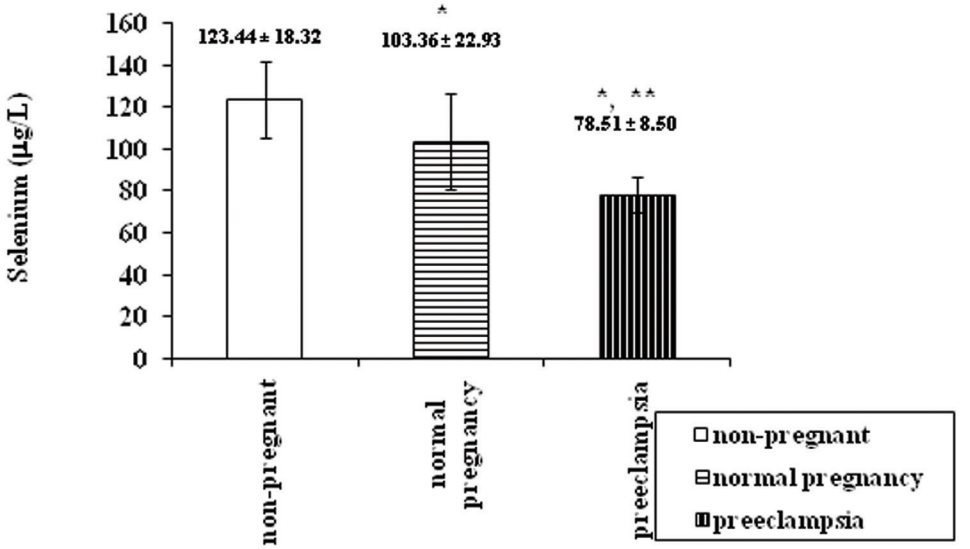

Figure 2. Mean Serum selenium level in non-pregnant women, normal pregnancy at 32 - 34 weeks and pre-eclamptic pregnancy of same gestational period.
*** indicates - P < 0.001 compared with non-pregnant group**
**** indicates - P < 0.001 compared with normal pregnancy**

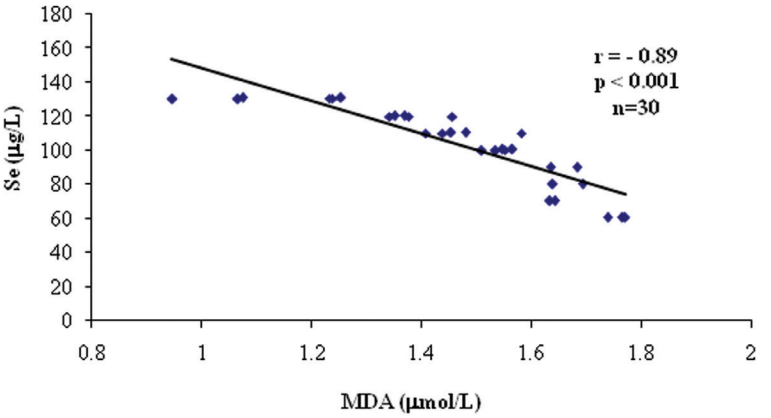

Figure 3. Correlation between serum MDA and Se level in normal pregnant women at 32 to 34 weeks of gestational period

Discussion

Pre-eclampsia remains one of the most serious complications of pregnancy, and its pathophysiology is still poorly understood. Several studies have reported that there is an imbalance between lipid peroxides and antioxidants in preeclampsia and suggest that this factor may contribute to the damage of endothelium, one of the pathophysiological features of the disease[15, 16, 17].

The present study was designed to evaluate serum level of lipid peroxides (in terms of MDA) and serum concentration of antioxidant factor (selenium) in women with preeclamptic pregnancy, with normal apparently healthy pregnancy and non-pregnant women acting as controls.

In the present study, the mean serum MDA level in preeclamptic pregnant women was (2.33 ± 0.23) µmol/l. It was significantly higher than that of normal pregnancy of the same gestational period and that of non-pregnant women ($P < 0.001$). This supports the well known fact that the higher lipid peroxidation level is present in pre-eclamptic pregnancies. These findings of present study were similar to that of previous Western studies[10, 11]. There were some documentary evidence of lipid peroxidation in PE such as higher plasma concentration of free radical oxidation products in PE[18] and significantly elevated plasma lipid peroxides in PE as compared to those of normotensive pregnancy[19]. The findings on serum MDA level found in present study indicate that there might be increased lipid peroxidation in normal pregnancy and pre-eclampsia. Higher MDA in PE provides further evidence of inappropriate or excessive lipid peroxidation in the pathophysiology of PE.

In the present study, serum Se level was significantly lower in normal pregnancy and PE. This finding is similar to those of previous Western studies[10, 11]. Decreased serum Se level in normal pregnancy and PE indicate that there might be an impaired anti-oxidant

defense mechanism in normal pregnancy and pre-eclampsia. Thus it could be postulated that Se might be utilized to great extent to counter free radical-mediated cell disturbance.

Although there are anti-oxidant systems in PE, many researchers were in agreement with high lipid peroxide levels and they found different results in anti-oxidant enzyme activity in PE[20, 21, 22]. Some studies also reported that PE women have an inadequate antioxidant status, i.e low vitamin C[23], low vitamin E[24], low β-carotene or low anti-oxidant trace element zinc[16].

During normal pregnancy, increased MDA and decreased Se indicated that significant negative correlation between serum MDA and Se level. Since there was increased lipid peroxidation and impaired anti-oxidants defense mechanism during normal pregnancy and Se dependent anti-oxidant system seems to play an important role in occurrence of oxidative stress during normal pregnancy.

There are very few reports on anti-oxidant activity and lipid peroxidation status in healthy pregnant women according to gestational weeks. In the present study, the serum Se level significantly decreased and the serum MDA significantly increased in normal healthy pregnancies while gestation progressed. Therefore, it might be concluded that oxidative stress, of which lipid peroxidation represents a major manifestation is involved in pregnancy. And then, selenium may be utilized to greater extent to counteract free radical-mediated cell disturbances, resulting in a reduction in its plasma level.

In the present study there is a significant negative correlation between serum level of Se and MDA in the normal healthy pregnancy. Therefore, increased peroxidation and impaired antioxidants defense mechanism demonstrate the presence of a possible relationship between them. There is no correlation between serum Se and MDA levels in pre-eclamptic pregnancy and non-pregnant women. This may be due to other anti-oxidant like vitamin E, vitamin C etc.., playing some roles in physiological defense in lipid peroxidation process.

Human plasma protection against free radical injury is offered by a wide spectrum of anti-oxidants with synergic action so that individual measurements of anti-oxidant concentrations in blood do not always reflect the level of antioxidant status. Llurba *et al* [25] showed that global plasma antioxidant activity was not decreased in sera of PE women, despite low levels of vitamin C and protein thiol groups, which demonstrates an adequate capacity of plasma to protect its environment from free radical aggression.

Human plasma protection against free radical injury is offered by a wide spectrum of anti-oxidants with synergic action so that individual measurement anti-oxidant activity was not decreased in sera of PE and non-pregnant women. Therefore, other anti-oxidant enzyme systems like SOD, catalase, G6PD etc, might also have some roles in physiological defense in lipid peroxidation process.

Conclusion

In conclusion, increased MDA and decreased Se levels indicated increased oxidative stress in PE, which could be one of the possible factors of lipid peroxidative damage to vascular endothelial cells in pre-eclampsia. Se dependent gluthathione peroxidase enzyme system seems to be involved as one of the anti-oxidant defense systems during normal pregnancy and PE. However, in the pathogenesis of PE, not only selenium dependent anti-oxidant system but also other enzymes might have certain extent of contribution.

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