

**Serum Trace Elements between Pre-eclamptic Women
and Apparently Healthy Pregnant Women**

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Pre-eclampsia is a leading cause of maternal mortality and morbidity. This hospital- and laboratory-based comparative study was conducted at Department of Medical Research (Pyin Oo Lwin Branch) and Central Women's Hospital (Mandalay) from September 2010 to February 2011. The purpose of this study was to find out the role of serum trace elements in the diagnosis of pre-eclampsia. Fifty pre-eclamptic pregnant women of any gestational period admitted to the Obstetric Wards of Central Women's Hospital (Mandalay) were enrolled as participants (50 cases) and fifty apparently healthy pregnant women attending the antenatal clinics with those same age, parity and gestational period were also enrolled as control group (50 cases) and systematic sampling procedure was used in this study. Diagnosis and severity of disease was classified according to American College of Obstetrician and Gynaecologist (ACOG) guideline. The serum trace elements levels were determined by Atomic Absorption Spectrophotometer. Serum magnesium and zinc levels in normal, mild and severe patients were 34.38±8.28 mg/l, 35.40±11.55 mg/l, 35.79±11.34 mg/l and 0.57±0.14 mg/l, 0.63±0.14 mg/l, 0.63±0.17 mg/l, respectively. There was no significant difference in mean serum magnesium and zinc levels between normal women and women with mild and severe pre-eclampsia ($p>0.05$). Areas under the receiver operating characteristics curve (ROC curve) for serum magnesium and zinc were 0.43 and 0.37 and these markers did not reach the acceptable limit for applying as the diagnostic test.

Key words: Pre-eclampsia, Pregnant women, Magnesium, Zinc

INTRODUCTION

Pre-eclampsia is a condition that occurs during mid-term pregnancy and potentially dangerous complication of pregnancy. The more severe the condition becomes, the greater the risk that complications will develop.¹ A widely accepted definition is a diastolic blood pressure equal to or greater than 90 mmHg before the onset of labor, or an increase in systolic blood pressure of 30 mmHg or more, or in diastolic blood pressure of 15 mmHg or more. Hypertension and significant proteinuria (2+ by dipstick testing, equal to or greater than 300 mg per 24 hours, or equal to or greater than 500 mg per liter) usually indicate the

presence of pre-eclampsia. Predictors of poor outcome include low gestational age and high level of proteinuria.² The incidence of pre-eclampsia is 4-8% of pregnancy worldwide.³ Hypertension has been estimated to complicate 5% of all pregnancies and 11% of first pregnancy, half associated with pre-eclampsia, and accounting up to 40,000 maternal deaths annually.⁴

In Myanmar, pre-eclampsia is one of the leading causes of maternal death next to haemorrhage and abortion. According to

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statistics of Mandalay Central Women's Hospital (CWH), about 10% of mothers who were delivered by caesarean section and 6% of the total deliveries were cases of pre-eclampsia and eclampsia.⁵ Furthermore, in CWH (Mandalay), from 1st July 2004 to 30th June 2005, 33% of maternal deaths and 71.4% of near-miss cases were due to severe pre-eclampsia and eclampsia.⁶

Pre-eclampsia is also known as the "Disease of Theories" because various hypotheses in the occurrence of the pre-eclampsia have been set including the increase of the thromboxane levels and the decrease of the prostacyclin levels as well as the imbalance of the lipid peroxidase and antioxidants. However, the exact etiology of pre-eclampsia is still unknown.⁷ There are many studies regarding the relationship between the aggravation of the complication of hypertension and the changes in concentrations of various chemicals in mother's serum.⁸⁻¹⁰ Malnutrition is also a risk factor in the etiology of pre-eclampsia and implicates it by deficit intake of calcium and zinc.¹¹ Deficient or excessive levels of blood trace elements can be an adverse factor in human pregnancy.¹² Zinc requirements are greatest during times of rapid growth such as infancy, adolescence, pregnancy, and lactation.

On the pathophysiological basis, magnesium plays an important role in neurochemical transmission as well as vasodilatation and it is an important co-factor for many enzyme systems.¹³ Magnesium may also act by opposing calcium dependent arterial constriction and may also antagonize the increase in intracellular calcium concentration. Blood magnesium has a relaxant effect on the blood vessels of pregnant women.¹⁴

Many biochemical markers such as uric acid, lactate dehydrogenase, serum calcium, magnesium, copper and zinc are used for the prediction of pre-eclampsia. One study described that serum and cellular magnesium levels show significant difference between the pre-eclamptic mothers and

normal pregnant women. Some authors found that changes of serum calcium, magnesium and trace elements levels have significant effect on the pathophysiology of pre-eclampsia.¹⁵ It was found that serum calcium and magnesium levels of pre-eclamptic women is significantly lower than that of normal pregnant women ($p=0.001$).¹⁶ The incidence and complication of pre-eclampsia is still high in Myanmar. Therefore, studies are required to explore the role of the trace elements levels and to propose these elements which may or may not be involved clinically in pathogenesis of gestational hypertension and to use them as early predictor of the estimation of the pre-eclampsia and its complications.

By using these simple biochemical tests for the early prediction and diagnosis of pre-eclampsia, this study would help the clinicians to improve case detection of pre-eclampsia at an early stage and timely referral to the hospital to receive appropriate treatment. This study would provide a framework upon which further advancements of knowledge could be made.

MATERIALS AND METHODS

This hospital- and laboratory-based, comparative study was conducted at the Pathology Research Division, Department of Medical Research (Pyin Oo Lwin Branch) and Central Women's Hospital (Mandalay), Department of Medical Services. Fifty pre-eclamptic pregnant women of any gestational period admitted to the Obstetric Wards of Central Women's Hospital (Mandalay) were enrolled as participants (50 cases) and fifty apparently healthy pregnant women attending the antenatal clinics with those same age, parity and gestational period were also enrolled as control group (50 cases). A total of 100 pregnant women within the age of 18-45 years were recruited and urine and blood samples were taken after getting informed consents from the participants after explaining the purpose, risks and benefits of the research.

Pre-eclampsia was defined according to the American College of Obstetricians and Gynaecologists (ACOG) guidelines. After that, urinary protein was tested by dipstick test (Urine Insta Test, 2 parameters, Cortez Diagnostics, USA) for both normal and pre-eclamptic pregnant women and these screening test were confirmed by using 24-hour urinary protein test with biochemical analyser (AE 600). Blood 3 cc was centrifuged at 3500 rpm for 30 minutes, which separated the serum and stored at -80°C until analysis. Then, serum magnesium and zinc were determined in triplicates by the standard atomic absorption spectrophotometric method at University Research Centre, University of Yangon.

Regarding the ethical consideration, this study was approved by the Institutional Ethical Committee of the Department of Medical Research (Upper Myanmar).

Data entry was done by using SPSS software and analysis was done by using SPSS software 11.05 version. Serum magnesium and zinc levels were calculated and shown with frequency (percentage) and the demographic factors of the participants, the clinical presentations and risk factors of the disease were analyzed with descriptive data analysis and shown with mean, SD, and frequency.

RESULTS

Among 100 pregnant women, according to the guidelines of the American College of Obstetricians and Gynecologists, 25 women with mild pre-eclampsia and 25 women with severe pre-eclampsia were identified as patient group and 50 apparently healthy pregnant women were used as control group. This study assessed the serum trace elements, magnesium and zinc levels of these pre-eclamptic pregnant women and healthy pregnant women.

In this study, all the pregnant women were in the age group 18-45 years, 8% were teenage pregnancies (less than 20 years) and 62% were within 20-34 years age group. Thirty-five percent of pregnant women were

between 35-45 years. The range of gravida of the participants varied from one to six, where 36% of pregnant women were primigravida, 8% were grand multipara and 56% were within the gravida 2 to 4 groups. The gestational age between 29-32 weeks was 34%, 33-36 weeks was 54% and above 37 weeks was 12%. The mean gestational age of the total pregnancy was 33 weeks (Table 1).

Table 1. Reproductive characteristics of the study group

Characteristics	Normal (n=50)	Mild PE (n=25)	Server PE (n=25)
Age (year)	31.36±6.97	32.00±6.40	30.72±7.57
Gravida (No.)	2.32±1.33	2.36±1.15	2.28±1.51
Gestational age by ultrasound (week)	33.58±2.30	33.64±2.91	33.04±2.01

PE=Pre-eclampsia

The mean levels of the serum trace elements of the pregnant women were depicted in Table 2.

Table 2. Mean serum magnesium and zinc levels of pre-eclamptic and healthy pregnant women

	Magnesium level (mg/l) (Mean±SD)	Zinc level (mg/l) (Mean±SD)
Normal pregnant women	34.38±8.28	0.57±0.14
Pre-eclamptic women	35.59±11.33	0.63±0.15
Mild pre-eclampsia	35.40±11.55	0.63±0.14
Severe pre-eclampsia	35.79±11.34	0.63±0.17

In this study, serum magnesium levels in pre-eclamptic women ranged from 8.31 to 55.63 mg/l (mean of 36.59±11.33 mg/l) and 13.92-46.82 mg/l for the control group (mean 34.38±8.28 mg/l). Serum magnesium level of mild pre-eclamptic women ranged from 8.31 to 55.63 mg/l (mean 35.40 ±11.55 mg/l) and 20.09-50.03 mg/l (mean 35.79±11.34 mg/l) for severe pre-eclampsia. No significant difference in serum magnesium level was observed between normal and pre-eclamptic cases. The mean serum magnesium level of mild pre-eclamptic women was lower than that of the severe ones. However, it was not statistically significant (p>0.05).

In this study, regarding the serum zinc level, the range of this trace element level in

apparently healthy pregnant women was from 0.32 to 1.09 mg/l and the mean level was 0.57 ± 0.14 mg/l. The mean serum zinc level in pre-eclampsia was 0.63 ± 0.15 mg/l within the limit of 0.30-1.00 mg/l. Among the pre-eclamptic women, the lower limit of the mild disease patients was 0.38 mg/l and the upper limit was 0.90 mg/l with a mean of 0.63 ± 0.14 mg/l. For the severe cases, the range of serum zinc level was 0.30 mg/l to 1 mg/l with a mean of 0.63 ± 0.17 mg/l. However, there was no significant difference in serum zinc levels compared in mild and severe pre-eclampsia.

Receiver Operating Characteristics (ROC) curves were used to identify the reliable cut-off point for the prediction of pre-eclampsia in this study. At the serum magnesium level (56.63 mg/l), this trace element level showed high sensitivity (100%), however, the specificity was not good (0%). Moreover, at the cut-off point (21.73 mg/l), the specificity was very good (90%) and sensitivity was 10% only. When the mean serum magnesium level 36.32 mg/l for the pre-eclamptic women was used as the cut-off point, the sensitivity and specificity were 48% and 52%, respectively for the prediction of disease.

Table 3. Birth outcomes of pre-eclamptic pregnant mothers (n=50)

Outcomes	Mild PE (n=25) (%)	Severe PE (n=25) (%)
<i>Maternal outcome</i>		
Normal vaginal delivery	8(16)	5(10)
Instrumental delivery	2(4)	2(4)
LSCS	15(30)	18(36)
<i>Foetal outcome</i>		
Normal	8(16)	0
Preterm	13(26)	16(32)
LBW	4(8)	7(14)
IUFD	0	2(4)

PE=Pre-eclampsia, LSCS=Lower segment caesarean section, LBW=Low birth weight, IUFD=Intrauterine foetal death

The accuracy of the serum zinc level to predict the pre-eclampsia, the sensitivity and specificity of the mean levels of that trace element were 40% and 46%, respectively. At the serum zinc level of 1.05 mg/l, this predictive test yields high sensitivity (100%)

and low specificity (0%). Moreover, at the serum zinc level of 0.41 mg/l, the test showed poor sensitivity (8%) but good specificity (90%).

For maternal outcomes, only 13 cases (26%) were delivered by normal vaginal delivery, instrumental delivery 4 cases (8%) and caesarean section 33 cases (66%) in pre-eclamptic women. The foetal outcomes showed 29 preterm deliveries, 11 low birth weight and intrauterine foetal death 2 cases (Table 3).

DISCUSSION

Pre-eclampsia is a condition that occurs after 20 weeks of pregnancy and potentially causes dangerous complications. In this study, 36% of first pregnancy were identified among the pre-eclamptic cases and 30% of pre-eclamptic women were over 35 years of age. One study showed that unadjusted relative risks of developing pre-eclampsia were nulliparity and women aged >40 years.¹ Some authors also described that this disease is primarily regarded as a disease of first pregnancy and the protective effect of multiparity is said to be lost with the change of partner.²

In this study, the mean maternal age, gestational age and gravida were not significantly different between the mild and severe pre-eclamptic pregnant women. One study described that the predictors of poor outcome include low gestational age and high level of proteinuria.³ The findings in the present study were not similar to the results of other studies. It may be due to the small sample size. There is a need to conduct the research with larger sample size.

Regarding the pathogenesis of pre-eclampsia, there are many studies exploring the relationship between the aggravation of the complication of hypertension and the changes in concentrations of various chemicals in mother's serum.⁴⁻⁶ Deficient or excessive levels of blood trace elements

could be adverse factor in human pregnancy.⁷ On the patho-physiological basis, magnesium plays an important role in neurochemical transmission as well as vasodilatation and it is an important cofactor for many enzyme systems. Moreover, magnesium sulphate has been used as the drug of choice in severe pre-eclampsia and eclampsia treatment.⁵ Zinc deficiency has been associated with complications during pregnancy, growth retardation, congenital abnormalities and low birth weight.⁸

According to results of the serum trace elements in the present study, there were no significant differences in serum concentration of zinc between the patients with both type of pre-eclampsia and normal pregnancy ($p>0.05$). A study with 45 Chinese patients found that no differences exist in either plasma or erythrocyte zinc levels between pre-eclamptic women and control group.⁹ One study also reported that there was no significant association between plasma zinc concentration and several types of hypertension during pregnancy.¹⁰ Thus, the present findings were similar to many previous studies which explored that there were no significant associations between this zinc and pre-eclamptic diseases.^{4, 11-13}

Contrary to the results of the present study, a study described that serum and cellular magnesium levels showed significant difference between the pre-eclamptic and normal pregnant women.¹⁴ One study found that changes of serum calcium, magnesium and trace elements levels have significant effect on the pathophysiology of pre-eclampsia.¹⁴ Many authors also reported that the mean serum magnesium in pre-eclampsia was lower than that noted in normal pregnancy.¹⁵⁻¹⁷

A study showed that serum zinc concentration was 43% lower in pre-eclamptic women than normotensive controls ($p<0.0001$).⁷ In a study, 21 pre-eclampsia pregnant women in their third trimester had a significant decrease in serum zinc concentration compared to normotensive women¹⁸ ($p<0.005$). Because

of 66% of third trimester participants in the present study, serum zinc level reduces in both normal and pre-eclamptic women.

Other study also assessed the serum copper and zinc levels in apparently healthy pregnant women in each trimester at the time of delivery and within three days of post partum. Serum zinc level is found to decrease towards delivery and became lowest compared to the other trimesters ($p<0.01$). In this study, serum zinc level of normal and pre-eclamptic pregnant women were 111 ± 0.48 $\mu\text{g/dl}$ and 88 ± 0.42 $\mu\text{g/dl}$, respectively. Comparison between serum trace elements levels of the normal and pre-eclamptic pregnant women showed significant difference ($p<0.05$).¹⁹

Other researchers described serum copper, zinc and magnesium levels in apparently healthy pregnant women in each trimester, in the pre-eclamptic pregnancies and in the pre- and post-delivery cases of pre-eclamptic pregnancies. Serum zinc and magnesium levels were found to decrease towards delivery and comparison between the serum trace elements levels of the normal and pre-eclamptic pregnant women showed significant difference.²⁰

Both maternal and perinatal outcomes were more complicated in severe pre-eclamptic cases than that of mild one. These findings were similar to one study which discussed that the maternal and perinatal outcomes were better in mild pre-eclampsia that developed after 36 weeks of gestation than in cases that are symptomatic before 33 weeks of gestation.²¹ Therefore, the present study highlighted the importance of severity of the diseases which is the determinant factor regarding the outcome of the patients.

The present study found that the areas under the ROC curve (AUC) for serum magnesium and zinc level were 0.43 and 0.37. Some authors noted that AUC was widely recognized as the measure of a diagnostic test's discriminatory power and ROC curves with an AUC of 0.97 had a very

high clinical value and an AUC<0.75 were not clinically useful.²² This study could not identify the valuable cut-off point for that serum trace elements level to apply as the predictive test in the clinical practice.

Conclusion

The present study showed that there was no significant difference in serum magnesium and zinc among normal pregnancy and both groups of pre-eclampsia. However, future studies with larger sample size are needed to explore the role of the serum trace elements levels and to propose that these elements which may or may not be involved in pathogenesis of gestational hypertension and to use as one of the predictive tests of pre-eclampsia and its complications in the clinical practice.

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