**Original Article**

# Serum Cholesterol and Ceruloplasmin Levels in Second Trimester can Predict Development of Pre-eclampsia

## Madhusudan Dey, Devendra Arora, Nagarja Narayan, Reema Kumar

*Department of Obstetrics and Gynaecology, Armed Forces Medical Services, India*

## Abstract

**Background:** Pre-eclampsia is one of the leading causes of high rates of maternal and perinatal mortality and morbidity. Pathophysiology of pre-eclampsia is still obscure. Currently, there are no screening tests for pre-eclampsia that are reliable, valid, and economical. Parameters of oxidative stress could be early markers of endothelial dysfunction that predates clinical pre-eclampsia. **Aim:** This study was to study ceruloplasmin in nulliparous women as marker of oxidative stress and lipid profile to evaluate their value in prediction of pre-eclampsia. **Materials and Methods:** Prospective observational study. 306 nulliparous women had their serum lipid profile and ceruloplasmin levels measured at 14-16 weeks period of gestation as sample 1 and at 18-20 weeks as sample 2. All cases were followed up till the end of pregnancy for development of pre-eclampsia. **Results:** There was no statistically significant difference between the normals and pre-eclampsia cases at 14-16 week for all the oxidative stress parameters ($P > 0.05$), but at 18-20 week, there was statistically significant difference between the normals and pre-eclampsia cases in cholesterol and ceruloplasmin parameters ($P < 0.05$). **Conclusion:** Cholesterol and ceruloplasmin levels in second trimester (18-20 weeks) can predict the development of pre-eclampsia.

**Keywords:** Ceruloplasmin, Cholesterol, Lipid profile, Oxidative stress, Pre-eclampsia

## Introduction

Hypertensive disorders of pregnancy continue to be one of the leading causes of high rates of maternal and perinatal mortality and morbidity. Hypertensive disorders are common and form one of the deadly triad along with hemorrhage and infection that results in much of the maternal morbidity and mortality related to pregnancy. Worldwide, 50,000 women die each year because of pre-eclampsia. Pre-eclampsia is defined as systolic/diastolic blood pressure values over 140/90 mm of Hg associated with proteinuria greater than 0.3 g/l in a 24-hour urine collection or exceeding 1 g/l in a random sample. The incidence of pre-eclampsia is commonly cited to be about 5-10% and mainly seen in late pregnancy that is at more than 30-32 weeks period of gestation.[1] New onset non-proteinuric hypertension during pregnancy termed-gestational hypertension, is followed by signs and symptoms of pre-eclampsia almost half the time, and pre-eclampsia is identified in 3.9% of all pregnancies.[2]

Hypertension is the established risk factor for cardiovascular and renal disease, and there is a strong association between microalbuminuria and hypertension.[3] Changes in human lifestyle, such as delayed childbirth and diets customs, have increased the global incidence of placental-related disorders over the last decades.[4] Pre-eclampsia is an important cause of both maternal and fetal morbidity and mortality in pregnant women. Therefore, identification of an effective strategy to prevent pre-eclampsia is a priority and a challenge for research in obstetrics.[5] Unfortunately, the precise etiology of pre-eclampsia remains unknown.[5,6] It is important that appropriate treatment is initiated

early in patients at highest risk and they are closely monitored. There is continuing controversy over the optimal treatment of severe early onset pre-eclampsia in pregnant patients remote from term, for whom the desire to avoid maternal organ damage is in direct conflict with the desire to avoid neonatal complications of prematurity. Proponents of expectant treatment of severe pre-eclampsia remote from term advocate the avoidance of poor neonatal outcomes by delaying delivery for women with severe pre-eclampsia until serious maternal complications are imminent or until neonatal survival can be assured reasonably.

Increasing evidence suggests that oxidative stress may play a key role in the etiology of pre-eclampsia.[7,8] It is conjectured that the parameters of oxidative stress could be early markers of endothelial dysfunction that predates clinical pre-eclampsia. Markers of oxidative stress like serum triglycerides, free fatty acids, and malondialdehyde are increased in pre-eclampsia, are positively correlated, and decrease within 48 hours postpartum.[9] A significant elevation of fasting serum triglyceride level was found at 10 weeks of gestation in mild and severe pre-eclampsia.[10] Placental ceruloplasmin, a protein with antioxidant properties, expression is up-regulated in the pre-eclampsia group vs. patients matched for gestational age.[6] Possibly, placental hypoxia associated with pre-eclampsia increases placental ceruloplasmin expression as has been noted for macrophages and monocytes.[11] This study aims to assess the lipid profile and ceruloplasmin as markers for predicting the development of pre-eclampsia. The results may give an insight into the pathophysiology of gestational hypertension and pre-eclampsia. Early markers will further help us in screening those cases that have a high probability of developing pre-eclampsia, which is one of the major causes of maternal and fetal mortality.

## Materials and Methods

This study is a prospective observational study of nulliparous women attending antenatal outpatient department (OPD) and was performed over a period of two years starting from February 2010 till January 2012 at a tertiary care hospital of armed forces, India. The local ethics committee approved the study protocol.

The study included 306 nulliparous women attending antenatal OPD. The inclusion criteria were: Antenatal cases registered before 14 weeks of gestation and who were willing to participate in the study. The exclusion criteria were: 1. Multipara, 2. Pre-existing hypertension, 3. Connective tissue disorder like systemic lupus erythematosus (SLE), 4. Anemia (Hb < 8 gm%), 5. Multiple pregnancies.

Total 332 patients were given consent for the study, and by using exclusion criteria, 26 patients were excluded from the study (anemia-16, pre-existing hypertension-05, multiple pregnancy-04, SLE-01). Remaining 306 booked nulliparous patients meeting the inclusion criteria were studied. Detailed history-taking and examination was done. Period of gestation (POG) was confirmed by dates and correlated with ultrasonography (USG). In case of unsure dates, ultrasonography-expected date of delivery (USG-EDD) of first trimester was taken to calculate POG. All 306 patients underwent serum lipid profile and ceruloplasmin levels estimation at 14-16 weeks POG as sample 1 and at 18-20 weeks as sample 2. All cases were then followed up till the end of pregnancy for development of pre-eclampsia.

Pre-eclampsia was defined as a rise in blood pressure combined with proteinuria after 20 weeks' gestation. Hypertension was defined as a sustained blood pressure (BP) reading of ≥140/90 on at least two occasions 6 h apart in a previously normotensive woman. Blood pressure was recorded in the right arm with a random zero sphygmomanometer; the reported values represented the mean of two readings taken at one-minute intervals. Proteinuria was defined as a protein dipstick measurement of ≥1+ on a midstream urine sample at least twice (24 h apart) or a 24-h urine excretion ≥0.3 g protein in the absence of a urinary infection. Maternal fasting blood samples were collected after an overnight fast of at least 8 h. All samples were analyzed for triglycerides (TG), total cholesterol, high-density lipoprotein (HDL), low-density lipoprotein (LDL), and ceruloplasmin concentrations. Cholesterol and triglyceride concentrations were measured with the enzymatic colorometric method (Cobas Integra 800, Roche, Germany). HDL cholesterol was separated from LDL and very LDL cholesterol by a chemical precipitation technique and ultrasound.[12] Ceruloplasmin was measured by automatable assay for ceruloplasmin as feroxidase.[13] Body mass index was calculated as weight in kilograms divided by the square of height in meters. The height and pre-gestational weight of each patient were reported; if the patients did not know their weight or if it had been measured a long time before conception, they were weighed during their initial visit. For socio-economic status, monthly income was taken into consideration and divided in three groups namely poor (<₹ 15,000), middle class (₹ 15,000-30,000), and rich (>₹ 30,000).

The first value for the biochemical markers was done in all subjects at 14 to 16 weeks gestation. The second value of biochemical markers at 18-20 weeks period of gestation in maternal blood for oxidative stress for three cases cannot be performed as they had mid-trimester abortion.

Twenty-four patients developed pre-eclampsia, and they are taken as cases of interest, and others who did not develop pre-eclampsia are taken as normals for statistical analysis. Baseline value of the biochemical markers for statistical analysis has been taken at 14-16 weeks period of gestation.

## Statistical analysis

The collected data so obtained was statistically analyzed by SPSS statistical software using *t* test for comparison of average values, Chi-square test for evaluation of qualitative variables. The results were expressed as percent and mean with standard deviation. The *P* values of less than 0.05 was considered as statistically significant. Univariate analysis was carried out for all the categorical variables using Chi square test and for quantitative variables using student '*t*' test at 14-16 weeks and at 18-20 weeks of POG. Logistic multivariate analysis was carried out to assess the effect of demographic and oxidative stress parameters at 18-20 weeks simultaneously on the dependent outcome variable of pre-eclampsia.

Findings of the study were compared with the other studies on biochemical markers of oxidative stress as predictors of pre-eclampsia given in literature, and conclusion was drawn about the prediction of pre-eclampsia in early gestation.

## Results

The univariate analysis [Table 1] revealed that there was no statistically significant difference between the normals and pre-eclampsia cases at 14-16 week for all the oxidative stress parameters.

The univariate analysis [Table 2] revealed that there was statistically significant difference between the normals and pre-eclampsia cases at 18-20 week in cholesterol and ceruloplasmin parameters.

The regression model revealed that only cholesterol was statistically significant parameter ($P$ = 0.034, Odds ratio 1.034, 95% CI = 1.002-1.067). All the other variables that is age, BMI, socio-economic status, triglyceride, HDL, LDL, ceruloplasmin was not significant as revealed in Table 3.

Maternal and fetal characteristics are shown in table 4 and table 5 is showing the total number of pre-eclampsia cases, which developed during different period of gestation. In this study, incidence of development of pre-eclampsia was 8%. Two cases had early onset pre-eclampsia, i.e., pre-eclampsia developed before 32 weeks period of gestation. Between 32-34 weeks

period of gestation, 5 cases developed pre-eclampsia, and 17 cases had pre-eclampsia after 34 weeks period of gestation. Most of the pre-eclampsia cases (70%) developed after 34 completed weeks. There was 1 case of eclampsia, which developed at 29 weeks period of gestation.

## Discussion

A potential role for oxidative stress in the pathophysiology of pre-eclampsia was first proposed by Stark in 1993.[14] Raised oxidative stress markers glutathione peroxidase, superoxide dismutase, and malondialdehyde in maternal

### Table 1: Comparison between normals and cases at 14-16 weeks period of gestation

| Parameters | Normal (*n*=282) (Mean±SD) | Cases (*n*=24) (Mean±SD) | Significance (2 tailed) |
|---|---|---|---|
| Cholesterol (mg/dl) | 197.84±35.53 | 200.38±44.48 | 0.742 |
| TG (mg/dl) | 166.65±55.59 | 166.88±43.24 | 0.984 |
| HDL (mg/dl) | 48.80±5.52 | 49.13±8.18 | 0.772 |
| LDL (mg/dl) | 117.27±29.85 | 119.13±34.12 | 0.787 |
| Ceruloplasmin (IU/L) | 1257.49±376.23 | 1345.63±350.47 | 0.269 |

TG: Triglycerides; HDL: High-density; lipoprotein; LDL: Low-density lipoprotein

### Table 2: Comparison between normals and cases at 18-20 weeks period of gestation

| Parameters | Normal (*n*=279) (Mean±SD) | Cases (*n*=24) (Mean±SD) | Significance (2 tailed) |
|---|---|---|---|
| Cholesterol (mg/dl) | 191.68±35.91 | 229.63±30.71 | 0.001 |
| TG (mg/dl) | 171.55±68.86 | 177.38±42.51 | 0.683 |
| HDL (mg/dl) | 47.14±5.01 | 48.13±5.14 | 0.354 |
| LDL (mg/dl) | 117.49±26.67 | 118.88±29.83 | 0.808 |
| Ceruloplasmin (IU/L) | 1185.87±343.19 | 1559.88±208.45 | **0.001** |

TG: Triglycerides; HDL: High-density; lipoprotein; LDL: Low-density lipoprotein

### Table 3: Logistic multivariate analysis

| Variables | Significance |
|---|---|
| Age | *P*=0.135 |
| Socio-economic status | *P*=0.67 |
| BMI | *P*=0.41 |
| Cholesterol | ***P*=0.034** |
| TG | *P*=0.95 |
| LDL | *P*=0.226 |
| HDL | *P*=0.06 |
| Ceruloplasmin | *P*=0.16 |

TG: Triglycerides; HDL: High-density; lipoprotein; LDL: Low-density lipoprotein; BMI: Body mass index

**Table 4: Maternal and fetal characteristics**

| Parameters | Normal (*n*=279) (Mean±SD) (%) | Cases (*n*=24) (Mean±SD) (%) | Significance (2 tailed) |
|---|---|---|---|
| Age | 24.10±4.52 | 23.82±3.86 | 0.371 |
| BMI | 22.16±3.52 | 22.39±4.12 | 0.247 |
| Gestational age at delivery | 37.4±3.56 | 36.5±3.43 | 0.879 |
| IUGR | 9 (3.2) | 3 (12.5) | 0.091 |
| Oligohydramnios | 21 (7.5) | 5 (20.8) | 0.063 |
| PROM | 24 (8.6) | 2 (8.3) | 0.737 |
| CS | 79 (28.3) | 10 (41.6) | 0.253 |
| Birth weight (gm) | 3026±436 | 2726±320 | 0.879 |
| 1st min Apgar | 7.20±1.7 | 6.69±1.6 | 0.764 |
| 5th min Apgar | 8.71±1.3 | 7.73±1.2 | 0.673 |

CS: Cesarean section; IUGR: Intrauterine growth restriction;
PROM: Premature rupture of membrane; BMI: Body mass index

**Table 5: Number of preeclampsia cases at different period of gestation**

| Period of gestation | No. of cases |
|---|---|
| <32 weeks | 2 |
| 32-34 weeks | 5 |
| 35-37 weeks | 10 |
| >37 weeks | 7 |

serum of patient of pre-eclampsia suggest that oxidative stress markers play a significant role in pathophysiology of pre-eclampsia.[15] The incidence of pre-eclampsia in this study was 8% as most of the patients were primigravidas, and it was comparable with other studies done at same local population.[16,17]

Triglyceride-rich remnants are known to cause endothelial dysfunction, and because the triglyceride content of intermediate dense lipoproteins (IDL) was positively correlated with elevated blood pressure and proteinuria, triglyceride-rich remnant lipoproteins might contribute to the pathophysiology of pre-eclampsia.[18] In our study, triglyceride level was not found to be significant between the normals and pre-eclampsia cases at 14-16 weeks and 18-20 weeks. However, Hubel, *et al.,* in 1996, found that triglycerides and fatty acid levels are elevated in patients with pre-eclampsia, and these changes antedate clinically evident disease by weeks to months,[9] which is not supported by our findings. The levels of HDL, LDL were not found to be significant between the normals and pre-eclampsia cases at 14-16 weeks and 18-20 weeks in our observation. Bainbridge and associates, in 2005,[19] stated increased levels of lipid peroxidase like malondialdehyde and variety of pro-oxidants or potentiators of pro-oxidants, including iron, trasferrin, and ferritin; blood lipids, including triglycerides, free fatty acids, and lipoproteins are increased, and antioxidants, including ascorbic acid and vitamin E, are decreased in pre-eclampsia.

Cekmen, *et al.* concluded that in the pre-eclamptic group, plasma total triglyceride, low-density lipoprotein cholesterol (LDL-C), malondialdehyde (MDA), and apolipoprotein B (apo-B) were significantly increased, while plasma high-density lipoprotein cholesterol (HDL-C) was significantly decreased

compared to that of control group. There was no significant difference in total cholesterol and apolipoprotein A1 concentrations,[20] which is contrary to our study, in which cholesterol is found to be significantly increased in pre-eclamptic cases.

Total cholesterol concentrations were significantly higher in pre-eclamptic patients compared with controls in the present study. Van den Elzen, *et al.* reported that first trimester serum total cholesterol concentrations were significantly associated with the risk of pre-eclampsia, and that those with such elevated levels of cholesterol experienced a 5-fold increase in the risk of pre-eclampsia after accounting for confounding factors like maternal body mass index and gestational age.[21] Enquobahrie, *et al.* also found that there was a 3.60-fold increase in the risk of pre-eclampsia among women with total cholesterol of >205 mg/dL before 16 weeks' gestation.[22]

The interactions among a disordered lipid profile, endothelial cells, and oxidative stress have been hypothesized to be of major significance to the pathophysiology of pre-eclampsia by several investigators.[9,23,24] High concentrations of circulating triglyceride-rich lipoproteins may induce endothelial dysfunction through the generation of small dense LDL sub-fractions, which have been found to be oxidized more readily than their larger counterparts. It was indicated that small dense LDL fractions had a greater capacity to stimulate the thromboxane synthesis by endothelial cells and an increase in intracellular calcium in vascular smooth muscle, which might be relevant to vasospasm in pre-eclampsia.[25] Studies have shown this endothelial cell damage in the kidney and placenta.[23]

In the present study, there was no statistically significant difference between the normals and pre-eclampsia cases at 14-16 week for all the oxidative stress parameters, but there was statistically significant difference between the normals and pre-eclampsia cases at 18-20 weeks in ceruloplasmin parameter. This shows that ceruloplasmin level increases during the middle of second trimester in patients who destined to develop pre-eclampsia. Aksoy, *et al.,* in 2003, support our findings as they also observed that the plasma antioxidant potential and transferrin levels in pre-eclampsia patients were reduced and the malondialdehyde with ceruloplasmin levels were increased as compared to the normotensive pregnant

women.[26] Shamsi AZ and associates reported that serum level of ceruloplasmin significantly lower in normal pregnancy than mild and severe pre-eclampsia.[27]

The strengths of our study include the prospective design and the high follow-up rate. In addition, the collection of blood early in pregnancy enabled us to show that increased levels of serum cholesterol and ceruloplasmin concentrations precede the clinical manifestations of pre-eclampsia. We used logistic regression to adjust for a number of confounders. We also used fasting blood samples to determine lipid concentrations. However, in studies of non-pregnant individuals, investigators have found a strong correlation between fasting and post-prandial lipid concentration measurements.[28] In addition, almost all our patients had similar socio-economic situations, education, and smoking status.

Several important limitations must be considered when interpreting the results of our study. First, two measurement has resulted in some misclassification of maternal lipid profiles and ceruloplasmin levels during pregnancy. Longitudinal studies with serial measurements of maternal lipid and ceruloplasmin concentrations are needed to elucidate patterns of their levels, the determinants of these changes, and the pathophysiologic consequences of such changes during pregnancy. Second, in our study, only 24 patients had pre-eclampsia. Relatively small number of pre-eclampsia patients hindered inferences from some of our analyzes. Third, although we compared for many potential confounders, we cannot exclude the possibility of the other confounding from unmeasured covariates. Finally, another limitation of the present study is that it was done in only one medical center.

This study suggests that cholesterol and ceruloplasmin levels in second trimester at 18-20 weeks POG can predict the development of pre-eclampsia. Information from this study may contribute to the development and evaluation of behavioral and medical interventions aimed at reducing the occurrence of pre-eclampsia.

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