

Evaluation Of Serum Calcium And Lipid Profile In Women With Pre-Eclampsia, Eclampsia And Normal Pregnancy

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Abstract : Introduction: Background & objective: Pre-eclampsia and eclampsia are the most common obstetrical complications during pregnancy. The objective of this work was to quantitate and find significance of serum calcium and lipid profile levels in pre-eclamptic and eclamptic pregnancy in comparison with normal pregnancy. Methods: Study includes 105 subjects divided into three groups. Estimation of Calcium, Triglycerides, Total-Cholesterol and HDL-C were analyzed by O-Cresolphthalein-Complexone, GPO-PAP, CHOD-PAP and Phosphotungstic acid precipitation method, using Erba Chem-5 plus semi-autoanalyser. Results: Mean serum calcium and HDL-C levels were significantly decreased in group-II and group-III in comparison to group-I, $p < 0.05$. The mean triglyceride, VLDL-C and LDL-C levels were significantly increased in group-II as compared to group-I, $p < 0.05$. There was no difference in the mean values of total-cholesterol between cases (group-II and group-III) and control (group-I), $p > 0.05$; but the mean level of VLDL-C was higher in group-III in comparison to group-I, $p < 0.05$. Non significant ($p > 0.05$) difference was found in mean values of triglyceride and LDL-C in group-III cases in comparison to group-I. Interpretation & conclusion: Women having pre-eclampsia and eclampsia had low levels of serum calcium and disturbed lipid profile. These levels may have cause and effect relationship with these disorders. [Das B et al NJIRM 2013; 4(4) : 85-91]

Key Words: pre-eclampsia, eclampsia, calcium, lipid profile, normal pregnancy.

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Introduction: Pre-eclampsia and eclampsia are very common pregnancy associated disorder in this part of the country mostly affecting primigravida of early ages. These disorders are associated with adverse prenatal outcomes such as stillbirth, preterm and small for gestational age babies¹⁻³. Pre-eclampsia is a transient but potentially dangerous disorder that is characterized by triad of high-blood pressure to the extent of 140/90 mm Hg or more, edema, and proteinuria, developing after 20 weeks of pregnancy. Its incidence is 4-8% of pregnancies^{4,5}. Eclampsia is a convulsive form of pre-eclampsia. Convulsion may occur 38% in antepartum, 18% in intrapartum or 44% in postpartum⁶. The incidence of pre-eclampsia and eclampsia is decreasing in the developed countries. In India the incidence of eclampsia has been quoted as 1.5%⁷. Majority of the cases of pre-eclampsia and eclampsia are the patients belonged to poor socio-economic class and have not received proper medical attention during their antenatal period⁸.

Calcium is one of the most abundant elements in the human body and it plays a critical role in the function of the cardiac and vascular smooth muscles. Deficiency of calcium may lead to tetanic

convulsions, bleeding diathesis, capillary haemorrhages, tissue exudation and osteomalacia⁹. Some studies have concluded that the increase in the intracellular calcium causes vasoconstriction, increase in the peripheral resistance and therefore, an increase in the blood pressure¹⁰.

Epidemiological and clinical studies have shown that an inverse relationship exists between calcium intake and development of hypertension in pregnancy¹¹. Many trials have been conducted to observe the protective effect of preventive calcium supplementation in pregnant women¹². A previous review by Hofmyer et al. has shown that calcium supplementation during pregnancy had a significant effect in reducing risk of gestational hypertension and pre-eclampsia¹³.

Most authors believe dyslipidemia in early pregnancy are associated with increased risk of pre-eclampsia and eclampsia. Women with a history of pre-eclampsia have significant difference in lipid parameters. Increase in plasma triglyceride in pre-eclampsia has been reported in several studies¹⁴, raised plasma triglyceride may be a potential contributor to hyperlipidemia¹⁵. On the

basis of some studies which claim that triglyceride mediated endothelial dysfunction and vasculopathy may contribute to the patho physiologic mechanisms of preeclampsia that warrants further analysis¹⁶. There is a positive correlation between serum triglycerides and systolic blood pressure as well as diastolic blood pressure in pre eclampsia cases¹⁷.

Serum calcium and lipid profile appear to be of immense value in understanding the pathogenesis of pre-eclampsia and eclampsia. In this context, the present study has been undertaken to evaluate the changes in serum level of calcium and lipid profile (Total Cholesterol, triglycerides, HDL-C, VLDL-C and LDL-C) in normal pregnancy, and pregnant women complicated with pre-eclampsia and eclampsia and also to find if there is any importance of serum calcium and lipid profile levels in management of pre-eclampsia and eclampsia.

Material & Methods: Case control study was conducted at the Department of Biochemistry, Rohilkhand Medical College and Hospital (RMCH), Bareilly, Uttar Pradesh after obtaining clearance from institutional ethical committee. The duration of study was 12 months, from September 2011 to August 2012. Total 105 women were recruited for the study. They were divided in the following three groups, each group consisting of 35 subjects.

Group –I: Normotensive pregnant women with age's ranges from 20-38 years receiving antepartum care at the outpatient department of Obstetrics and Gynecology, RMCH, Bareilly.

Group- II: Diagnosed pre-eclamptic women with age's ranges from 21-36 years admitted to the department of Obstetrics and Gynecology, RMCH Bareilly.

Group-III: Diagnosed eclamptic women with age's ranges from 20-34 years who were admitted to the department of Obstetrics and Gynecology, RMCH, Bareilly.

While selecting the subjects, care was taken that none of them was suffering from diabetes mellitus, cardio-vascular diseases, renal diseases, pre-

existing hypertension, hemophilia and also not under the treatment of antifolate drug therapy. Before performing the various tests, subjects consent had been taken. All the procedures reported here in the study have followed the guidelines approved by the locally appointed ethical committee.

Pre-eclampsia was diagnosed by i) Blood pressure for at least 140/90 mmHg measured on two occasions each 6 hours apart, ii) 24 hours urinary protein > 300mg. Patients developing convulsion or coma superimposed on pre-eclampsia were regarded as eclampsia.

Venous blood samples were collected from antecubital vein after an overnight fasting from all participant's with aseptic precautions. Blood samples were allowed to clot at room temperature and the serum was separated by centrifugation. The estimation of these parameters was carried out within 4-6 hrs. The following tests were done in each sample during the study.

- Serum Calcium by O-Cresolphthalein-Complexone method¹⁸
- Serum Total Cholesterol by CHOD-PAP method¹⁹
- Serum Triglycerides was measured by GPO-PAP method²⁰
- HDL- Cholesterol Estimation by Phosphotungstic acid precipitation method²¹
- Estimation of Serum VLDL cholesterol and LDL cholesterol

For the estimation of VLDL-C and LDL -C indirect method has been used in accordance with the outline of Freidewald's Formula. (Freidewald W.T. et al 1972). Here VLDL cholesterol can be indirectly ascertained as 1/5th of the triglyceride value. VLDL results are expressed as mg/dl or mmol/L. The LDL cholesterol has been calculated from the estimated values of Total cholesterol (TC), triglyceride and HDL cholesterol which were directly measured in serum by enzymatic method as described above.

The value of LDL cholesterol is calculated as LDL-Cholesterol= Total Cholesterol – [(Triglycerides/5) + (HDL-Cholesterol)]

LDL results are expressed as mg/dl or mmol/L.

Statistical Analysis: Data were presented as mean \pm SD. Comparison of serum levels of the parameters between cases and control was performed by student's t test and $p < 0.05$ was considered as statistically significant.

Result: Total 105 women have participated in our study which is divided into three groups. Each group consisting of 35 subjects. Table- 1, 2, 3 and Fig. 1 and 2 shows our results.

Table-1 showing the mean maternal age \pm SD, gestational age \pm SD and socio-economic status of group I, II and III cases. In this study a large number of the cases (57.1%) belonged to lower socio-economic class.

Table: 1: Showing the mean \pm SD of maternal ages, gestational ages and socio-economic status of group I, II and III.

Groups	Maternal age (Year) Mean \pm SD	Gestational age (Week) Mean \pm SD	Socio-economic status	
			Lower	Middle
Groups-I (n=35)	26.8 \pm 5.8	30.4 \pm 4.7	14 (40.0%)	21 (60.0%)
Groups-II (n=35)	27.2 \pm 5.1	28.5 \pm 4.05	22 (62.9%)	13 (37.1%)
Groups-III (n=35)	25.8 \pm 4.8	29.5 \pm 4.7	24 (68.6%)	11 (31.4%)

Table-2(a): Showing the number and percentage of primigravida and multigravida patients participated in our studies.

Groups	Primigravida		Multigravida	
	No.	Percentage	No.	Percentage
Group- I	16	45.8	19	54.2
Group-II	22	62.9	13	37.1
Group-III	20	57.1	15	42.9

62.9 % and 57.1% of primigravida belong to group-II (pre-eclampsia) and group-III (eclampsia) cases shown in the above table.

Table 2(b): shows that systolic, diastolic & mean arterial blood pressure.

Groups	Systolic BP (mmHg) Mean \pm SD	Diastolic BP (mmHg) Mean \pm SD	Mean Arterial Pressure (mmHg) Mean \pm SD
Group- I	108.5 \pm 7.9	70.6 \pm 7.8	83.2 \pm 5.6
Group- II	154.7 \pm 6.3*	100.9 \pm 6.05*	119.8 \pm 7.1*
Group- III	157.7 \pm 5.6*	104.02 \pm 6.5*	122.9 \pm 6.9*

* Statistically significant Vs. Control group, $P < 0.05$

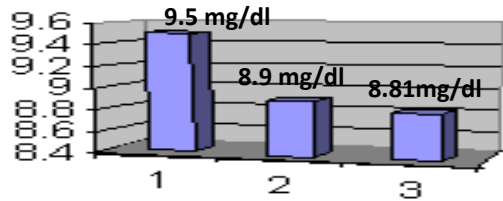
The mean systolic, diastolic and arterial blood pressure were significantly higher in eclamptic and in pre-eclamptic women as compared to normotensive women.

Table-3: Showing serum calcium & lipid profile values in study groups.

Parameters	Group -I (n=35)	Group -II (n=35)	Group -III (n=35)	Statistical relationship of Gr-II & Gr-III with Gr-I
	Mean \pm SD	Mean \pm SD	Mean \pm SD	
Calcium (mg/dl)	9.50 \pm 0.70	8.90 \pm 0.65	8.81 \pm 0.63	$P < 0.05$ in Gr-II; $P < 0.05$ in Gr-III
Triglyceride (mg/dl)	174.0 \pm 11.2	203.2 \pm 15.7	179.6 \pm 14.1	$P < 0.05$ in Gr- II; $P > 0.05$ in Gr-III
Total Cholesterol (mg/dl)	190.9 \pm 13.3	197.4 \pm 14.4	195.74 \pm 13.8	$P > 0.05$ in Gr-II; $P > 0.05$ in Gr-III
HDL-C (mg/dl)	53.58 \pm 4.43	49.22 \pm 6.25	47.94 \pm 5.7	$P < 0.05$ in Gr-II; $P < 0.05$ in Gr-III
VLDL-C (mg/dl)	34.8 \pm 2.24	36.02 \pm 2.9	40.7 \pm 3.2	$P < 0.05$ in Gr-II; $P < 0.05$ in Gr-III
LDL-C (mg/dl)	102.6 \pm 13.8	112.3 \pm	107.0 \pm 9	$P < .05$ in Gr-II; $P >$

		17.23	16.31	0.05 in Gr-III
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Figure1 :Showing the Mean Serum Calcium Values in Group-1 (Normotensive women), Group-II (Pre-eclampsia) and Group-III (Eclampsia)



1. **Group-1 (Normotensive women)**
2. **Group-II (Pre-eclamptic women)**
3. **Group-III (Eclamptic women)**

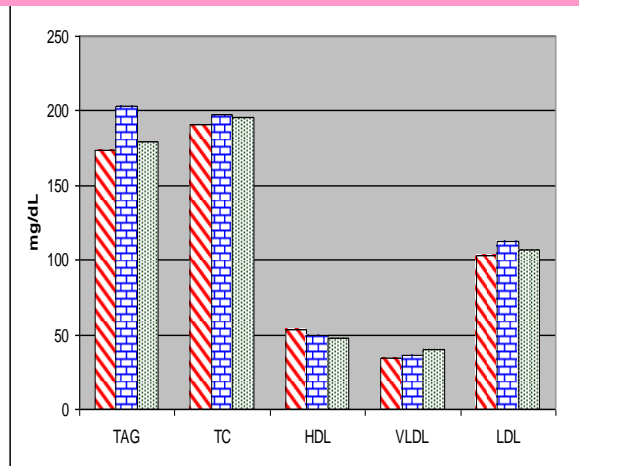


Table-3, Figure1 & 2 shows significantly lower mean serum calcium & HDL-C in group-II and group-III ($p < 0.05$) compared to group-I. The mean value of serum triglyceride was significantly higher ($p < 0.05$) in group-II cases as compared to group-I; but non significantly higher ($p > 0.05$) in group-III in comparison to group-I. There was also non significant ($p > 0.05$) difference between mean values of total cholesterol between cases (group-II and group-III) and control (group-I). The mean value of VLDL-C was significantly higher ($p < 0.05$) in group-II and group-III cases as compared to group-I. Table-3 also shows the mean value of LDL-cholesterol which was highly significant ($p < 0.05$) in group-II cases and non significant ($p > 0.05$) in group-III cases in comparison to group-I cases.

Discussion: Age and parity of the pre-eclampsia and eclampsia patients were statistically significant risk factors²². In this study the mean \pm SD maternal ages and mean \pm SD gestational ages of group-II cases were 27.2 ± 5.1 year and 28.5 ± 4.05 week and also majority of the patients (69.2%) were primigravida. In group III cases we observed that the mean \pm SD maternal ages and mean gestational ages were 25.8 ± 4.8 year and 29.5 ± 4.7 week and here is also primigravida having maximum (57.1%) incidence of the disease (Table 1 and 2a). This finding is in agreement with other authors^{22,23}. On analyzing the results of our study, we observed that majority of patients (Group-II and Group-III) belonged to poor socio-economic status and coming from far flung areas, hence unable to seek and get proper medical advice. In previous study, two authors namely Halimi et al.²³ and Begum et al.²⁴ also reported similar results. Systolic, Diastolic, and Mean arterial pressure in our study were (108.5 ± 7.9 , 154.7 ± 6.3 , 157.7 ± 5.6), (70.6 ± 7.8 , 100.9 ± 6.05 , 104.02 ± 6.5), (83.2 ± 5.6 , 119.8 ± 7.1 , 122.9 ± 6.9) mm Hg in Group-I (Control), Group-II (Pre-eclampsia), and Group-III (Eclampsia) respectively (Table-2b). There was significant difference of all blood pressure parameter between cases and controls ($p < 0.05$). These results are in agreement with study of NAF Islam et al.²⁵.

Our study has also shown that the mean \pm SD serum calcium of group II and III were (8.90 ± 0.65 and 8.81 ± 0.63 mg/dl) less than the mean serum calcium of group I (9.50 ± 0.70 mg/dl). There was a statistically significant difference between the cases and control (Table-3). This finding matches with previous studies conducted by Idogun E.S. et al.²⁶, J. Moodley et al.²⁷, Punthumapol C et al.²⁸. This result supported the hypothesis that low serum calcium level might be a cause in the development of pre-eclampsia. The effect of serum calcium on changes in blood pressure could be explained by the level of intracellular concentration of calcium²⁸. Belizan hypothesized that a low calcium intake results in high parathyroid hormone levels and increased membrane permeability. As a result, calcium is released from the mitochondria and it enters the

cytoplasm, thus resulting in increased intracellular free calcium levels and decreased serum calcium levels. The elevation of cytoplasmic calcium levels triggers smooth muscle contraction, thus resulting in vascular constriction and increased blood pressure²⁹. As seen in table-1; majority of the pregnant women in group-II and group-III in this study lives in places of low socioeconomic status. Socioeconomic status may be correlated with calcium intake. Women from the low income group were more likely to have less than the recommended dietary allowance (RDA) for calcium. However, the present finding was contradictory to some other studies where the mean serum calcium levels in group-II and group-III were not different from normal pregnancy^{30,31}.

Normal human pregnancy results in pronounced physiological hyperlipidemia involving rise in blood triglycerides and cholesterol. The current study we analyzed, the role of lipid parameters in pre-eclampsia and eclampsia cases and found that the patients of group-II (preeclampsia) have significant difference in serum triglyceride as compare to group-I (control) subject ($p < 0.05$). In this study the mean \pm SD serum triglyceride of group-II participant was (203.22 ± 15.7) more than the mean \pm SD serum triglyceride of group-I (174.02 ± 11.2) participants. These findings are in agreement with work of many authors^{25, 32, 33}. The principle modulator of this increase in triglyceride is estrogen, as pregnancy is associated with hyperestrogenaemia. Estrogen inhibits the hepatic lipid oxidation so the net effect is increased delivery of free fatty acids into hepatic biosynthesis of endogenous triglycerides which carried by VLDL (Jayanta et al. 2006)³⁴. But some authors believed that the increase level of triglycerides in preeclampsia is probably not due to hyperestrogenaemia as the levels of estrogen decreases in pre-eclampsia. Another hypothesis for increase level of triglycerides in pre-eclampsia is that hyper-triglyceridemia is probably a consequence of competition between the substrates chylomicron and very low-density lipoprotein cholesterol for the enzyme lipoprotein lipase. Classically, chylomicron clearance occurs in two sequential steps: (a) Triglyceride hydrolysis by

the enzyme lipoprotein lipase, (b) Uptake of the remnant by the liver. Delay in the second step leads to accumulation of remnants in plasma and is generally thought to represent the atherogenic risk of hyper-triglyceridemia¹⁶. Elevation in triglyceride, found in preeclampsia is likely to be deposited in predisposed vessels, such as uterine spiral arteries and contributes to the endothelial dysfunction, both directly and indirectly through generation of small dense low-density lipoprotein cholesterol (Sattar et al. 1997)³⁵. But in our study, the group-III (eclampsia) cases were not statically significant in comparison to group-I (control) cases ($p > 0.05$). Similar finding was observed by NAF Islam et al.²⁵. Possible causes for this finding is eclamptic process is very frequently associated with aggravated hepatic damage which inhibits the de novo synthesis of triglyceride in liver.

In the present study we could not observe any significant change in TC (total cholesterol) level in any of the studied groups. This finding is similar to previous studies conducted by NAF Islam et al²⁵, Jayanta et al³⁴, Sattar et al³⁵. In table-3, Fig-2. ; we also found that the mean \pm SD of serum HDL-C level (53.5 ± 4.43) increases and the mean \pm SD serum LDL-C level (102.6 ± 13.8) decreases in group-I (control) cases as compared to mean \pm SD serum HDL-C and LDL-C levels in group-II (preeclampsia; HDL-C 49.2 ± 6.25 and LDL-C 112.3 ± 17.2 mg/dl) and group-III (eclampsia; HDL-C 47.9 ± 5.7 and 107.09 ± 16.3) cases. In normotensive pregnant women, the increase in HDL-C and decrease in LDL-C levels is due to hyperestrogenaemia. But in preeclampsia, the estrogen level is decreased, so reduced serum HDL-C level and increased serum LDL-C levels were observed. This finding is supported by other authors^{25, 36}. The mean \pm SD of VLDL-C level increased in present study in both group-II and group-III cases in comparison to group-I. Same result was reported in another study³⁷. Hubel et al. reported that VLDL-C is increased in pre-eclamptic patients. Significantly higher values of VLDL-C were found in pre-eclamptic groups as compare to normal pregnant women³⁸.

Conclusion: Based on the results of the present study and data available from literature, it is clear that the women who develop pre-eclampsia and eclampsia had low level of maternal total serum calcium and disturbed lipid profile due to abnormal lipid metabolism. This association may be significant in understanding the pathological processes of pre-eclampsia and eclampsia and may help in developing strategies for prevention and early diagnosis of pre-eclampsia and eclampsia.

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