

Original Article

A Comparative Study of Serum Lipid Levels in Pregnant Normotensive and Pre-eclamptic Women in Dalhatu Araf Specialist Hospital Lafia, Nigeria.

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Abstract

Background: Pre-eclampsia is associated with significant maternal and perinatal mortality and morbidity. Increased oxidative stress due to endothelial dysfunction in pre-eclampsia has been linked with lipid abnormality. This study compared the fasting serum lipid levels in pre-eclamptic and normotensive pregnant women.

Methodology: A case-control study in which venous blood samples (5mls) were collected from 50 consenting pregnant healthy normotensive women and 50 women with pre-eclampsia accessing care at the hospital. Study participants were matched for maternal age, parity, and gestational age after 8-12 hours of fasting. The fasting serum levels of total cholesterol (TC), low-density lipoprotein (LDL-C), very low-density lipoprotein (VLDL-C), high-density lipoprotein (HDL-C) and triglycerides (TGs) of the participants were evaluated using standard enzymatic methods.

Result: Of the 50 pre-eclamptic and 50 pregnant normotensive women who participated in the study, the mean maternal age was 24.92±4.38 and 24.90±4.27 years respectively (p=0.98).

There were statistically significant higher mean levels of TC, triglycerides, and VLDL-C among women with pre-eclampsia compared to normotensive pregnant women (p<0.001, p<0.001, p=0.007 respectively). The mean HDL-C level was significantly reduced among women with pre-eclampsia compared to controls (p<0.001). However, there was no statistically significant difference in the mean serum level of LDL-C in both groups (p=0.068). The serum lipid profile did not significantly change with the severity of pre-eclampsia.

Conclusion: Women with pre-eclampsia have increased serum levels of TC, triglycerides, and VLDL-C and decreased levels of HDL-C compared to normotensive pregnant women. However, the lipid profile of women with severe pre-eclampsia did not differ significantly from those with mild pre-eclampsia.

Keywords: Pre-Eclampsia; Triglycerides; HDL-C; LDL-C; VLDL-C; Cholesterol; Hypertension.

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Quick Response Code:



Introduction

Pre-eclampsia is a pregnancy-specific disease entity characterised by the occurrence of hypertension and significant proteinuria after 20 weeks of gestation in a previously normotensive and non-proteinuric woman.^[1] It is a multisystem disorder that is peculiar to pregnancy, and it complicates 2-8% of pregnancies.^[2]

Despite considerable research, the aetiology and mechanism of pre-eclampsia is yet to be elucidated and there are no clinically useful screening tests to detect women in which it will develop. Evidence of increased oxidative stress due to endothelial dysfunction in pre-eclampsia has been well established and the increased oxidative stress has been linked with lipid abnormality.

Several studies have looked at the variations in the serum lipid profiles (TC, TG, LDL-C, HDL-C, and VLDL-C) in pre-eclamptic compared to normotensive pregnant women with inconsistent findings. Some studies^[3, 4] found significantly higher serum TG, LDL-C, and VLDL-C in the pre-eclamptic group compared to normotensive pregnant women while other studies^[5] didn't find any significant difference in the lipid profiles (TG, LDL-C). Accumulation of lipids has been proposed to contribute to the oxidative stress and generation of free radicals resulting from endothelial dysfunction which is a key mechanism in the pathophysiology of pre-eclampsia.^[6] The increased triglyceride is hypothesized to be deposited in predisposed vessels thereby resulting in pre-eclampsia.^[7] The significantly higher level of triglycerides in women with pre-eclampsia found can also be linked to hyperoestrogenaemia seen in pregnancy.^[8] Oestrogen is thought to induce hepatic biosynthesis of endogenous triglycerides which is mediated by VLDL-C.^[8]

Though a large number of studies^[3,5,9,10,11] found significantly lower HDL levels among the pre-eclamptic when compared to normotensive pregnant women, some studies either did not find any significant difference^[4,12] in the HDL in both groups or recorded a higher HDL level^[13] in the pre-eclamptic group when compared to the pregnant normotensive group. The objectives of this study are to compare the fasting serum lipid levels in pre-eclamptic and normotensive pregnant women and also to assess the relationship between serum lipid profile changes and the severity of pre-eclampsia.

Subjects and Methods

This case-control study was conducted among pre-eclamptic and normotensive pregnant women receiving antenatal care in the Department of Obstetrics and Gynaecology after obtaining informed consent. The study was conducted between 3rd February 2020 and 31st May 2020. It was approved by the ethical committee of the hospital.

Eligible participants for the study were pregnant women between the ages of 18 and 34 with singleton pregnancies and a diagnosis of pre-eclampsia and pregnant normotensive, non-proteinuric women from 20 weeks of gestation till term. Both groups were matched for maternal age, parity, and gestational age to eliminate confounding factors. Pregnant women were excluded from the study if they had multiple pregnancies, molar pregnancies, personal or family history of dyslipidemia, or medical disorders such as cardiac disease, liver disease, renal disease, hypertension, diabetes mellitus, or HIV infection. Other exclusion criteria were previous history of hypertensive disorders in pregnancy, patients in labour or with membrane rupture, clinical or laboratory evidence of urinary tract infection, and those who declined consent to the study.

Sample size determination

The sample size was determined using the formula^[14] below

$$\text{Where: } N = \frac{(Z_{\alpha} + Z_{1-\beta})^2 (\sigma_1 + \sigma_2)}{(\mu_1 - \mu_2)^2}$$

N = minimum sample size required in each group.

Z_{α} = Standard normal deviate corresponding to 5% level of significance = 1.96 (obtained from normal distribution table)

$Z_{1-\beta}$ = Standard normal deviate corresponding to a power of 80% = 0.84 (obtained from normal distribution table)

σ_1 = Standard deviation of serum lipids among patients with severe pre-eclampsia from a previous study^[15] (0.23mg/dl)

σ_2 = Standard deviation of serum lipids among controls from a previous study^[15] (0.11mg/dl)

μ_1 = the mean of serum lipids among patients with severe pre-eclampsia from a previous study^[15] (0.6mg/dl)

μ_2 = the mean of serum lipid among controls from a previous study^[15] (0.36mg/dl)

$$N = \frac{(1.96 + 0.84)^2 (0.23 + 0.11)}{(0.6 - 0.36)^2}$$

N = 46 is the minimum sample size in each group. To allow for attrition, 10% of the sample size will be added.

Therefore, 50 patients per each group were recruited for the study making a total of 100 women.

Group A: Normotensive Pregnant Women (Control)

Group B: Women with pre-eclampsia (Cases)

Sampling Techniques

Eligible, consenting pre-eclamptic women were recruited into the study consecutively from the antenatal clinic, antenatal ward, and obstetric emergency of the hospital by convenience sampling method. A pre-tested, structured, interviewer-administered questionnaire was used to obtain information on age, marital status, parity, gestational age, weight, educational status, socioeconomic status (Olusanya's classification),^[16] past medical history, urinalysis, and blood pressure.

Pre-eclampsia was defined as the occurrence of blood pressure of 140/90 mmHg or more taken twice at least four hours apart with proteinuria of 2+ or more or 1+ in the presence of PH < 8.0 and specific gravity of < 1.030 after 20 weeks of gestation in a previously normotensive and non-proteinuric woman^[1]. Severe pre-eclampsia in this study was defined as elevated blood pressure of $\geq 160/110$ mmHg with significant proteinuria while mild pre-eclampsia was elevated blood pressure of less than 160/110mmHg with significant proteinuria. A control group of normotensive pregnant women from the same obstetric population was recruited into the study.

Data collection

Data were collected from each subject using a well-designed questionnaire and the information was entered into the pro forma.

For the case group (Pre-eclampsia)

The arterial blood pressure in the brachial artery was measured using a mercury sphygmomanometer (made by Accoson-England) on the left arm in a comfortable sitting position after 5 minutes of rest. Both palpation and auscultation methods were used to measure the blood pressure. Korotkoff sound V was used to get the diastolic blood pressure because it is more reproducible than the fourth heart sound.^[17] However, where there was no disappearance of the sound, the muffling sound (Korotkoff sound IV) was used as the diastolic blood pressure. The reported values represent the mean of two readings taken at 5 minutes intervals. Confirmation of hypertension was done by repeating blood pressure measurements at least four hours later.

For urinalysis, each patient was given a labeled sterile urine sample bottle for mid-stream urine collection. Urine sample estimation for protein was done using Combi 10 urinary strip.

Blood samples for fasting serum lipid levels were collected after 8-12 hours of fasting. Five milliliters (ml) of a blood sample for fasting serum lipids estimation was drawn from the cubital vein using a sterile disposable syringe and needle with the application of a tourniquet after proper aseptic precautions. The blood was emptied immediately into a labeled sterile plain vacutainer glass tube and allowed to clot at room temperature. When the clot was formed, the sample was transported in a cool vaccine box to the laboratory within 3 hours of collection. The vacutainer tubes were centrifuged for 15 minutes at 3000 revolutions per minute to separate sera using centrifuge 80-3 made by Techmel & Techmel, USA. The separated sera were obtained using pipettes and the samples were emptied into appropriately labeled cuvette tubes and analyzed immediately.

For the control group (normotensive)

The controls were obtained after matching for maternal age, parity, and gestational age consecutively as the cases were recruited. A similar method of blood pressure measurement as explained for the case group was used, however, the blood pressure was measured once and once it was normal, there was no need for a repeat blood pressure check after 4 hours as done for the cases. The procedures for urine samples, fasting blood samples collection, and preparations of samples collected were the same as done for the pre-eclamptic group. Rescue stickers (logo) were tagged on the upper right corner of the antenatal folders and or case notes of all patients for identification and to avoid recruiting such patients into the study over again.

Laboratory lipids estimation

The biochemical analysis was done for total cholesterol (TC), triglycerides (TG), and High-density lipoproteins (HDL) using chemical precipitation reagents viz Randox kit HDL, and Biosystem kits for TGs and TC. The chylomicron fraction was separated from the sample with the aid of a photo tungstate contained in the reagents before the supernatant was used for the final spectrophotometric analysis. A Chemwell autoanalyzer machine was used to run the blood samples. The serum LDL-C and VLDL-C were calculated using Frederickson-Friedward's formula^[18] according to which.

$$\text{LDL-C} = \text{TC} - (\text{HDL-C} + \text{VLDL-C}).$$

$$\text{VLDL-C} = \text{TG}/5.$$

Data analysis

Data management and analysis were performed using a statistical package for social sciences (IBM-SPSS version 23.0). Continuous variables were presented as mean (\pm SD) and compared using Student's t-test. A P-value of less than 0.05 at a 95% confidence interval was regarded as statistically significant.

Results

Almost all the participants in the normotensive and pre-eclamptic group were married (96% vs 98%). About a third of normotensive women were Hausa/Fulani while the majority of the mothers in the pre-eclamptic group 30 (60%) were Hausa/Fulani. Just over half (54.4%) of the normotensive women had at least primary education but 50% of women with pre-eclampsia had at least secondary education.

Table 1: Socio-demographic variable table showing the characteristics of respondents.

Socio-demographic characteristic	Normotensive n (%)	Pre-eclamptic n (%)
Marital status		
Single	2(4.0)	1(2.0)
Married	48(96)	49(98)
Total	50(100.0)	50(100.0)
Tribe		
Hausa	12(24.0)	23(46.0)
Fulani	5(10.0)	7(14.0)
Igbo	0(0.0)	2(4.0)
Alago	7(14.0)	2(4.0)
Eggon	13(26.0)	2(4.0)
Others	13(26.0)	14(28.0)
Total	50(100.0)	50(100.0)
Education		
None	16(32.0)	17(34.0)
Primary	11(22.0)	8(16.0)
Secondary	10(20.0)	9(18.0)
Tertiary	13(26.0)	16(32.0)
Total	50(100.0)	50(100.0)
Social class		
Upper	27(54.0)	18(36.0)
Middle	7(14.0)	9(18.0)
Lower	16(32.0)	23(46.0)
Total	50(100.0)	50(100.0)

The mean age of women in the normotensive group was 24.90 ± 4.27 years and 24.92 ± 4.38 years in the pre-eclamptic group. The mean parity among normotensive women was 1.52 ± 1.90 while the mean parity in women with pre-eclampsia was 1.30 ± 1.95 . The mean gestational age at recruitment among cases was 35.66 ± 3.94 weeks and 35.36 ± 3.64 weeks for normotensive women. There was no statistically significant difference in age, parity, and gestational age of women in the two study groups. (Table 2)

Table 2: Obstetric characteristics of study participants

Characteristics	Normotensive Mean(SD)	Pre-eclampsia Mean(SD)	Statistics
Age (years)	24.90 ± 4.27	24.92 ± 4.38	$t = -0.023, p = 0.98$
Parity	1.52 ± 1.90	1.30 ± 1.95	$t = 0.572, p = 0.57$
Gestational age at recruitment (weeks)	35.36 ± 3.64	35.66 ± 3.94	$t = -0.395, p = 0.69$

The mean serum levels of TC, TG, VLDL-C and LDL-C were higher in women with pre-eclampsia compared with normotensive control. The HDL-C levels was however lower in cases than in control. (Figure 1)

Mean serum levels among study group

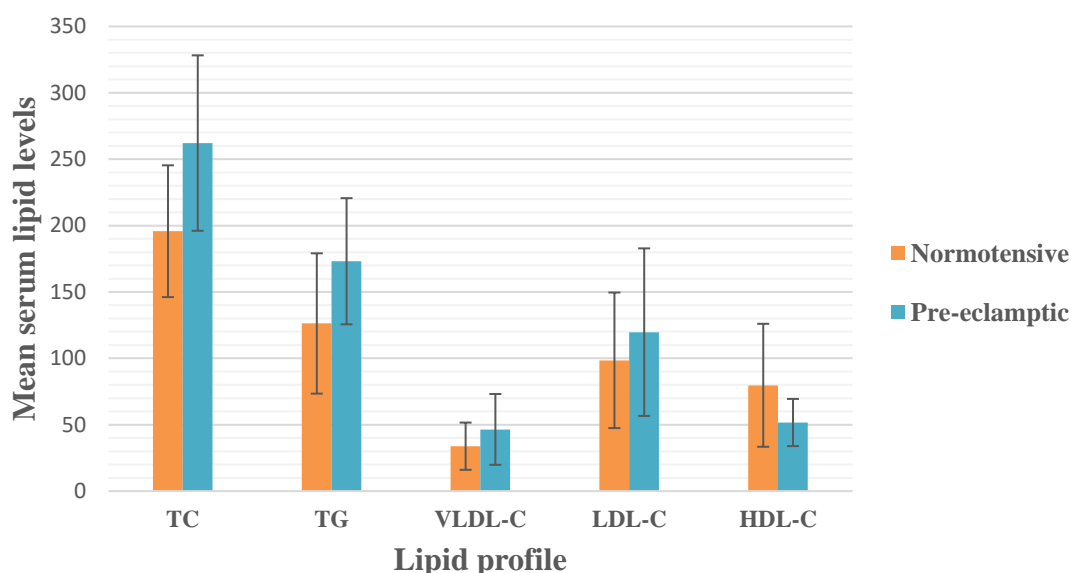


Figure 1: Mean serum levels of lipid among study group

There was a statistically significant higher level of serum total cholesterol, (262.16 ± 66.07 mg/dl vs 195.76 ± 49.64 mg/dl, $p < 0.001$), triglycerides (173.14 ± 47.51 mg/dl vs 126.26 ± 52.86 mg/dl, $p < 0.001$) and VLDL-C (46.44 ± 26.68 mg/dl vs 33.82 ± 17.79 mg/dl, $p = 0.007$) in women with pre-eclampsia compared to the normotensive group. However, there was no statistically significant difference in the LDL cholesterol (119.72 ± 63.11 mg/dl vs 98.52 ± 51.05 mg/dl, $P = 0.068$) among women in both groups. Women with pre-eclampsia had significantly lower HDL-C (51.66 ± 17.78 mg/dl vs 79.70 ± 46.32 mg/dl, $p < 0.001$). (Table 3).

Table 3: Comparison of mean serum lipid levels among normotensive and pre-eclamptic

Serum lipid levels	Normotensive	Pre-eclampsia	Statistics
	Mean (SD)	Mean (SD)	
TC (mg/dl)	195.76(49.64)	262.16(66.07)	$t = -5.681$, $p < 0.001$
TG (mg/dl)	126.26(52.86)	173.14(47.51)	$t = -4.664$, $p < 0.001$
VLDL-C(mg/dl)	33.82(17.79)	46.44(26.68)	$t = -2.735$, $p = 0.007$
LDL-C (mg/dl)	98.52(51.05)	119.72(63.11)	$t = -1.847$, $p = 0.068$
HDL-C(mg/dl)	79.70(46.32)	51.66(17.78)	$t = 3.997$, $p < 0.001$

TC-Total cholesterol, TG – triglycerides, LDL-C-low-density lipoprotein, VLDL-Very low-density lipoprotein, HDL-C-High-density lipoprotein

The mean serum total cholesterol (265.20 ± 13.80 mg/dl vs 234.80 ± 13.81 mg/dl, $p = 0.33$), triglyceride (177.92 ± 48.83 mg/dl vs 158.67 ± 16.17 mg/dl, $p = 0.42$), VLDL-C (47.44 ± 27.95 mg/dl vs 37.40 ± 3.72 mg/dl, $p = 0.43$), and HDL-C (52.29 ± 18.10 mg/dl vs 46.00 ± 14.97 mg/dl, $p = 0.46$) were higher in women with severe pre-eclampsia compared to women with mild pre-eclampsia. However, this finding was not statistically significant. In contrast, the serum LDL-C was higher in women with mild pre-eclampsia compared to women with severe pre-eclampsia (137.40 ± 29.30 mg/dl vs 117.76 ± 65.71 mg/dl, $p = 0.52$). This finding was also not statistically significant. (Table 4)

Table 4: Relationship between serum lipid levels and severity of pre-eclampsia

Serum lipid levels	Mild pre-eclampsia n=5	Severe pre-eclampsia n=45	Statistics
	Mean (SD)	Mean (SD)	
TC (mg/dl)	234.80 (13.81)	265.20 (13.80)	t= -0.98, p=0.33
TG (mg/dl)	158.67 (16.17)	177.92 (48.83)	t= -0.81, p=0.42
VLDL-C(mg/dl)	37.40 (3.72)	47.44 (27.95)	t= -0.80, p=0.43
LDL-C(mg/dl)	137.40 (29.30)	117.76 (65.71)	t= 0.68, p=0.52
HDL-C(mg/dl)	46.00 (14.97)	52.29 (18.10)	t= -0.75, p=0.46

TC-Total cholesterol, TG – triglycerides, LDL-C-low-density lipoprotein, VLDL-Very low-density lipoprotein, HDL-C-High-density lipoprotein

Discussion

One hundred women (50 pre-eclamptic and 50 normotensives) were recruited in this case-control study conducted between the 3rd of February 2020, and the 31st of May 2020.

The age range of participants was between 19 and 34 years but there was no difference in the mean age of normotensive women and women with pre-eclampsia (24.90 ± 4.27 years vs 24.92 ± 4.38 years). The obstetric characteristics of the cases and controls were similar as the participants were matched for age, parity, and gestational age at the time of recruitment. This eliminated confounders that may have arisen from these variables.

This study showed that there were significantly higher levels of serum total cholesterol, triglycerides, and VLDL-C in women with pre-eclampsia compared to normotensive pregnant women (Table 3). This was similar to findings by Sangeeta et al^[9] and Thathagari and colleagues.^[19] In contrast, Baksu et al^[20] and Irinyenikan et al^[13] did not find a statistically significant difference in the serum TC levels among the cases and controls.

In our study, the mean serum triglycerides in women with pre-eclampsia (173.14 ± 47.51 mg/dl vs 126.26 ± 52.86 mg/dl, $t = -4.664$, $p = <0.001$) were significantly higher than in a normotensive group. Similarly, Siddiqui et al^[12] also found significantly higher serum triglyceride levels in women with pre-eclampsia compared to normotensive pregnant women. A meta-analysis also found significantly high triglyceride levels in women who subsequently developed pre-eclampsia compared to women who remained normotensive during pregnancy.^[21] This supports the finding of a higher triglyceride level in pre-eclamptic women than in normotensive women in our study. This elevated level of triglycerides may have been present in early pregnancy and continued to the time of onset of pre-eclampsia. In contrast, Zinat and colleagues found no statistically significant difference in the level of triglycerides in women with pre-eclampsia and normotensive pregnant women.^[5] The inconsistent findings in these studies might be because some of the studies^[5,12,22] did not use fasting serum samples for lipid analysis. This gives room for falsely elevated lipid profiles from the post-prandial effect.^[23] Similarly, most of the above studies^[4,12,13] didn't match participants for maternal age, parity, and gestational age. These factors were put into consideration in our study as fasting serum samples were used for lipid analysis and our subjects were also matched for maternal age, parity, and gestational age in order to eliminate confounding factors. The serum VLDL-C was significantly higher in the pre-eclamptic group compared to the normotensive group ($t = -2.735$ $p = 0.007$). This was similar to the reports from Ibadan^[13] and Kano.^[15] The elevated level of VLDL-C is perhaps due to hypertriglyceridemia leading to the enhanced entry of VLDL-C that carries endogenous triglycerides into circulation.^[8] Also, increased levels of VLDL-C have been noted to accumulate within the maternal vascular endothelium particularly those of uterine and renal vessels causing injury to the endothelium.^[8]

In our study however, the LDL cholesterol (119.72 ± 63.11 mg/dl vs 98.52 ± 51.05 mg/dl, $p= 0.068$) in both groups was comparable. This is similar to the study by Baksu and colleagues^[20] that found no statistically significant difference in the LDL-C levels among women with pre-eclampsia and pregnant normotensive women.

Women with pre-eclampsia were discovered to have significantly lower HDL-C levels (51.66 ± 17.78 mg/dl vs 79.70 ± 46.32 mg/dl, $p<0.001$). This finding was like works done by other researchers.^[11,15,24,25] However, Thomas et al^[4] and Musa et al^[26] found no statistically significant difference in the HDL-C levels between the cases and the controls. In contrast, some studies showed significantly higher HDL-C levels among the pre-eclamptic group compared with the normotensive pregnant group.^[13, 19] Unlike LDL-C which is atherogenic, HDL-C is an antiatherogenic lipoprotein. The reduced HDL-C observed in our study may be useful in understanding the pathogenesis of pre-eclampsia in this regard to the possible risk of cardiovascular complications which could occur considering the cardio-protective function of HDL-C.

Despite the atherogenic properties known with LDL-C, our study did not find any statistically significant difference in serum LDL-C levels among pre-eclamptic when compared to normotensive women (t -test = -1.847, $p=0.068$). This finding further buttressed previous studies that found similar results.^[13, 24] On the contrary, few studies demonstrated significant rise in serum LDL among pre-eclamptic compared to healthy pregnant women.^[11, 25] The variations in these findings might be due to differences in the socio-demographic variables of the subjects used in these studies.

It was also discovered in our study that the total serum cholesterol was significantly elevated in pre-eclamptic compared to the normotensive group (t -test = -5.681, $p=0.000$). This was comparable to the studies by Udenze,^[25] Yakubu^[24] and Attah^[15] et al. This hypercholesterolemia may cause deposition of excess cholesterol in the spiral arterioles or glomerular capillaries thereby narrowing the lumen of these vessels resulting in pathological changes seen in pre-eclampsia.^[7]

Our study clearly revealed that the mean serum levels of TC, TGs, LDL-C and VLDL-C were higher in pre-eclamptic group than the normotensive pregnant group (Figure 1) while the mean serum level of HDL-C was lower in the pre-eclamptic group as compared to the normotensive pregnant group.

The relationship between serum lipid profile changes and severity of pre-eclampsia was also assessed in our study (Table 4). It was observed that the mean serum TC, TGs, VLDL-C and HDL-C in severe pre-eclamptic patients were higher than those of mild pre-eclampsia, but this was not statistically significant. Conversely, LDL-C levels were lower in severe pre-eclamptic compared to those with mild pre-eclampsia; this difference was also not statistically significant. These findings were different from what was obtained in a study done in Kano by Attah et al^[15] which compared the serum lipid profiles of 19 mild pre-eclamptic to 51 severe pre-eclamptic patients. Attah and colleagues reported that TC, TGs, and VLDL-C levels were significantly higher in the severe pre-eclamptic group compared to the mild pre-eclamptic group while HDL-C was significantly lower among women with mild pre-eclampsia than those with severe pre-eclampsia. He also noted that there was no significant difference in the LDL-C levels between the two groups.^[15]

Conclusion

Women with pre-eclampsia have increased serum levels of total cholesterol, triglycerides, and VLDL-C and decreased level of HDL-C compared to normotensive pregnant women. However, the lipid profile of women with severe pre-eclampsia did not differ from those with mild pre-eclampsia. The dyslipidaemia found among the pre-eclamptic group in this study, may play an important role in the aetiopathogenesis of pre-eclampsia. This finding may also be responsible for the increase in cardiovascular risk later in life in this group of patients.

Limitation

The laboratory estimation of LDL-C and VLDL-C requires lipoprotein electrophoresis and ultracentrifugation, which are very complex and expensive procedures. These were however estimated using Friedwald's formula.^[18]

Recommendation

A large multicenter study may be required to determine the normal reference range for lipid profile in pregnant women in our obstetric population.

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