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Original Article

Comparative study of serum lipid levels in normotensive and pre-eclamptic Nigerian women

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ABSTRACT

Background: Pre-eclampsia is a common syndrome that occurs in the second half of pregnancy and often manifest with hypertension and significant proteinuria. It occurs in up to 10% of pregnancies and is the second cause of maternal mortality worldwide accounting for 17% of maternal deaths in Nigeria. **Aim:** This study was performed to compare the serum lipid levels of pregnant normotensive and pre-eclamptic women. **Methods:** It was a case control study involving 50 normotensive and 50 pre-eclamptic women at the University College Hospital, Ibadan, making a total of 100 participants. Their venous blood (5mls) was collected after an overnight fast of 8-12 hours and this was analysed for cholesterol, triglyceride, high density lipoprotein-cholesterol (HDL-C), very low density lipoprotein-cholesterol (VLDL-C) and low density lipoprotein-cholesterol (LDL-C) using standard enzymatic methods. **Results:** The study showed that there was a significantly higher levels of triglyceride, VLDL-C and HDL-C in the pre-eclamptic compared to the normotensive group (P<0.05). Also on Spearman´s Correlation, there was a significant positive correlation between these lipids and pre-eclampsia. **Conclusion:** Lipids therefore have an important role in the aetiopathogenesis of pre-eclampsia and could be estimated during the conservative management of patients with pre-eclampsia.

Key Words: Serum lipids, hypertension, pre-eclampsia, normotensive women, Nigerian women

INTRODUCTION

Pre-eclampsia is a non-convulsive form of pregnancy induced hypertension occurring in 5 to 10% of gestations.[1] It is a major cause of both maternal and fetal morbidity and mortality. In Nigeria it accounts for 17% of maternal deaths.[2] Although the exact cause

of pre-eclampsia is unknown, however endothelial injury is a key factor in the pathogenesis of the disease.[3] Studies have shown that lipids accumulate in arterial intima cells and in macrophages causing endothelial injuries.[4] An altered lipid profile is therefore associated with arteriosclerotic cardiovascular disease and with endothelial dysfunction.[5]

Recent papers have suggested that a maternal predisposition to pre-eclampsia may be explained by abnormal lipid metabolism.[1] The role of dyslipidaemia in the pathophysiology of pre-eclampsia has therefore stimulated interest. Although few studies have been carried out in our locality, however studies done among Caucasians have consistently showed marked hyperlipidaemia in pre-eclamptic patients compared with women with normal pregnancies.[1] Evidence has also shown that there is a rise in LDL-C and a fall in HDL-C in hypertensive pregnant women compared with their normotensive counterparts.[6]

This study was carried out to investigate the serum lipid levels of pre-eclamptic women in comparison with their normotensive counterparts and to also find out if there is a relationship between serum lipids and pre-eclampsia.

METHODOLOGY

It was a case control study conducted at the department of Obstetrics and Gynaecology of University College Hospital, Ibadan in Oyo State between July 2010 and December 2010. The studied population included 50 consenting pregnant healthy normotensive women and 50 women diagnosed to have pre-eclampsia accessing care at the University College Hospital, Ibadan. The study was approved by the Ethics Committee of University of Ibadan/University College Hospital. Informed consent was obtained from each of the subjects.

Inclusion criteria

Healthy normotensive pregnant women who were more than 20 weeks pregnant and women diagnosed to have pre-eclampsia.

Exclusion criteria

- Women with multiple gestation
- Women with molar pregnancy
- Women with chronic hypertension
- women with previous history of cardiac disease, HIV, renal disease and liver disease or who were currently diagnosed in this pregnancy to have any of the disease
- Women with known history of dyslipidaemia
- Obesity

Determination of sample size

The women were divided into 2 groups:
Group A: Normotensive Pregnant Women
Group B: Women with pre-eclampsia.
The sample size was calculated using the Leslie Fischer's formula.[16]

$$N = \frac{(Z\alpha + Z\beta)^2 (P_O(1-P_O)+P_1(1-P_1))^2}{(P_1-P_O)^2}$$

where:
N = required minimum sample size for each group.
Zα = % of normal distribution corresponding to the
required significant level of 5%=1.96
Zβ = point of normal distribution corresponding to the
statistical power of 80% = 0.842
p_o = response in the first group from previous study
= 0.90
P1 = expected response in the second group
= 0.80

$$n = \frac{(1.96 + 0.842)^2 (0.80 (1-0.80) + 0.90 (1.0 – 0.90))^2}{(0.9 -0.8)^2}$$

≈ 49 (minimum sample size in each group)
This was rounded up to 50 subjects in each group with a total of 100 women for the study.

Diagnosis/sample collection

The diagnosis of pre-eclampsia was based on two consecutive measurements of systolic and diastolic blood pressure equal to or greater than 140/90mmHg 6hours apart, one measurement of diastolic blood pressure of 110mmHg or more or a rise of 30mmHg or 15mmHg above the normal pre-pregnancy systolic and diastolic blood pressures after the 20th week of pregnancy. Persistent elevation of the blood pressure on two occasions with proteinuria was used to make a diagnosis of pre-eclampsia. Patients with a

history of chronic hypertension, renal disease and/or cardiovascular disease, dyslipidaemia, diabetes mellitus were all excluded.

5mls of venous blood was drawn from the ante cubital vein from all the subjects following an overnight fast of 8-12 hours. The sample was analysed in the chemical pathology laboratory of the University College Hospital, Ibadan using Roche Hitache 902 auto-analyser which takes up to 30 samples at a time. The sample was first centrifuged at 4,000 revolutions per minute over 4 minutes to separate the red cells from the serum. Sera were stored at 2-8^{0}C in the refrigerator or analysed immediately.

Analysis was done for serum triglycerides (TG), total cholesterol (TC) and HDL-cholesterol (HDL-C) by using chemical precipitation reagents: Randox kits for HDL, triglyceride Roche Kit for the triglycerides, Fortress Diagnostic Cholesterol Kit for the cholesterol. The Chylomicron fractions were precipitated quantitatively from the serum by the addition of phosphotungstic acid and magnesium ions which are contained in the above kits. After centrifugation at 4,000 revolution per minute over 10 minutes to separate the chylomicrons and phospholipids, the Total cholesterol, the HDL and the Triglycerides were then determined from the supernatant using the different kits.

Serum LDL-cholesterol (LDL-C) was calculated by Frederickson-Friedwald's formula according to which LDL-cholesterol = total cholesterol - (HDL-cholesterol + VLDL-cholesterol). VLDL cholesterol was calculated as 1/5 of triglycerides.[7]

Statistical analysis

Statistical Package for Social Science (SPSS) for Windows Version 16 was used to analyse data. Categorical variables were tested for statistical significance using Chi-square test. The differences in the mean serum lipid level of the two groups was statistically tested using the student ´t´ test, while the one way ANOVA was used to test for differences in the means of three groups. Spearman's correlation was used to find a correlation between the lipid fractions and Pre-eclampsia. The level of statistical significance was set at P<0.05.

RESULTS

Table 1 shows the clinical characteristics of the participants. The age range of the participants was 20 -42 years. The mean age of the normotensive group was 29.28 ± 4.57(SD) years while the mean age of the pre-eclamptic group was 29.42 ± 5.21(SD) years. There was no statistically significant difference in the mean ages of the two groups (t = 2.333, P>0.05). Table 1 also shows that in the normotensive group, 17(34%) of the participants were primigravidas, 31(62%) were primipara while 2(4%) were multipara. The median parity was 1. In the pre-eclamptic group, 25(50%) were primigravidas, 18(36%) were primipara and 7 (14%) were multipara. Their median parity was 0. There was a statistically significant difference in the parity distribution of the two groups. (X^2= 11.449, P<0.05). In the normotensive group, the mean body mass index was 20.58 ± 3.07(SD) kg/m^2 while that of the pre-eclamptic group was 21.81 ± 2.66(SD) kg/m^2. There was no statistically significant difference in the two groups (t= 0.87, P>0.05).

The normotensive group 31(62%) were unskilled, 6(12%) were semi-skilled workers and 13(26%) were skilled workers. In the pre-eclamptic group, 35(70%) were unskilled, 11(22%) were semi-skilled workers and 4(8%) were skilled workers. In the normotensive group 2(4%) of the participants were in their 2nd trimester while 48(96%) were in their 3rd trimester. In the pre-eclamptic group, 4(8%) were in their 2nd trimester while 46(92%) were in their 3rd trimester. The mean gestational age for the normotensive participants was 34.04 ± 3.25(SD) weeks and 34.76± 4.98 (SD) weeks for the hypertensive. There was no statistically significant difference in the gestational ages (t=1.953, P>0.05). The mean birth weight of the babies for the normotensive participants was 3.38±0.27 (SD)kg and 2.46±0.79(SD) kg for the pre-eclamptic group. There was a statistically significant difference in the babies' weights. (X^2=7.795, P< 0.05).

Figure 1 shows the serum lipid levels of the normotensive and the pre-eclamptic participants, in the normotensive group the mean cholesterol level was 184.02 ±

40.47(SD) mg/dl while in the pre-eclamptic group it was 192.36 ± 44.40(SD) mg/dl. There was no statistically significant difference in the levels. (t=0.982, *P*>0.05). In the normotensive group, the mean triglyceride level was 149.70± 47.42(SD) mg/dl, while that of the pre-eclamptic group was 199.68 ± 82.13(SD) mg/dl. There was a significantly higher level of triglyceride in the pre-eclamptic group (t=3.726, *P*<0.05).

Table 1: The clinical characteristics of the participants

Characteristics	Percentage (%)	
Age (years)	Normotensive(n=50)	Pre-eclamptic(n=50)
20-24	10.0	16.0
25-29	44.0	34.0
30-34	30.0	30.0
35-39	14.0	14.0
40-44	2.0	6.0
Parity *		
0	34.0	50.0
1	62.0	36.0
≥2	4.0	14.0
Occupation		
Unskilled	62.0	70.0
Semi-Skilled	12.0	22.0
Skilled	26.0	8.0
Gestational Age (weeks)*		
20-26	4.0	8.0
≥27	96.0	92.0
Mean Body mass index(kg/m^2)	20.58±3.07(SD)	21.81±2.66(SD)
*Mean Babies weight	3.38±0.27(SD)	2.46±0.791(SD)

*Significant finding (*P*<0.05)

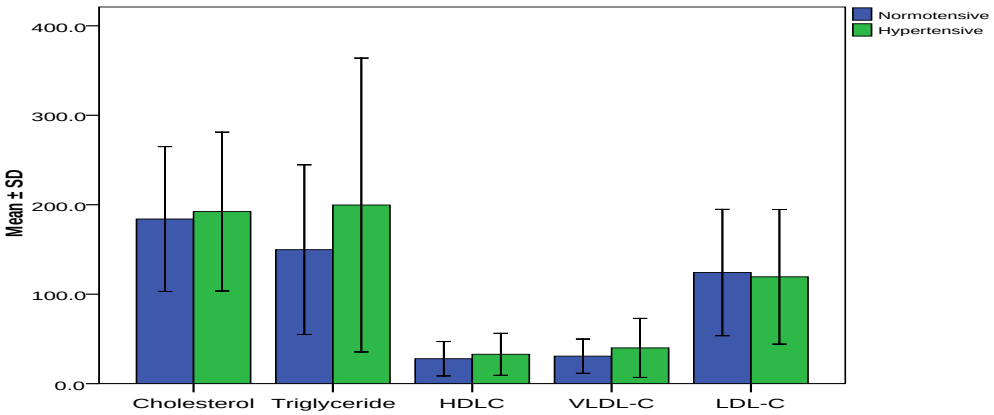

Figure 1: A bar chart showing the serum lipids in the normotensive and pre-eclamptic (hypertensive) participants.

Table 2: Showing the mean serum lipids of the participants according to the severity of pre-eclampsia compared with the normotensive.

Serum Lipid	Normotensive n=50	Mild pre-eclampsia n=3	Severe pre-eclampsia n=47
Cholesterol	184.02±40.47	153.00±33.96	194.87±44.07
Triglycerides*	149.70±47.42	94.67±40.08	206.38±79.69
HDL-C*	27.78±9.60	41.00±9.54	32.26±11.74
VLDC-C*	30.70±9.57	18.93±8.02	41.30±16.01
LDL-C	124.18±35.33	93.00± 23.30	121.09±37.89

*Significant difference using one way ANOVA

Table 3a: Serum lipids in normotensive and pre-eclamptic participants in the 2nd trimester

	Cholesterol (mg/dl)	Triglycerides (mg/dl)	HDL-C (mg/dl)	VLDL-C (mg/dl)	LDL-C (mg/dl)
Normotensive	148.00±94.75	117.00±43.84	20.00±0.00	23.40±8.77	54.50±14.85
Pre-eclamptic	236.25±54.93	235.00±125.81	32.00±5.89	47.30±25.75	157.00±51.19
P-value	0.20	0.29	0.05	0.29	0.06

Level of significance at $P<0.05$

Table 3b: Serum lipids in normotensive and pre-eclamptic participants in the 3rd trimester

	Mean value				
	Cholesterol (mg/dl)	Triglyceride* (mg/dl)	HDL-C* (mg/dl)	VLDL-C* (mg/dl)	LDL-C (mg/dl)
Normotensive	185.52±38.19	151.06±47.50	28.10±9.66	31.00±9.56	127.08±32.88
Pre-eclamptic	188.54±41.95	196.61±78.55	32.85±12.14	39.32±15.71	116.13±35.06
P-value	0.72	0.00	0.03	0.00	0.12

*Level of significance at $P<0.05$

Table 4: Serum lipids in the pre-eclamptic in 2nd and 3rd trimesters of pregnancy

Trimester	*Cholesterol (mg/dl)	*Triglyceride (mg/dl)	HDL-C (mg/dl)	*VLDL-C (mg/dl)	*LDL-C (mg/dl)
2nd	236.25±54.93	235.00±125.81	32.00±5.89	47.30±25.75	157.00±51.19
3rd	188.54±41.95	196.61±78.55	32.85±12.14	39.32±15.71	116.13±35.06
P value	0.01	0.02	0.71	0.00	0.03

*Significant difference ($P<0.05$).

Table 5: The correlation between serum lipids and Pre-eclampsia using Spearman's correlation coefficient

Lipid fraction	Spearman's correlation coefficient (R)	*P*-value
*Triglyceride	0.358	0.000
Cholesterol	0.082	0.417
HDL-C	0.193	0.550
*VLDL-C	0.323	0.001
LDL-C	-0.089	0.378

*Level of significance at $P<0.05$

The normotensive group the mean high density lipoprotein cholesterol (HDL–C) was 27.78±9.60 (SD) mg/dl, while in the pre-eclamptic group it was 32.78 ± 11.73(SD)mg/dl. There was a significantly higher level of HDL-C in the pre-eclamptic group (t=2.333, $P<0.05$).In the normotensive group the mean level of VLDL-C was 30.70 ± 9.57(SD) mg/dl, while that of the pre-eclamptic group was 39.96±16.49 (SD)mg/dl.

There was a significantly higher level of VLDL-C in the pre-eclamptic group (t=3.435, $P<0.05$). Also in the normotensive group the mean level of LDL-C was 124.18 ± 35.33(SD) mg/dl, while that of the pre-eclamptic group was 119.40 ± 37.62(SD) mg/dl. There was no statistically significant difference in the level of LDL-C in the 2 groups (t=0.655, $P>0.05$).

Table 2 shows the mean serum lipids of the participants according to the severity of pre-eclampsia compared with the normotensive. In the mild pre-eclamptic the mean serum cholesterol was 153.00 ± 33.96(SD)mg/dl, in the severe pre-eclamptic group it was 194.87±44.07(SD)mg/dl while in the normotensive group it was 184.02± 40.47(SD) mg/dl. Using one way ANOVA to compare the differences in the means of the groups, there was no statistically significant difference (F=1.886, $P>0.05$).

In the mild pre-eclamptic group the mean serum triglyceride level was 94.67 ± 40.08 (SD) mg/dl, in the severe pre-eclamptic group it was 206.38±79.69 (SD)mg/dl, while in the normotensive it was 149.70±47.42(SD)mg/dl. Using one way ANOVA, there was a statistically significant difference in the groups (F=11.677, $P<0.05$).

In the mild pre-eclamptic, the mean HDL-C was 41.00± 9.54(SD) mg/dl, in the severe pre-eclamptic it was 32.26±11.74 (SD)mg/dl, while in the normotensive it was 27.78±9.60(SD) mg/dl. Using a one way ANOVA, there was a statistically significant difference (F=3.694, $P<0.05$).

In the mild pre-eclamptic the mean serum VLDL-C was 18.93 ± 8.02 (SD)mg/dl, in the severe pre-eclamptic it was 41.30±16.01(SD)mg/dl, while in the normotensive it was 30.70±9.57(SD)mg/dl. Using the one way ANOVA, there was a statistically significant difference in the groups (F=10.513, $P<0.05$). In the mild pre-eclamptic the mean serum LDL-C was 93.00 ± 23.30(SD) mg/dl, in the severe pre-eclamptic it was 121.09±37.89(SD)mg/dl, while in the normotensive group, it was 124.18±35.33(SD)mg/dl. Using the one way ANOVA to compare their means there was no statistically significant difference (F=1.057, $P>0.05$).

Using a 2 tail t- test as a Post-hoc test to identify the point of differences in the mean serum lipid as the blood pressure rises. It showed that as the blood pressure rises the level of triglyceride, VLDL-C and HDL-C increased with the severity of pre-eclampsia.

Table 3a shows the serum lipids of the participants in the second trimester of pregnancy. The mean cholesterol in the normotensive was 148.00 ± 94.75 (SD) mg/dl while in the pre-eclamptic group it was 236.25±54.93 (SD) mg/dl. There was no statistically significant difference in their level ($P>0.05$). The mean triglyceride was 117.00± 43.84 (SD) mg/dl in the normotensive and 235.00 ± 125.81 (SD) mg/dl in the pre-eclamptic group. There was no statistically significant difference in their level ($P>0.05$). The mean HDL-C was 20.00±0.00 (SD)

mg/dl in the normotensive and 32.00 ± 5.89 (SD) mg/dl in the pre-eclamptic group. There was no statistically significant difference in their levels (*P*>0.05). The mean VLDL-C was 23.40±8.77 (SD) mg/dl in the normotensive and 47.30±25.75 (SD) mg/dl in the pre-eclamptic group. There was no statistically significant difference in the values (*P* >0.05).

The mean LDL-C was 54.50±14.85 (SD) mg/dl in the normotensive and 157.00± 51.19 (SD) mg/dl in the pre-eclamptic group. There was also no statistically significant difference in their values (*P*>0.05).

Table 3b shows the serum lipid levels in the normotensive and the pre-eclamptic participants in the third trimester of pregnancy. The mean cholesterol in the normotensive was 185.52± 38.19 (SD) mg/dl while in the pre-eclamptic it was 188.54±41.95 (SD) mg/dl. There was no statistically significant difference in their values (*P*>0.05). The mean triglyceride was 151.06 ± 47.50 (SD) mg/dl in the normotensive group and 196.61±78.55 (SD) mg/dl in the pre-eclamptic group. There was a statistically significant difference in the mean values (*P*<0.05).

The mean HDL-C was 28.10 ± 9.66 (SD) mg/dl in the normotensive group and 32.85±12.14 (SD) mg/dl in the pre-eclamptic group. There was a statistically significant difference in the mean values (*P*<0.05). The mean VLDL-C was 31.00±9.56 (SD) mg/dl in the normotensive and 39.32±15.71 (SD) mg/dl in the pre-eclamptic group. There was a statistically significant difference in the mean values (*P* <0.05). The mean LDL-C was 127.08±32.88 (SD) mg/dl in the normotensive group and 116.13± 35.06 (SD) mg/dl in the pre-eclamptic group. There was no statistically significant difference in the mean value (*P*>0.05).

Table 4 shows serum lipids of the pre-eclamptic participants in the 2nd and 3rd trimesters. When the serum lipids of the pre-eclamptic participants were compared in the second and third trimesters, there was a statistically significant difference in the lipid fractions except in the level of HDL-C.

Using Spearman´s correlation, the study showed that triglyceride and VLDL-C had a significant positive correlation with pre-eclampsia (*P*<0.05). There was no significant correlation with the other lipids (table 5).

DISCUSSION

Pre-eclampsia is a disease of theories whose pathogenesis is not clearly understood. However studies among the Caucasian women have suggested that maternal predisposition to pre-eclampsia may be explained by abnormal lipid metabolism.[1] This study was perform to verify this claim especially due to the paucity of studies in our locality. The socio-demographic characteristics of the patients were similar which showed that the participants are within the same environment. The ages of the participants were within the reproductive age group when they are not likely to have an underlying vascular disease. Most of the women in the pre-eclamptic group were young and mostly primigravidas which is in keeping with the fact that pre-eclampsia is more frequent in first gestation young women.[1] Also the finding of a lower birth weight of the babies in the pre-eclamptic group which may be due to the complication of the disease is in keeping with other studies.[8]

Some studies have shown that the most dramatic damage in the lipid profile of pregnancy is serum hypertriglyceridaemia which is even higher in toxaemia of pregnancy.[9] In this study, this observation holds true. The mean serum triglyceride was significantly higher in the pre-eclamptic group compared to the normotensive. The mean value being raised almost two folds in those with severe pre-eclampsia. The finding of hypertriglyceridaemia is in keeping with the findings of Enquobahrie and Cekmen.[10]

The principle modulator of this hypertriglyceridaemia is oestrogen as pregnancy is associated with hyperestrogenaemia. Oestrogen induces hepatic biosynthesis of endogenous triglycerides, which is carried by very low density lipoprotein-cholesterol (VLDL-C).[4] It is therefore thought that the increased triglyceride found in pre-eclampsia, is likely to be deposited in predisposed vessels, such as the uterine spiral arteries and

consequently contributes to the endothelial dysfunction both directly and indirectly.[11]

In this study, no statistically significant difference was found in the level of cholesterol in the two groups. This is in keeping with the study of Sattar *et al.*[11] On the contrary, others have found significant rise in serum cholesterol in toxaemia of pregnancy.[12,13] This may be due to variation in races and nutrition or possibly due to the methodology of these studies.

Winny *et al.*[1] compared the serum lipids of pregnant women aged 35years or more suffering from pre-eclampsia with their age matched normotensive pregnant women and reported that HDL-C was significantly higher in women with pre-eclampsia compared to the normotensive counterpart. This is in keeping with the findings in this study.

A major modifiable risk factor for cardiovascular diseases is the concentrations of plasma lipoprotein. Increased levels of atherogenic lipoproteins especially LDL contribute to the development of atherosclerosis, therefore lowering LDL-C reduces fatal and nonfatal heart attacks.[14] Similarly, low HDL-C levels also predispose to atherosclerosis.[15] This study showed a lower level of LDL-C (119.40 ± 37.62 vs 124.18 ± 35.33) and a significantly higher level of HDL-C (32.78 ± 11.73 vs 27.78 ± 9.60) in the pre-eclamptic participants when compared to the normotensive (figure 1). This is reassuring as it means that the lower level of LDL-C and the higher level of HDL-C are protective of cardiovascular diseases in patients with pre-eclampsia.

In this study, the serum VLDL-C was significantly higher in the pre-eclamptic group which is in keeping with the study of Sattar *et al.*[11] The elevated level of VLDL-C is perhaps due to hypertriglyceridaemia leading to enhanced entry of VLDL-C that carries endogenous triglyceride into circulation.[14] Also increased level of VLDL-C have been noted by other researchers to accumulate over the maternal vascular endothelium particularly those of uterine and renal vessels causing injury to the endothelium.[14] This study showed that the levels of triglyceride, HDL-C and VLDL-C increased significantly with the severity of pre-eclampsia. This further suggests increase endothelial damage in those with severe pre-eclampsia.

A large number of clinical and biochemical tests have been employed to predict women at the risk of developing pre-eclampsia in an effort to improve outcome in high risk women. However, no single biochemical test has been identified to predict this condition. In this study, triglyceride and VLDL-C showed significant positive correlation with pre-eclampsia. Therefore assaying for the level of triglycerides and the lipoprotein sub-fractions in high risk pregnant women could be used in detecting women who may likely develop pre-eclampsia and this could subsequently improve their outcome.

CONCLUSION

Lipid fractions increase during pregnancy and this is more profound in the pre-eclamptic especially the levels of triglycerides, VLDL-C and HDL-C. The rise in lipid levels is parallel to the severity of the disease which is improved in the normotensive but worse in women with severe pre-eclampsia. Therefore, lipids have an important role in the aetiopathogenesis of pre-eclampsia and could be estimated during the conservative management of patients with pre-eclampsia.

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