REVIEW ARTICLE
PREGNANCY AND CVD RISK FACTORS

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ABSTRACT

Elevated concentrations of total cholesterol, particular lipoproteins (e.g., LDL) and homocysteine are risk factors for cardiovascular disease and vascular dysfunction that can adversely affect the health of a pregnant woman and her fetus. Although it has been documented in many populations worldwide that the serum total cholesterol, LDL-cholesterol, HDL-cholesterol, and triglyceride levels increase substantially by the third trimester, there are few reports of the levels of these risk factors in pregnant women in sub-Saharan Africa. We therefore compared the levels of these lipids and homocysteine in the serum of third-trimester pregnant women (n=18) and healthy, age-matched non-pregnant controls (n=38) in Abuja, Nigeria. Relative to the controls, the following substances were significantly elevated in the pregnant women: total cholesterol, 212 vs. 191 mg/dL (p=0.02); triglycerides, 153 vs. 89.5 mg/dL (p=0.004); and HDL, 67.0 vs. 56.6 mg/dL (p=0.004). The mean LDL-cholesterol levels of the pregnant (116 mg/dL) and non-pregnant controls (110 mg/dL) were not significantly different. However, the mean homocysteine concentration of the pregnant women was about 30% lower compared with the control group (7.1 vs. 10.1 μmol/L, p<0.001). The mean folate level of the pregnant women, 16/18 of whom were taking a multivitamin supplement, was more than twice that of the non-pregnant controls (10.4 vs. 4.4 ng/mL, p=0.002), whereas the vitamin B_{12} level was nearly two-fold higher in the controls than in the pregnant women (620 vs. 343 pg/mL, p=0.001). These results indicate that pregnant women in Nigeria we studied show the same trend that most women in other parts of the world exhibit as they enter the third trimester of pregnancy; that is, their serum cholesterol and triglyceride levels increase, while their homocysteine levels decrease. The lower homocysteine levels in the pregnant women relative to the non-pregnant women were likely due to the use of folate supplements by the pregnant women. Despite the difference in the serum lipid profiles of the pregnant and non-pregnant women, both groups had values of serum concentrations of lipids, folate, vitamin B_{12} and homocysteine that were well within the reference range of values provided by the American Heart Association (AHA).

Key Words: cholesterol, LDL, HDL, lipid ratios, homocysteine, pregnant, and CVD risk factors

INTRODUCTION

Hyperhomocysteinemia and elevated serum lipid concentrations, especially total cholesterol and LDL-cholesterol, are risk factors for cardiovascular disease and endothelial dysfunction (1). Hypercholesterolemia promotes functional and structural injury to the vascular wall (2). Even mild hyperhomocysteinemia has been identified as a risk factor for arterial disease and venous thrombosis (3). More than a decade ago, elevated homocysteine concentrations were also found to be associated with neural tube defects (4). Pregnancy in populations worldwide is associated with increases in plasma total cholesterol, triglycerides, and HDL-cholesterol (5). These changes are thought to serve the nutritional needs of the fetus (6). On the other hand, extreme hypercholesterolemia increases artery resistance to blood flow and can result in fetal growth retardation (7). In addition, the formation of fatty streaks, precursors of advanced atherosclerotic lesions, are markedly increased in fetuses whose mothers are hypercholesterolaemic during pregnancy (8). Moreover, there is evidence that the fetal onset of atherogenesis greatly influences the rate of its progression throughout childhood and adolescence (8). Furthermore, in a study conducted in Lima, Peru (9), high triglyceride and low HDL-cholesterol concentrations were found to be important risk factors for preeclampsia. Similarly, an elevated plasma homocysteine level in pregnancy may increase the risk of a pregnant woman developing preeclampsia by as much as three-fold (10). Similarly, a study by Berge and coworkers (5) indicated that changes in the serum lipid profile during pregnancy can influence the risk of cardiovascular disease (CVD) development in women (5). Following birth, most of the gestational changes return to pre-pregnancy levels except total cholesterol and triglycerides where elevated levels of both of these lipid classes are associated with CVD (5). The significance of the atherogenic lipid profile that develops during normal gestation are unclear, but is widely regarded as having important implications for both the mother and the fetus (11).

To our knowledge, despite the significance of the levels of various lipoproteins and homocysteine to the health of the pregnant woman and her fetus and in light of the high rates of maternal and neonatal mortality in many countries in sub-Saharan Africa (12,13), the literature contains few reports of studies of the concentrations of homocysteine, triglycerides, total cholesterol, or LDL-cholesterol in pregnant women in that part of the world. Ekeke (14), Taylor (15), Ojo and Sogbanmu (16), and Ahaneku and coworkers (17) investigated the changes in serum cholesterol and various cardiovascular disease risk factors during normal pregnancy in Nigerian women; however, they did not report homocysteine data. The results of these studies indicated an increase in the total cholesterol level, LDL-cholesterol, and triglyceride concentrations late in pregnancy.

We collected blood serum from 18 pregnant Nigerian women in the third trimester and 38 healthy non-pregnant, age-matched controls and compared their lipid profiles and homocysteine levels between the two groups. Because homocysteine levels are influenced by dietary factors, particularly one's folate and vitamin B_{12} status, we also determined serum levels of these two water-soluble vitamins in these same subjects.

METHODS

Study population. This study was conducted in the summer of 1999 in the city of Abuja in northern Nigeria. The subjects, 18 pregnant and 36 non-pregnant women, were recruited from among the relatives and the patients attending the National Hospital, Abuja, which provides care mainly to patients from the upper socioeconomic stratum. Only women in their third trimester were enrolled in the study. Both the pregnant and non-pregnant women were similar in

terms of diet and smoking and drinking patterns with the exception that 16/18 of the pregnant women acknowledged they were taking multivitamin supplements. None of the subjects reported the use of alcohol or tobacco and all were in good health. This study was approved by the Human Research Review Committee of the University of New Mexico School of Medicine and was in accordance with the Helsinki Declaration.

Anthropometric measurements. The height and weight of each subject was determined using a portable stadiometer and battery-operated scale (accurate to 0.5 kg), respectively.

Biochemical analyses. Blood samples were obtained by venipuncture and the samples were allowed to clot at room temperature for 45 minutes before centrifugation to separate the serum. Samples were then aliquoted into cryovials and stored at - 40°C until they were transported in a frozen state to Albuquerque, NM for analysis.

Total cholesterol was determined by the end-point colorimetric method of Allain *et al.* (18) using a Vitros 950 analyzer. HDL-cholesterol was assayed using Kodak Vitros Cholesterol slides and a Vitros 250 analyzer (19). Triglycerides were determined by the method of Spayd *et al.* (20) using a Vitros Analyzer Clinical Chemistry Slide (TRIG) and a Vitros 950 analyzer. LDL-cholesterol was calculated using the following equation: LDL-cholesterol = total cholesterol (HDL-cholesterol + VLDL-cholesterol). Serum homocysteine levels were determined using the IMx Homocysteine assay kit (Abbott Diagnostics Division, Abbott Laboratories, Abbott Park, IL, USA). Serum folate and vitamin B_{12} levels were assayed immunologically by competitive magnetic separation assays using the Bayer Immuno 1 System (Bayer Corporation, Tarrytown, NY, USA).

Statistical analysis. Descriptive statistics, group comparisons and correlations were made using the Number Cruncher Statistical Software (NCSS, version 6, Kaysville, UT, U.S.A.). Results are expressed as mean ± 1 standard deviation. Group comparisons were made using the two-sample t-test to determine the statistical significance of various parameters between the different groups. A p-value of 0.05 was considered statistically significant.

RESULTS

Comments on study population. Characteristics of the 18 pregnant and 36 non-pregnant women are summarized in Table 1. The mean ages of the pregnant and non-pregnant women were 28.0 and 34.7 years, respectively. The pregnant women were comparable to the non-pregnant women in terms of height, but were, on average, about 10 kg heavier than the non-pregnant controls.

Serum lipid profiles. As shown in Table 2, a significant difference was observed between the various cardiovascular disease risk factors in the pregnant and non-pregnant women. With respect to lipids, the pregnant women had the poorer CVD risk profile; relative to the non-pregnant controls, they had significantly higher total cholesterol and triglyceride concentrations. However, the LDL-cholesterol levels of the two groups were not different (110-116 mg/dL). The pregnant women had about 15% more ($p=0.004$) cardioprotective HDL-cholesterol than their non-pregnant counterparts. The LDL-cholesterol/HDL-cholesterol and total cholesterol/HDL-cholesterol ratios of the two groups were not different. Furthermore, these two ratios were well within the range of values prescribed by Columbia University and the Altruis Biomedical Network, respectively (21, 22).

Serum homocysteine, vitamin B_{12}, and folate concentrations. The mean homocysteine level was about 30% lower in the pregnant women than in the non-pregnant controls (7.1 versus 10.1 μmoles/L, $p<0.001$). This difference could be accounted for by the fact that the serum folate levels were about 2.5-fold higher in the pregnant women. In contrast, however, the vitamin B_{12} levels were nearly twice as high in the non-pregnant women relative to the pregnant women.

Both of these vitamins are intimately involved in homocysteine metabolism.

We analyzed our data to determine if any correlations existed between homocysteine levels and the folate or vitamin B_{12} concentration. A relatively strong negative correlation between homocysteine and folate values was observed in the non-pregnant controls ($r= 0.47$, $p=0.02$); however, no such correlation was seen in the pregnant women. No correlation was observed between homocysteine and vitamin B_{12} levels in either of the study populations. Finally, a positive correlation between homocysteine and age was observed, but only in the non-pregnant women ($r=0.46$, $p=0.006$).

DISCUSSION

We were interested in the lipid profiles of pregnant women in Nigeria because elevations of certain lipids - triglycerides, total cholesterol and LDL-cholesterol in particular - during pregnancy can increase not only the woman's risk of cardiovascular disease, but also that of her fetus later in life (23). Our findings are consistent in some respects with the findings of other investigators, yet different in one other respect. As others have reported (5,14,23 - 25), we found a nearly two-fold increase in the serum triglyceride level in pregnant Nigerian women in the third trimester, and a 10-15% increase in total cholesterol (Table 2). However, whereas most other investigators have reported significant increases in LDL-cholesterol in women in the last few weeks of pregnancy (17), we did not find any such difference between our pregnant and non-pregnant subjects.

As other researchers have noted (5), we observed that the HDL-cholesterol level was elevated about 15% during the third trimester. The results of these lipid analyses indicate that pregnancy did not worsen the HDL-cholesterol and LDL-cholesterol levels with respect to cardiovascular disease risk. This finding contradicts a previous study conducted by Berge and coworkers (5), who found that pregnancy-related changes in the serum lipid profile increase the risk of CVD development in these women (5). Nevertheless, the large apparent pregnancy-associated increases in atherogenic triglycerides and total cholesterol we found in the third-trimester Nigerian women we studied should raise the same concerns Berge and coworkers did about the implications for cardiovascular health in pregnant women and their fetuses elsewhere in the world (23).

On the other hand, with regard to the issue of homocysteine levels and pregnancy, our finding in the Nigerian women we studied should be reassuring. The homocysteine concentrations we found in the pregnant women in their third trimester were significantly lower, by about 30%, than they were in the non-pregnant control group. Homocysteine has been identified as a risk factor for neural tube defects in the fetus and as a cardiovascular disease risk factor for both the mother and the fetus (3). Although homocysteine levels were lower in the pregnant women in our study, nevertheless, the mean serum homocysteine concentration we observed for the 18 pregnant subjects was 7.1 μmoles/L, a value that is higher than that reported by other investigators. For example, the mean plasma homocysteine concentration of Canadian women reported by Walker and colleagues (26) was 5.6 μmoles/L at 36-42 weeks gestation compared to a value of 7.9 μmoles/L for non-pregnant women. Similarly, in a study conducted in Florida, Bonnette and coworkers (27) reported plasma homocysteine levels of 5.4 μmoles/L for pregnant women in the third trimester versus 8.7 μmoles/L for the non-pregnant controls. Because the study by Bonnette and coworkers was one in which the folate intake was controlled, they speculated that the lower homocysteine concentrations observed in pregnancy compared with non-pregnant controls could be the result of a physiologic response to pregnancy. Since we did not control for folate or vitamin B_{12} intake in our study, and because most of the pregnant women in the present study acknowledged that they were taking multivitamin

supplements, we cannot comment on whether or not the lower levels of homocysteine we observed in the pregnant women relative to the control group were due to their being well advanced in their pregnancies or due to the multivitamin supplements. The lower homocysteine concentrations could also be due to a physiologic response to pregnancy. In addition, decreased homocysteine levels can also be due to haemodilution, plasma volume expansion, or the heightened remethylation of homocysteine due to an increased demand of methionine by the developing fetus (28).

In conclusion, our overall finding is that while some of the cardiovascular disease risk factors (e.g., serum total cholesterol, triglycerides) of the Nigerian women in our study were markedly increased by pregnancy, these risk factors were in line with values reported for other populations worldwide and do not seem to be exceptional or remarkable. On the other hand, for the 18 pregnant Nigerian women who participated in our study, the picture with respect to pregnancy-associated changes in serum levels of LDL-cholesterol, HDL-cholesterol and homocysteine looks favorable: pregnancy was associated with a reduction in the homocysteine level, no increase in the level of atherogenic LDL-cholesterol, and an increase in the concentration of cardioprotective HDL-cholesterol.

Several limitations may affect our findings and conclusions. Statistical power was compromised due to the relatively small number of subjects we studied. In addition, dietary data, including specific information regarding vitamin intake, was not available.

Our next investigation of pregnancy-associated risk factors for neural tube defects and cardiovascular disease in Nigerian women will control for vitamin supplements and will utilize a longitudinal design in which cardiovascular disease risk factors are monitored in individual subjects from conception throughout pregnancy.

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Table 1. Summary of study population characteristics

Parameter	Mean ± S.D	
	Pregnant (n=18)	non-Pregnant (n=36)
Age (years)	28 ± 3	34.7 ± 11.1
Height (m)	1.64 ± 4.68	1.62 ± 7.56
Weight (Kg)	78.3 ± 13.2	68.8 ± 16.0
BMI (Kg/m^2)	29.2 ± 4.9	26.4 ± 6.6

Table 2. Comparison of the serum concentrations of biochemical risk factors of cardiovascular disease in pregnant versus non-pregnant women in the city of Abuja, Nigeria

Parameter	Mean ± S.D Pregnant	Non-pregnant	p-Value
Total cholesterol (mg/dL)	212 ± 51.5	191 ± 42.7	0.022
Triglycerides (mg/dL) (Range)	153* (50-236)	89.5* (29-302)	0.004
High density lipoprotein (mg/dL)	67.0 ± 16.2	56.6 ± 13.2	0.004
Low density lipoprotein (mg/dL)	116 ± 42.4	110 ± 42.5	NS[a]
Total cholesterol/HDL	3.2	3.4	NS
LDL/HDL	1.7	1.9	NS
Homocysteine (µmol/L)	7.13 ± 2.03	10.1 ± 3.93	<0.001
Vitamin B_{12} (pg/mL) (Range)	343* (94 - 901)	620* (221 - 1482)	0.001
Folate (ng/mL) (Range)	10.4* (2.6 - 64)	4.4* (1.6 - 30)	0.002

[a]NS, not significant.
*median; reported due to non-distributed curves.

Table 3. Summary of reference range values for the serum concentrations of various parameters related to the risk of cardiovascular disease

Parameters	Reference range
Age (years)	20-50
Total cholesterol (mg/dL)	120-201[a]
Triglycerides (mg/dL)	31.0-175[a]
High density lipoproteins (mg/dL)	28.2-63.0[a]
Low density lipoproteins (mg/dL)	78.1-176[a]
Homocysteine (μmol/L)	4.5-12.4[c]
Vitamin B_{12} (pg/mL)	201-849[b]
Folate (ng/mL)	1.6-12.0[b]

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