

# Evaluation of Cardiovascular Markers in Normotensive Pregnant Women

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## Abstract

Pregnancy is associated with series of biochemical changes such as changes in cardiovascular markers. These changes may contribute to risk of developing cardiovascular disease among this group of people. Therefore, this work was aimed at evaluating changes in cardiovascular biochemical markers in pregnancy. This research was a cross-sectional study with 150 female subjects divided into three groups; control (non-pregnant women), 2<sup>nd</sup> trimester and 3<sup>rd</sup> trimester. Each of the group had 50 subjects each. Subjects were selected randomly from Rivers State University and Rivers State University Teaching Hospital. Fasting blood was collected using venipuncture technique (WHO, 2010). The collected blood was carefully dispensed into plain vacutainer tubes and assayed for uric acid, Apo A1 and B, CRP, TG, HDL and TC while VLDL and LDL were calculated. The data obtained from the study were analyzed using GraphPad Prism Version 8.0.2.263. ANOVA results revealed that there were significant differences in TC, TG, LDL, HDL, VLDL, CRP, ApoA1, and ApoB levels among non-pregnant, 2<sup>nd</sup> trimester and 3<sup>rd</sup> trimester groups of normotensive pregnant women ( $P < 0.05$ ) except for uric acid (UA) that showed no significant difference. Post hoc analysis revealed significant difference in studied parameters between groups except in HDL, TG and VLDL in non-pregnant and 2<sup>nd</sup> trimester group while UA remained non-significant between compared groups. This study has shown that pregnancy causes significant changes in cardiovascular risk markers in healthy pregnant women.

**Keywords:** pregnancy; lipoprotein; apolipoproteins; uric acid; C-reactive protein

## Introduction

Cardiovascular disease such as atherosclerosis remains the leading cause of mortality and morbidity in Western countries. Atherosclerosis which is a disease of large arteries (Hussain *et al.*, 2013), results from the interaction between genetic and environmental factors modulating the functions of various cell types and inflammatory molecules within the arterial wall (Dashti *et al.*, 2011). The major risk factor of this disease is hyperlipidaemia, which can arise due to either overproduction of lipoproteins and/or their reduced clearance from the plasma. Plasma lipoproteins are responsible for the transport and delivery of lipids throughout the body. The primary function of plasma lipoproteins is to deliver hydrophobic lipids such as triglycerides and cholesterol to peripheral tissues for storage as sources of energy and substrates for steroidal hormone synthesis (Farkas-Epperson and Le, 2012). The liver and the intestine are the two major organs that assemble lipoproteins. The liver synthesizes VLDLs (mainly apoB100- containing lipoproteins in humans) to transport endogenous fat to peripheral tissues. These particles are catabolised in the circulation, thereby producing a generation of plasma LDL: It is known that LDL cholesterol is atherogenic, therefore,

it is assumed that hepatic lipoproteins are the main and only contributors to atherosclerosis (Hussain *et al.*, 2013). ApoB100 is independently associated with ischaemic heart disease and identifies high-risk phenotypes in normocholesterolaemic diabetic patients. This could be used to evaluate the lipidaemic pattern of these patients (American Diabetes Association (ADA), 2010).

C-reactive protein (CRP) is an acute phase reactant synthesized in the liver in response to the cytokine interleukin-6 (Casas *et al.*, 2008). It has been anticipated that even cells of the smooth muscles present in the human coronary arteries can also produce C-reactive protein in response to cytokines that have inflammatory actions (Liu *et al.*, 2013). According to a study, CRP plays an active role in atherosclerosis in addition to being a marker for vascular inflammation. This is a factor responsible for the progression of atherosclerotic plaque.

Human pregnancy also known as gestation or gravidity is a period when a woman has one or more progenies in her womb. It is typically separated into three trimesters based on gestational age which is measured in weeks and months. The first trimester is from conception to 12 weeks (2 months and 3 weeks). The second trimester is from 13-27 weeks, (3 months to 6

months and 2 weeks); while the third trimester starts about 28 weeks and lasts until birth (7 months to 9 months) (Huda *et al.*, 2009).

According to Global Burden of Disease (2014), deaths resulting from complications of pregnancy reduced from 377,000 in 1990 to 293,000 in 2013, and the general causes include arterial blood pressure of pregnancy, maternal bleeding, complications of abortion, obstructed labour and maternal sepsis.

Pregnancy is characterized by changes in maternal metabolism and body composition in order to provide sufficient energy and nutrients to the developing fetus and later for lactation. There are marked increases in plasma lipid concentrations as gestation advances, with plasma cholesterol and triglyceride concentrations rising typically by 25-50% and 20-40% respectively. Therefore, this study is focused on evaluating changes in cardiovascular markers in normotensive pregnant women.

## Materials and Methods

### Study Design

This research was a cross-sectional study with 150 female participants recruited from Rivers State University Teaching Hospital, Port Harcourt and the Rivers State University, in Rivers State, Nigeria. The participants were grouped into three major groups: control (non-pregnant women), 2<sup>nd</sup> trimester and 3<sup>rd</sup> trimester. Each of the group had 50 subjects.

### Study Location

This study was carried out jointly in the Rivers State University and the Rivers State University Teaching Hospital (formerly called Braithwaite Memorial Specialist Hospital) in Port Harcourt, the capital city of Rivers State in Nigeria. Port Harcourt is a cosmopolitan city with a population of about two million residents.

### Eligibility Criteria

#### Inclusion criteria

All apparently healthy pregnant women attending antenatal care were eligible for inclusion in this study, except the control subjects. Other inclusion criteria are no history of surgery and blood transfusions, not diagnosed diabetic and subjects gave written consent after counseling.

#### Exclusion Criteria

These included those who were apparently ill or with known history of any of the infectious diseases, underlying chronic illness for example; gastric and intestinal illness, history of prenatal bleeding, malignancy, tuberculosis, diagnosed diabetes, and cardiovascular disease. A history of blood transfusion, surgery or an inability to provide informed consent was also noted for exclusion.

### Ethical Consideration and Informed Consent

Ethical clearance for the study was obtained from the Ethics Committee of Rivers State Ministry of Health, Port Harcourt, Nigeria and upon the study approval, the work was conducted.

### Subject Selection

Subjects were selected randomly from Rivers State University and Rivers State University Teaching Hospital, having ensured that they met the criteria on the questionnaire that made them suitable for inclusion and exclusion.

### Sample Collection Method

Subjects were selected using simple random method as described by Fyneface *et al.*, (2018) and Fyneface *et al.*, (2020). Blood was collected using venipuncture technique. The collected blood was carefully

dispensed into plain vacutainer tubes, left to clot and centrifuged at 1500rpm for 10 minutes. Serum was separated and stored at -4°C until it was assayed for CRP, apoA1, apo B, uric acid, total cholesterol, triglycerides and high density lipoprotein cholesterol, while the values of LDL and VLDL were calculated (Friedewald *et al.*, 1972).

### Biochemical Determinations

Fasting blood samples were used for the biochemical investigations for uric acid, Apo A1 and B, CRP, TG, HDL and total Cholesterol. All biochemical analyses were carried out in the Department of Medical Laboratory Science, Rivers State University.

### Determination of High Sensitive C-reactive Protein Concentration in Human Serum.

The method of Nazir & McQueen, 1993 was used.

#### Procedure

2µl of sample was added to the test tubes, and 5 other tubes for calibration. 250µl of R1 assay buffer (see composition in appendix) was added to all the tubes. It was mixed by tilting the bottom of the tubes and then incubated at 37°C for 5 minutes and read as absorbance OD1. 50µl of R2 antibody reagent (see composition in appendix) was added to all the tubes. It was mixed by tilting the bottom of the tubes and then incubated at 37°C for 3 minutes and read as absorbance OD2.

### Determination of Apo Lipoprotein A1 in Human Serum

The method of Nazir and McQueen, 1993 was used.

#### Procedure

2µl of serum was placed in the test tubes and 5 other tubes for calibration. 250µl of buffer (R1) was added to all the tubes, mixed by tilting the bottom of the tubes, and allowed for 5 minutes at 37°C in a water bath. It was then read in a spectrophotometer at 340 nm. The absorbance was recorded as OD1. 50µL of the antibody reagent (R2) was added to the reaction and allowed for 5 minutes at 37°C in a water bath. It was then read at 340nm using the spectrophotometer. The absorbance was recorded as OD2. The absorbance was taken as [OD2– OD1] of standard and sample.

### Determination of Apolipoprotein B in Human Serum

The method of Nazir and McQueen, 1993 was used.

#### Procedure

2µl of serum was placed in the test tubes and 5 other tubes for calibration. 250µl of buffer (R1) was added to all the tubes, mixed by tilting the end of the tubes and allowed for 5 minutes at 37°C in a water bath. It was then read in a spectrophotometer at 340 nm wavelength. The absorbance was recorded as OD1. 50 µl of the antibody reagent (R2) was added to the reaction and allowed for 5 minutes at 37°C in a water bath. It was then read at 340nm, using the spectrophotometer. The absorbance was recorded as OD2. The absorbance was taken as (OD2 – OD1) for samples and standard. A standard curve was plotted and the concentration of controls, standard and sample was read.

### Determination of Total Cholesterol in Serum

The method of Allain *et al.*, 1974 was used.

#### Procedure

The assay conditions were considered. The instrument was zeroed with distilled water. One ml of the cholesterol reagent was transferred by pipetting into clean dry test tubes labelled as blank, standard and tests and 10 µl of distilled water, standard and sample were added to their respective tubes. It was properly mixed, by tilting the bottom of the tubes

and incubated in a waterbath at 37°C for 5 minutes. The absorbance of the standard and test samples was measured against the blank in a spectrophotometer at 540nm wavelength.

### Determination of High-Density Lipoprotein (HDL) Cholesterol in Serum

The method of Tietz, 1987 was used.

#### Procedure

The blood samples were transferred into tubes and centrifuged for five minutes at 12,000rpm. The supernatant (sera) was separated and arranged according to the labelled tubes as control, standard and samples. 200µl of precipitating reagent (R) and 20µl of sample were transferred into the tubes for test, 20µl of standard for standard tube and distilled water for blank. It was mixed properly by tilting the bottom of the tubes and allowed to stand for 10 minutes at room temperature. The contents of the tubes were centrifuged for 2 minutes at 12,000rpm. Thereafter, the clear supernatant was separated and determined for HDL cholesterol.

### Determination of Triglycerides in Serum

The method of Fraser & Hearne, (1981) was used.

#### Procedure

The assay conditions were considered. The instrument was zeroed with distilled water. 1ml of triglyceride reagent was added to the tubes as blank, standard and test. 10 µl of standard and sample were added to the tubes, mixed and incubated for 5 minutes at 37°C. The absorbance was read using 1cm light path (cuvette) for samples against blank at 505nm wavelength.

### Determination of Low-Density Cholesterol (LDL-C)

The method of Friedwald *et al.*, 1972 was used.

#### Calculation

LDL cholesterol values in the serum sample were calculated as a difference in the results of the total cholesterol, triglycerides and HDL.

$$\text{LDL} - \text{Cholesterol} = \text{Total Cholesterol} - (\text{TG}/2.2) - \text{HDL} \\ (3.8 - 4.9)\text{mmol/l}$$

### Determination of Uric Acid in Serum

The enzymatic method of Barr (1990) was used.

#### Procedure

Tubes were arranged according to labels as blank, standard and test. 20µl of distilled water was added into the blank tube, 20µl of standard to standard tube and 20µl of serum to test tubes and properly mixed by tilting the bottom of the tubes. It was incubated for 5 minutes at 37°C. It was then read in a spectrophotometer at 520nm wavelength.

### Quality Control

Two levels of normal and abnormal controls were used to verify the performance of the procedures for each batch of analysis. Other control measures followed were checking instrument settings, and light source, cleanliness of all equipment used, ensuring the water used was free of contaminants to avoid interferences, checking reaction temperature and checking expiry date of kit and contents.

### Statistical Analysis

The data obtained from the study were analyzed using the GraphPad Prism Version 8.0.2.263. The data were expressed as mean and standard deviation. Comparison of the means was done using the one-way analysis of variance (ANOVA). The Turkey comparison test was used to verify significant differences between the groups at P<0.05.

### Results

Comparing cardiovascular markers in Normotensive Pregnant Women

Parameters	Normotensive Women			P-value	F-value
	Non-pregnant n = 50	2 <sup>nd</sup> Trimester n = 50	3 <sup>rd</sup> Trimester n = 50		
TC(mmol/l)	3.80 ± 0.45	4.37 ± 0.46	4.72 ± 0.50	TC(mmol/l)	3.80 ± 0.45
HDL(mmol/l)	0.82 ± 0.19	0.87 ± 0.20	0.94 ± 0.21	HDL(mmol/l)	0.82 ± 0.19
TG(mmol/l)	1.19 ± 0.27	1.30 ± 0.24	1.43 ± 0.31	TG(mmol/l)	1.19 ± 0.27
LDL(mmol/l)	2.45 ± 0.41	2.94 ± 0.40	3.16 ± 0.28	LDL(mmol/l)	2.45 ± 0.41
VLDL(mmol/l)	0.54 ± 0.13	0.59 ± 0.11	0.65 ± 0.14	VLDL(mmol/l)	0.54 ± 0.13
UA(mg/dl)	4.87 ± 0.53	5.08 ± 0.42	4.89 ± 0.80	UA(mg/dl)	4.87 ± 0.53
CRP(mg/dl)	1.11 ± 0.40	4.30 ± 1.45	5.31 ± 1.92	CRP(mg/dl)	1.11 ± 0.40
APoA1(mg/dl)	320.50 ± 25.49	342.40 ± 35.45	350.00 ± 33.38	APoA1(mg/dl)	320.50 ± 25.49
APoB(mg/dl)	97.60 ± 11.47	130.20 ± 23.97	152.60 ± 33.04	APoB(mg/dl)	97.60 ± 11.47

The results above showed that there were significant differences in TC, TG, LDL, HDL, VLDL, CRP, ApoA1, and ApoB levels among non-pregnant, 2<sup>nd</sup> trimester and 3<sup>rd</sup> trimester groups of normotensive pregnant women (P<0.05) except for uric acid (UA) that showed no significant difference.

Parameters	NP vs 2 <sup>ND</sup> T	NP vs 3 <sup>RD</sup> T	2 <sup>ND</sup> T vs 3 <sup>RD</sup> T
TC (mmol/l)	<0.0001	<0.0001	0.0008
HDL (mmol/l)	0.5925	0.0106	0.1304
TG (mmol/l)	0.1326	<0.0001	0.0516

LDL (mmol/l)	<0.0001	<0.0001	0.0113
VLDL (mmol/l)	0.1326	<0.0001	0.0516
UA (mg/dl)	0.1985	0.9892	0.2611
CRP (mg/dl)	<0.0001	<0.0001	0.0013
APoA1 (mg/dl)	0.0018	<0.0001	0.2221
APoB (mg/dl)	<0.0001	<0.0001	<0.0001

**Table 3.2:** The ANOVA Post – Hoc Findings Using Turkey Multiple Comparison Test for Maternal Characteristics (Normotensive) Within the Study Groups

NP- Non-Pregnant, 2nd T – second Trimester, 3<sup>rd</sup> T- third trimester.

The above Post hoc analysis revealed significant difference in studied parameters between groups except in HDL, TG and VLDL in non-pregnant and 2<sup>nd</sup> trimester group while UA remained non-significant between compared groups.

## Discussion

The analysis of the mean values of the maternal characteristics of the Normotensive women in the 2<sup>nd</sup> and 3<sup>rd</sup> Trimester and comparing with Non-pregnant showed that there was a significant difference in TC, LDL, HDL, TG, VLDL, CRP, ApoA1, and Apo B at  $P < 0.05$ . There was no significant difference in UA at  $P < 0.05$ , but UA in the 2<sup>nd</sup> trimester was higher than 3<sup>rd</sup> trimester and non- pregnant. TC, TG, LDL VLDL and HDL progressively increased during the course of the pregnancy such that values in the 3<sup>rd</sup> trimester were highest in them all. This work differed with Shen (2018). For normotensives in this study, HDL was increased in 3<sup>rd</sup> trimester, quite different from 2<sup>nd</sup> trimester, though within normal range, even if first trimester was not done, as opposed to a decrease in 3<sup>rd</sup> trimester by Shen. It is a known fact, that during pregnancy, there is an increase in both low and high density lipoprotein cholesterol and triglyceride which provide energy to the growing fetus according to Harvey *et al*, 2015; this difference in HDL and LDL, could suggest that, pregnancy as a stress test for the cardiovascular system may have simply revealed a pre-existent cardiovascular dysfunction rather than cause it, as obesity and dyslipidemia are shared between cardiovascular disease and pregnancy disorders (Wenger, 2014).

For TC, it has been determined several decades ago that the average total cholesterol level in the Nigerian population is  $3.54 \pm 0.14$  mmol/L. In the present study, the serum total cholesterol levels obtained for the normotensive subjects were  $3.80 \pm 0.45$  mmol/L (non-pregnant women),  $4.37 \pm 0.46$  mmol/L (2<sup>nd</sup> trimester pregnant women) and  $4.72 \pm 0.50$  mmol/L (3<sup>rd</sup> trimester pregnant women). Both trimesters in the normotensive subjects were significantly higher than the non-pregnant women. This implied that pregnancy induced the rise in TC level. The third trimester was also significantly higher than the second trimester, thereby pointing to the fact that the longer the duration of pregnancy, the higher the total level of cholesterol. Conversely, another study reported that pregnancy does not affect the concentration of TC and other lipid parameters (Oladapo-Akinfolarin, *et al.*, 2018).

CRP is known to be slightly elevated during pregnancy, due to maternal inflammatory reaction to the pregnancy. However, Ernest *et al.* (2011) suggested that CRP of 2.5 mg/dl and above could be considered high for pregnant women. In this study, the level of CRP was significantly higher ( $P < 0.05$ ) in the pregnancy both second and third trimester  $4.30 \pm 1.45$  mg/dl and  $5.31 \pm 1.92$  mg/dl respectively than the non-pregnant control of  $1.11 \pm 0.40$  mg/dl. The values for second and third trimester are higher than normal levels for pregnant women and this is in agreement with a study conducted in 2017 that reported CRP value higher than normal in non-hypertensive pregnant women (Oladapo-Akinfolarin *et al.*, 2017). Elevated levels of CRP in pregnancy may be a marker for complications, but more studies are necessary to fully understand the role of CRP and pregnancy.

Previous studies showed that measures of unhealthy lifestyle habits such as cigarette smoking are also associated with the elevated CRP levels whereas moderate alcohol consumption and increased physical activity are associated with lower CRP levels (Fredrikson *et al.*, 2004; Oliveira *et al.*, 2010). Normal people with CRP levels greater than or equal to 2mg/dl likely need more intense management and treatment for heart disease. Elevated levels of CRP may have an important role in identifying those who might need closer follow-up or more intensive treatment after heart attacks or heart procedures. The CRP levels may also be useful in uncovering those at risk of heart disease where cholesterol levels alone may not be helpful.

The two lipoproteins investigated in this work were ApoA1 and Apo B. The Apo A1 levels for normotensive subjects were; non-pregnant  $320.50 \pm 25.49$  mg/dl, second trimester  $342.40 \pm 35.45$  mg/dl and third trimester  $350.00 \pm 33.38$  mg/dl. The results were far above the normal level of 225 mg/dl for all the three groups. This could indicate that the subjects are protected from CAD. Apo A1 is the major component of HDL and has been shown to predict short term and long term risk in patients with normal HDL according to May *et al*, 2013. The HDL values in this work were lower than normal, and could be as a result of change in HDL composition, but Apo A1, is the major protein component of HDL, which is largely responsible for reverse cholesterol transport. In essence, the functional-state of the HDL molecule is reflected by Apo A1. May *et al*, 2013 also stated that Apo A1 has a constant inverse relationship with CAD events, that is, an increase in Apo A1 will always lead to a decrease in CAD events.

The Apo B values in this work were within the normal range of 117 mg/dl for non-pregnant normotensives ( $97.60 \pm 11.47$  mg/dl). However, the values for 2<sup>nd</sup> trimester ( $130.20 \pm 23.97$  mg/dl) and 3<sup>rd</sup> trimester ( $152.60 \pm 33.04$  mg/dl) were higher than the normal range. The Apo B concentration reflects the number of atherogenic particles (VLDL, IDL and LDL) according to American Diabetes Association (2010).

There is only one Apo B molecule for each VLDL-C and LDL-C particle and because VLDL-C particles are cleared much faster than are LDL-C, Apo B levels in essence reflect LDL-C number and high Apo B levels reflect a relative reduction in cholesterol level, resulting in small dense LDL-C particles derived from VLDL-C overproduction. These could be the reasons why the cholesterol values were in the normal range for 2<sup>nd</sup> and 3<sup>rd</sup> trimester, though LDL levels were unusually below the normal reference range of 4.9mmol/L.

## Conclusion

This study has shown that changes in pregnancy period such as second and third trimesters have significant effect in cardiovascular markers. Thus, pregnancy could become a risk factor for cardiovascular disease

among women especially when the changes in these biomarkers are not corrected after pregnancy.

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