

Original Research Article

Effect of Gravidity on Cardiovascular Markers in Normotensive Pregnant Women

ABSTRACT

Gravidity, which is the number of time that a woman has been pregnant 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. The aim of this research was to evaluate the effect gravidity has on some cardiovascular markers among normotensive pregnant women. A cross-sectional study of 100 women of reproductive age was carried out. The subjects were selected randomly from Rivers State University and the Rivers State university Teaching Hospital. Blood samples were collected for the following biochemical analysis; total cholesterol, triglyceride, high density lipoprotein, Uric acid, Apolipoprotein A1 and B and analyzed. low density lipoprotein and very low density lipoprotein were calculated. The data obtained from the study were analyzed using GraphPad Prism Version 8.0.2.263. Result gotten from the study showed that Gravidity had no significant effect on biochemical parameters of pregnant subjects, but had a level of significant increase on HDL with the values 0.87 ± 0.21 (1-2), 0.93 ± 0.21 (3-4), 0.86 ± 0.12 (5-6) and 1.30 ± 0.00 for (7-8). the ANOVA post hoc using Turkey multiple comparison test of the effect of gravidity (1-2, 3-4, 5-6, 7-8) on the biochemical parameters showed a significant effect on HDL (1-2 vs 7-8) ($P= 0.0204$) and (5-6 vs 7-8) ($P= 0.0250$). There was no recorded significant effect on TC, TG, UA, LDL, Apo A1, Apo B, CRP and VLDL) at $P<0.05$. This study demonstrated that gravidity had little or no effect on the biochemical parameters but increases the HDL cholesterol level of normotensive pregnant women. It is therefore recommended that C – Reactive protein and lipid should be suggested as a screening test for all women of child bearing age, as a prognosis for cardiovascular disease.

Keywords: *Gravidity, pregnancy, C-reactive protein lipoproteins, apolipoproteins, uric acid,*

1.0 Introduction

Human pregnancy which is also known as gestation or gravidity is a period when a woman has one or more progenies in her womb. It is typically divided 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. 28weeks and lasts until birth (7 months to 9 months) (Wenger, 2014).

According to Global Burden of Disease (GBD), (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, abortion complications, 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 200-40% respectively (Wenger, 2014).

Normal pregnancy is characterized by increase in cardiac output and blood volume, generalized vasodilation, decrease in blood pressure and resistance to stress factor agents such as norepinephrine and angiotensin II (Garovic and Hayman, 2007). Metabolic changes in normal pregnancy, including hyperlipidaemia and hypercoagulable and inflammatory states are further accentuated in preeclampsia and are similar to those associated with an unfavourable risk profile for cardiovascular disease. According to Neboh and his co-researchers (2012).

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. Lipoproteins, which are a group of lipids that circulate in plasma in complexes not bound to albumin, include total cholesterol (TC), low density lipoprotein (LDL), high density lipoprotein (HDL), triglyceride (TG) and very low-density lipoprotein (VLDL) (Rifai and Wanick, 2006). Plasma lipoproteins are responsible for the transport and delivery of lipids throughout the body.

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 (Casas *et al.*, 2008). This is a factor responsible for the progression of atherosclerotic plaque.

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).

Following the fact that metabolic changes occur during pregnancy and possible changes in cardiovascular markers occur, the study focused on evaluating the impact of gravidity on cardiovascular risk markers among normotensive pregnant women in Rivers State.

2.0 Materials And Methods

2.1 Study Design

This research was a cross-sectional study of 100 female subjects. Subjects were selected randomly from the Rivers State University and the Rivers State University Teaching

Hospital, and placed in different groups based of the number of pregnancy having ensured that they met the criteria on the questionnaire that made them suitable for inclusion and exclusion.

2.2 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. The case studies were from the Rivers State University Teaching Hospital (Braithwaite), a specialist Hospital located in the old Government Reserved Area (GRA) axis Port Harcourt, where samples were collected from women who were pregnant and normotensive after ascertaining their medical conditions based on the reports in their folders and physical observations during ward rounds by the attending Physician.

2.3 Eligibility Criteria

Inclusion criteria

All apparently healthy pregnant women attending antenatal care were eligible for this study. Also pregnant women with medical history of no surgery and blood transfusions, not diagnosed diabetic were also included in this study. Those that gave their informed consent after counseling were selected.

Exclusion Criteria

These following groups of people were excluded from this study: those who were apparently ill or with known history of any of the infectious diseases, underlying chronic illness for example; gastric and intestinal illness, those with history of prenatal bleeding, malignancy, tuberculosis, diagnosed diabetes, and cardiovascular disease. Those with history of blood transfusion, surgery or an inability to provide informed consent were also excluded from this study.

2.4 Ethical Consideration and Informed Consent

The Ethics Committee of the Rivers State Ministry of Health, Port Harcourt, Nigeria approved the protocol for this study. After counseling the women about the hospital policy of screening every antenatal woman for HIV and the attendance benefits to those who may be zero – positive, verbal informed consent for this study was obtained. The aim of the research was also made clear to all the non-pregnant women and the pregnant normotensive women. Relevant confidentiality was maintained throughout the study period. All relevant demographic data were also obtained from all the women in this study.

2.5 Subject Selection

Subjects were selected randomly from the Rivers State University using a number selection approach as described by Fyनेface *et al.* (2018) and Fyनेface *et al.* (2020). The selection was made at Rivers State University Teaching Hospital, having ensured that they met the criteria on the questionnaire that made them suitable for inclusion and exclusion.

2.6 Sample Collection

Blood was collected by venepuncture technique (WHO, 2010). The 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).

2.7 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. 250ul 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 37⁰C for 5 minutes and read as absorbance OD1. 50ul 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 37⁰C 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 37⁰C 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 37⁰C 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 37⁰C 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 37⁰C 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 37⁰C 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.

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

$$(3.8 - 4.9)\text{mmol/l}$$

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.

2.8 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.

2.9 Statistical Analysis

The data obtained from the study were analysed 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 Tukey comparison test was used to verify significant differences between the groups at $P < 0.05$.

3.0 RESULT

Table 1: Effect of Gravity on Biochemical Parameters in Normotensives pregnant women

Parameters	1 – 2	3 – 4	5 – 6	7-8	P Value	F Value
	n = 53	n = 32	n = 13	n = 2		
TC(mmol/l)	4.49 ± 0.47	4.57 ± 0.56	4.54 ± 0.50	5.40 ± 0.00	0.0987	2.152
TG(mmol/l)	1.34 ± 0.29	1.37 ± 0.28	1.42 ± 0.24	1.80 ± 0.00	0.1267	1.950
HDL(mmol/l)	0.87 ± 0.21	0.93 ± 0.21	0.86 ± 0.12	1.30 ± 0.00	0.0215	3.375
LDL(mmol/l)	3.04 ± 0.30	3.05 ± 0.42	3.05 ± 0.44	3.30 ± 0.00	0.7992	0.3362
UA(mg/dl)	4.88 ± 0.70	5.16 ± 0.60	5.00 ± 0.47	4.70 ± 0.00	0.2454	1.407
APoA1(mg/dl)	345.90 ± 32.28	345.40 ± 39.19	354.90 ± 33.35	385.00 ± 0.00	0.3718	1.055
APoB(mg/dl)	139.70 ± 27.07	141.30 ± 31.35	144.20 ± 44.52	170.00 ± 0.00	0.5822	0.6543
CRP(mg/dl)	4.52 ± 1.58	5.39 ± 2.10	4.52 ± 1.45	4.80 ± 0.00	0.1586	1.767
VLDL(mmol/l)	0.61 ± 0.13	0.62 ± 0.13	0.64 ± 0.11	0.81 ± 0.00	0.1267	1.950

Table 1 above shows the effects of gravidity (1-2, 3-4, 5-6, 7-8) on the biochemical parameters (TC, TG, HDL, UA, LDL, Apo A1, Apo B, CRP and VLDL) in normotensive pregnant women. Gravidity showed a significant effect on HDL with the values 0.87 ± 0.21 (1-2), 0.93 ± 0.21 (3-4), 0.86 ± 0.12 (5-6) and 1.30 ± 0.00 for (7-8). There was no significant effect on TC, TG, LDL, UA, CRP, VLDL, Apo A1 and Apo B.

Table 2: The ANOVA Post – Hoc Findings Using Turkey Multiple Comparison Test for Gravidity on Biochemical Parameters in Normotensives pregnant women

Parameters	1-2 vs 3-4	1-2 vs 5-6	1-2 vs 7-8	3-4 vs 5-6	3-4 vs 7-8	5-6 vs 7-8
TC(mmol/l)	0.9002	0.9919	0.0648	0.9970	0.1130	0.1139
TG(mmol/l)	0.9642	0.7943	0.1036	0.9486	0.1500	0.2737
HDL(mmol/l)	0.5752	0.9974	0.0204	0.7165	0.0628	0.0250
LDL(mmol/l)	0.9983	0.9993	0.7483	>0.9999	0.7837	0.8054

UA(mg/dl)	0.2124	0.9288	0.9799	0.8734	0.7585	0.9264
APoA1(mg/dl)	0.9999	0.8363	0.4030	0.8361	0.4006	0.6637
APoB(mg/dl)	0.9955	0.9667	0.5297	0.9924	0.5848	0.6921
CRP(mg/dl)	0.1261	>0.9999	0.9961	0.4387	0.9672	0.9968
VLDL(mmol/l)	0.9642	0.7943	0.1036	0.9486	0.15	0.2737

Table 2 above shows the ANOVA post hoc using Turkey multiple comparison test for the effect of gravidity (1-2, 3-4, 5-6, 7-8) on the biochemical parameters (TC, TG, HDL, UA, LDL, Apo A1, Apo B, CRP and VLDL) in Normotensives. Gravidity showed a significant effect on HDL (1-2 vs 7-8) ($P= 0.0204$) and (5-6 vs 7-8) ($P= 0.0250$). There was no recorded significant effect on TC, TG, UA, LDL, Apo A1, Apo B, CRP and VLDL) at $P<0.05$.

4.0 Discussion

This study examined and evaluated CRP, TC, TG, LDL, HDL, VLDL, UA, Apo A1, ApoB, among normotensive pregnant women. The subjects that participated in this study were pregnant women that attended the health facility where the research was conducted. The result from this study shows that gravidity had no significant effect on biochemical parameters, but there was a significant effect on HDL value 0.87 ± 0.21 mmol/L (1-2), 0.93 ± 0.21 mmol/L (3-4), 0.86 ± 0.12 mmol/L (5-6) and 1.30 ± 0.00 mmol/L (7-8) at $p < 0.05$. This could mean that more pregnancies lead to increase in HDL level in normotensive pregnant women.

It is a known fact, that during pregnancy there are changes in both low and high density lipoprotein cholesterols, and triglyceride which provide energy to the growing fetus according to Harvey *et al.* (2015). However, this work was not comparing cardiovascular risk markers between pregnant and non-pregnant women but among number of pregnancies (gravidity). It has been determined several decades ago that the average total cholesterol level in Nigeria population is 3.54 ± 0.14 mmol/L. In the present study, gravidity brought about a slight increase above the normal range in total cholesterol level 4.49 ± 0.47 mmol/L (1-2), 4.57 ± 0.56 mmol/L (3-4), 4.54 ± 0.50 mmol/L (5-6), and 5.40 ± 0.00 mmol/L (7-8). But this increase was not statistically significant at $p < 0.05$. This implies that number of pregnancies (gravidity) does not have any impact on total cholesterol level in pregnant normotensive women.

Uric acid is a chemical produced when the body breaks nucleic acids that contain organic compounds called purines. Most Uric acid is dissolved in the blood, filtered through the kidney and expelled in the urine. Sometimes the body produces too much uric acid or does not filter out enough of it. High levels of uric acid are associated with several conditions such as diabetes, gout, kidney stone and acute kidney failure. Normal values obtained for normotensive subjects are within the normal range values and there was no significant difference in uric acid levels among the gravidity groups ($p > 0.05$). Thus, gravidity does not have an impact on uric acid level.

CRP is known to be slightly elevated during pregnancy, due to the 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 also slightly higher than the normal range 4.52 ± 1.58 mg/dl (1-2), 5.39 ± 2.10 mg/dl (3-4), 4.52 ± 1.45 mg/dl (5-6) and 4.80 ± 0.00 mg/dl (7-8). But these values were not statistically significant. 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 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 2 mg/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 Apo-lipoproteins investigated in this work were ApoA1, the values were 345.90 ± 32.28 mg/dl (1-2), 345.40 ± 39.19 mg/dl (3-4), 354.90 ± 33.35 mg/dl (5-6), and 385.00 ± 0.00 mg/dl (7-8) were above the normal level of 225 mg/dl though not statistically significant at $p < 0.05$. 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 139.70 ± 27.07 mg/dl (1-2), 141.30 ± 31.35 mg/dl (3-4), 144.20 ± 44.52 mg/dl (5-6), and 170.00 ± 0.00 mg/dl (7-8), were slightly higher than the normal range though not statistically significant at $p < 0.05$. The Apo B concentration reflects the number of atherogenic particles (VLDL, IDL and LDL) according to American Diabetes Association (2010). According to Harvey *et al.* (2015) this reflection is closely associated with atherosclerosis. 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 level and high Apo B levels reflect a relative reduction in cholesterol level, resulting in small dense LDL-C particles derived from VLDL-C overproduction.

Conclusion

The findings in this study have shown that gravidity has little or no effect on the biochemical parameters of normotensive pregnant women but induces higher levels of HDL in these women. This means the higher the number of pregnancy, the higher the HDL value among pregnant women.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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