Effects of Gravidity on Artheriogenic Indices in Normotensive ~~and Hypertensive~~and Hypertensive Second Trimester Pregnant Women

Abstract

Background

Pregnancy results in certain physiological and metabolic changes that results to shift in certain biochemical markers and could even result in hypertension in some women, thus predisposing them to risk of cardiovascular disease. While this is true, some women express these predisposing risk factors in subsequent pregnancies. Therefore, it may be a significant contribution to understand the dynamics of artherogenic indices with increasing number of pregnancies.

Aim

This~~e~~ study ~~i~~was aimed at evaluating the effects of gravidity on artherogenic indices in normotensive and hypertensive second trimester pregnant women.

Materials and Methods

A ~~The~~ cross-sectional study was conducted with ~~among~~ 100 women at Rivers State University Teaching Hospital (RSUTH), Port Harcourt, Rivers, Nigeria. The consenting subjects having met the inclusion criteria were randomly selected and were divided into two main groups: normotensive group having 50 normotensive pregnant women in their second trimester (NPW2T) and hypertensive group having 50 hypertensive pregnant women (HPW2T). ~~Subjects~~ The participants in each of the groups were further divided into three groups based on gravidity; primigravida (number of pregnancy=1), multigravida (number of pregnancy>1) and grand multigravida (number of pregnancy≥5). Fasting blood samples were collected by venepuncture technique for the determination of TC, TG, HDL and LDL. Artheriogenic indices (AIP, CR-I, CR-II, AC and APoB/APoA1) were mathematically calculated. Data generated were analyzed for ANOVA and Tukey comparison test at P-value<0.05.

Results

In the normotensive group, the result showed that there was no significant difference in the artheriogenic indices among the gravidity groups, *P*-value>0.05, which was also the cases among those in the ~~. Similar finding was shown in the~~ hypertensive group.

Conclusion

The study has shown that gravidity does not have any effect on artheriogenic indices among normotensive and hypertensive pregnant women in the second trimester in Rivers State University Teaching Hospital.

Keywords: *normotensive, hypertensive, artheriogenic, pregnancy, second trimester*

1.0 Introduction

Hypertension also called high blood pressure or arterial blood pressure is a persistent medical state whereby there is an increase in the pressure of blood in the arteries. Blood pressure levels are usually ≥140/90 mm/Hg prior to pregnancy or sooner than the 20th week of gestation (Braunthal,& Brateanu, 2019; Leeman *et al*.,

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2016). Over the years, it has been considered a major risk factor associated with disease of the cardiovascular system (Lewinton *et al.,* 2002). Hypertensive disorders in pregnancy are among the leading causes of maternal and neonatal morbidity worldwide; this include pregnancy induced hypertension (PIH) and preeclampsia (Burdorf *et al.,* 2012). Preeclampsia occurs in 2-4% of pregnancies and is a leading cause of maternal and neonatal morbidity and mortality in the developed world (Freeman *et al.,* 2009).

Pregnancy is the term used to describe the period in which a fetus develops inside a woman's womb or uterus. It usually lasts about 40 weeks, or just over 9 months, as measured from the last menstrual period to delivery (National Institutes of Health, 2017). Health care providers refer to three segments of pregnancy, called trimesters. Normal pregnancy 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. 28weeks and lasts until birth (7 months to 9 months) (Huda *et al*., 2009). Gravidity is referred to the number of times a woman has been pregnant. (Huda *et al.,* 2009).

Pregnancy induced hypertension and preeclampsia complicate about 7% of all pregnancies and severe preeclampsia is a major cause of severe maternal morbidity such as stroke and liver rupture (Seegers *et al.,* 2010). Preeclampsia usually develops after 20 weeks of gestation and is characterize by chronic or gestational hypertension combined with proteinuria which results from defective placentation (Hromadnikova, 2015); eliciting inadequate uteroplacental blood perfurin and ischaemia (Khan, 2006). The causes of preeclampsia are unknown, but thought to be an implantation disorder (Miko *et al.,* 2013).

Several efforts have been made in seeking emergent or new cardiovascular risk factors to improve cardiovascular disease prediction. ~~And, l~~In an attempt to optimize the predictive capacity of the lipid profile, several lipoprotein ratios or “atherogenic indices” have been defined in health literature (Millan *et al*., 2009). These indices could prove to be a better alternative to the routine investigations. One of them is Cardiac Risk Ratio (CRR) which is frequently used for risk assessment of cardiovascular disease (CVD) and is given by the total cholesterol to High Density Lipoprotein cholesterol (HDL) ratio (Bafna *et al*., 2012). Another index is Atherogenic Index Of Plasma (AIP), calculated as log (Tryglyceride(TG)/High Density Lipoprotein-Cholesterol). It has recently been proposed as a marker of plasma artheriogenecity because it is increased in people at higher risk for coronary heart disease and is inversely correlated with Low Density Lipoprotein particle size (Millan *et al*., 2009). The association of TGs and HDL-C in this simple ratio theoretically reflects the balance between risk and protective lipoprotein forces, and both TGs and HDL-C are widely measured and available (Dobiasova *et al*., 2011). Artheriogenic Coefficient (AC) is another index which is given by the ratio of non HDL cholesterol to HDL cholesterol. Non HDL-c is easily calculated, with no need for previous fasting of the

patient. It is essentially the cholesterol analogue to an apo B level, having a higher correlation coefficient in comparison with the LDL cholesterol concentration (Deric *et al*., 2008). Many studies have considered the evaluation of lipid and artheriogenic markers in pregnancy (Alahakoon *et al*, 2020; Geraghty *et al*., 2017; Parlakgumus *et al*., 2014;) but this study is focused on evaluating the effects of gravidity on artherogenic indices in normotensive and hypertensive second trimester pregnant women.

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2.0 Materials and Methods

2.1 Study Design

A ~~The~~ cross-sectional study design involved 100 women which included both pregnant and non-pregnant women. Fifty participants ~~50~~ were normotensive subjects and another 50 were hypertensive subjects based on the clinical history available in their clinical folder. The both groups (normotensive and hypertensive groups) had three sub-groups based on gravidity (number of pregnancy); primigravida (number of pregnancy=1), multigravida (number of pregnancy>1) and grand multigravida (number of pregnancy≥5). In normotensive group, primigravida subgroup had 15 subjects, multigravida group had 27 subjects and grand multigravida group had 8 subjects in participation. In hypertensive group, primigravida subgroup had 21 subjects, multigravida group had 25 subjects and grand multigravida group had 4 subjects in participation. Their artheriogenic parameters were separately assayed to determine the effect of gravidity on artheriogenic indices among 2nd trimester pregnant women in Rivers State University Teaching Hospital.

2.2 Study Area

This study was carried out Rivers State University Teaching Hospital (formerly called Braithwaite Memorial Specialist Hospital) in Port Harcourt, the capital city of Rivers State in Nigeria.

2.3 Study Population

The population of interest is 2nd trimester pregnant women who were further sub-divided into two sub-population of interest; normotensive 2nd trimester pregnant women and hypertensive 2nd trimester pregnant women.

2.4 Ethical Clearance and Consent

Ethical clearance was obtained from The Ethics Committee of Rivers State Ministry of Health. Eligible subjects provided a written informed consent before they were allowed to participate in the study.

2.5 Eligibility criteria

All apparently healthy pregnant women and hypertensive pregnant women including those on medication attending antenatal care for the first time during the current pregnancy were eligible for inclusion in this study. However, recent history of blood

transfusion, surgery, or inability to provide informed consent was criterion for exclusion.

2.6 Selection method

Participants ~~Subjects~~ who have met the inclusion criteria and provided consent for study participation were selected through simple random technique using a numbering system described by some researchers in a study on pregnant women (Catherine *et al.,* 2021; Faith *et al.,* 2021)

2.7 Sample Collection method

Fasting blood samples were collected by venepuncture technique for total cholesterol (TC), triglyceride (TG), high density lipoprotein (HDL) and low density lipoprotein (LDL). 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^{o}C until it was time for analysis (Oladapo- Akinfolarin *et al*., 2017; Oladapo-Akinfolarin *et al*., 2018).

2.8 Laboratory methods

Determination of Total Cholesterol in Serum
Total cholesterol was measured quantitatively by enzymatic method (Allain *et al.,* 1974).

Principle
The cholesterol is determined after enzymatic hydrolysis and oxidation. The indicator quinoneimine is formed from hydrogen peroxide and 4-aminoantipyrine in the presence of phenol and peroxidase. The amount of colour formed is proportional to the concentration of cholesterol in the serum.

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~~water bath at 37^{0}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
HDL-C was measured quantitatively by enzymatic method (Tietz, 1987)

Principle
Low density lipoprotein (LDL and VLDL) and chylomicron fractions are precipitated quantitatively by the addition of phosphotungstic acid in the presence of magnesium

ions. After centrifugation, the cholesterol concentration in the HDL fraction which remains in the supernatant is determined by enzymatic method.

Procedure

The blood samples were transferred into tubes and centrifuged for five minutes at 12,000 rpm. The supernatant (sera) was separated and arranged according to the labelled tubes as control, standard and samples. 200 µl of precipitating reagent (R) and 20 µl of sample were transferred into the tubes for test, 20ul 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 12,000 rpm. Thereafter, the clear supernatant was separated and determined for HDL cholesterol.

Determination of Triglycerides in Serum

Triglycerides are determined quantitatively by enzymatic method (Fraser and Hearne, 1981).

Principle

Triglycerides are determined after enzymatic hydrolysis with lipases and oxidation. The indicator is a quinoneimine formed from hydrogen peroxide, 4-aminophenazone and 4-chlorophenol under the catalytic influence of peroxidase. The amount of colour formed is proportional to the triglyceride concentration in the sample.

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 37^0C. The absorbance was read using 1cm light path (cuvette) for samples against blank at 505 nm wavelength.

Determination of Low-Density Cholesterol (LDL-C)

LDL cholesterol was calculated from the Friedewald's equation (Friedwald *et al*., 1972).

LDL – Cholesterol = Total Cholesterol – (TG/2.2) – HDL

The atherogenic index and lipid ratios were calculated using the following established formulas:

AIP = Log (TG/ HDL-C): Reference Range **=** Low risk (-0.3 – 0.1), Moderate risk (0.1 – 0.24), High risk (>0.24) (World Health Organization (WHO), 2014)

CRI-1 = TC/HDL-C: Reference Range **=** Low risk (< 1-3), Moderate risk (3-5), High risk (>5) (WHO, 2014).

CRI-II = LDL-C /HDL-C: Reference Range **=**Low risk (< 1-3), Moderate risk (3- 5), High risk (> 5) (WHO, 2014).

AC = TC – HDL-C/ HDL-C: (Reference >3.0) (WHO, 2014)
Apo B/ Apo A1: Reference range = (low risk 0.30, moderate risk 0.6 and high risk 0.8) (WHO, 2014).

2.9 Statistical analysis

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

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3.0 Results

Tables 1.0 (a) and 1.0 (b) show the effect of gravidity on atherogenic indices (AIP, CRI1, CRI2, AC and apo B/apo A1) in normotensive 2nd trimester pregnant women. Gravidity showed no significant effect on the indices in hypertensive pregnant women at 2nd trimester (p>0.05).

Table 1.0 (a): Effect of Gravidity on Artheriogenic Indices in Normotensives 2nd Trimester

Parameters		Normotensive women		P-value	F-value
	Primigravida (1) n = 15 (30%)	Multigravida(>1) n = 27 (54%)	Grand Multigravida (≥ 5) n = 8 (16%)		
AIP	0.18 ± 0.04	0.18 ± 0.06	0.20 ± 0.05	0.7611	0.2746
CRI 1	5.82 ± 0.82	5.19 ± 1.28	4.77 ± 0.78	0.0739	2.7550
CRI 2	4.16 ± 0.80	3.54 ± 1.21	3.07 ± 0.77	0.0521	3.1490
AC	4.82 ± 0.82	4.19 ± 1.28	3.77 ± 0.78	0.0739	2.7550
APoB/APoA1	0.36 ± 0.03	0.38 ± 0.05	0.36 ± 0.06	0.4512	0.8096

P-value<0.05 is statistically significant

Table 1.0 (b): The ANOVA Post – Hoc Findings Using Turkey Multiple Comparison Test for Effect of Gravidity on Artheriogenic Indices (Normotensive 2nd Trimester)

Parameters	Primagravida Vs. Multigravida	Primagravida Vs. Grand multigravida	Multigravida Vs. Grand Multigravida

AIP	0.9783	0.8548	0.7405
CRI 1	0.1865	0.0836	0.6090
CRI 2	0.1650	0.0521	0.5203
AC	0.1865	0.0836	0.6096
APoB/APoA1	0.9475	0.4390	0.5233

P-value<0.05 is statistically significant

Tables 2.0 (a) and 2.0 (b) show the effect of gravidity on atherogenic indices (AIP, CRI1, CRI2, AC and apo B/apo A1) in hypertensive 2nd trimester pregnant women. Gravidity showed no significant effect on the indices in hypertensive pregnant women at 2nd trimester (p>0.05).

Table 2.0 (a): Effect of Gravidity on Artheriogenic Indices in Hypertensive 2nd Trimester

Parameters		**Hypertensive women**		**P-value**	**F-value**
	Primigravida (1) n = 21 (42%)	Multigravida(>1) n = 25 (50%)	Grand Multigravida (≥ 5) n = 4 (8%)		
AIP	0.22 ± 0.05	0.21 ± 0.07	0.18 ± 0.04	0.6246	0.4753
CRI 1	5.39 ± 0.98	5.33 ± 1.79	4.38 ± 0.25	0.4292	0.8612
CRI 2	3.64 ± 0.95	3.57 ± 1.58	2.79 ± 0.34	0.4115	0.9050
AC	4.39 ± 0.98	4.33 ± 1.79	3.38 ± 0.25	0.4292	0.8612
APoB/APoA1	0.34 ± 0.03	0.34 ± 0.03	0.35 ± 0.00	0.4629	0.7828

P-value<0.05 is statistically significant

Table 2.0 (b): The ANOVA Post – Hoc Findings Using Turkey Multiple Comparison Test for Effect of Gravidity on Atherogenic Indices (Hypertensive 2nd Trimester)

Parameters	**Primagravida Vs. Multigravida**	**Primagravida Vs. Grand multigravida**	**Multigravida Vs. Grand Multigravida**
AIP	0.8596	0.6181	0.7856
CRI 1	0.9914	0.4104	0.4399
CRI 2	0.9800	0.3865	0.4354

AC	0.9914	0.4110	0.4399
APoB/APoA1	0.9469	0.5406	0.4295

P-value<0.05 is statistically significant

4.0 Discussion

This study had examined and evaluated atherogenic Index of Plasma (AIP), castelli Risk Index (CRI 1), (CRI 2), Artherogenic Coefficient (AC) and ApoB/ApoA1 among pregnant women in Rivers State University and Rives State University Teaching Hospital. Evaluation of these parameters ~~could~~ can help in prognostication of remote cardiometabolic pathology among this category of women.

From the result obtained in this study, it showed that there was no significant effect of gravidity on the artheriogenic indices of normotensive pregnant women in their second trimester. Gravidity also showed no significant effect on the artheriogenic indices of hypertensive pregnant women in their second trimester. The results from this study does not agree with the study carried out by Serrano and Casas (2018), in their study they discovered that an increase in TG and ApoB / ApoA1 ratio are associated with an increased risk of pre-eclampsia. In this case there was no significant increase in ApoB/ ApoA1. This difference may be due to the age of pregnancy as this study focused on pregnant women in their second trimester while several other studies focused on pregnant women in their first and third trimesters. However, a result pattern was observed in this study although the changes were not statistically significant when test. The pattern showed a gradual drop in the CRI 1, CRI 2 and AC values as the number of pregnancies increased, suggesting that the higher the number of pregnancy, the lower the CRI 1 CRI 2 and AC values may be for normotensive pregnant women in their second trimester.

Similarly, in hypertensive pregnant women in their second trimester stage of pregnancy showed slight and gradual decline in their CR-I, CR-2 and AC levels among the gravidity groups. Although not statistically significant, but owing to similar or repeated artheriogenic pattern among these groups in both normotensive and hypertensive pregnant women in their second trimester of pregnancy, it suggest that increase in number of pregnancy could lead to decrease in artheriogenic indices especially CR-1, CR-2 and AC markers. The loss of significant difference may be due to inequality in the number of subject's distribution among the gravidity groups. A relatively equal number of subject distribution may have provided more logical explanation. This study finding is not in consonance with the study by Meenakshi *et al*. (2015), who reported a significant increase in artheriogenic indices (AIP, CRI and AC) in case group as compared to the control group ($p < 0.05$). The level of significance was $p < 0.0001$, and evaluation of the artheriogenic indices during pregnancy may help prevent the risk of cardiovascular disease (CVD). It also does not agree with a study by Aksonova *et al.* (2016), which also demonstrated an increase in AIP, CRI indices in pregnant women with PE in second trimester of

pregnancy. There is a dearth of research on the study of artheriogenic indices among pregnant women in their second trimester.

Generally, based on World Health ~~Organisation~~Organization (WHO), (2014) reference values for artheriogenic risk classifications, most pregnant women in this study, both normotensive and hypertensive pregnant women among the various groups of gravidity were moderately at risk of artheriosclerosis and cardiovascular disease. Subjects in the primigravida and multigravida groups in both normotensive and hypertensive subjects had high CR-2 level above the WHO cut-off limit (>5.0) which made subjects in this group at high risk of artheriosclerosis and CVD.

Conclusion

Normotensive and hypertensive women in their second trimester stage of pregnancy may not have expressed significant changes in artheriogenic indices following multiple pregnancies but caution should be emphasized among these women as many artheriogenic indices were at moderate risk level.

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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