Journal of Advances in Medicine and Medical Research

34(14): 57-63, 2022; Article no.JAMMR.86191
ISSN: 2456-8899
(Past name: British Journal of Medicine and Medical Research, Past ISSN: 2231-0614, NLM ID: 101570965)

Evaluation of Cardiovascular Risk Markers in Hypertensive Pregnant Women

Tomaziga Tomiloba Oladapo-Akinfolarin [a*], Oladapo Mayowa Akinfolarin [b], Stella Urekweru Ken-Ezihuo [a] and Ebirien-Agana Samuel Bartimaeus [a]

[a] *Department of Medical Laboratory Science, Rivers State University, Port Harcourt, Nigeria.*
[b] *Department of Chemistry, Rivers State University, Port Harcourt, Nigeria.*

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JAMMR/2022/v34i1431388

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/86191

Original Research Article

Received 10 February 2022
Accepted 18 April 2022
Published 09 May 2022

ABSTRACT

Background: In developed countries, heart disease and stroke are the major causes of death. One of the main risk factors of cardiovascular disease is dyslipidemia, and this could be due to increased lipoproteins and/or decreased plasma clearance. Pregnancy is marked with changes in metabolism in preparation for the developing fetus and lactation after delivery. Pregnancy causes long-term metabolic and vascular changes, which may raise the general risk of heart disease.
Aim: The goal of this study was to see how cardiovascular indicators changed during pregnancy in hypertensive pregnant women at Rivers State University Teaching Hospital.
Methodology: The study included 150 people who were divided into three groups: Non-pregnant hypertensive women (50), second trimester pregnant hypertensive women (50), and third trimester pregnant hypertensive women (50). After giving their consent, subjects were chosen using a simple random procedure. Total cholesterol (TC), high density lipoprotein (HDL), triglyceride (TG), low density lipoprotein (LDL), very low density lipoprotein (VLDL), uric acid (UA), C-Reactive protein (CRP), apolipoprotein A1 (APoA1), and apolipoprotein B (APoB) were all measured in the lab using the venipuncture technique under fasting conditions (APoB).
Results: The findings revealed that there was a significant difference in each of the examined parameters (P<0.05) between hypertensive non-pregnant, 2nd trimester, and 3rd trimester hypertensive pregnant women, with the exception of HDL, TG, and VLDL (P>0.05).

**Corresponding author: E-mail: ola_atomaziga@yahoo.com;*

Conclusion: This study has shown that hypertensive pregnant women are potential future candidates of cardiovascular disease.

Keywords: Cardiovascular; pregnancy; cholesterol; lipoprotein.

1. INTRODUCTION

In developed countries, atherosclerosis, a cardiovascular disease, is the leading cause of death. It is a disease which affects large arteries [1]. According to the research conducted by Dashti et al., [2], atherosclerosis disease results from environment and genetic interaction which modulate the activity of various types of cell and inflammatory molecules within the arterial wall [2]. Because of elevated lipoproteins and/or impaired plasma clearance, dyslipidemia has been identified as a major risk factor for cardiovascular disease. Lipoproteins play an important function in the transport of hydrophobic lipid molecules [3]. Lipoprotein synthesis is thought to occur in the gut and liver. VLDLs (primarily apoB100-containing lipoproteins in humans) are synthesized in the liver and then transported and catabolized to form LDL. LDL cholesterol is an atherogenic lipoprotein, which is well known. As a result, it's thought that hepatic lipoproteins are the only ones responsible for atherosclerosis [1]. ApoB100 has been shown to be an individual risk marker for ischemic heart disease and to identify high-risk phenotypes in diabetic patients with normal cholesterol levels. This could be used to determine the patients' lipidaemic profile [4].

The liver produces C-reactive protein (CRP), a positive acute phase protein, when there is inflammation [5]. It was proposed that CRP can be synthesized by smooth muscles in the coronary arteries in response to cytokine-induced inflammation [6]. CRP's role in atherosclerosis qualifies it as a vascular inflammatory marker.

Pregnancy is marked with changes in metabolism in preparation for the developing fetus and lactation after delivery. Pregnancy causes long-term metabolic and vascular changes that may raise the risk of cardiovascular, cerebrovascular, and renal disease, as well as diabetes later in life [7]. Women with gestational hypertension have been linked to an increased risk of ischemic heart disease, myocardial infarction, heart failure, and ischaemic stroke [7]. Changes in nutrient composition were reported in lactating mothers due to changes in maternal metabolism and environment [8,9]. Changes in lipid and lipoprotein concentrations have been implicated during pregnancy and these changes are reported to be positively related with gestation such that increase in gestation should result to increase in lipid and lipoprotein concentrations. Hypertension in pregnancy is a common phenomenon among pregnant women which may present with varying metabolic changes compared to healthy normotensive women. Therefore, this study was conducted to evaluate changes in cardiovascular markers in hypertensive pregnant women in Port Harcourt with the view of assessing the impact or effect of gestation on the cardiovascular risk markers.

2. MATERIALS AND METHODS

2.1 Study Design

A total of 150 females were divided into two groups for the cross-sectional study: a control group of 50 non-pregnant hypertensive women and a case group divided into second and third trimester groups. 50 hypertensive pregnant women were included in each trimester.

2.2 Study Location

This research was carried out at Rivers State University and the Rivers State University Teaching Hospital in Port Harcourt, Nigeria's capital city. Port Harcourt, the capital of Rivers State, has a population of about two million people.

2.3 Eligibility Criteria

Inclusion criteria: Participants were all hypertensive pregnant women who had registered for antenatal care, however control subjects were hypertensive non-pregnant women. Subjects between the ages of 20 and 45 who signed a written informed permission form were also chosen.

Exclusion Criteria: Subjects in prehypertension stage or unconfirmed hypertensive by clinician were excluded. Also, subjects with previous record of cardiovascular disease or other origin

were excluded. Subjects with history of other metabolic syndrome were equally excluded.

2.4 Sampling Method

Using Catherine et al. [9] numbering system and Faith et al. [10], test candidates were chosen at random from Rivers State University and Rivers State University Teaching Hospital.

2.5 Sample Collection Method

Venipuncture technique was employed for blood sample collection into plain vacutainer tubes and samples were spun for 10 minutes at 1500rpm after clotting to separate serum from clotted blood [11,12]. CRP, apoA1, apoB, uric acid, total cholesterol, triglycerides, and high density lipoprotein cholesterol were all measured in serum and stored at -4ºC until laboratory analysis. LDL and VLDL levels were determined [13]. Blood was obtained in a fasting state to estimate uric acid, Apo A1 and B, CRP, TG, HDL, and total Cholesterol.

2.6 Biochemical Determinations

All laboratory examination was conducted at Rivers State University's Department of Medical Laboratory Science's Chemical Pathology Laboratory.

Human Serum High Sensitive C-reactive Protein Concentration Determination: Nazir & McQueen, [14] method was applied.

Principle: CRP is assessed turbidimetrically in a spectrophotometer at 340nm wavelength when complexed human anti-C-reactive protein antibodies react with antigen in the serum to generate antigen/antibody complexes, which are then measured turbidimetrically in a spectrophotometer after agglutination.

Procedure: 2µl of material was put to the test tubes for CRP determination, as well as 5 additional tubes for calibration. 250µl of R1 assay buffer was added to each of the tubes. It was blended by tilting the bottoms of the tubes, then incubated at 37ºC for 5 minutes, and the absorbance was measured as OD1. All of the tubes received 50µl of R2 antibody reagent once again. It was mixed by tilting the bottoms of the tubes, then incubated at 37ºC for 3 minutes, and the absorbance was measured as OD2.

Determination of Apo Lipoprotein A1 in Human Serum: The technique of Nazir and McQueen, [14] was employed.

Principle: Apo –A is determined after anti- apo-A antibodies reacts with the antigen in the sample to form antigen/antibody complexes which following agglutination is measured turbidimetrically in a spectrophotometer at 340nm wavelength.

Procedure: 2µl of serum was deposited in the test tubes and 5 additional tubes for calibration in the laboratory determination of Apo B. Then 250µl of buffer (R1) was added to all of the tubes, mixed by tilting the tubes' ends, and allowed to sit in a water bath for 5 minutes at 37ºC. It was then measured with a spectrophotometer at a wavelength of 340 nm. OD1 was used to represent the absorbance. In a water bath, another 50µl of antibody reagent (R2) was added to the reaction and left to sit for 5 minutes at 37ºC. The spectrophotometer was then used to read it at 340nm. The absorbance was represented by OD2. [OD2– OD1] was used to compute the absorbance of the standard and sample.

Apolipoprotein B Determination in Human Serum: Nazir and McQueen, [14] method was used.

Principle: Anti-Apo-B antibodies react with the antigen in the sample to generate antigen-antibody complexes, which are then quantified turbidimetrically in a spectrophotometer at 340nm after agglutination.

Procedure: 2µl of serum was deposited in the test tubes and 5 additional tubes for calibration in the laboratory determination of Apo B. Then 250µl of buffer (R1) was added to all of the tubes, mixed by tilting the tubes' ends, and allowed to sit in a water bath for 5 minutes at 37ºC. It was then measured with a spectrophotometer at a wavelength of 340nm. OD1 was used to represent the absorbance. In a water bath, another 50µl of antibody reagent (R2) was added to the reaction and left to sit for 5 minutes at 37ºC. The spectrophotometer was then used to read it at 340nm.

Determination of Total Cholesterol in Serum: Allain et al., [15] technique was used.

Principle: After enzymatic hydrolysis and oxidation, the cholesterol is measured. In the presence of phenol and peroxidase, the indicator quinoneimine is produced from hydrogen peroxide and 4-aminoantipyrine. The quantity of color produced is proportional to the amount of cholesterol present in the blood.

Procedure: The assay circumstances were taken into account. Using distilled water, the instrument was zeroed. One milliliter of cholesterol reagent was pipetted into clean dry test tubes labeled blank, standard, and tests, with 10 milliliters of distilled water, standard, and sample added to their corresponding tubes. It was thoroughly mixed by tilting the bottoms of the tubes and incubated for 5 minutes in a waterbath at 37°C. In a spectrophotometer, the absorbance of the standard and test samples was measured against the blank at 540nm wavelength.

Determination of High-Density Lipoprotein (HDL) Cholesterol in Serum: The technique of Tietz, [16] was employed.

Principle: The addition of phosphotungstic acid in the presence of magnesium ions quantitatively precipitates low density lipoprotein (LDL and VLDL) and chylomicron fractions. The cholesterol concentration in the HDL fraction that remains in the supernatant is measured using an enzymatic technique after centrifugation.

Procedure: The blood samples were placed in tubes and centrifuged at 12,000 rpm for five minutes. The supernatant (sera) was separated and organized into control, standard, and sample tubes according to the labels. Then 200µl of precipitating reagent (R) and 20µl of sample were placed into test tubes, 20µl of standard into standard tubes, and distilled water into the blank tubes. It was properly blended by tilting the bottoms of the tubes and let to stand at room temperature for 10 minutes. The contents of the tubes were centrifuged at 12,000 rpm for 2 minutes. After that, the clear supernatant was collected and HDL cholesterol was measured.

Determination of Triglycerides in Serum: Fraser & Hearne, [17] method was used.

Principle: After enzymatic hydrolysis using lipases and oxidation, triglycerides are measured. Under the catalytic effect of peroxidase, a quinoneimine is produced from hydrogen peroxide, 4-aminophenazone, and 4-chlorophenol. The amount of color generated in the sample is related to the triglyceride concentration.

Procedure: Conditions for the assay were taken into account. Distilled water was used to zero the instrument. The tubes were then filled with 1ml each of blank, standard, and test triglyceride reagent. The tubes were filled with 10µl of standard and sample, mixed, and incubated at 37°C for 5 minutes. At a wavelength of 505 nm, the absorbance of samples was measured using a 1cm light path (cuvette).

Low-Density Cholesterol (LDL-C) Determination: The method of Friedwald et al. [13] was applied.

Calculation: The difference between the results of total cholesterol, triglycerides, and HDL in the serum sample was used to calculate LDL cholesterol levels.

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

(3.8 - 4.9**)**mmol/l

Determination of Uric Acid in Serum: Barr [18] enzymatic method was used.

Principle: After uricase's enzymatic action, uric acid was measured. Under the catalytic effect of peroxidase, a quinoneimine is produced from hydrogen peroxide, 3, 5-dichloro-2-hydroxybenzenesulfonic acid, and 4-aminophenazone.

Procedure: Tubes were labeled blank, standard, and test and were placed accordingly. Then, tilting the bottoms of the tubes, 20µl of distilled water was added to the blank tube, 20µl of standard to the standard tube, and 20µl of serum to the test tubes, and properly mixed. At 37°C, it was incubated for 5 minutes. The wavelength was then measured with a spectrophotometer at 520nm.

3. RESULTS

Table 1 represents the result of maternal characteristics of hypertensive pregnant women. There were significant differences in the level of the markers TC, LDL, LDL,UA, CRP, ApoA1, and ApoB in the non-pregnant, 2nd trimester and 3rd trimester groups of normotensive pregnant women ($P<0.05$), whereas, the levels of HDL, VLDL and TG remained constant, showing no significant difference ($P>0.05$).

Except for APoA1, which was significant across all groups analyzed, the post hoc analysis indicated no significant differences in studied parameters between the 2nd and 3rd trimester groups. The differences in HDL and TG values between the groups were entirely insignificant.

Table 1. Maternal characteristics of hypertensive pregnant women

Parameters	Non-pregnant n = 50	Hypertensive Women		P-value	F-value
		2nd Trimester n = 50	3rd Trimester n = 50		
TC(mmol/l)	4.58 ± 0.65	4.91 ± 0.34	4.90 ± 0.45	0.0012	6.949
HDL(mmol/l)	0.95 ± 0.21	0.98 ± 0.21	0.95 ± 0.20	0.7034	0.3527
TG(mmol/l)	1.46 ± 0.30	1.57 ± 0.30	1.53 ± 0.35	0.2248	1.508
LDL(mmol/l)	3.00 ± 0.65	3.21 ± 0.26	3.27 ± 0.30	0.0064	5.227
VLDL(mmol/l)	0.66 ± 0.14	0.71 ± 0.14	0.70 ± 0.16	0.2248	1.508
UA(mg/l)	5.074 ± 0.444	4.70 ± 0.37	4.68 ± 0.40	<0.0001	14.82
CRP(mg/l)	3.70 ± 1.06	7.44 ± 1.82	7.86 ± 2.26	<0.0001	82.57
APoA1(mg/l)	346.80 ± 21.74	361.30 ± 27.35	379.70 ± 19.94	<0.0001	25.24
APoB(mg/l)	118.60 ± 11.87	122.00 ± 12.75	137.90 ± 15.08	<0.0001	29.90

Table 2. The ANOVA Post – Hoc findings using Turkey multiple comparison test for maternal characteristics (hypertensive) within the study groups

Parameters	NP vs 2ND T	NP vs 3RD T	2ND T vs 3RD T
TC(mmol/l)	0.0041	0.0046	0.9991
HDL(mmol/l)	0.7215	0.9949	0.7787
TG(mmol/l)	0.2066	0.493	0.8371
LDL(mmol/l)	0.0434	0.0073	0.7981
VLDL(mmol/l)	0.2066	0.493	0.8371
UA(mg/dl)	<0.0001	<0.0001	0.9534
CRP(mg/dl)	<0.0001	<0.0001	0.4711
APoA1(mg/dl)	0.0061	<0.0001	0.0003
APoB(mg/dl)	0.4056	<0.0001	<0.0001

NP- Non Pregnant, 2nd T – second Trimester, 3rd T- third trimester

4. DISCUSSION

There was a significant difference in TC, LDL, UA, CRP, Apo A1 and Apo B when hypertensive women in the second and third trimesters were compared to non-pregnant women. This finding agrees with a study conducted by Raghuram et al., [19]. The study found that pregnant women's lipid profiles changed significantly between the second and third trimesters [19]. The changes in the concentrations of these lipids and lipoproteins above or below the normal range are potential indication of CVD candidacy. This supports ADA's [4] findings that a rise in Apo B is linked to Ischaemic heart disease, and that women with severe prenatal hypertension (≥160/110 mm Hg) are more likely to have pre-eclampsia and may have a higher risk of having cardiovascular disease later in life.

The HDL level was lower in 3rd trimester than non-pregnant and second trimester, but was not significantly increased in this work. The TG level was also not significant but within the normal range. Also the non-pregnant, 2nd trimester, and 3rd trimester groups' VLDL levels did not vary significantly. These findings are not in agreement with different studies that have reported significant changes in lipid profile [20,21,19]. According to Shen et al., [22], there were no persistent significant changes in TG among pregnant women in the first, second, and third trimesters [22], Also, another work by Oladapo-Akinfolarin et al. [12] showed that certain lipid levels may not necessarily change following pregnancy.

The LDL was significantly increased but was within the normal range. This also agrees with Charlton, [21] and Islam, [20] that LDL increases in pregnant hypertensive women compared to non-pregnant women and as such are at risk of CAD.

As the pregnancy continued, the level of uric acid in this group of women reduced dramatically. Non-pregnant women had considerably greater uric acid levels than pregnant women in both the second and third trimesters, but the second trimester did not differ significantly from the third trimester. All of the numbers were within normal limits. In agreement with Richard et al. [23], the drop in uric acid in pregnancy may be due to estrogen activity and rise in renal blood flow. This

may be different for pregnant women with renal disease affecting the GFR.

All of the participants in this group had abnormally high CRP levels. This outcome is consistent with previous research on pregnant women [11]. This supports Bock's [24] hypothesis that CRP, in addition to being a marker for vascular inflammation, plays an active role in atherosclerosis. The results were significantly higher than Ernest et al. [25]'s 2.50mg/dl proposed value. This means that hypertension favored the inflammatory response in addition to being a sign for inflammation.

5. CONCLUSION

Finally, it can be seen that if hypertensive pregnant women are not effectively controlled, they may be at the risk of developing cardiovascular disease later in life, according to this study.

CONSENT

As per international standard or university standard, patients' written consent has been collected and preserved by the author(s).

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Hussain MM, Leung TM, Zhou L, Abu-Merhi S. Regulating intestinal function to reduce Atherogenic Lipoproteins. Clinical Lipidology. 2013;8(4):481-490.
2. Dashti M, Kulik W, Hoek F, Veerman EC, Peppelenbasch MP, Rezaee F. A phospholipidomic analysis of all defined human plasma lipoproteins. Scientific Reports. 2011;1:139.
3. Farkas-Epperson M, Le N. Lipoproteins and biosensors of endothelial oxidative status. Clinical Lipidology. 2012;7(1):49-63.
4. American Diabetes Association. Diagnosis and classification of diabetes mellitus. Diabetes Care. 2010;33(1):562–569.
5. Casas JP, Shah T, Hingorani AD, Danesh J, Pepys MB. C-reactive protein and coronary heart disease: A critical review. Journal of Internal Medicine. 2008;264(4):295-314.
6. Liu Y, Salter HK, Johnson CM, Stephens E, Lakarvsky PJ, Walshaw J, Bullock SL. Bi caudal- D uses a parallel, homodimer coiled coil with heterotypic registry to coordinate recruitment of cargos to dyeing. Genes Development. 2013;27(11):1233-1246.
7. Mannisto T, Mendola P, Vaarasmaki M, Jarvelin MR, Hartikainen AL, Pouta A. Elevated blood pressure in pregnancy and subsequent chronic disease risk. Circulation. 2013;127(6):681-690.
8. Biambo KG, Nyebuchi J, Roseline E, Fyneface AC, Laurretta N. Zinc composition in breast milk of lactating mothers in urban and Sub-urban Areas in Rivers State. Asian Journal of Research in Nursing and Health. 2021;4(4):165-169.
9. Catherine I, Biambo KG, Nyebuchi J, Fyneface AC, Goodnews N. Evaluation of nutrient composition in breast milk of breast feeding mothers in urban and Sub-urban Subjects in Rivers State. Asian Journal of Pediatric Research. 2021;7(2):39-44
10. Faith D, Kalaotaji GB, Nyebuchi J, Chikadibia FA, Felix EK. Comparative study of heavy metals in breast milk of breast feeding mothers in urban and sub-urban subjects in Rivers state. Journal of Applied Life Sciences International. 2021;28(8):31-36.
11. Oladapo-Akinfolarin TT, Bartimeaus ES, Nwachukwu EO, Nduka N. Assessment of C-reactive protein levels in normotensive and hypertensive pregnant subjects in Port Harcourt, Nigeria. World Journal of Pharmaceutical and Medical Research. 2017;3(10):292-296.
12. Oladapo-Akinfolarin TT, Bartimeaus ES, Nwachukwu EO, Nduka N. Assessing lipoprotein levels in normotensive and hypertensive pregnant women in Port Harcourt, Nigeria. American Journal of Biomedical Sciences. 2018;10(1):18-27.
13. Friedwald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low density lipoprotein cholesterol in plasma, without use of preparative ultracentrifuge. Clinical Chemistry. 1972;18:499-502.
14. Nazir DJ, McQueen MJ. Evaluation of a turbidimetric procedure for Apolipoproteins A1 and B on the CIBA corning 550

express. Clinical Biochemistry. 1993;26(6): 477-482.

15. Allain CC, Poon LS, Chan CSG, Richmond W, Fu PC. Enzymatic determination of total serum cholesterol. Clinical Chemistry. 1974;20(4):470-475.
16. Tietz NW. Laboratory methods. In: Fundamentals of Clinical Chemistry, 3rd Edition, W.B. Saunders Company, Philadelphia. 1987;927-962.
17. Fraser CG, Hearne CR. Assessment of colorimetric enzymatic determination of triglyceride, by manual and centrifugal analyzer techniques, and comparison with a CDC standardized method. Clinical Biochemistry. 1981;14(1):28-31.
18. Barr WG. Uric acid. In: Walker, H.K., Hall, W.D & Hurst, J.W, editors. Clinical methods: The History, Physical and Laboratory Examinations. 3rd edition, Boston: Butterworths. 1990;16:165.
19. Raghuram P, Arjun SS, Prakash KK, Babita G, Varshil M, Kunal B, Aadhijaya B. Evaluation of lipid profile in second and third trimester of pregnancy. Journal of Clinical and Diagnostic Research. 2016; 10(3):12-16.
20. Islam NAF, Chowdhurry MAR, Kibria GM, Akhter S. Study of serum lipid profile in preeclampsia and eclampsia. Faridpur Medical College Journal. 2010;5:56-59.
21. Charlton F, Tooher J, Rye KA, Hennessy A. A cardiovascular risk, lipids and pregnancy: preeclampsia and the risk of later life cardiovascular disease. Heart Lung Circulation. 2014;23:203-212.
22. Shen H, Liu X, Chen Y, He B, Cheng W. Associations of lipid levels during gestation with hypertensive disorders of pregnancy and gestational diabetes mellitus: A prospective longitudinal cohort study. British Medical Journal Open. 2016;6: e013509.
    DOI: 10,1136.
23. Richard JJ, Mehmet K, Dukhee K, Laura GL, Daniel F. Uric acid: A clinically useful marker to distinguish preeclampsia from gestational hypertension. Hypertension. 2011;58(4):548-549.
24. Bock JL. Cardiac Injury, Atherosclerosis and thrombotic Disease, In Mcpherson, R.A. & Pincus, M.R. (22nd Edn) Henry's Clinical Diagnosis and Management by Laboratory Methods (Pp 249-259), Philadelphia, USA: Elservier, Saunders; 2011.
25. Ernest GDS, de Jonge LL, Hofman A, Lindemans J, Russcher H, Steegers EAP, Jaddoe VWV. C-reactive protein levels in early pregnancy, fetal growth patterns, and the risk for neonatal complications: The Generation R Study. American Journal of Obstetrics and Gynecology. 2011;205(2):132.e1–132.e12.

© 2022 Oladapo-Akinfolarin et al.; This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle5.com/review-history/86191