Original Research Article

Evaluation of cardiovascular risk markers in hypertensive pregnant women

Abstract

Cardiovascular disease is the leading cause of death in developed countries. Dyslipidemia has been the main risk factor for cardiovascular disease which may 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 induces long term metabolic and vascular abnormalities that might increase the overall risk of cardiovascular. This study was aimed at evaluating changes in cardiovascular markers in different stages in pregnancy among hypertensive pregnant women in Port Harcourt. This study was aimed at comparing levels of cardiovascular risk markers in different changes in pregnancy among hypertensive pregnant women in Rivers state University Teaching Hospital. A total of 150 subjects participated in the study and were classified into three groups; non-pregnant hypertensive women (50subjects), 1st trimester (50 hypertensive pregnant women) and 2nd trimester (50hypersive pregnant women). Subjects were selected in a simple random technique after they gave their consent. Blood sample were collected into plain bottles under fasting condition via venipuncture technique for laboratory analysis of TC, HDL, TG, LDL, VLDL, UA, CRP, APoA1 and APoB. Results showed that there were significant differences in the studied parameters ($P<0.05$) except in HDL, TG and VLDL ($P>0.05$). This study has shown that hypertensive pregnant women are potential future candidates of cardiovascular disease.

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

1.0 Introduction

Atherosclerosis, a cardiovascular disease is the leading cause of death in developed countries. Atherosclerosis, a disease affecting large arteries (Hussain *et al.,* 2013), occurs due to environment and genetic interaction which modulate the activity of various cell types and inflammatory molecules within the wall of the arteries (Dashti *et al.,* 2011). Dyslipidemia has been the main risk factor for cardiovascular disease which may be due to increased lipoproteins and/or decreased plasma clearance. One key role of lipoproteins is the transport of hydrophobic lipid compounds (Farkas-Epperson and Le, 2012). The intestine and liver are implicated in assembling lipoproteins. The liver is responsible for the synthesis of VLDLs (mainly apoB100-containing lipoproteins in humans) which is transported and eventually catabolized to produce LDL. It is a known fact that LDL cholesterol is an atherogenic lipoprotein. Therefore, it is believed that lipoproteins of hepatic origin are the sole contributors to atherosclerosis (Hussain *et al.,* 2013). ApoB100 is reported to be an independent risk marker for ischaemic heart disease and identifies high-risk phenotypes in normocholesterolaemic diabetic patients. This could be used to assess the lipidaemic pattern of these patients (American Diabetes Association (ADA), 2010).

C-reactive protein (CRP), a positive acute phase protein is synthesized by the liver in the events of inflammation (Casas *et al.,* 2008). It suggested that smooth muscles of the coronary arties can synthesize CRP in response to cytokines inflammatory action (Liu *et al.,* 2013). The implication of CRP in atherosclerosis makes it serve as a marker for vascular inflammation.

Pregnancy is marked with changes in metabolism in preparation for the developing fetus and lactation after delivery. Pregnancy induces long term metabolic and vascular abnormalities that might increase the overall risk of cardiovascular, cerebrovascular and kidney diseases as well as diabetic mellitus later in life (Mannisto *et al.,* 2013). Increased risk for ischaemic heart disease, myocardial infarction, heart failure and ischaemic stroke has also been observed among women with gestational hypertension (Mannisto *et al.,* 2013). Changes in nutrient composition were reported in lactating mothers due to changes in maternal metabolism and environment (Biambo *et al.,* 2021; Catherine *et al.,* 2021). 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.

2.0 Materials and Methods

2.1 Study Design

The cross-sectional study was designed is such a way that the total participant of 150 females were divided into two groups; control group which composed 50 non-pregnant hypertensive women and case group which was divided into second and third trimester groups. Each of the trimesters comprised 50 hypertensive pregnant women.

2.2 Study Location

This study was conducted at Rivers State University and Rivers State University Teaching Hospital in Port Harcourt, the capital city of Rivers State in Nigeria. Port Harcourt is the capital city of Rivers State with population strength of roughly two million people.

2.3 Eligibility Criteria

Inclusion criteria

All hypertensive pregnant women registered for antenatal care were eligible for participation but control subjects were hypertensive non-pregnant women. Subjects who were within the ages of 20 and 45 years and gave written informed consent were also selected.

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

With the aid of a numbering system as described by Catherine *et al.* (2021) and Faith *et al.* (2021), subjects were randomly selected 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 (Oladapo- Akinfolarin et al., 2017; Oladapo- Akinfolarin et al., 2018). Serum was stored at -4^{o}C until the time for laboratory analysis of the studied parameters; CRP, apoA1, apo B, uric acid, total cholesterol, triglycerides and high density lipoprotein cholesterol. LDL and VLDL were calculated (Friedewald *et al.,*1972). For the estimation of uric acid, Apo A1 and B, CRP, TG, HDL and total Cholesterol blood was taken in fasting condition.

2.7 Biochemical Determinations

All laboratory tests were done in Chemical pathology laboratory of 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 was added to all the tubes. It was mixed by tilting the bottom of the tubes and then incubated at 37^{0}C 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 37^{0}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

2 µl of serum was placed in the test tubes and 5 other tubes for calibration. 250 µl of buffer (R1) was added to all the tubes, mixed by tilting the bottom of the tubes, and allowed for 5 minutes at 37^{o}C in a water bath. It was then read in a spectrophotometer at 340 nm. 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^{o}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
2 µl of serum was placed in the test tubes and 5 other tubes for calibration. 250 µl of buffer (R1) was added to all the tubes, mixed by tilting the end of the tubes and allowed for 5 minutes at 37^{o}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^{o}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^{o}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 12,000 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
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^{o}C. The absorbance was read using 1cm light path (cuvette) for samples against blank at 505 nm wavelength.

Determination of Low-Density Cholesterol (LDL-C)

Parameters	**Hypertensive Women**

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.

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

(3.8 - 4.9**)**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. 20 µl of distilled water was added into the blank tube, 20 µl of standard to standard tube and 20 µl of serum to test tubes and properly mixed by tilting the bottom of the tubes. It was incubated for 5 minutes at 37^{o}C. It was then read in a spectrophotometer at 520 nm wavelength.

3.0 Results

	Non-pregnant n = 50	2nd Trimester n = 50	3rd Trimester n = 50	P-value	F-value
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

The results in Table 1. showed that there were significant differences in TC, LDL, LDL,UA, CRP, ApoA1, and ApoB levels among non-pregnant, 2nd trimester and 3rd trimester groups of normotensive pregnant women ($P<0.05$) except for HDL, VLDL and TG that showed no significant difference.

Table 1.: Maternal Characteristics of Hypertensive Pregnant Women

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.

The above Post hoc analysis revealed no significant difference in studied parameters between 2nd and 3rd trimester groups except APoA1 that was significant between all groups compared. HDL and TG levels were completely non-significant between the groups compared.

4.0 Discussion

The analysis of the mean values of the maternal characteristics of the hypertensive women in the 2nd and 3rd trimester, and comparing with non-pregnant showed that there was a significant difference in TC, LDL, UA, CRP, Apo A1 and Apo B. This finding is in consonance with a study conducted in 2016. The study reported significant changes in lipid profile between 2nd and 3rd trimester among pregnant women (Raghuram *et al.*, 2016). The changes in the concentrations of these lipids and lipoproteins above or below the normal range are potential indication of CVD candidacy. This agrees with ADA, (2010), that an increase in Apo B is associated with Ischaemic heat disease and that women who suffer from severe gestational hypertension (≥ 160 / 110 mm Hg) are at a greater risk of progressing to the development of pre-eclampsia and may be at increased risk for developing 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. Again, VLDL levels showed no significant change in the non-pregnant, 2nd trimester and 3rd trimester groups. These findings are not in agreement with different studies that have reported significant changes in lipid profile (Islam, 2010; Charlton, 2014; Raghuram *et al.*, 2016). A study by Shen et al. 2016 reported that there were no consistent significant changes in TG in 1st, 2nd and 3rd trimester among pregnant women. Also, another work by Oladapo-Akinfolarin *et al.* (2018) showed that certain lipid levels may not necessarily change following pregnancy.

Historically, adverse CAD effects of pre-eclampsia and other hypertensive pregnancy disorders were limited to the time of pregnancy, but there is a growing consensus that cardiovascular disease risks persist to later in life far beyond the affected pregnancy.

The LDL was significantly increased but was within the normal range. This also agrees with Charlton, (2014) and Islam, (2010) that LDL increases in pregnant hypertensive women compared to non-pregnant women and as such are at risk of CAD.

The level of uric acid was consistently significantly decreased as the pregnancy progressed in this group of subjects. The level of uric acid in the non-pregnant women was significantly higher than both second and third trimesters while the second trimester was not significantly higher than the third trimester. The values were all within the normal range. In agreement with Richard *et al.* (2011), the drop in uric acid in pregnancy may be due estrogen activity and rise in renal blood flow. This may be different for pregnant women with renal disease affecting the GFR.

The CRP was outrageously high in all the subjects in this group. This finding is in consonance with related work on pregnant women (Oladapo-Akinfolarin *et al.,* 2017). This agrees with Bock, (2011), that CRP plays an active role in atherosclerosis in addition to being a marker for vascular inflammation. The values were far above the value of 2.50 mg/dl as suggested by Ernest *et al*. (2011). This implies that, in addition to being a marker for inflammation, hypertension favoured the inflammatory response.

Conclusion

This study has revealed that hypertensive pregnant women are at risk of developing cardiovascular disease later in life if not properly managed.

References

Allain, C.C.., Poon, L.S., Chan, C.S.G.,Richmond, W and Fu, P. C. (1974).Enzymatic Determination of Total Serum Cholesterol. *Clinical Chemistry, 20 (4), 470-475*.

American Diabetes Association (2010). Diagnosis and Classification of Diabetes Mellitus. *Diabetes Care, 33 (1), 562 – 569.*

Barr, W.G. (1990). 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; 16,165.

Biambo, K. G., Nyebuchi, J., Roseline, E., Fyneface, A. C., & Laurretta, N. (2021). 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,* 4(4), 165-169

Bock, J.L. (2011). 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.

Casas, J.P.,Shah, T.,Hingorani, A.D, Danesh, J. and Pepys, M.B. (2008). C-reactive protein and Coronary Heart Disease: A critical Review. *Journal of Internal Medicine, 264(4), 295-314.*

Catherine, I., Biambo, K.G., Nyebuchi, J., Fyneface, A. C., & Goodnews, N. (2021). 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,* 7(2), 39-44

Charlton, F., Tooher, J., Rye, K.A. and Hennessy, A. (2014). A cardiovascular risk, lipids and pregnancy: preeclampsia and the risk of later life cardiovascular disease. *Heart Lung Circulation, 23, 203-212*.

Dashti, M., Kulik, W., Hoek, F., Veerman, E. C., Peppelenbasch, M. P. and Rezaee, F. (2011). A phospholipidomic analysis of all defined human plasma lipoproteins. *Scientific Reports*, 1,139.

Ernest, G. D. S., de Jonge, L. L., Hofman, A., Lindemans, J., Russcher, H., Steegers, E. A. P., and Jaddoe, V. W. V. (2011). 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, 205(2), 132.e1–132.e12*.

Faith, D., Kalaotaji, G. B., Nyebuchi, J., Chikadibia, F. A. and Felix, E. K. (2021). 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*, 28(8), 31-36

Farkas-Epperson, M. & Le, N. (2012). Lipoproteins and biosensors of endothelial oxidative status. *Clinical lipidology*, 7(1), 49-63.

Fraser, C. G and Hearne, C. R. (1981). Assessment of colorimetric enzymatic determination of triglyceride, by manual and centrifugal analyzer techniques, and comparison with a CDC standardized method. *Clinical Biochemistry, 14 (1), 28-31.*

Friedwald, W.T, Levy, R. I. and Fredrickson, D.S. (1972). Estimation of the concentration of low density lipoprotein cholesterol in plasma, without use of preparative ultracentrifuge. *Clinical Chemistry,* 18, 499-502.

Hussain, M.M., Leung, T.M., Zhou, L., and Abu- Merhi, S. (2013). Regulating intestinal function to reduce Atherogenic Lipoproteins. *Clinical Lipidology. 8(4), 481-490.*

Islam, N. A. F., Chowdhurry, M.A.R., Kibria, G.M. and Akhter, S. (2010). Study of serum lipid profile in preeclampsia and eclampsia. *Faridpur Medical College Journal, 5, 56-59.*

Liu, Y., Salter, H.K., Johnson, C.M., Stephens, E., Lakarvsky, P.J., Walshaw, J. and Bullock, S.L. (2013). Bi caudal- D uses a parallel, homodimer coiled coil with heterotypic registry to coordinate recruitment of cargos to dyeing. *Genes Development, 27 (11), 1233-1246.*

Mannisto, T., Mendola, P., Vaarasmaki, M., Jarvelin, M. R., Hartikainen, A. L. and Pouta, A. (2013). Elevated blood pressure in pregnancy and subsequent chronic disease risk. *Circulation, 127(6), 681-690.*

Nazir, D. J. and McQueen, M.J. (1993). Evaluation of a turbidimetric procedure for Apolipoproteins A1 and B on the CIBA Corning 550 Express. *Clinical Biochemistry, 26 (6), 477-482.*

Oladapo- Akinfolarin, T. T., Bartimeaus, E. S., Nwachukwu, E. O. and Nduka, N. (2017). Assessment of C-reactive protein levels in normotensive and hypertensive pregnant subjects in Port Harcourt, Nigeria. *World Journal of Pharmaceutical and Medical Research,* 3(10), 292-296

Oladapo- Akinfolarin, T. T., Bartimeaus, E. S., Nwachukwu, E. O. and Nduka, N. (2018). Assessment lipoprotein levels in normotensive and hypertensive pregnant women in Port Harcourt, Nigeria. *American Journal of Biomedical Sciences,* 10(1), 18-27

Raghuram, P., Arjun, S. S., Prakash, K. K., Babita, G., Varshil, M., Kunal, B. and Aadhijaya, B. (2016). Evaluation of lipid profile in second and third trimester of pregnancy. *Journal of Clinical and Diagnostic Research,* 10(3), 12-16

Richard, J. J., Mehmet, K., Dukhee, K., Laura, G. L. and Daniel, F. (2011).Uric acid: A clinically useful marker to distinguish preeclampsia from gestational hypertension. *Hypertension*, 58(4), 548-549

Shen, H., Liu, X., Chen, Y., HE, B. and Cheng, W. (2016). Associations of lipid levels during gestation with hypertensive disorders of pregnancy and gestational diabetes mellitus: A prospective longitudinal cohort study. *British medical Journal Open, 6, e013509.doi:10, 1136.*

Tietz, N.W. (1987).Laboratory Methods. In: Fundamentals of Clinical Chemistry, 3rd Edition, W.B. Saunders Company, Philadelphia, Pp927-962