Original Research Article

Effect of Gravidity on Biochemical Parameters in Normotensives and Hypertensive 2nd Trimester Pregnant Women

Abstract

The study was aimed at evaluating the effect of gravidity on selected biochemical parameters of cardiovascular disease in normotensive and hypertensive 2nd trimester pregnant women attending antenatal clinics at Rivers State University Teaching Hospital. The 100 consenting subjects who participated in the study were pregnant women in their second trimester. These subjects were randomly selected and divided into two main groups; normotensive pregnant women and hypertensive pregnant women groups. The groups were further divided based on gravidity; primigravida (number of pregnancy=1), multigravida (number of pregnancy>1) and grand multigravida (number of pregnancy≥5). Fasting blood sample was collected using venipuncture method and dispensed into plain bottles for TC, TG, HDL, LDL, ApoA1, ApoB, UA, VLDL and CRP determination. The result represented showed that gravidity had significant impact only on TG, HDL and VLDL among the subgroups, P-value<0.05. While in hypertensive subjects, gravidity had no effect on cardiovascular markers, uric acid level was significant, P-value<0.05. This study has shown that certain lipids are affected due to metabolic changes in 2nd trimester of pregnancy especially in normotensive subjects and these changes increase with increase in gravidity.

Keywords: *Gravidity, pregnancy, cardiovascular marker, hypertensive women, normotensive women*

1.0 Introduction

Gravidity is the number of times a woman has been pregnant. (Huda *et al.,* 2009). Normal pregnancy 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) (Huda *et al*., 2009).

Pregnancy comes with physiological changes to support foetal growth and development such as lipid metabolic changes (Brizzi *et al.,* 1999). Natural rising of plasma lipids is seen in normal pregnancy, but this event is not artheriogenic and it is believed this process is under hormonal control (Rovinsky & Jaffin, 1966), but in complicated pregnancy, there is a possible defect in the mechanism of adjusting physiologic hyperlipidaemia (Cunningham *et*

al., 2005). Lipoproteins are a group of lipids that circulate in plasma in complexes not bound to albumin, low density lipoprotein (LDL), high density lipoprotein (HDL) and very low-density lipoprotein (VLDL) (Rifai & Wanick, 2006). Plasma lipoproteins are responsible for the transport and delivery of lipids throughout the body (Rifai & Wanick, 2006). Plasma lipid profiles in the first trimester of pregnancy may predict the incidence and severity of pre-eclampsia.(Setareh *et al*., 2009). The anabolic phase of early pregnancy encourages lipogenesis and fat storage in preparation for rapid foetal growth in late pregnancy. (Kaaja, 1998). Lipolysis is increased as a result of insulin resistance, leading to increased flux of fatty acids to the liver promoting the synthesis of very low-density lipoproteins (LDLs) and increased triglyceride (TG) concentrations. Because of a decrease in the activity of lipoprotein lipase, very-LDL remains in the plasma for longer and leads to the accumulation of LDL. An increase in LDL is associated with the development of atherosclerosis. (Ross, 1999) Abnormal lipid metabolism also seems important in the pathogenesis of pregnancy-induced hypertension (PIH). Obviously, the association of serum lipids with gestational proteinuric hypertension is highly suggestive of a role for lipid profile analysis as a diagnostic tool. (Jayanta *et al*., 2006)

During the course of normal pregnancy, plasma triglyceride and cholesterol concentrations rise and as pregnancy progresses both become normal (Patrizia *et al.,*1999) .
Another hypothesis is that hypertriglyceridemia is probably a consequence of competition between chylomicrons and very LDL cholesterol for the lipoprotein lipase. The conclusion of another study also indicated that there exists a consistent positive association between elevated maternal TG and the risk of pre-eclampsia. (Ray *et al*., 2006).

Hypertension in 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 (Manisto *et al.,* 2013). It is therefore imperative to investigate the effect of gravidity on most of the biochemical parameters in normotensive and hypertensive 2nd trimester pregnant women.

2.0 Materials and Methods

2.1 Study Design

The cross-sectional study conducted at Rivers State University Teaching Hospital, Port Harcourt was designed with the participation of 100 pregnant women in their second trimester of pregnancy. These 100 subjects were divided into two main groups; normotensive (n=50) and hypertensive (n=50) based on their clinical folder report. Each of the main groups were further divided into three subgroups; primigravida (number of pregnancy=1), multigravida (number of pregnancy>1) and grand multigravida (number of pregnancy≥5). Normotensive subgroups had the following subject participation; primigravida (n=15), multigravida (n=27) and grand multigravida (n=8) while hypertensive subgroups had the following; primigravida (n=21), multigravida (n=21) and grand multigravida (n=4). The data generated were compared among the subgroups for each group.

2.2 Ethical consideration and consent

The Ethics Committee of Rivers State Ministry of Health provided the ethical approval for the study while the subjects provided written consent to participate in the study.

2.3 Study Eligibility

All subjects registered in the facility (Rivers State University Teaching Hospital) for antenatal purpose were included in the study having provided written consent to participate in the study but subjects assessing other form of healthcare service other than antenatal care were not included. Also, subjects with unconfirmed pregnancy such as fibroid growth perceived to be pregnancy were excluded.

2.4 Sampling Method

Simple sampling technique that employed the use of selection of numbers between "0" and "1" was adopted, such that subjects who picked '0" were not selected while subjects who picked "1" were selected (Fyneface *et al.,* 2018; Fyneface *et al.,* 2020).

2.5 Sample collection method

Sample for analysis collected was blood. The sample was collected in fasting condition via venipuncture technique. The collected sample was dispensed in plain bottle, allowed to cloth, spun to separate the serum and store in refrigerator at $4^{o}C$ until the time for analysis (Oladapo- Akinfolarin *et al*., 2017; Oladapo- Akinfolarin *et al*., 2018).

2.6 Laboratory Methods

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

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 $37^{0}C$ 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)

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).

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

Determination of Apo Lipoprotein A1 in Human Serum

Apolipoprotein A1 was measured quantitatively by turbidimetric method (Nazir and McQueen, 1993) as described by Fortress Diagnostics Limited (United Kingdom).

Procedure

20 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^oC 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^oC 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

Apolipoprotein B was measured quantitatively by turbidimetric method (Nazir and McQueen, 1993) as described by Fortress Diagnostics Limited (United Kingdom).

Procedure

20 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^oC 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^oC 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 Uric Acid in Serum

Uric acid was determined quantitatively by enzymatic method (Barr, 1990) as described by Randox Laboratories Limited (United Kingdom).

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 37^0C. It was then read in a spectrophotometer at 520 nm wavelength.

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

C-reactive protein was measured quantitatively by turbidimetric method (Nazir and McQueen, 1993) as described by Fortress Diagnostics Limited (United Kingdom).

Procedure

20µl of sample was added to the test tubes, and tubes used for calibration. 250ul of assay buffer (see composition in appendix) was added to all the tubes. It was mixed by tilting the bottom of the tubes and then incubated at 37^0C for 5 minutes and read as absorbance OD1. 50ul of 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 37^0C for 3 minutes and read as absorbance OD2.

2.6 Statistical Analysis

The data gathered in this study was statistically analyzed for descriptive statistics for mean±SD and inferential statistics (ANOVA) using SPSS version 23.0. Level of significance was set at $P<0.05$.

3.0 Results

Table 1.0 (a) shows the effect of gravidity on biochemical parameters among normotensive pregnant women in their 2^{nd} trimester. The result show that there was a significant difference in TG among the gravidity groups (primigravida, multigravida and grand multigravida), P-value <0.05. Also there was a significant difference in HDL and VLDL among the gravidity groups, P-value <0.05. Other parameters studied had no significant difference, P-value >0.05.

Table 1.0(a): Effect of Gravidity on Biochemical Parameters in Normotensives 2^{nd} Trimester

Parameters	Normotensive	P-value	F-

		women			value
	Primigravida (1) N=15	Multigravida (>1) N=27	Grand Multigravida (≥5) N=8		
TC(mmol/l)	4.31 ± 0.33	4.37 ± 0.50	4.46 ± 0.59	0.7706	0.2617
TG (mmol/l)	1.15 ± 0.18	1.32 ± 0.22	1.50 ± 0.28	**0.0023**	0.9320
HDL(mmol/l)	0.76 ± 0.15	0.89 ± 0.20	0.96 ± 0.23	**0.0441**	0.9320
LDL (mmol/l)	3.05 ± 0.22	2.92 ± 0.47	2.84 ± 0.37	0.3990	0.9369
APoA1 (mg/dl)	340.90 ± 20.71	341.50 ± 40.90	348.50 ± 40.92	0.8737	0.1354
APoB (mg/dl)	131.40 ± 15.43	130.40 ± 26.83	136.30 ± 21.13	0.8803	0.1278
CRP(mg/L)	3.85 ± 1.17	4.63 ± 1.76	4.05 ± 0.82	0.2155	1.5860
VLDL (mmol/l)	0.52 ± 0.08	0.60 ± 0.10	0.68 ± 0.13	**0.0023**	6.9310
UA (mg/dl)	5.03 ± 0.40	5.13 ± 0.42	4.99 ± 0.49	0.6183	0.4857

Table 1.0(b) shows the comparison of gravidity between groups. The comparison shows that comparison between primigravida and grand multigravida was statistically significant in TG values between both groups, P-value<0.05. Similarly, the comparison between primigravida and grand multigravida was statistically significant in VLDL values between both groups, P-value<0.05. Other comparisons were not statistically significant, P>0.05.

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

Parameters	Primagravida vs. Multigravida	Primagravida vs Grand multigravida	Multigravida vs Grand Multigravida
TC(mmol/l)	0.9253	0.7515	0.8786
TG (mmol/l)	0.0511	**0.0019**	0.1160
HDL(mmol/l)	0.1224	0.0543	0.5867
LDL (mmol/l)	0.5263	0.4322	0.8784
APoA1 (mg/dl)	0.9988	0.8816	0.8796
APoB (mg/dl)	0.9952	0.8801	0.8948
CRP(mg/L)	0.2173	0.9432	0.5776
VLDL (mmol/l)	0.0511	**0.0019**	0.1161
UA (mg/dl)	0.7355	0.9761	0.6879

Table 2.0 (a) shows the effect of gravidity on biochemical parameters among hypertensive pregnant women in their 2nd trimester. The result show that there was no significant difference in the studied parameters among the gravidity groups (primigravida, multigravida and grand multigravida), P-value >0.05. However, there was a significant difference in UA among the gravidity groups, P-value <0.05.

Table 2.0 (a): Effect of Gravidity on Biochemical Parameters in Hypertensive 2nd Trimester

Parameters	**Hypertensive Women**			**P-value**	**F-value**
	Primigravida (1) n = 21	Multigravida(>1) n = 25	Grand Multigravida (≥ 5) n = 4		
TC(mmol/l)	4.86 ± 0.37	4.96 ± 0.32	4.80 ± 0.23	0.5109	0.6812
TG (mmol/l)	1.53 ± 0.33	1.57 ± 0.28	1.70 ± 0.35	0.6074	0.5039
HDL(mmol/l)	0.93 ± 0.20	0.99 ± 0.22	1.10 ± 0.12	0.2919	1.6240
LDL (mmol/l)	3.23 ± 0.29	3.24 ± 0.24	2.95 ± 0.06	0.1148	2.2670
APoA1 (mg/dl)	361.60 ± 26.96	361.40 ±29.82	358.50 ±15.59	0.9789	0.0214
APoB (mg/dl)	122.30 ± 14.41	121.00 ± 12.24	127.00 ± 5.77	0.6904	0.3735
CRP(mg/L)	7.61 ± 2.04	7.28 ± 1.75	7.50 ± 1.27	0.8293	0.1879
VLDL (mmol/l)	0.70 ± 0.15	0.71 ± 0.13	0.77 ± 0.16	0.6074	0.5039
UA (mg/dl)	4.81 ± 0.30	4.67 ± 0.40	4.25 ± 0.29	**0.0200**	4.254

Table 2.0(b) shows the comparison of gravidity between groups. The comparison shows that comparison between primigravida and grand multigravida was statistically significant in UA values between both groups, P-value<0.05. Other comparisons were not statistically significant, P>0.05.

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

Parameters	**Primagravida vs. Multigravida**	**Primagravida vs Grand multigravida**	**Multigravida vs Grand Multigravida**
TC(mmol/l)	0.5938	0.9454	0.6671
TG (mmol/l)	0.9054	0.5841	0.7209
HDL(mmol/l)	0.5699	0.3155	0.6250
LDL (mmol/l)	0.9993	0.1176	0.1072
APoA1 (mg/dl)	0.9999	0.9778	0.9791
APoB (mg/dl)	0.9433	0.7826	0.6700
CRP(mg/L)	0.8159	0.9930	0.9736
VLDL (mmol/l)	0.9054	0.5841	0.7209
UA (mg/dl)	0.5030	**0.0152**	0.0625

4.0 Discussion

From the result gotten, triglyceride (TG) was significantly higher in grand multigravida compared with primigravida and multigravida (p=0.0023) of normotensive pregnant women at 2nd trimester. High Density Lipoprotein (HDL) was also significantly higher in grand multigravida compared with primigravida and multigravida (p=0.0441) of the normotensive pregnant women at 2nd trimester. Very Low Density Lipoprotein (VLDL) was significantly higher in grand multigravida compared with primigravida and mutigravida (p=0.0023). This indicates that the higher the number of pregnancy, the higher the TG, HDL and VLDL concentrations in normotensive pregnant women at 2nd trimester. Using Turkey multiple comparison, it showed that TG was significantly higher when grand multigravida was compared with primigravida of Normotensive pregnant women at 2nd trimester (p=0.0019). VLDL was also significantly higher in grand multigravida of Normotensive pregnant women at 2nd trimester compared with primigravida of Normotensive pregnant women at 2nd trimester (p=0.0019) indicating that the higher the number of pregnancy, the higher the concentration of TG and VLDL. The result also showed that gravidity had no effect on other biochemical parameters such as Total Cholesterol (TC), Low density Lipoprotein (LDL), ApoA1, ApoB, C-reactive protein (CRP) and Uric Acid (UA). Gravidity were found to be positively associated with prevalence of metabolic syndrome and multiparus women have increased risk of developing metabolic syndrome according to Sharmimma *et al*., 2013.

The results from this work agrees with Siddiqui, (2014) that there are no statistical differences in TC, and LDL in Normotensive women and therefore not related with the disease, and that TG increased statistically in normotensives and that the increase is involved in endothelial damage leading to preeclampsia.

In this study, TG, HDL and VLDL were statistically increased in Normotensive at $p < 0.05$. It also disagrees with Bayhan *et al*. (2005) who assessed 25 pregnant women that developed mild preeclampsia, 28 pregnant women that developed severe preeclampsia and 25 pregnant women in a control group, during the third trimester and found a significant decrease in HDL levels in patients that developed preeclampsia.

The result further showed that gravidity brought about a significant reduction in UA concentration in multigravida and grand multigravida of pregnant women at 2nd trimester compared with primigravida of pregnant women at 2nd trimester (p=0.0200). This suggests that the higher the number of pregnancy, the lower the Uric acid Concentration. Using Tukey multiple comparison, UA was significantly lower in grand multigravida of hypertensive pregnant women at 2nd trimester compared with primigravida of hypertensive pregnant women at 2nd trimester (p=0.0152) at $p<0.05$ while gravidity had no effect on other biochemical parameters.

This work disagrees with Enquobahrie *et al*. (2004) that there was a significant rise in LDL concentration in the preeclamptic women than in normal pregnant women. However, this work agrees with Enquobahrie (2009) and Clausen (2001) that there was no significant difference in mean total cholesterol concentration in the preeclamptic group when compared with that in normal pregnant group. The finding in this work agrees with Tam *et al*. (2018) that maternal serum uric acid concentration was a good prognostic factor for monitoring, and prognosis of fetal/neonatal outcomes in women with preeclampsia/eclampsia. There was a relationship between high uric acid level and the risk of preterm birth, low Apgar

index, and neonatal death, but not fetal death. Contrarily, the level of significance in this work was a decreased value of uric acid in hypertensive pregnant women.
Preeclampsia is characterized by inflammation, although the onset is placental origin, a network of unfavourable responses is released, this inflammatory responses is complex involving multiple cytokines such as interleukin 6 and tumor necrosis factor– α which are elevated in preeclampsia. These cytokines support the expression of the acute – phase protein C-reactive protein. According to Mohaupt (2015), an increase in CRP is associated with preeclampsia. The author demonstrated the placenta as the production site for CRP in addition to the liver in non-pregnant conditions but infused CRP at a concentration comparable to those found in the circulation of preeclamptic women into mice, which led to hypertension, glomerular damage and associated proteinuria as well as to features of premature atherosclerosis within the placenta. This disagrees with the result of this research that the CRP values increases, but there was no significant effect in the CRP concentration. Rebelo *et al*. (2013) also showed a positive association between CRP levels and development of preeclampsia, though admonished that other factors should be considered.
The parameters Apo A1, Apo B and Apo B / ApoA1 were not significant at $p < 0.05$. These values disagree in part with Timur *et al.* (2016). In their work on the Apolipoprotein levels in women with preeclampsia, they found out that Apo B and Apo B / Apo A1 were significantly increased, but Apo A1 was significantly decreased and advocated that Apo A1 and Apo B/Apo A1 ratio be useful markers in patients with preeclampsia.

Conclusion

Changes in lipid profile in pregnancy is a long existing fact, however, this study has not only pointed to the fact that these lipid cardiovascular markers change in pregnancy but some of these markers increase with increasing gravidity, therefore, it is important that cardiovascular risk assessment be included in antenatal clinics.

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.

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.

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.

Bayhan, G., Koiyigit, Y., Atamar, A., Atamar, Y. & Akkus, Z. (2005).Potential Atherogenic Roles of Lipids, Lipoprotein (a) and Lipid Peroxidation in Preeclampsia.*Gynecology and Endocrinology, 21, 1 -6.*

Clausen, T., D., Djurovic, S.A. & Henriksen, T. (2001).Dyslipidemia in Early Second Trimester.*Journal of Obstetric Gynecology, 108, 1081-1087*.

Cunningham, F.G., Leveno, K..J. & Bloom, S.L. (2005). Williams Obstetrics. 22nd ed. New York: McGraw-Hill, pp 762–808 and pp 126–32*.*

Enquobaline, D.A., Williams, M.A., Butler, C.L., Frederick, J.D., Muller, R.S. & Luthy, D. (2004).Maternal Plasma Lipid Concentrations in Early Pregnancy and Risk of Preeclampsia.*American Journal Hypertension, 17, 574 – 581.*

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.

Fyneface, C. A., Joel, B. K. and Felix, E. K. (2020). Assessment of creatinine levels in blood and saliva of haemodialysed subjects. International Journal of Advances in Nephrology Research, 3(1), 21-25

Fyneface, C. A., Onengiyeofori, I. and Davies, T. (2018). Evaluation of Saliva for Monitoring Renal Function in Haemodialysis Patients at University of Port Harcourt Teaching Hospital. Asian Journal of Biochemistry, Genetics and Molecular Biology, 1(2), 1-6Jayanta, D., Ananda, K.M. & Pradip, K.S. (2006). Study of serum lipid profile in pregnancy-induced hypertension. *Ind ian Journal of Clinical Biochemistry,* 21, 165–8*.*

Kaaja, R. (1998). Insulin resistance syndrome in pre-eclampsia. *Seminal Reproduction and Endocrinology* 16, 41–6*.*

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), 18-27Patrizia, B., Giancarlo, T. & Franca, E. (1999), Lipoprotein metabolism during normal pregnancy. *American Journal of Obstetrics and Gynecology,* 181(2), 430-4.

Pederson, E.B., Johannesen, P, (1984). calcium parathyroid hormone and calcitonin in normal pregnancy and preeclampsia ,Gynecology and obstelogy Investigation. 18, 156-64.

Ray, J.G., Diamond, P., Singh, G. & Bell, C.M. (2006). Brief overview of maternal triglycerides as a risk factor for pre-eclampsia. *British Journal of Obstetrics and Gynaecology,* 113, 379–86.

Rifai, N. and Warnick, G.B. (2006).Lipids, lipoproteins, Apolipoproteins and other Cardiovascular Risk factors.In Tietz, Textbook of Clinical chemistry and molecular Diagnostics, edited by Carl, A.Burtis, Edward, R. Ashwood and David, E. Bruns. 4th Edition, Elsevier, Pp 962-963.

Ross, R. (1999). Atherosclerosis: an inflammatory disease. *New England Journal of Medicine,* 340, 115–26.

Rovinsky JJ, Jaffin H. Cardiovascular hemodynamics in pregnancy. III. Cardiac rate, stroke volume, total peripheral resistance, and central blood volume in multiple pregnancy. Synthesis of results. *American Journal of Obstetrics and Gynaecology,* 95, 787–94.

Sattar, N., Greer, I.A., Louden, J. (1997). Lipoprotein subfraction changes in normal pregnancy: threshold effect of plasma triglyceride on appearance of small dense low density lipoprotein. *Journal of Clinical Endocrinology and Metabolism* 1997; 82: 2483–91.

Setareh, A., Mitra, M.G. & Sedigheh, B. (2009). Maternal plasma lipid concentrations in first trimester of pregnancy and risk of severe pre-eclampsia. *Pak Journal of Medical Science,* 25: 563–7

Siddiqui, I.A. (2014). Maternal Serum Lipids in Women with Preeclampsia.*Annals of Medical Health Sciences Research, 4 (4), 638 – 641.*

Tam, M., Lelong, H., Nguyennam, L., Phan, D.D., Lehuy, V.Q. and Nguyen A. (2018).Maternal Serum Uric Acid Concentration and Pregnancy Outcomes in Women with Preeclampsia/Eclampsia.*International Journal of Gynecology and Obstetrics, 144, 1.*

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