LIPID PROFILE IN NORMAL PREGNANCY AND IN PREGNANCY INDUCED HYPERTENSION

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JUNE 2018
DECLARATION

This thesis is my original work and has not been presented for a degree in any other University or for any other award.

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Signature .................................................. Date 10-07-2018

We confirm that the work reported in this thesis was carried out by the candidate under our supervision.

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DEDICATION

This work is dedicated to my daughter Abigail Mwongeli Muindu.
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### ABBREVIATIONS AND ACRONYMS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
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<tbody>
<tr>
<td>ACOG</td>
<td>American College of Obstetricians and Gynecologists</td>
</tr>
<tr>
<td>ANC</td>
<td>Ante Natal Care</td>
</tr>
<tr>
<td>BP</td>
<td>Blood pressure</td>
</tr>
<tr>
<td>CETP</td>
<td>Cholesterol Ester Transfer Protein</td>
</tr>
<tr>
<td>DBP</td>
<td>Diastolic Blood Pressure</td>
</tr>
<tr>
<td>DHEAS</td>
<td>Dehydroepiandrosterone sulphate</td>
</tr>
<tr>
<td>ERC</td>
<td>Ethical review committee</td>
</tr>
<tr>
<td>GA</td>
<td>Gestational Age</td>
</tr>
<tr>
<td>HBsAg</td>
<td>Hepatitis B surface Antigen</td>
</tr>
<tr>
<td>HDL-C</td>
<td>High Density Lipoprotein Cholesterol</td>
</tr>
<tr>
<td>HDP</td>
<td>Hypertensive disease in pregnancy</td>
</tr>
<tr>
<td>HELLP</td>
<td>Hemolysis, elevated liver enzymes, and low platelets</td>
</tr>
<tr>
<td>HIV</td>
<td>Human Immunodeficiency Virus</td>
</tr>
<tr>
<td>HPL</td>
<td>Human placental lactogen</td>
</tr>
<tr>
<td>KNH</td>
<td>Kenyatta national hospital</td>
</tr>
<tr>
<td>LDL-C</td>
<td>Low Density Lipoprotein Cholesterol</td>
</tr>
<tr>
<td>LPL</td>
<td>Lipoprotein lipase</td>
</tr>
<tr>
<td>MAP</td>
<td>Mean Arterial Pressure</td>
</tr>
<tr>
<td>OR</td>
<td>Odds Ratio</td>
</tr>
<tr>
<td>P1*</td>
<td>Probability of exposure given disease presence</td>
</tr>
<tr>
<td>P2*</td>
<td>Probability of exposure given disease absence</td>
</tr>
<tr>
<td>SBP</td>
<td>Systolic Blood Pressure</td>
</tr>
<tr>
<td>SPSS</td>
<td>Statistical Package for Social Sciences</td>
</tr>
<tr>
<td>TC</td>
<td>Total Cholesterol</td>
</tr>
<tr>
<td>TG</td>
<td>Triglycerides</td>
</tr>
<tr>
<td>VDRL</td>
<td>Venereal Disease Research Laboratory</td>
</tr>
<tr>
<td>VLDL</td>
<td>Very Low Density Lipoprotein</td>
</tr>
<tr>
<td>WHO</td>
<td>World Health Organization</td>
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</table>
### DEFINITION OF TERMS

- **Third trimester:** Gestational age ≥26 weeks.
- **Dyslipidemia:** Abnormally high lipids in blood.
- **Chronic hypertension:** Systolic blood pressure ≥140 or diastolic blood pressure ≥90 mmHg at <20 weeks gestation, and/or hypertension diagnosed at any time during pregnancy.
- **Mild preeclampsia:** Systolic blood pressure ≥140 or diastolic blood pressure ≥90 mmHg on two occasions two hours apart with 1+ proteinuria on dipstick.
- **Severe preeclampsia:** Systolic blood pressure ≥160 or diastolic blood pressure ≥90 mmHg on two occasions two hours apart with 3+ proteinuria on dipstick and end-organ signs.
- **Gestational hypertension:** Systolic blood pressure ≥140 or diastolic blood pressure ≥90 mmHg in the absence of proteinuria.
- **Hypercholestoraemia:** Serum or plasma cholesterol level >5.7mmol/l
- **Case:** A pregnant woman in the third trimester diagnosed with hypertensive disease in pregnancy in the absence of comorbid conditions.
- **Control:** A healthy pregnant woman in the third trimester having normal blood pressure.
Hypertensive disease in pregnancy (HDP) is a major cause of morbidity for both mother and fetus. Currently HDP is diagnosed by elevated blood pressure (BP) \( \geq 140/90 \) mmHg and end organ damage including proteinuria both of which can occur due to preexisting conditions. The potential to lower lipids could help to reduce the maternal mortality and complications associated with HDP. The objective of the study was to determine and compare the changes in lipid profile in hypertensive and normal pregnancies during the third trimester of pregnancy. The effects of parity and maternal age on the lipid profiles of cases of HDP were also evaluated. This was a matched case control study comprising of 85 cases with an equal number of controls matched on maternal age, gestational age and parity of the cases. Fasting blood samples were collected from the participants by venipuncture and allowed to clot to obtain serum which was analysed for TC, TG and HDL-C using enzymatic and spectrophotometric methods in an autoanalyzer. The serum LDL-C level was calculated using the Friedewald’s formula. Three of the four parameters TC, TG and LDL-C were statistically significantly different between hypertensive cases and normotensive controls \((p<0.05)\). In contrast there was no statistically significant difference for HDL-C between the hypertensive and control groups \((p = 0.11)\). The mean value of the ratio of TC to HDL-C in the cases was 4.52 ± 2.21 while the mean value for controls was 3.50 ± 0.68 showing statistically significant value, \( t = 4.07, P = 0.0001 \). The mean serum levels of TC, TG, HDL-C and LDL-C were not statistically significant when one-way ANOVA and post-ANOVA was performed on cases of varying categories of HDP. Pearson correlation analysis results showed that parity and maternal age do not influence the lipid profiles of cases of hypertensive disease in pregnancy. This study observed that the serum levels of TC, TG and LDL-C are elevated in hypertensive pregnant women relative to normotensive pregnant women. This study recommends that fasting lipid profile test should be considered as a screening test for HDP in addition to the testing for proteins in urine. The management of HDP should not only focus on lowering blood pressure but also in lowering the circulating levels of TC, TG and LDL-C.
CHAPTER ONE
INTRODUCTION

1.1 Background Information

Hypertension during pregnancy is a major obstetric problem and a leading cause of maternal and perinatal morbidity and mortality (Swati et al., 2014). Hypertension in pregnancy is defined as a systolic blood pressure of 140 mmHg or greater or a diastolic blood pressure of 90 mmHg or greater occurring after the 20th week of pregnancy until 6-12 weeks postpartum. It is known that high levels of steroid hormones occur as pregnancy advances. The circulating steroids are derived from cholesterol and therefore lipid metabolism is very intriguing as cholesterol is a major factor in the development of atherosclerosis (Deepak and Digisha, 2011). Foetal growth and development leads to increased demand for metabolic substrates such as essential fatty acids and long chain polyunsaturated fatty acids. Maternal lipid metabolism leads to accumulation of fats and hyperlipidemia (Vani et al., 2015).

The lipid profile test is used to detect disorders of lipid metabolism and the analytes assayed are total cholesterol (TC), triglycerides (TG), high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C) and very low density lipoprotein (VLDL). The ratio TC:HDL is a determinant of the predisposition to the risk of atherosclerosis (De et al., 2006).
Previous studies have reported a positive correlation between increased levels of TC, TG, LDL and VLDL and the occurrence of hypertension whereas, at the same time, the levels of HDL are decreased. The TC, TG, LDL and VLDL are known as bad cholesterols as they are involved in the pathophysiology of several diseases (Anjum et al., 2013). Hypercholestaemia and hypertriglyceridemia are risk factors for the development of atherosclerotic plaques with hypertriglyceridemia being more common. The primary source of cholesterol is diet; however the liver and other body tissues have the capacity to synthesize cholesterol (Murray et al., 2003). Abnormal lipid metabolism has been reported to cause atherosclerosis in cases of chronic hypertension. The outcome of hypertensive disease in pregnancy (HDP) is affected by factors such as gestational age at onset, severity of disease, and the presence of co-occurring conditions such as diabetes mellitus, renal disease or preexisting hypertension (Heard et al., 2004). The outcomes of hypertensive pregnancies range from uneventful pregnancy in women with chronic uncontrolled hypertension to death in cases of eclampsia. The major adverse maternal outcomes include central nervous system injuries such as seizures occurring in eclampsia, hemorrhagic and ischemic strokes, hepatic damage ranging from transaminase elevation, hemolysis, elevated liver enzymes and low platelet count (HELLP) syndrome, hepatic failure, renal dysfunction as well as increased frequency of cesarean delivery, preterm delivery, and abruptio placentae (Mackay et al., 2001).

The effects of controlled hypertension in pregnancy on the fetus are minimal. However, gestational hypertension, preeclampsia and eclampsia can lead to
increased cases of induced labor, fetal growth restriction and neonatal respiratory distress. Hypertensive disease in pregnancy, even in its mild forms contributes to increased risk for perinatal or fetal death (Lindheimer, 2009). Although HDP is usually a pregnancy specific problem that resolves itself with delivery, it posses significant risk complications such as infants born small and premature who often face developmental delays (Hauth et al., 2000).

1.2 Problem Statement

Pregnancy is characterized by increased levels of steroid hormones which are derived from cholesterol. Hypercholestoraemia leads to an abnormal lipid profile which is strongly associated with atherosclerotic cardiovascular diseases such as hypertension during pregnancy and is a global health problem accounting for 10–15% of maternal deaths and it complicates 18% of all pregnancies in Kenya. Up to 696 cases of HDP have been reported at KNH from January to November 2014, 26 of which resulted in death (KNH health information department). Studies in different populations show that hyperlipidemia in pregnancy is a normal finding although the change in lipid parameters differs in each population. It is therefore not possible to conclude that there is a certain pattern of dyslipidemia which leads to HDP all over the world. In Kenya, clinical laboratories do not have specific reference values for the pregnant women population and this further complicates the diagnosis of abnormal lipid profile during pregnancy. Currently HDP is diagnosed by elevated blood pressure ≥140/90 mmHg and end organ damage including proteinuria both of which can occur due to preexisting conditions. The current management of HDP is
by delivery, administration of anti-hypertensives to lower the BP and administration of magnesium sulfate to prevent convulsions in women already diagnosed with the disease.

1.3 Justification and significance

This study will reveal the population specific changes in lipid metabolism during normal and hypertensive pregnancies. The findings of this study will be used to determine the role of lipid metabolism in the ethiopathogenesis of HDP. If lipids are found to be implicated in this pathological process, intervention strategies can be focused not only to lower the blood pressure but also to lower the serum levels of the implicated lipids. The determination of fasting serum lipid profile can be used together with other screening tests for HDP. The potential to lower the implicated lipids will help to reduce maternal mortality and complications associated with HDP.

1.4 Hypotheses

i. Serum levels of TC, TG, HDL-C and LDL-C in hypertensive and normotensive pregnant women are not different.

ii. Parity and maternal age do not influence lipid profiles of women with pregnancy induced hypertension.

iii. The TC:HDL-C ratio is not affected by the severity of pregnancy induced hypertension.
1.6 Objectives

1.6.1 General Objective

To determine and compare the changes in the lipid profile of women with normal blood pressure and those with pregnancy induced hypertension.

1.6.2 Specific Objectives

i. To determine and compare the lipid profile changes of both hypertensive and normotensive women during their third trimester of pregnancy.

ii. To evaluate the association between parity, maternal age and lipid profile changes and the predisposition to pregnancy induced hypertension during the third trimester of pregnancy.

iii. To evaluate the association between TC:HDL-C ratio and severity of HDP during the third trimester of pregnancy.
CHAPTER TWO
LITERATURE REVIEW

2.1 The disease

Hypertensive disease in pregnancy is the commonest medical disorder of pregnancy and one of the major causes of pregnancy-related maternal deaths and relatively little is known about the role of lipids in its pathogenesis. The principal concern about elevated blood pressure (BP) relates to the possible negative effects on both mother and fetus which range in severity from mild to life threatening. The risk of developing hypertensive disease in pregnancy (HDP) is greater in women who have a family history of pre-existing vascular disease, obesity, underage pregnancy, nulliparity, multiple gestation and maternal age 35 years or more. Intrauterine growth restriction, death and pre-maturity are the likely risks for the fetus (Rubina et al., 2007).

2.2 Classification and diagnosis of hypertensive disease in pregnancy

The international classification of diseases (WHO, 2008) classifies hypertensive disorders occurring during pregnancy into five sub-groups depending on its clinical presentation and severity.
2.5.1 Gestational hypertension

Gestational hypertension is the new onset of hypertension in the absence of proteinuria. The SBP ≥140 mmHg and/or DBP ≥90 mmHg at ≥20 weeks GA (ACOG 2013). The BP readings are taken twice at least four hours apart.

2.5.2 Chronic hypertension with superimposed preeclampsia

This is characterized by chronic elevated BP documented either prior to pregnancy or before the 20th week GA which is later complicated by visual disturbances, thrombocytopenia, edema, renal insufficiency and elevated blood levels of liver transaminases (ACOG, 2013).

2.5.3 Preeclampsia

Preeclampsia is characterized by BP ≥ 140 / 90 mmHg accompanied by the presence of proteins in urine exceeding 300 mg/24 hours which is for the first time detected after 20 weeks gestation (ACOG, 2013).

2.5.4 Severe preeclampsia

Blood pressure is ≥ 160/110 mmHg, and proteinuria exceeds 2.0 g/24 hours or 3 + dipstick. Other features diagnostic of severe preeclampsia are onset of cerebral or visual disturbances, thrombocytopenia, pulmonary edema, renal insufficiency,
intrauterine growth restriction and elevated blood levels of aminotransferases (ACOG, 2013). The risk of developing preeclampsia appears to be greater in women who have family history of essential hypertension (Packer, 2005).

2.5.5 Eclampsia

The occurrence of convulsions in a woman having severe preeclampsia characterizes the progression of the disease to eclampsia and this facilitates its diagnosis. Early detection, timely and appropriate management of preeclampsia and severe preeclampsia are critical to lower the risk of eclampsia. During the management of eclampsia the main goal is usually to stop the convulsions, to lower the elevated BP to normal, to promptly deliver the baby while monitoring closely the onset of organ failure (ACOG, 2013).

2.3 Physiological changes during pregnancy

Normal pregnancy is accompanied by vascular physiology changes notably the reduction in the mean arterial pressure (MAP) which reaches its lowest point between the 16th and 20th weeks of gestation with the decrease in diastolic blood pressure (DBP) being higher than that in systolic blood pressure (SBP). The reduction of BP is typically 8-10 mmHg or less than 10% decline from pre-pregnancy levels. After the 20th week, MAP slowly returns to pre-pregnancy levels (Reem et al., 2012).
The female hormonal cycle is an exquisitely controlled system that includes the hypothalamo-pituitary system, gonads, adrenal and thyroid endocrine glands with both positive and negative feedback loops. Steroid hormone levels are affected by thyroid hormones and they themselves contribute to regulation of lipid metabolism. Estrogen and progesterone have been reported to cause expansion of the plasma volume which in turn leads to the decrease in TC (Manju et al., 1998).

During the first trimester the metabolic processes are influenced by the increased levels of progesterone and estrogen as well as the higher insulin levels. Hyperinsulinaemia leads to an increase in peripheral glucose utilization, hypoglycaemia, glycogenesis, increased storage of fat deposits and decreased lipolysis. Maternal metabolic adjustments during late pregnancy involve a sparing of glucose for use by the fetus and an increased concentration of fatty acids in plasma (Freinkel, 1964). The concentrations of total cholesterol (TC), high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C) and triglycerides (TG) are dependent on insulin resistance and estrogen stimulation from the 12th week of gestation. As pregnancy advances a metabolic shift occurs to ensure that the growing fetus has adequate supply of nutrients from the mother. The metabolic shift is characterized by the utilization of fats by maternal tissues while the fetus depends on glucose and amino acids. The fetal tissues use the available cholesterol to build cell membranes while in the maternal system it is used to synthesize steroid hormones after uptake by the placenta. The hypertriglyceridemia
that occurs in pregnancy contributes to growth and development of the fetus as well as acting as an energy reserve for maternal dietary fatty acids (Ghio et al., 2011).

High density lipoprotein cholesterol increases by 12 weeks of gestation in response to estrogen and remains elevated throughout pregnancy. Total cholesterol and LDL-C levels decrease initially, but then increase in the second and third trimesters. Very low density lipoprotein (VLDL) and triglycerides decrease in the first 8 weeks of gestation and then progressively increase until delivery. The clearance of VLDL is altered in the second half of pregnancy due to the reduced activity of lipoprotein lipase (LPL) in adipose tissues (Nancy, 2000).

Human placental lactogen (HPL) is a protein secreted by the placenta and it is important for growth of the foetus. Human placental lactogen increases the rate of lipolysis leading to increased plasma free fatty acids and increases glucose uptake and inhibits gluconeogenesis. The increase of HPL during pregnancy is responsible of the considerable increase of TG, free fatty acids and phospholipids (Cuneyt et al., 2004).

2.4 Lipid metabolism during pregnancy

Early pregnancy is characterized by the accumulation of fat deposits associated with both maternal hyperphagia and increased lipogenesis while the third trimester is marked by the breakdown of fat depots in the body, which is important for fetal development. Therefore the plasma concentration of lipids increases as gestation
progresses (Hadden, 2009). The increased circulating maternal TC is beneficial to the fetus during the first trimester but during late pregnancy fetal tissues are able to synthesize cholesterol. Maternal hypertriglyceridemia occurring during pregnancy correlates with the accumulation of VLDL-C, HDL-C and LDL-C (Herrera and Ortega, 2010).

Lipogenesis during pregnancy occurs due to the enhanced activities of insulin as well as adipose tissue lipoprotein lipase. The fats which are produced are transported across the placenta and metabolized causing elevation of TC and TG throughout pregnancy but the increase of TG is not proportional with gestational age compared to other lipid fractions. It increases up to 2-3 times to pre-pregnancy levels. Belo et al. (2002) reported that these changes are not responsible for induction of any atherogenic process and revert to pre-pregnancy levels after childbirth. The composition and size of LDL-C particles is altered during normal pregnancy. Studies have demonstrated that as TG levels increase, overall LDL size decreases with an increase in proportion of smaller LDL particles which are denser and reported to favour atherogenesis (Belo et al., 2002). The HDL-C levels also increase during normal gestation and this presents a potential protective effect to the mother because it offsets the elevation of atherogenic LDL-C and TG. Multiparous women tend to have relative decrease in HDL-C levels in comparison to their primiparous counterparts (Brizzi et al., 1999).
Increased activity of hepatic lipase and decreased lipoprotein lipase activity characterise normal pregnancies. Hepatic lipase synthesizes TG in the liver, whereas the decrease in the activity of lipoprotein lipase decreases the breakdown of TG in the adipose tissues thus both processes lead to an increase in circulating TG (Rubina et al., 2007). Because of the decrease in LPL activity, VLDL-C remains in the plasma for longer and leads to the accumulation of LDL-C which plays a key role in the development of atherosclerosis (Ross, 1999). Similarly, hyperestrogenaemia in normal gestation increases endogenous production of VLDL and TG by reducing the activity of LPL in the adipose tissue and inhibiting the activity of hepatic lipase. The enhanced hepatic production of VLDL decreases the clearance of these particles from maternal circulation due to reduced LPL activity, high plasma concentration of Cholesterol Ester Transfer Protein (CETP) activity, and low hepatic lipase activity. This may be responsible for the accumulation of VLDL and LDL-C which are rich in triglycerides (Sasa et al., 2012).

The change in concentration of lipoprotein subclasses such as LDL-C during pregnancy is dependent on TG levels. These lipoproteins are composed of subfractions of varying sizes, densities and compositions, whose ability to trigger atherogenesis differs (Rajman et al., 1994). One such sub-fraction of LDL is LDL-3 which is small, dense and does not bind readily to the LDL receptors and thus remains in the circulation for longer time, thus is able to penetrate the arterial intima easily and better than the larger sub-fractions. After penetration they readily undergo oxidation, probably because they contain less vitamin E and other antioxidants. They
initiate atherogenesis after the creation of form cells which follows their uptake into macrophages (Campos et al. 1992).

2.5 Pathogenesis of pregnancy-induced hypertension

Alteration of blood circulation in the uteroplacental system has been the focus of the pathophysiology of hypertensive disease in pregnancy although pregnancy is characterized by changes in vascular physiology. Women with HDP have been reported to present arterial lesions at the uteroplacental implantation site which resembles the atherosclerosis present in chronic hypertensive patients. Alteration of lipid profile may be responsible for the development of these endothelial lesions. The acute clinical symptoms of HDP usually resolve after delivery and this suggests that the presence of the placenta could be linked to the pathogenesis of HDP. To facilitate circulation between the mother and fetus in normal pregnancies the placenta undergoes a process of vascularization involving angiogenesis, vasculogenesis and remodeling of spiral arteries. This process requires that both proangiogenic and antiangiogenic components involved are properly balanced and their imbalance has been reported to trigger abnormal vascularization of the placenta and onset of HDP. The reduction in blood circulation in the uteroplacental system is the key contributing factor in the pathophysiology of this disease (Agarwal and Karumanchi, 2011).

Women with HDP have abnormal placentae with the key pathology appearing to be at the maternal-fetal interface which is characterized by poor trophoblastic invasion of the uterus and incomplete endovascular invasion of the spiral arteries. Venuto and
Lindheimer (2009) hypothesized that the failure of the cytotrophoblasts to infiltrate deep and widen the pathway explains the corelative reduction of blood flow in the uteroplacental system.

2.5 The role of maternal endothelial dysfunction

Abnormal lipid metabolism has been linked with the occurrence of atherosclerotic cardiovascular disease and has an effect on endothelial function. An elevated plasma LDL-C is a known risk factor for atherosclerosis and cardiovascular disease (Manten et al., 2005). The resemblance of the lesions noted in cases of HDP and atherosclerosis lead to speculations of a common pathophysiological pathway. Although studies have indicated the origin of HDP is the placenta, maternal endothelium is the mostly affected tissue and this is demonstrated by the extensive endothelial dysfunction accompanied by vasoconstriction and end organ ischemia. Inappropriate vasoconstriction which tends to favour the hypercoagulable state and the widespread microvascular thrombi are reported uniformly in the placenta of women with preeclampsia and eclampsia (Taylor et al., 2009).

2.6 Lipid profiles in normotensive and hypertensive pregnancies in different population

Hyperlipidemia during pregnancy has been the common finding in studies investigating the association of HDP and abnormal lipid metabolism. Cases of HDP among Indian women were found to have markedly increased serum TC and TG levels. Total cholesterol was highly elevated in the third trimester while the serum TG level increased gradually in the second and third trimesters up to 2.8 times higher
than that of non-pregnant women (Deepak and Digisha, 2011). The hypertriglyceridemia observed in HDP cases in this population is different from other hypertriglyceridemia since it was accompanied by an increase of HDL-C.

Jayanta et al. (2006) while comparing the lipid profile in normotensive and cases of HDP in India found significantly increased serum TG and decreased LDL-C in normotensive pregnancies while TC and HDL-C levels did not depict significant change. From this study it is postulated that the increased TG, found in HDP cases, is likely to be deposited in the uterine spiral arteries and cause endothelial dysfunction through the production of LDL-C particles which could be associated with hypercoagulability. In eclamptic subjects, the mean serum TG value was a bit lower than those observed in preeclamptic subjects. This finding can be accounted for by the fact that eclamptic women are not able to feed orally and also due to the eclamptic process which is linked with liver damage which interrupts the production of TG in liver (Jayanta et al., 2006).

Findings of a different study conducted in India by Lorentsen et al. (1998) revealed that preeclampsia was characterized by increased TG concentration and decreased HDL-C while eclampsia was marked by a fall in HDL-C and a rise in LDL-C. These changes are different from those observed in normotensive pregnancies suggesting that in the Indian population there exists an association between an abnormal lipid profile and the pathophysiology of HDP. The TC:HDL-C ratio was decreased in
normal pregnancy but significantly increased in hypertensive pregnancies which indicates an increased risk for atherosclerosis. The interaction of endothelial cells with endothelial disturbing factors of the placental origin such as lipid peroxidases or a combination of placental factors with the small dense lipoproteins could be regarded as feasible contributors for the pathogenesis of HDP (Lorentsen et al., 1998).

Comparison of lipid profile in cases of HDP and normotensive pregnant women in Turkey (Cuneyt et al., 2004) showed that there is an increase in all lipid profile parameters parallel to the increase in gestational age (GA). The increase in lipid profile parameters analytes in the cases was higher compared to the control group with the increase in serum levels being found to parallel the severity of the disease with a major improvement in the normotensive pregnant women compared to cases of gestational hypertension, chronic hypertension with superimposed preeclampsia, preeclampsia and worst in the eclamptic women. In this population endothelial dysfunction was found to be a predominant event in the pathogenesis of HDP, and lipids were implicated in this event. Free fatty acids, TG, LDL-C, HDL-C, TC and VLDL values were reported to be increased during HDP with lipid peroxidase and cytokines increasing secondary to the increase in lipids which directly or indirectly could disturb endothelial cells leading to vasoconstriction throughout the body. However there was no established correlation between the plasma levels of lipids and prognosis of the disease (Sattar et al., 1996).
A study to assess the lipid profile in Sudanese pregnant women showed that there is a significant decrease in HDL-C and an increase in LDL-C level. The increase in LDL-C was postulated to increase the risk of development of diseases associated with dyslipidemia in pregnancy. However, the concentration of TC and TG were not different from the non-pregnant state. Therefore in this population it's concluded that there is no significant alteration in serum TC during the third trimester (Abdelhai et al., 2013).

Different findings documented by Festus et al. (2011) who carried out research on serum lipid profile in Nigerian women found that the concentration of serum TC, TG, HDL-C and LDL-C in normal pregnancy increased with GA while HDL-C level was low in the second trimester with the concentration of serum TG being elevated in late gestation of normotensive pregnancies. The formation of zygote and subsequent implantation on the uterine wall were responsible for the increased levels of TC and TG in the first trimester. Similar findings of progressively increased TC, TG and LDL-C throughout pregnancy with significantly higher values being recorded after the third trimester were recorded by Munoz et al. (1999). The level of HDL-C in the Nigerian population was observed to be slightly lower in the second trimester compared to the first trimester and this was attributed to the increase in resistance to insulin (Munoz et al., 1999).

Rubina et al. (2007) observed significantly increased serum TG and decreased HDL-C levels in Pakistan women with HDP while in the normotensive counterparts the
plasma TG and TC increased as pregnancy progressed they both become normal. This is brought about by the endogenous female sex hormones. This finding is further attributed to the increased hepatic synthesis of TG occasioned by the activity of hepatic lipase during pregnancy and decreased catabolic activity at the adipose tissue resulting in hypertriglyceridemia. The reduced activity of LPL lowers the rate of catabolism at the adipose tissue which coupled with the delayed hepatic uptake of remnant chylomicrons lead to accumulation of TG in plasma (Patrizia et al., 1999). Increased TG plays a role to decrease the HDL-C particles which transport cholesterol from peripheral tissues to liver. According to Pirzado et al., (1999) there is a direct correlation between adipose tissue LPL activity and plasma HDL-C level which may be responsible for reduced plasma levels of HDL-C.

In India, Pradnya and Jyoti (2013), observed significantly increased serum TC, TG and LDL-C and decreased HDL-C in cases of preeclampsia compared to normotensive pregnant women in whom hyperlipidemia characterized by elevation of TG and TC which is considered physiological was observed. Musa et al. (2014) found a significant decrease in HDL-C as preeclampsia progresses to eclampsia accompanied by a significant increase in TG indicating worsening of lipid derangement with change of disease severity. Therefore from results of this study it is evident that increase in TG levels and reduction of HDL-C levels could be characteristic lipid profile changes as the HDP process becomes severe.
2.8 Causes and consequences of hyperlipidemia

Lipid profile changes in normotensive pregnancies differ in each trimester according to Shital (2012). Normal gestation is characterized by hyperestrogenaemia which triggers hepatic synthesis of endogenous TG, which is transported by VLDL. Progesterone has been observed to oppose the effects of estrogens on lipoprotein metabolism leading to elevation of LDL-C and decreased HDL-C level in circulation (Winny et al., 2008).

Pro-atherogenic patterns in lipid concentration precede clinical manifestations of HDP. Preeclamptic pregnancies are marked by high levels of serum TG, low HDL-C and elevated LDL-C. The elevation of all lipid profile parameters parallel to GA is likely to be secondary to the increase in steroids during pregnancy (Potter and Nestle, 1994). These derangements of elevated LDL-C and low HDL-C are more pronounced in women with HDP.

Elevated TG levels and not TC during the first trimester is associated with unfavourable outcomes for both the mother and the baby according to a study by Virjkotte et al. (2012). Similar findings are documented by Fanshave and Ibrahim, (2013) who concluded that preeclamptic pregnancies are marked by high TG levels, lower HDL-C and increased LDL-C levels. The atherogenic role of dyslipidemia in the pathophysiology of HDP is due to high LDL-C and TG which presents a risk factor for atherosclerosis among preeclamptic women (Siddiqui, 2014).
Hypertriglyceridemia occurs most likely as a consequence of competition between chylomicrons and VLDL for the LPL. The hydrolysis of TG by LPL and the uptake of remnants by the liver are responsible for chylomycin clearance. Delay in the uptake step leads to accumulation of remnants in plasma and it presents the atherogenic risk of hypertriglyceridemia (Rubina and Tabassum, 2007).

2.9 Management and prevention of hyperlipidemia

The management and prognosis of the different hypertensive disorders in pregnancy is very different and it is important that every effort is made to accurately categorize women with hypertension in pregnancy so as to ward off adverse maternal and prenatal outcomes associated with the onset of hemolysis, elevated liver enzymes, low platelet count (HELLP) syndrome and convulsions in women with gestational hypertension. About 15-25% of pregnant women with gestational hypertension are likely to develop preeclampsia with 50% probability of progression in those that develop gestational hypertension before reaching 32 weeks gestation. Therefore cases of gestational hypertension are clinically managed while focusing on close maternal and fetal monitoring for the development of preeclampsia, extremely high BP, maternal end organ involvement and fetal compromise. Blood pressure measurement and urine analysis are performed every time the patient attends antenatal care clinic (Chancellor and Thorp, 2008).
Medical therapy of mild hypertension has not been shown to improve neonatal outcomes and masks the diagnosis and recognition of progression to severe disease. In the absence of comorbid conditions, antihypertensive drugs are used to keep SBP at 120-140 mmHg and DBP at 80-90 mmHg. At 37 weeks GA a clinical evaluation which includes the woman's symptoms, severity of the HDP, wellbeing of the fetus and the favourability of the cervix is done and after which induction of labour is considered because the fundamental treatment of preeclampsia is delivery of the baby.

All antihypertensive drugs used for the treatment of mild to severe HDP cross the placenta but to variable degrees. Labetalol is able to lower maternal BP without any marked fetal side effects; however, it is contraindicated in women with asthma. Methyldopa's stimulation of alpha-2 adrenergic receptor results in a decrease in sympathetic outflow and decreased BP; however, it takes 24 hours to achieve therapeutic levels and therefore not appropriate for fast control of hypertension. Methyldopa has not been linked with any adverse effects on the uteroplacental circulation; however, increase in dosage causes adverse effects such as sedation and depression. Nifedipine, a calcium channel antagonist is a potent antihypertensive which is not administered sublingually as it can reduce the BP, leading to fetal distress. Nifedipine is a long acting antihypertensive without any adverse effects on the uteroplacental circulation. The dose may be increased incrementally if the initial dose fails to satisfactorily control BP until the maximum dose is reached. Adequate control of BP can also be achieved if a second antihypertensive agent is introduced.
(Clare and Louise, 2013). Cases of severe preeclampsia are treated with magnesium sulphate to prevent seizures.

Modification of nutritional components and consumption of specific foods influences hyperlipidemia in non-pregnant women (Kelly, 2010). The most beneficial approach involves reducing the intake of saturated fats while regular aerobic exercise particularly 120 minutes per week has a smaller benefit. Lifestyle modification with a focus on reduction of weight and increase in physical activity during pregnancy prevents the complications of HDP (Dempsy et al., 2004). Lipid metabolism during pregnancy is affected by dietary habits and therefore controlling maternal diet during gestation is likely to influence the synthesis of cholesterol thus alter the plasma cholesterol concentration (Di Ciann et al., 2003).
CHAPTER THREE
MATERIALS AND METHODS

3.1 Study area
The study was carried out at Kenyatta National Hospital between January 2015 and August December 2015. Kenyatta National Hospital is a teaching, research and referral hospital with a bed capacity of 1800. The study participants were chosen from among patients admitted in the antenatal ward and among pregnant women who attended the ANC clinic at KNH during the study period.

3.2 Study design
The study design was matched case control with an equal number of cases and controls. The controls were matched according to the maternal age, gestational age and parity of the cases. The participants were grouped into two groups: pregnant hypertensive women (cases) and normal pregnant women (controls).

3.3 Study population
The study subjects comprised of eligible consenting pregnant women who presented for antenatal care at Kenyatta National Hospital in the third trimester. The average number of pregnant women attending the clinic per month is 2110 with an average of 405 new clients being registered per month. The types of hypertension recorded
among the women attending the clinic range from pre-existing hypertension complicating pregnancy to eclampsia (KNH Health Information Department, 2014).

3.4 Sample size

The sample size for the study was determined using the statistical method by Stanley et al. (1990) used to calculate the adequacy of sample size in health studies for case control studies. The ratio of cases to controls was 1:1 and the odds ratio (OR) was computed using the expression by Gerstman (2008): OR = OT/OC. Where; OT = odds of exposure in the cases and OC = odds of exposure in the controls. Where OT = PT/1-PT and OC = PC/1-PC. PT (Probability of exposure in the cases) = 0.16, PC (Probability of exposure in the controls) = 0.1 Therefore OR = 0.19/0.1 = 1.9 OR=2.

The minimum sample size was calculated using the formula,

\[ n = \frac{Z^2 \cdot \frac{1}{1 - \alpha/2} \cdot \left\{ \frac{1}{\left[ P_1^* \cdot (1 - P_1^*) \right]} + \frac{1}{P_2^* \cdot (1 - P_2^*)} \right\} \cdot \ln(1 - \epsilon)^2 }{ \ln(1 - \epsilon) \}^2 \]

Where: z (confidence interval) = 95%

\[ P_1^* = \frac{(OR) \cdot P_2^*}{(OR) \cdot P_2^* + (1 - P_2^*)} \]

Therefore \((2) \cdot 0.18/(2) \cdot 0.18 + 0.82 = 0.36\)

\[ P_2^* = 0.20\]

\[ \epsilon = 0.50 \] (Relative precision) Therefore, \((1.960)^2 \cdot \left\{ \frac{1}{0.36 \cdot 0.64} + \frac{1}{0.20 \cdot 0.80} \right\} \cdot \ln(1 - 0.50)^2 = 85.\]

A total of 170 study subjects aged 18-45 years in the third trimester were included in the study and they were distributed in two groups: Group I (cases): 85 pregnant
women already diagnosed with pregnancy induced hypertension and Group II (controls): 85 healthy pregnant women.

3.5 Screening and recruitment

Screening forms (Appendix III and IV) were used to identify suitable controls and cases, respectively. Cases were identified both in the ANC clinic and wards while the screening and selection of controls was conducted in the ANC clinic. Screening of cases in the wards was done in the morning before patients were served breakfast and those found to fit the criteria of inclusion were recruited and upon consenting their fasting blood samples were collected then. Gestational age was determined based on the last menstrual cycle. Matching controls were identified within three weeks after locating a case and they were given appointments when fasting blood samples were collected. The screening, recruitment, consenting and specimen collection and analysis process is represented by the following flow chart diagram.
3.6 Inclusion and exclusion criteria

3.6.1 Inclusion criteria of cases

Pregnancy induced hypertension was defined as new onset of hypertension after 20 weeks of pregnancy. Cases were characterized by blood pressure ≥140/90 mm Hg after the 20th week of gestation with or without proteinuria. In addition to elevated blood pressure, inclusion criteria also included consenting pregnant women with no history of pre-existing hypertension aged 18-45 years.

3.6.2 Exclusion criteria of cases

Pregnant women with diabetes mellitus, chronic hypertension before pregnancy, renal diseases, obesity, hyperthyroidism, taking diuretics or any digoxin treatment and familial hypercholesterolaemia were excluded from the study. Women over 45 years were also excluded because pregnancy in those age groups is considered unsafe since the incidence of miscarriage and ectopic pregnancies goes up substantially with age (Andersen et al., 2000).

3.6.3 Inclusion criteria of controls

The control group comprised of consenting healthy normal pregnant women in the third trimester having normal blood pressure (120/80 – 140/90 mmHg) with no
history of pre-existing hypertension, diabetes mellitus, obesity and on diet which is not likely to affect lipid metabolism.

3.6.4 Exclusion criteria of controls

Pregnant women with gestational diabetes mellitus, obesity, hyperthyroidism, renal failure, nephrotic syndrome, familial hypercholesterolaemia, blood pressure ≥140/90 mm Hg and on digoxin and diuretic treatment were excluded from the study.

3.7 Sample collection

All subjects were asked not to eat or drink anything except water for 8-10 hours before their blood samples were drawn. Fasting blood samples were obtained by venipuncture from the antecubital vein under aseptic conditions. Blood samples for the cases were collected by the phlebotomist in the ward while the controls were bled at the phlebotomy area in ANC laboratory. The controls also had an option to have their blood specimens collected in a laboratory of a health facility of their choice near their residence so as to prevent hypoglycaemia and hypotension. In this case a phlebotomist met the participants in the health facility they chose in the morning and the blood specimens were collected in the laboratory.

3.8 Specimen processing and storage

The clotted samples were centrifuged at 4000 rpm for 5 minutes to obtain the sera. The specimens were transported in a cool box from the ward to the laboratory. The serum samples were stored at 4°C for up to one week before being analyzed.
3.9 Equipment for analysis

HumaStar 100 analyzer (Human Diagnostics, Germany) was used. It is a Random Access clinical chemistry autoanalyzer which performs various biochemical tests. It has multiple reading modes including multi point calibration, flow cell or direct cuvette reading.

3.10 Reagents preparation

The analyzer uses commercially prepared reagents which are supplied in reagent bottles ready for use. Each reagent was placed in its respective reagent tank and placed on the reagent rack. The stability of the reagents was guaranteed by the inbuilt reagent refrigeration feature of the autoanalyer.

3.11 Operation of the analyzer

After placing the samples on the sample tray the order of performing the tests was selected on the test panel. The analyzer carried out the tests and generated results following the selected order.

3.12 Calibration of tests

To ensure accuracy and precision of the results the analyzer performed calibration procedure using Autocal® calibrator solution which is supplied together with the reagents.
3.13 Quality control

Humatrol N (normal) and Humatrol P (pathological) quality control sera from Human Diagnostics were the quality control materials used during the study period. Before use, a QC bottle was carefully opened and exactly 5 ml distilled water pippeted carefully into the bottle, closed, and carefully dissolved by gentle swirling within 30 minutes. This was then aliquoted into six eppendorf tubes and stored at -20°C. Quality control used the same types of tubes as the samples but designated positions in the sample tray. The analyzer performed controls automatically according to the specifications in the test panel and generated QC reports.

3.14 Analytical methods for the parameters

3.14.1 Serum total cholesterol

Cholesterol liquicolor reagent was used to measure serum total cholesterol level. The method determined cholesterol level by the enzymatic colorimetric test with lipid clearing factor. The reaction involved enzymatic hydrolysis by cholesterol esterase and oxidation by cholesterol oxidase. The indicator quinoneimine was formed from hydrogen peroxide and 4-aminophenazone in the presence of phenol and peroxidase. The autoanalyzer aliquoted 10µl of serum sample and 1000 µl of the cholesterol reagent into a reaction cuvette. After 5 minutes incubation at 37°C the absorbance of the coloured compound produced was measured at 500 nm against the reagent blank and compared to the standard. Serum cholesterol level was expressed in mmol/l.
Reaction principle

Cholesterol ester + H₂O \[ \xrightarrow{\text{CHE}} \] Cholesterol + fatty acid

Cholesterol + O₂ \[ \xrightarrow{\text{CHO}} \] Cholestene-3-one + H₂O₂

2H₂O₂ + 4-aminophenazone + phenol \[ \xrightarrow{\text{POD}} \] Quinoneimine + 4H₂O

3.14.2 Serum HDL-C

Determination of Serum HDL-C levels was done using the HDL liquicolor reagent. The method was a direct homogeneous enzymatic assay involving two steps: in the first step chylomicrons, very low density lipoproteins and low density lipoproteins were specifically eliminated and destroyed by enzymatic reactions. In the second step remaining cholesterol from the HDL-C fraction was determined by well-established specific enzymatic reactions in the presence of specific surfactants for HDL-C. The autoanalyzer automatically mixed 10 µl of serum sample and 750 µl of the HDL reagent in a reaction cuvette maintained at 37°C. After 5 minutes incubation the absorbance was read at 593 nm against a reagent blank. The HDL-C concentration was expressed in mmol/l.

Reaction principle

1st step:

LDL, VLDL and Chylomicrons \[ \xrightarrow{\text{CHE + CHO}} \] cholestenone + H₂O₂

specific conditions

2 H₂O₂ \[ \xrightarrow{\text{catalase}} \] 2 H₂O
2\textsuperscript{nd} step:

\[
\begin{align*}
\text{HDL} \quad \text{CHE + CHO} & \quad \text{cholestenone + H}_2\text{O}_2 \\
\text{specific surfactants} & \\
\text{H}_2\text{O}_2 + \text{Chromogen} \quad \text{Peroxidase} & \quad \text{quinone pigment}.
\end{align*}
\]

3.14.3 Serum triglycerides

Triglycerides level determination was done using triglyceride liquicolor reagent. The method was an enzymatic colourimetric test involving enzymatic hydrolysis with lipases. This assay used the indicator quinoneimine formed from hydrogen peroxide, 4-amino-antipyrine and 4-chlorophenol under the catalytic influence of peroxidase. The autoanalyzer aliquoted 10 μl of serum sample and 1000 μl of the triglycerides liquicolor reagent into a reaction cuvette. After 5 minutes incubation at 37°C the absorbance of the coloured compound produced was measured at 546 nm against the reagent blank and compared to the HUMAN\textsuperscript{®} triglycerides standard. Triglycerides level was expressed in mmol/l.

**Reaction principle**

\[
\begin{align*}
\text{Triglycerides} \quad \text{lipases} & \quad \text{glycerol + fatty acids} \\
\text{Glycerol} + \text{ATP} \quad \text{GK} & \quad \text{glycerol-3-phosphate + ADP} \\
\text{Glycerol-3-phosphate} + \text{O}_2 \quad \text{GPO} & \quad \text{dihydroxyacetone phosphate + H}_2\text{O}_2 \\
\text{H}_2\text{O}_2 + 4\text{-aminoantipyrine} \quad \text{POD} & \quad \text{quinoneimine + Hcl + H}_2\text{O} + 4\text{-chlorophenol}
\end{align*}
\]
3.14.4 Serum LDL-C

The serum low density lipoprotein levels were calculated using the Friedewald’s formula; LDL-C = TC – HDL-C - (Tg/2.2).

3.14.5 The TC: HDL-C ratio

This was obtained by dividing the TC value by the HDL-C value. The normal values used by the laboratory are TC 0-5.9 mmol/l, TG 0.3-2.3 mmol/l, HDL-C 0.9-1.42 mmol/l, LDL-C 1-3.9 mmol/l and ratio 0-5.

3.15 Ethical approval

The study was approved by Kenyatta National Hospital – University of Nairobi (KNH-UoN) ethical research committee, Protocol number P53/02/2015 (Appendix VI).

3.16 Data analysis

The collected analytical data was entered into the Excel spreadsheet and then transferred to Statistical Package for Social Sciences (SPSS) software version 20 IBM for statistical analysis. The data was subjected to normality testing using Kolmogorov - Smirnov test and was found to be normally distributed. The results of serum lipid profiles in this study were presented as Mean ±SD. A paired student’s t-test was used for comparison of lipid profiles of cases and controls and ANOVA and post-ANOVA tests were used for multiple comparisons of means. P values ≤0.05 were considered statistically significant.
CHAPTER FOUR

RESULTS

4.1 Socio-demographic and clinical characteristics of the study population

Between January to December 2015 a total of 258 pregnant women were screened, of whom 170 (65.8%) were recruited for the study. Of the 88 (34.1%) women who did not meet the eligibility criteria, 14 (14.9%) were HIV infected, 28 (31.8%) did not attend antenatal clinic regularly, 2 (2.3%) had Syphilis (VDRL) test positive results, 2 (2.3%) Hepatitis B Surface Antigen (HBsAg) positive results, 13 (14.7%) had pre-existing chronic medical conditions and 29 (32.9%) were either below 19 years or above 41 years of age. The 170 eligible pregnant women were categorized by case status into two groups of hypertensive (85 cases) and normotensive (85 controls) and enrolled into the study. Of the cases; 17 had gestational hypertension, 17 chronic hypertension with superimposed preeclampsia, 17 preeclampsia, 17 severe preeclampsia and 17 had eclampsia. Cases and controls were matched on maternal age, parity and gestational age.

4.1.1 Socio-demographic characteristics of the study population

Out of the 85 cases, majority of the participating women (78.8%) were married, while 7.1% were separated while 14.1% were single women. Most of the women with hypertensive cases, 43.5% had average monthly income of less than Kenya shillings (KSh) 20,000, while 34.1% had between Ksh 20,000 to 50,000 while only 3.5% had average monthly income of above Ksh 50,000. It was noted that, 18.8% of
the cases did not indicate their average monthly income and maybe did not have a quantifiable monthly income. The result showed that 47.1% of these women had above secondary level of education. However, 38.8% had secondary education while 14.1% had attained only primary level of education. Comparison of the demographic characteristics using chi-square test showed there were no significant differences in the cases with the control group (p > 0.05).

Table 4.1 Socio-demographic characteristics of the study population

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Cases Mean (SD) n = 85</th>
<th>Controls Mean (SD) n = 85</th>
<th>$\chi^2$ value</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parity</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Para 0+0</td>
<td>19 (22.4%)</td>
<td>19 (22.4%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Para 1+0</td>
<td>26 (30.5%)</td>
<td>26 (30.5%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Para 2+0</td>
<td>21 (24.7%)</td>
<td>21 (24.7%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Para 3+0</td>
<td>13 (15.3%)</td>
<td>13 (15.3%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Para 4+0</td>
<td>6 (7.0%)</td>
<td>6 (7.0%)</td>
<td>0.000</td>
<td>1.000</td>
</tr>
<tr>
<td>Marital status</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Single</td>
<td>12 (14.1%)</td>
<td>14 (16.5%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Married</td>
<td>67 (78.8%)</td>
<td>65 (76.5%)</td>
<td></td>
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</tr>
<tr>
<td>Separated</td>
<td>6 (7.1%)</td>
<td>6 (7.1%)</td>
<td>0.184</td>
<td>0.912</td>
</tr>
<tr>
<td>Average monthly income (KSh)</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 20,000</td>
<td>37 (43.5%)</td>
<td>39 (45.8%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>20-50,000</td>
<td>29 (34.1%)</td>
<td>29 (34.1%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;50,000</td>
<td>3 (3.5%)</td>
<td>4 (4.7%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>N/S</td>
<td>16 (18.8%)</td>
<td>13 (15.3%)</td>
<td>0.506</td>
<td>0.918</td>
</tr>
<tr>
<td>Education level attained</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Primary</td>
<td>12 (14.1%)</td>
<td>13 (15.3%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Secondary</td>
<td>33 (38.8%)</td>
<td>27 (31.8%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Above secondary</td>
<td>40 (47.1%)</td>
<td>45 (52.9%)</td>
<td>0.934</td>
<td>0.627</td>
</tr>
</tbody>
</table>

4.1.2 Clinical characteristics of the study population

Clinical characteristics of the hypertensive women showed that the systolic blood pressure measurement of the hypertensive women was 166.96 mmHg with a
standard deviation of 17.91 mmHg. This was significantly higher than in the normotensive women who had a systolic blood pressure of 115.93 mmHg and a standard deviation of 13.24 mmHg (t = 10.87, P < 0.05). Similarly, diastolic blood pressure measurement of 103.91 mmHg was recorded in women with hypertension and this was significantly higher than the diastolic BP measurement in the control group which was 74.52 mmHg with a standard deviation of 8.82 mmHg (t = 9.52, P < 0.05).

Table 4.2: Clinical characteristics of the study population

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Cases Mean (SD)</th>
<th>Controls Mean (SD)</th>
<th>t-value</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal age (years)</td>
<td>29.22 ± 5.51</td>
<td>29.11 ± 5.39</td>
<td>0.16</td>
<td>0.870</td>
</tr>
<tr>
<td>Gestational Age (weeks)</td>
<td>35.31 ± 2.70</td>
<td>35.31 ± 2.70</td>
<td>0.04</td>
<td>0.969</td>
</tr>
<tr>
<td>Blood pressure (mmHg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic</td>
<td>166.96 ± 17.91</td>
<td>115.93 ± 13.24</td>
<td>10.87</td>
<td>0.001*</td>
</tr>
<tr>
<td>Diastolic</td>
<td>103.91 ± 12.11</td>
<td>74.52 ± 8.82</td>
<td>9.52</td>
<td>0.001*</td>
</tr>
</tbody>
</table>

*mean values between cases and control parameters are significantly different at P ≤ 0.05

4.2 The lipid profile changes of both hypertensive and normotensive women during the third trimester of pregnancy

4.2.1 Comparison of lipid profile of hypertensive cases and normotensive controls

The lipid profiles comprising serum levels of total cholesterol (TC), triglycerides (TG), high density lipoprotein cholesterol (HDL-C) low density lipoprotein cholesterol (LDL-C) were compared using two sample t-test analysis between hypertensive and normotensive women (Table 4.3). The mean serum TC (6.80 ±2.28 versus 5.22 ±0.59 mmol/l), TG (2.59 ± 0.87 versus 2.22 ± 0.47 mmol/l) and LDL-C
(3.97 ±2.17 versus 2.65 ± 0.63 mmol/l) levels in the cases were significantly higher than those of the controls (p<0.05). However, the mean serum HDL-C (1.66 ± 0.66 versus 1.54 ± 0.30 mmol/l) in the cases did not significantly differ from those of the controls (p>0.05). In addition, the TC:HDL-C ratio (4.52 ±2.21 versus 3.50 ±0.68 mmol/l) in the hypertensive cases was significantly higher than that of the controls (p>0.05).

Table 4.3. Comparison of lipid profile of hypertensive group and controls

<table>
<thead>
<tr>
<th></th>
<th>Hypertension in pregnancy (n=85)</th>
<th>Normotensive Controls (n=85)</th>
<th>t-value</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>TC (mmol/l)</td>
<td>6.80 ±2.28</td>
<td>5.22 ±0.59</td>
<td>6.17</td>
<td>0.0001*</td>
</tr>
<tr>
<td>TG (mmol/l)</td>
<td>2.59 ±0.86</td>
<td>2.21 ±0.47</td>
<td>3.54</td>
<td>0.001*</td>
</tr>
<tr>
<td>HDL-C (mmol/l)</td>
<td>1.66 ±0.66</td>
<td>1.53 ±0.30</td>
<td>1.59</td>
<td>0.115</td>
</tr>
<tr>
<td>LDL-C (mmol/l)</td>
<td>3.97 ±2.17</td>
<td>2.65 ±0.63</td>
<td>5.38</td>
<td>0.0001*</td>
</tr>
<tr>
<td>TC:HDL Ratio</td>
<td>4.52 ±2.21</td>
<td>3.50 ±0.68</td>
<td>4.07</td>
<td>0.0001*</td>
</tr>
</tbody>
</table>

*indicate mean values in the row were significantly different at p ≤ 0.05 by t-test.

4.2.2 Association of abnormal lipid profile and hypertensive disease in pregnancy

Using the clinical chemistry laboratory reference intervals, parameters were categorized as normal or high using the following reference intervals; TC 0-5.7 mmol/l, TG 0.3 - 2.3 mmol/l, HDL-C 0.9 -1.68 mmol/l, LDL-C 1.0-3.9 mmol/l and TC/HDL upto 5.0. Using these reference intervals, proportions, of having elevated TC, TG, HDL-C, LDL-C and TC:HDL-C were obtained for hypertensive pregnant women compared to normotensive pregnant women. Based on the odds ratio, the odds of a pregnant women developing HDP when the measured serum TC was above the reference interval was 4.43 times significantly higher if they were in the
hypertensive pregnant women group than if they were in the normotensive pregnant women group ($\chi^2$ value of 21.321, Fishers’ exact test value of <0.001 and p<0.05).

Further, the odds of pregnant women developing HDP when the measured serum TG values were above the reference interval were 2.06 times significantly higher if they were in the hypertensive pregnant women group than if they were in the normotensive pregnant women group ($\chi^2$ value of 5.341, Fishers’ exact value of 0.031 and p<0.05).

In addition, the odds of pregnant women developing HDP when the measured serum LDL-C value were above the reference interval were 57 times significantly higher if they were in the hypertensive group than if they were in the normotensive pregnant women group ($\chi^2$ value of 32.546, Fishers’ exact test value of <0.001 and p<0.05).

Finally, the odds of pregnant women developing HDP when the calculated TC:HDL-C ratio were above 5.0 were 16.32 times significantly higher if they were in the hypertensive pregnant women group than if they were in the normotensive pregnant group ($\chi^2$ value of 21.976, Fishers’ exact test value of <0.001 and p<0.05).

However, the odds of pregnant women developing HDP when the measured HDL-C values were above the reference interval were 1.48 times higher if they were in the
hypertensive pregnant women group than if they were in the normotensive pregnant group (χ² value of 0.717, Fishers’ exact test value of 0.498) and p>0.05).

Table 4.4: Association of abnormal lipid profile in hypertensive and normotensive women.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Hypertension in pregnancy (N/%)</th>
<th>Normotensive controls (N/%)</th>
<th>Fishers exact test</th>
<th>Pearson chi-square</th>
<th>Odds Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>TC&gt;5.7 mmol/l</td>
<td>54 (69.2%)</td>
<td>24 (30.8%)</td>
<td>0.000*</td>
<td>21.321</td>
<td>4.42**</td>
</tr>
<tr>
<td>TG&gt;2.3 mmol/l</td>
<td>46 (59.7%)</td>
<td>31 (40.3%)</td>
<td>0.031*</td>
<td>5.341</td>
<td>2.05**</td>
</tr>
<tr>
<td>HDL-C &gt;1.68 mmol/l</td>
<td>27 (55.1%)</td>
<td>22 (44.9%)</td>
<td>0.498</td>
<td>0.717</td>
<td>1.48</td>
</tr>
<tr>
<td>LDL-C&gt;3.9 mmol/l</td>
<td>28 (96.6%)</td>
<td>1 (3.4%)</td>
<td>0.000*</td>
<td>32.546</td>
<td>57.00**</td>
</tr>
<tr>
<td>Ratio &gt;5.0</td>
<td>24 (92.3%)</td>
<td>2 (7.7%)</td>
<td>0.000*</td>
<td>21.976</td>
<td>16.32**</td>
</tr>
</tbody>
</table>

*indicate a significant Fishers exact test value in the row at P ≤ 0.05 ** indicates significant OR when odds of cases and compared with odds of matching controls.

4.2.3 Third trimester lipid profile differences in normotensive women and in cases with different categories of hypertension

Comparison of means of lipid profiles of cases among hypertensive cases showed there was no significant difference (P > 0.05) in; TC within the different categories of HDP (F = 0.093), in TG (F = 0.393), in HDL-C (F = 0.0.419), in LDL-C (F = 0.124) and also in the ratio TC/HDL (F = 0.258). The levels of TC, TG, LDL-C and ratio TC/HDL were however significantly higher (P < 0.05) than in the control (F = 7.606, 2.980, 5.869 and 3.651 respectively).
Table 4.5: Comparison of lipid profiles in different classes of HDP

<table>
<thead>
<tr>
<th>Lipid profile</th>
<th>Categories of hypertensive disease in pregnancy.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>GH (n=17) Mean± SD</td>
</tr>
<tr>
<td>TC</td>
<td>6.7± 2.66a</td>
</tr>
<tr>
<td>TG</td>
<td>2.4± 0.56a</td>
</tr>
<tr>
<td>HDL-C</td>
<td>1.5± 0.59a</td>
</tr>
<tr>
<td>LDL-C</td>
<td>4.0± 2.78a</td>
</tr>
<tr>
<td>RATIO</td>
<td>4.9± 3.07a</td>
</tr>
</tbody>
</table>

Mean values in the same row denoted by similar letters are not significantly different at P≤ 0.05. Mean separated by LSD.
4.3 Lipid profile changes for hypertensive cases and normotensive controls stratified by parity

To evaluate the lipid profile changes among the various parity, the lipid profiles were compared (Table 4.6). Using One-way ANOVA and post-ANOVA analysis the result indicated that there was no significant difference in the lipid profiles in various parity (P > 0.05).

Table 4.6: Lipid profiles levels in the different parity in the sampled population

<table>
<thead>
<tr>
<th>Parity</th>
<th>TC (mean ± SD)</th>
<th>TG (mean ± SD)</th>
<th>HDL-C (mean ± SD)</th>
<th>LDL-C (mean ± SD)</th>
<th>Ratio (mean ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>5.96 ±2.23</td>
<td>2.35 ±0.85</td>
<td>1.66 ±0.63</td>
<td>3.26 ±2.05</td>
<td>3.87 ±1.82</td>
</tr>
<tr>
<td>1</td>
<td>6.17 ±2.03</td>
<td>2.41 ±0.65</td>
<td>1.52 ±0.46</td>
<td>3.55 1.97</td>
<td>4.32 ±2.00</td>
</tr>
<tr>
<td>2</td>
<td>5.80 ±1.23</td>
<td>2.32±0.71</td>
<td>1.60±0.48</td>
<td>3.09 1.12</td>
<td>3.79 ±1.05</td>
</tr>
<tr>
<td>3</td>
<td>6.24±2.05</td>
<td>2.67±0.78</td>
<td>1.71±0.54</td>
<td>3.36 1.88</td>
<td>3.96 ±1.96</td>
</tr>
<tr>
<td>4</td>
<td>5.75 ±0.93</td>
<td>2.31±0.45</td>
<td>1.50±0.55</td>
<td>3.12 0.76</td>
<td>4.16±1.50</td>
</tr>
<tr>
<td>F-value</td>
<td>0.323</td>
<td>0.919</td>
<td>0.693</td>
<td>0.381</td>
<td>0.563</td>
</tr>
<tr>
<td>P value</td>
<td>0.899</td>
<td>0.470</td>
<td>0.629</td>
<td>0.861</td>
<td>0.728</td>
</tr>
</tbody>
</table>

Mean values in each column tested at P ≤ 0.05. Mean separated using LCD

4.4 Lipid profile changes for hypertensive cases and normotensive controls stratified by parity

Comparison of lipid profiles of hypertensive cases of varying parity showed that parity has no effect on the lipid profiles (Table 4.7). It was also established that the lipid profiles of the normotensive women having various parities (Table 4.8) were not significantly different (P > 0.05).
Table 4.7: Lipid profiles levels in hypertensive cases with different parity

<table>
<thead>
<tr>
<th>Parity</th>
<th>TC (mean ± SD)</th>
<th>TG (mean ± SD)</th>
<th>HDL-C (mean ± SD)</th>
<th>LDL-C (mean ± SD)</th>
<th>Ratio (mean ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>6.65± 3.00</td>
<td>2.54±1.02</td>
<td>1.71± 0.82</td>
<td>3.86±2.77</td>
<td>4.37±2.44</td>
</tr>
<tr>
<td>1</td>
<td>7.22± 2.37</td>
<td>2.49±0.77</td>
<td>1.56±0.60</td>
<td>4.52±2.34</td>
<td>5.08±2.55</td>
</tr>
<tr>
<td>2</td>
<td>6.37±1.49</td>
<td>2.48±0.88</td>
<td>1.70±0.63</td>
<td>3.56±1.29</td>
<td>4.01±1.28</td>
</tr>
<tr>
<td>3</td>
<td>7.30±2.42</td>
<td>3.15±0.82</td>
<td>1.80±0.63</td>
<td>4.05±2.36</td>
<td>4.54±2.53</td>
</tr>
<tr>
<td>4</td>
<td>6.07±1.09</td>
<td>2.54±0.54</td>
<td>1.57±0.78</td>
<td>3.25±0.99</td>
<td>4.50±2.14</td>
</tr>
</tbody>
</table>

F-value: 0.63; P value: 0.676

Mean values in each column tested at P ≤ 0.05. Mean separated using LCD

Table 4.8: Lipid profiles levels in normotensive controls of different parity

<table>
<thead>
<tr>
<th>Parity</th>
<th>TC (mean ± SD)</th>
<th>TG (mean ± SD)</th>
<th>HDL-C (mean ± SD)</th>
<th>LDL-C (mean ± SD)</th>
<th>Ratio (mean ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>5.28±0.47</td>
<td>2.17±0.60</td>
<td>1.62±0.36</td>
<td>2.67±0.41</td>
<td>3.37±0.57</td>
</tr>
<tr>
<td>1</td>
<td>5.12±0.70</td>
<td>2.33±0.51</td>
<td>1.47±0.24</td>
<td>2.58±0.69</td>
<td>3.56±0.67</td>
</tr>
<tr>
<td>2</td>
<td>5.24±0.47</td>
<td>2.16±0.44</td>
<td>1.51±0.24</td>
<td>2.61±0.65</td>
<td>3.56±0.70</td>
</tr>
<tr>
<td>3</td>
<td>5.19±0.70</td>
<td>2.20±0.31</td>
<td>1.62±0.42</td>
<td>2.66±0.86</td>
<td>3.38±0.94</td>
</tr>
<tr>
<td>4</td>
<td>5.43±0.69</td>
<td>2.09±0.20</td>
<td>1.43±0.23</td>
<td>2.99±0.51</td>
<td>3.82±0.46</td>
</tr>
</tbody>
</table>

F-value: 0.54; P value: 0.744

Mean values in each column tested at P ≤ 0.05. Mean separated using LCD

4.5 Lipid profile changes for hypertensive cases stratified by maternal age

Comparison of lipid profiles of hypertensive cases of different maternal age groups using One-way ANOVA and post-ANOVA showed that maternal age had no effect on the serum levels of TC, TG, HDL-C and LDL-C for hypertensive cases (p>0.05).

Table 4.9: Comparison of lipid profile of cases stratified by maternal age

<table>
<thead>
<tr>
<th>Maternal age</th>
<th>TC (mean ± SD)</th>
<th>TG (mean ± SD)</th>
<th>HDL-C (mean ± SD)</th>
<th>LDL-C (mean ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>19 – 23 years</td>
<td>7.89±3.16</td>
<td>2.53±0.85</td>
<td>1.61±0.53</td>
<td>5.09±2.89</td>
</tr>
<tr>
<td>24 – 29 years</td>
<td>6.48±2.27</td>
<td>2.38±0.83</td>
<td>1.67±0.69</td>
<td>3.78±2.22</td>
</tr>
<tr>
<td>30 – 35 years</td>
<td>6.61±1.86</td>
<td>2.89±0.93</td>
<td>1.70±0.71</td>
<td>3.61±1.76</td>
</tr>
<tr>
<td>36 – 41 years</td>
<td>6.74±1.69</td>
<td>2.64±0.75</td>
<td>1.65±0.71</td>
<td>3.86±1.48</td>
</tr>
</tbody>
</table>

F-value: 1.42; P value: 0.243

Mean values in each column tested at P ≤ 0.05. Mean separated using LCD
4.6 Relationship between maternal age and the lipid profile

To establish the relationship between the maternal age and the lipid profile, Pearson correlation analysis was conducted on these parameters. The result showed that there is no relationship between maternal age and the all the lipid profile parameters (p>0.05).

Table 4.10: Correlation table showing relationship between maternal age and lipid profile changes.

<table>
<thead>
<tr>
<th>Maternal age in years</th>
<th>TC</th>
<th>TG</th>
<th>HDL-C</th>
<th>LDL-C</th>
<th>TC/HDL ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal age Pearson Correlation</td>
<td>1</td>
<td>0.055</td>
<td>0.047</td>
<td>0.084</td>
<td>0.064</td>
</tr>
<tr>
<td>Sig. (2-tailed)</td>
<td>0.478</td>
<td>0.541</td>
<td>0.278</td>
<td>0.417</td>
<td>0.929</td>
</tr>
<tr>
<td>N</td>
<td>170</td>
<td>170</td>
<td>170</td>
<td>170</td>
<td>170</td>
</tr>
</tbody>
</table>

4.5.5 Relationship between parity and the lipid profile

The relationship between parity and the lipid profile, using Pearson correlation analysis result showed that there is no relationship between parity and lipid profile changes (p>0.05).

Table 4.11: Correlation table showing relationship between parity and lipid profile changes

<table>
<thead>
<tr>
<th>Parity</th>
<th>Pearson Correlation</th>
<th>TC</th>
<th>TG</th>
<th>HDL-C</th>
<th>LDL-C</th>
<th>TC/HDL Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parity</td>
<td>1</td>
<td>0.115</td>
<td>0.052</td>
<td>0.031</td>
<td>-0.007</td>
<td>-0.005</td>
</tr>
<tr>
<td>Sig. (2-tailed)</td>
<td>0.137</td>
<td>0.497</td>
<td>0.689</td>
<td>0.931</td>
<td>0.948</td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>170</td>
<td>170</td>
<td>170</td>
<td>170</td>
<td>170</td>
<td>170</td>
</tr>
</tbody>
</table>
4.7 Lipid profile changes for normotensive women of different maternal ages

Comparison of lipid profiles of normotensive women of different maternal ages showed that maternal age has no effect on the lipid profile of normotensive women (P > 0.05).

Table 4.12: Lipid profiles in normotensive women of varying maternal ages.

<table>
<thead>
<tr>
<th>Maternal age</th>
<th>TC (mean ± SD)</th>
<th>TG (mean ± SD)</th>
<th>HDL-C (mean ± SD)</th>
<th>LDL-C (mean ± SD)</th>
<th>Ratio (mean ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>19 – 23 years</td>
<td>5.52 ± 0.47</td>
<td>2.39 ± 0.36</td>
<td>1.60 ± 0.17</td>
<td>2.75 ± 0.54</td>
<td>3.48 ± 0.54</td>
</tr>
<tr>
<td>24 – 29 years</td>
<td>5.17 ± 0.62</td>
<td>2.26 ± 0.57</td>
<td>1.49 ± 0.30</td>
<td>2.59 ± 0.71</td>
<td>3.56 ± 0.67</td>
</tr>
<tr>
<td>30 – 35 years</td>
<td>5.16 ± 0.54</td>
<td>2.09 ± 0.40</td>
<td>1.54 ± 0.37</td>
<td>2.69 ± 0.52</td>
<td>3.49 ± 0.72</td>
</tr>
<tr>
<td>36 – 41 years</td>
<td>5.12 ± 0.71</td>
<td>2.18 ± 0.40</td>
<td>1.56 ± 0.26</td>
<td>2.57 ± 0.85</td>
<td>3.39 ± 0.86</td>
</tr>
<tr>
<td>F-value</td>
<td>1.57</td>
<td>1.45</td>
<td>0.46</td>
<td>0.30</td>
<td>0.18</td>
</tr>
<tr>
<td>P value</td>
<td>0.203</td>
<td>0.234</td>
<td>0.710</td>
<td>0.826</td>
<td>0.909</td>
</tr>
</tbody>
</table>

Mean values in each column tested at P ≤ 0.05. Mean separated using LCD

4.8 The effect of dyslipidemia and the severity of disease among hypertensive pregnant women

To investigate the association of lipid profile changes and the severity of disease among cases, the cases were grouped into two categories based on severity: mild and severe where mild disease was characterized by high blood pressure without any other symptoms while severe disease was characterized by elevated BP and other symptoms and signs of HDP. The lipid profiles of cases with mild and severe hypertensive disease in pregnancy were compared using two sample t-test. Serum levels of TC, LDL-C and the ratio TC/HDL-C were found to be significantly (P < 0.05) elevated in the severe disease cases (Table 4.13). Using one sample t – test the
TC/HDL-C ratio which is taken to be the indicator of the risk of atherosclerosis was found to be significantly lower in the mild disease category (3.74 ± 1.10) \( (t = 6.678, P < 0.05) \). In the severe disease category, the value of the ratio was not significantly different \( (t = 0.130, P = 0.897) \), Appendix VII.

### Table 4.13: Mean lipid profiles and severity of HDP among hypertensive cases

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mild disease</th>
<th>Severe disease</th>
<th>T value</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>TC</td>
<td>5.74 ± 1.24</td>
<td>7.51 ± 2.55</td>
<td>4.29</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>TG</td>
<td>2.39 ± 0.62</td>
<td>2.74 ± 0.98</td>
<td>1.97</td>
<td>0.052</td>
</tr>
<tr>
<td>HDL</td>
<td>1.66 ± 0.64</td>
<td>1.67 ± 0.68</td>
<td>0.08</td>
<td>0.939</td>
</tr>
<tr>
<td>LDL-C</td>
<td>2.96 ± 0.94</td>
<td>4.65 ± 2.48</td>
<td>4.41</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Ratio</td>
<td>3.74 ± 1.10</td>
<td>5.05 ± 2.60</td>
<td>3.18</td>
<td>0.002*</td>
</tr>
</tbody>
</table>

*indicate a significant difference in the lipid profile in the row using two sample t-test at \( P \leq 0.05 \)
CHAPTER FIVE
DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

5.1 Discussion

This case control study investigated the role of changes in lipid profile on the occurrence of hypertensive disease in pregnancy (HDP) during the third trimester in subjects aged 19-41 years. The third trimester was targeted because it is during this stage most patients present with signs and symptoms of hypertensive disease. Serum levels of total cholesterol (TC), triglycerides (TG) and low density lipoprotein cholesterol (LDL-C) were significantly higher in the hypertensive group compared to the normotensive control group while high density lipoprotein cholesterol (HDL-C) did not differ between the cases and controls. Similarly, when reference levels and laboratory cut offs were used, the risk of developing HDP was significantly higher in women who had TC > 5.7 mmol/l, TG > 2.3 mmol/l, LDL-C > 3.9 mmol/l and TC:HDL-C ratio > 5.0. These changes suggest that there is a specific lipid profile pattern which is associated with pregnancy induced hypertension. Lorentsen et al. (1998), Gohil et al. (2011), Pradnya et al. (2013), Fanshave et al. (2013) and Siddiqui (2014) reported similar findings of elevated TC, TG and LDL-C in studies conducted in India. The observed significant increase in TC could be due to the persistent increase in the plasma concentration of progesterone during pregnancy as well as the increased demand of lipids by the fetus for the building of cell membranes which results in hypercholestaemia.
Elevation of TC is expected during the third trimester of normal pregnancies because this stage is characterized by increased breakdown of maternal lipids (Vani et al., 2015). The findings of this study suggest that there could be accelerated breakdown of the maternal lipids to support foetal growth in strenuous conditions. Maternal hyperphagia which is normally exhibited during pregnancy could also be a contributing factor to lipogenesis according to Vani et al. (2015).

In this study hypertensive disease in pregnancy (HDP) was characterized by elevated LDL-C. This finding was reported by Abdelhai et al. (2013) in women at risk of developing pregnancy induced hypertension in Sudan. This study did not find statistically significant difference in the serum levels of HDL-C between hypertensive and normotensive pregnant women. This similarity may be accounted for by the human placental lactogen (HPL) synthesized during pregnancy since it leads to insulin resistance which in turn decreases HDL-C synthesis. The mean serum level of HDL-C for both cases and controls were above the normal range cut off value. The increase in serum HDL-C level could be responsible for exerting its protective effect from atherosclerosis. This finding contrasts that of Munoz et al. (1999) who reported lower plasma levels of HDL-C during pregnancy in Nigerian women. Human placental lactogen (HPL) synthesized during pregnancy may be responsible for the similarity since it leads to insulin resistance which in turn decreases HDL-C synthesis. The insulin resistance occasioned by HPL may be linked to the pathogenesis of HDP since it increases the levels of fats especially TG.
The observed significantly elevated serum TG levels in the hypertensive group suggests that there could be increased hepatic synthesis of TG by the action of hepatic lipase in the liver during hypertensive than in normotensive pregnancies. A similar observation of TG elevation in HDP was made by Patrizia et al. (1999), and Enquobahrie et al. (2004), who all concluded that a decrease in the activity of lipoprotein lipase led to decreased catabolism at the adipose tissue level and whose net effect was an increase in circulating triglycerides. The circulating TG is likely to be deposited in the predisposed spiral arteries and contribute to endothelial dysfunction (Patrizia et al., 1999), a known finding in pregnancies complicated by hypertensive disease. Hypertriglyceridemia may also be caused by the hepatic failure associated with HDP because the failure to remove the products of hydrolysis from circulation by the liver leads to the accumulation of TG (Rubina et al., 2007). Studies by Cekmen et al. (2003), Enquobahrie et al. (2004) and Jayanta et al. (2006), also reported significant rise in serum TG concentration in HDP. The hypertriglyceridemia could be due to stimulation of the liver by estrogen to synthesize endogenous TG which enter into circulation and may be deposited in the spiral arteries leading to endothelial dysfunction.

This study observed that the odds of developing HDP are significantly higher in hypertensive pregnant women who had elevated serum TG level. This finding is in agreement with that of Ray et al. (2006) who demonstrated an association between maternal hypertriglyceridemia and the risk of HDP. In a study by Kokia et al. (1990) hypertriglyceridemia was associated with hyper-coagulability. The
hypertriglyceridemia observed in this study may contribute to the enhanced coagulation which characterizes preeclampsia.

The statistically significant increase in both TG and LDL-C in hypertensive women compared to the normotensive controls could also be due to the decreased expression of VLDL receptors which leads to maternal hypertriglyceridemia and also due to the reduced uptake of LDL-C for the synthesis of Dehydroepiandrosterone sulphate (DHEAS) by fetal adrenal glands. Lawrence and Patricia, (2008) linked altered uteroplacental flow to hypoestrogenemia considering the DHEAS is the source of estrogen. Sally and Linda, (2006) reported that the reduction in fetoplacental perfusion led to increased TG.

The findings of this study revealed that there was no significant differences (p >0.05) in the lipid profiles of cases with varying categories of HDP when One-way ANOVA was performed. However, there was statistically significant difference in all the lipid profile parameters when each category of HDP was compared with matching controls. In the gestational hypertensive cases, significantly elevated serum levels of TC and LDL-C were observed in this study which is similar to the findings of Cuneyt et al. (2004) who reported significantly elevated levels of TC and LDL-C in women with gestational hypertension compared to normotensive pregnant women. This elevation of LDL-C in cases with the mild form of HDP implicates LDL-C in the pathogenesis of HDP.
Pearson correlation analysis results showed that there was no significant relationship between the lipid profile changes and parity as well as lipid profile changes and maternal age groups in both the hypertensive and normotensive groups. These findings are similar to that of Haichen et al. (2016) who observed that parity and maternal age had no impact on lipid metabolism during hypertensive and normotensive pregnancies in a study conducted on Chinese pregnant women. The extent of insulin resistance during hypertensive and normotensive pregnancies in the two population does not increase the serum levels of free fatty acids and therefore lipid metabolism remains unchanged.

This findings contrast those of Clapp and Capeless (1997) who reported significant association between maternal TC and parity as well as LDL-C and parity in a study conducted in India. This difference could be attributed to race and environmental factors. Kritz et al. (1992) also reported that parity is not related to lipid levels in women with lower parity. A study by Wolff, et al. (2005) reported high TC/HDL-C ratio and lower HDL-C level with increase in parity and maternal age while Humphries et al. (2001) reported that women with parities 1 and 2 had lower LDL-C levels.

In this study disease severity was associated with significant elevated TC, LDL-C as well as the TC:HDL-C value in cases with severe disease as compared to those with mild disease. There was no significant difference in the serum levels of TG and
HDL-C which suggests that their elevation is not associated with disease severity. Abnormally low levels of lipoprotein lipase could be responsible for the concurrent significant increase in serum TG and LDL-C level observed in this study which could be a contributing factor in the pathogenesis of HDP. The effect of such is increase in circulating TG level and accumulation of LDL-C which is associated with the development of atherosclerosis (Ross, 1999).

The TC:HDL value was significantly higher in the hypertensive women (4.52±2.21) compared to the normotensive controls (3.50±0.68). There are no previous published reports on the values of the ratio in hypertensive pregnancies. In this study the value was lower than 5.00, above which it is considered to favour atherosclerosis during the non-pregnant state. In the severe disease category the TC:HDL is significantly increased and only this category of cases of HDP recorded a value above 5.00. Therefore the application of the TC:HDL-C cut off value of 5.0 may not be used in the prediction or as an indicator of the risk of HDP.

5.2 Conclusions

i. The findings of this study reveal that in pregnancy induced hypertension serum levels of TC, TG and LDL-C are elevated significantly as compared to those of normotensive pregnancies and could be used as markers for HDP.
Parity and maternal age have no effect on the lipid profiles of hypertensive pregnant and normotensive pregnant women.

The TC:HDL-C ratio was significantly different in hypertensive women compared to the normotensive ones and the value increased with disease severity. Even then, in this study the value of TC:HDL-C was below 5.0 considered to favour atherosclerosis.

5.3 Recommendations

The recommendations of this study are:

i. Fasting lipid profile test should be considered as a screening test for HDP in addition to the testing of proteins in urine.

ii. Strategies of preventing and managing PIH should not only focus on lowering the blood pressure but also lowering the circulating levels of TC, TG and LDL-C.

5.4 Suggestions for further studies

i. Clinical trials should be conducted to determine the efficacy of current regimes in lowering lipids during pregnancy as well as their therapeutic safety during pregnancy.

ii. Studies should be carried out to establish the postpartum and long term effects of these lipid profile changes.
REFERENCES


APPENDICES

Appendix I: Consent information

Title of Study:
Lipid Profile in Normal Pregnancy and in Pregnancy Induced Hypertension.

Introduction
This study is on pregnancy induced hypertension which is a very common condition in our country. I am inviting you to participate in this study. The purpose of this study is to determine the changes which occur in lipid metabolism during pregnancy in both normal and hypertensive pregnancies. The study will be conducted at Kenyatta National Hospital and will include 170 participants who are pregnant. The study will provide useful information related to the lipid profile during pregnancy and help to reduce the cases of perinatal morbidity and mortality. If you have questions concerning your participation during and after the study please ask me or my supervisor.

Procedures to be followed
If you agree to take part in this study;

- You will be asked some questions related to your pregnancy
- Blood specimens will be collected at the third trimester for the determination lipid profiling.
- Your blood pressure and blood glucose level will be checked when the blood specimen will be collected.
- Your lipids will be analyzed to check whether there are any abnormalities.

Benefits of this study
There are benefits to you if you take part in this study. Your state of lipid metabolism during pregnancy will be monitored and medical attention will be accorded if
necessary. Information from this study will help in the management of pregnancy induced hypertension. This will go a long way in understanding hyperlipidemia during pregnancy and prevent pregnancy induced hypertension complications. You will be provided with a copy of your results when you attend the ANC clinic.

Risks

You may develop a scar during venous blood collection. Your blood sugar level may decrease as a result of fasting. Care will be taken during blood collection to avoid the development of a scar and your blood glucose will be checked. You are encouraged to ask questions in case you need any clarification.

Number of study participants

The study participants will be grouped into two groups. One group will include 85 pregnant mothers who are suffering from high blood pressure during pregnancy and the other comprising of 85 pregnant women who are normal. You will be in one of the two groups.

Referral system

The researcher will avail a copy of your laboratory results to you within five days after specimen collection. In case there is an abnormality in your results which necessitates immediate medical attention the research team will sent your laboratory report to your health provider immediately.

Confidentiality

All medical information which you will provide for the study will be solely used by the investigators. Your identity will not be disclosed to any other party. All data will be confidential.

Collection and storage of specimens

The blood specimens will be collected by aseptic methods. The amount of blood collected will be 5 mls which is equivalent to one table spoon. During and after the samples will be stored without patient identifiers.

Right of participation

Your participation in this study is voluntary. You may wish not to participate in the study at any time and in such an event your rights shall be respected.
Appendix II: Consent form

This is to certify that I………………………………………… have explained the purpose of the study to the participant. I have also answered all the questions she may have asked to her understanding.

Sign:………………… Date: …………………

I ……………………………………………………………………… have read/ been read and satisfactorily understood the information related to this study. I hereby give consent for my participation as explained to me.

Study Participant’s name: …………………… Sign:……………. Date: ……………

If you have any questions pertaining to your participation in the study you can contact me;

Shadrack M. Mweu  P.O Box 16700 – 00100 Nairobi.
Mobile: 0727 037731  Email: shadmuindu@gmail.com

Or my supervisor Dr. Alfred Osoti.  P.O Box 19676 – 00202 KNH
Mobile: 0733 886664 Email: alfosoti@yahoo.com

Or The Chairman KNH/UoN-Ethics and Research Committee. P.O Box 20723-00202 KNH
Telephone: 726300 - 9
Appendix III: Screening form for Cases

<table>
<thead>
<tr>
<th>Clinical characteristics</th>
<th>(Tick Yes or No)</th>
<th>YES</th>
<th>NO</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pregnancy in the third trimester (&gt;24 weeks)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aged 18-45 years</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BP $\geq 140/90$ mmHg</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diagnosis of hypertension at 20 weeks of pregnancy</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No history of pre-existing hypertension</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Not obese/BMI</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Regularly visits ANC clinic</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No history of renal diseases</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Absence of diabetes mellitus and gestational diabetes mellitus</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Not taking any medication for dyslipidemia</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Not suffering from sexually transmitted diseases</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gestational period between 24 -36 weeks</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No history of familial hypercholesteraemia</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Having gestational hypertension, pre-eclampsia or eclampsia</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Appendix IV: Screening form for controls

<table>
<thead>
<tr>
<th>Clinical characteristics</th>
<th>(Tick Yes or No)</th>
<th>YES</th>
<th>NO</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy Pregnancy in the third trimester (&gt;24 weeks)</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Aged 18-45 years</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal BP (120/80 -140/90) mmHg</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Not on diet likely to affect lipid metabolism</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No history of pre-existing hypertension before pregnancy</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Not obese/BMI</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Regularly visits ANC clinic</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No history of renal diseases and hyperthyroidism</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Absence of diabetes mellitus and gestational diabetes mellitus</td>
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</tr>
<tr>
<td>Not taking any medication for dyslipidemia</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Not suffering from sexually transmitted diseases</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gestational period between 24 -36 weeks</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No history of familial hypercholestoraemia</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Appendix V: Study Questionnaire

Date: ........................................

Study number: □ CT (Control) □ C (Case)

Social Demographic and Clinical Characteristics

Age: _______ Years

Highest level of education attained (Tick one)

College □ Secondary □ Primary □ None □

Occupation: _________________________________________

Average monthly income: (Tick one)

□ <Kes. 20,000 □ 21,000 - 50,000 □ > 51,000

Marital status: (Tick one) Single □ Married □ Separated □

Gestational Age (Weeks) _________

Parity: _______ Gravida _______ History of abortion/miscarriage (Yes/No) □

Obstetric History: (Tick one) □ Normal pregnancy □ Hypertensive pregnancy

Blood pressure: Diastolic _______ mmHg Systolic _______ mmHg

History of familial hypertension (Tick one) Yes □ No □

Classification of P.I.H: (Tick one)

□ Gestational hypertension

□ Chronic hypertension in pregnancy

□ Pre-eclampsia

□ Mild eclampsia

□ Chronic hypertension with superimposed preclampsia

□ Severe eclampsia
# Laboratory Results

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Result</th>
<th>Units</th>
<th>Normal values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Cholesterol (TC)</td>
<td>mmol/L</td>
<td></td>
<td>0 – 5.9 mmol/l</td>
</tr>
<tr>
<td>Triglycerides (TG)</td>
<td>mmol/L</td>
<td></td>
<td>0.3 – 2.3 mmol/l</td>
</tr>
<tr>
<td>HDL-C cholesterol (HDL-C)</td>
<td>mmol/L</td>
<td></td>
<td>0.9 – 1.42 mmol/l</td>
</tr>
<tr>
<td>LDL-Cholesterol (LDL-C)</td>
<td>mmol/L</td>
<td></td>
<td>1.0 – 3.9 mmol/l</td>
</tr>
<tr>
<td>Ratio (TC/HDL-C)</td>
<td></td>
<td></td>
<td>0 – 5</td>
</tr>
</tbody>
</table>
Appendix VI: KNH-UoN ERC approval letter

Dear Shadrack,

Research Proposal: Lipid profile in normal pregnancy and in pregnancy induced hypertension (P530212015)

This is to inform you that the KNH/UoN-Ethics & Research Committee (KNH/UoN-ERC) has reviewed and approved your above proposal. The approval periods are 18th May 2015 to 17th May 2016.

This approval is subject to compliance with the following requirements:

a) Only approved documents (informed consents, study instruments, advertising materials etc) will be used.
b) All changes (amendments, deviations, violations etc) are submitted for review and approval by KNH/UoN ERC before implementation.
c) Death and life threatening problems and severe adverse events (SAEs) or unexpected adverse events whether related or unrelated to the study must be reported to the KNH/UoN ERC within 72 hours of notification.
d) Any changes anticipated or otherwise that may increase the risks or affect safety or welfare of study participants and others or affect the integrity of the research must be reported to KNH/UoN ERC within 72 hours.
e) Submission of a request for renewal of approval at least 60 days prior to expiry of the approval period. (Attach a comprehensive progress report to support the renewal).
f) Clearance for export of biological specimens must be obtained from KNH/UoN-Ethics & Research Committee for each batch of shipment.
g) Submission of an executive summary report within 90 days upon completion of the study. This information will form part of the data base that will be consulted in future when processing related research studies so as to minimize chances of study duplication and/or plagiarism.

For more details consult the KNH/UoN ERC website www.erc.uonbi.ac.ke

Protect to discover
Yours sincerely,

PROF. M. L. CHINDIA
SECRETARY, KNUON-ERC

cc. The Principal, College of Health Sciences, UoN
The Deputy Director CS, KNH
The Chair, KNH/UoN-ERC
Supervisors: Prof. Joseph J. Ngeranwa, Prof. C. S. Kigondu, Dr. Alfred Osoti
**APPENDIX VII: One-Sample t-test for association of TC/HDL-C and severity of HDP**

One-Sample t-test for association of TC/HDL-C and severity of HDP

<table>
<thead>
<tr>
<th></th>
<th>Test Value = 5.0</th>
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<tr>
<td></td>
<td>t</td>
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<tr>
<td>Mild disease</td>
<td>-6.678</td>
</tr>
<tr>
<td></td>
<td></td>
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<tr>
<td>Severe disease</td>
<td>0.130</td>
</tr>
<tr>
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</table>