ADDIS ABABA UNIVERSITY

COLLEGE OF HEALTH SCIENCES

DEPARTMENT OF MEDICAL LABORATORY SCIENCES

Assessment of RFT, serum total protein, lipid profile tests and associated risk factors among pregnant women with pregnancy-induced hypertension attending in Asrade Zewudie Memorable hospital (ASZMPH), Gojjam, Ethiopia: Case-control study.

By: Haymanot Tewabe

Advisors: Dr. Mistire Woldie (Msc, Ph.D.)

Abebe Edao (Msc)

A thesis paper submitted to the Department of Medical Laboratory Sciences, College of Health Sciences, Addis Ababa University, in partial fulfillment of Master of Science Degree in Clinical Laboratory Sciences (Clinical chemistry track).

June, 2020

Addis Ababa, Ethiopia

Addis Ababa University

School of Graduate Studies

This is to certify that the thesis prepared by HAYMANOT TEWABE, entitled: **"Assessment of RFT, serum total protein, lipid profile tests and associated risk factors among pregnant women with pregnancy-induced hypertension attending in Asrade Zewudie Memorable hospital (ASZMPH), Gojjam, Ethiopia: Case-control study, 2020"** and submitted in partial fulfillment of the requirements for Master of Science degree in Clinical Laboratory Sciences (Clinical chemistry track) complies with the regulations of the University and meets the accepted standards concerning originality and quality.

Signed by the Examining Committee:

External Examiner_____ Signature _____Date _____

Internal Examiner_____ Signature _____ Date _____

Advisor_____ Signature _____ Date _____

Advisor_____ Signature _____Date _____

_____

Chairperson of the Department of Graduate Program Coordinator

Acknowledgment

I would like to thanks Addis Ababa University, College of Health Sciences, Department of Medical Laboratory Sciences for granting me to do the research. My deepest gratitude and heartfelt thanks go to my advisors Dr. Mistire Wolde (Msc, Ph.D., Associate Professor) and Mr. Abebe Edao (MSC, Ph.D. candidate) for their unreserved constructive advice, professional supports, suggestions, and technical guidance throughout the process of this research proposal without any reservation.

My sincere thanks also go for the Amhara health bureau and management members of Asrade Zewudie Memorable Primary hospital members for their permission to do this research in Asrade Zewudie Memorable Primary hospital. Also, I would like to address my heart full thanks to all Midwife professionals, Laboratory professionals in AZMPH for their support during data collection and sample analysis. My grateful thanks also extended to study participants for their willingness and voluntarism to participate in this study. Here also I would like to address my heart full thanks to Doctor Tebikew (Radiologist specialist) for his constructive advice and professional support during data collection. Finally, my family and friends are gratefully acknowledged for realizing my study next to my God.

Table of Contents

List of Tables

ABBREVIATIONS

AOR	Adjusted Odd Ratio
BMI	Body Mass Index
CDC	Center for Disease Control and prevention
DBP	Diastolic Blood Pressure
EDHS	Ethiopian Demographic Health Survey
GFR	Glomerular filtration rate
HDL	High Density Lipoprotein
HDP	Hypertension Disorder of Pregnancy
IQC	Internal Quality Control
LDL	Low Density Lipoprotein
LPT	Lipid Profile Test
PIH	Pregnancy Induced Hypertension
SBP	Systolic Blood Pressure
SOPs	Standard Operating Procedures
TC	Total Cholesterol
TG	Triglyceride
STP	Serum Total Protein
VLDL	Very low Density Lipoprotein
WHO	World Health Organization

Abstract

Background: - Pregnancy-induced hypertension has remained a significant global public health threat in both developed and developing countries. Therefore, this study aims to assess renal function tests, serum total protein, lipid profile tests and associated risk factors among pregnant women with pregnancy-induced hypertension attending in Asrade Zewudie Memorable hospital (ASZMPH), Gojjam, Ethiopia: Case-control study.

Methods: - A prospective case control study was done from January 24, 2020, to April 30, 2020. 200 pregnant women were recruited; 100
with pregnancy induced hypertension (case group) and 100 normotensives (control group). 5 ml of venous blood was collected on the SST test tube and analyzed for lipid profile, renal function and serum total proten using standard enzymatic methods, modified Jaffe method, urease and biu ret method respectively. The analysis was done by using SPPS software (version 20.0). Finally, the result was interpreted by using chi-square, Pearson's correlation, and multivariate logistic regression. The level of statistical significance was set at a 95% confidence interval and those variables in which P-value is less than 0.05 were considered clinically significant.

RESULTS:- In the present study, there was a significant increase in the serum level of serum total cholesterol, triglyceride, blood urea, serum creatinine, and low density lipoprotein ($p < 0.05$) and a significant decrease in high density lipoprotein in case groups as compared to normal groups ($p < 0.05$). The level of serum total protein was almost similar between the two groups even if the level was somewhat high in cases. However, the elevation of serum total protein was not significant ($p > 0.05$). Factors like body mass index (BMI) >24.9 k/m^2, absence of scheduled exercise, alcohol, did not consume enough fruit, to be in the 3rd trimester and pregnant women with multigravidia status have significant association with abnormal results of lipid profile tests relative with the reveres one. Factors like BMI >24.9 k/m^2 and multigravidia were have significant association with blood urea nitrogen and serum creatinine test results. Also pregnant women's in the third trimester have more chance to have abnormally low serum total protein compared with pregnant women in the second trimester in both groups($P< 0.05$).

CONCLUSION: - The findings of the present study indicated that abnormal results of lipid profile and RFT were significantly associated with pregnancy induced hypertension. Also pregnant women's with body mass index >24.9 k/m^2 ,not doing scheduled exercise, drunks alcohol, not consuming fruit, in the 3rd trimester, and with multigravidia were have high significant chance to have abnormal results of lipid profile test, renal function test and serum total protein relative with the opposite one. So it will better if clinicians use lipid profiles and renal function tests as screening test than requesting other costly tests. Further studies in different areas of the country by considering life style variation also recommended.

Keywords: Lipid profile, serum total protein, renal function test, PIH, and Ethiopia

1. INTRODUCTION

1.1. Background

Pregnancy-Induced Hypertension (PIH) also referred to as Gestational Hypertension (GH); is a multifactorial condition occurring during pregnancy, usually appears after the 20th week of gestation and characterized by high blood pressure (over 140/90) present during pregnancy(1-2). PIH can lead to more serious maternal complications called preeclampsia, which is characterized by a hypertensive state, systemic vascular disturbances, and reduced blood flow to multiple organs both for mothers and babies. If left untreated can prevent the placenta from getting enough blood and this limit the baby from getting enough oxygen and food (3-7).

Pregnancy-induced hypertension is classified into three main classes. The first one is Chronic Hypertension which is defined by women who have high blood pressure (over 140/90) before pregnancy, early in pregnancy (before 20 weeks), or persisting longer than 12 weeks after delivery. The second class is gestational hypertension characterized by a woman with high blood pressure that develops after 20 weeks' gestation in pregnancy and goes away after delivery. In addition, the third one preeclampsia which is defined both by chronic and gestational hypertension can lead to this severe condition after 20 weeks of pregnancy (3-4).

The incidence of preeclampsia globally is about 2 - 10 % of all pregnancies (8). Women whose parents had PIH, carrying multiples, younger than age 20 or older than age 40 and who had high BP or kidney disease before pregnancy have an increased risk of developing gestational hypertension **(9)**. PIH continues to be a major obstetric problem in the present-day (10). Currently, some studies show that elevation of plasma lipids level occurs during pregnancy due to alteration in hormones, and due to systemic vascular disturbances causes endothelial dysfunction. Usually, levels of lipids revert to normal shortly after delivery physiologically. But in the case of preeclampsia endocrinological alteration is more and change in serum lipids cannot easily revert to normal (11,12 ,14). An association of serum lipid profile with gestational protein uric hypertension is highly suggested to reflect some new diagnostic tool. Moreover, the hormon al imbalance is a prime factor for the pathogenesis of PIH and this endocrinal imbalance is well reflected in alteration of serum lipid profile (15-17).

Pre-eclampsia is a theory of complication during pregnancy; where the exact etiology is still unknown (18). Serum total protein is important in regulating blood volume by maintaining the oncotic pressure (colloid osmotic pressure) of the blood compartment. So alternation level of total protein can be identified as an early sign in developing preeclampsia, and many clinicians consider serum protein level as one of the important laboratory findings in the treatment of hypertensive disorders in pregnancy (19). During physiological pregnancy, in most women's urinary protein excretion normally increases from its normal valve because of increased GFR, the permeability of the glomerular basement membrane and reduction of tubular reabsorption of filtered protein; but if it exceeds 300 mg/24 hours; it is abnormal (20, 21).

Proteinuria in pregnancy can indicate primary preeclampsia, renal disease, or renal disease secondary to systemic disorders, such as diabetes or primary hypertension (20, 22). Additional information on pregnancy-related changes in renal function and the urinary tract can be found separately (23). During the assessment of a pregnant woman with proteinuria; determination of the time of onset and quantity of proteinuria should be considered and if the onset of proteinuria is also continuing for more than 20 weeks after gestation, preeclampsia is likely (24). On the other hand, if the onset of proteinuria is before 20 weeks gestation, the cause may be due to primary or secondary renal disease (25). In women with chronic hypertension, even mild proteinuria (50 to 300 mg/day) is associated with adverse pregnancy outcomes. Urinary protein excretion >3 g/day can be seen in preeclampsia (24, 26). With the dramatic hormonal and hemodynamic changes of pregnancy, renal function is altered and these changes must be considered when assessing renal function in pregnancy and the choice of medications provided through parturition. Renal function and filtration are also affected in preeclampsia, and recent advances have greatly expanded our understanding of the pathophysiological mechanisms of this pregnancy-related renal syndrome (27).

In general, the study aims to identify the association between RFT, total protein, and lipid profile with PIH, which have greater advantages for physicians in the management, and control of complications due to PIH happened on the pregnant mother. And if the clinically significant association is found in this study between those analytes with PIH the physician can simply request analysis of serum lipid profile, urea, creatinine, and total protein rather than requesting of 24-hour urine protein and hormonal test which has a more complicated procedure and high cost

1.2. **Statement of the Problem**

Pregnancy-induced hypertension has remained a significant global public health threat in both developed and developing countries. This disorder has a greater contribution to prenatal morbidity and mortality of mothers. Additionally, with its complication in mothers, it has a greater effect in a fetus, by resulting in various complications like intrauterine, preterm delivery, premature delivery, fetal growth retardation, abruption placenta, etc (27-28). PIH has been recognized for centuries; however, the etiology of this syndrome remains uncertain, limiting effective intervention (31). WHO goal regarding PIH was for the prevention and treatment of preeclampsia and eclampsia and to improve the quality of care and outcomes for pregnant women (32).

Hypertensive disorders represent the most common medical complications of pregnancy with a reported incidence between 5 and 10% and WHO estimates that at least one woman dies every 7 minutes due to PIH (33, 34). According to 2015 WHO reports approximately 25.7% women's died globally; of which, 99% of deaths occur in Latin-American, Asian and African countries and Sub-Saharan Africa. The maternal complication due to PIH in Sub-Saharan Africa account for about 56% of all maternal deaths which shows that pregnancy-related complications in developing countries are 14 times higher than in developed countries (35). In the United States; PIH accounts for approximately 15% (36) and in Ghana contributed to 8.9% for maternal mortality (37).

In Ethiopia; PIH is a common maternal medical complication during pregnancy (38). Studies in Ethiopia showed that the incidence of PIH is approximate 16% in association with preeclampsia/eclampsia and increased rapidly from time to time with an incidence between 5 and 10% (39, 40). When we see separately each study in Ethiopia regarding PIH; a systematic review study conducted on the causes of maternal mortality showed that the proportion of maternal mortality due to hypertensive disorders between 1980 and 2012 is increased from 4%-29% at different health facilities (41); and as Ethiopian Demographic Health Survey (EDHS) 2016 reports; maternal mortality ratio is 412 deaths per 100,000 live births (42, 43).

Proteinuria is one of the most important diagnostic and prognostic variables determining t he clinical significance of pregnancy-related hypertension (44). Some studies reported that decreased HDL cholesterol and delayed triglyceride clearance and high blood pressure are associated with the development of preeclampsia and eclampsia and this association can be used for understanding the pathologic processes of pre-eclampsia and eclampsia and May helpful for prevention PIH (45).

During physiological pregnancy, it is known that there are changes in the body metabolites due to increased demand for metabolic fuels for the fetal growth, development of its associated structures and also causes hormonal changes in the body which may lead to changes in lipid profile and total protein during different trimesters of the pregnancy. It has been noted that in the first trimester, the maternal metabolic environment gets modified due to a rise in serum levels of estrogen, and progesterone followed by pancreatic beta-cell hyperplasia leading to an increase in insulin secretion which changes different metabolites (46). The association of alteration of serum lipid profile is known to be strongly associated with atherosclerotic cardiovascular diseases and has a direct effect on endothelial dysfunction with the most important feature in toxemia of pregnancy of hypertension (47). During pregnancy glomerular filtration may be impaired and this leads to rising in serum urea and creatinine concentration. So that the association of serum lipid profile with gestational proteinuric hypertension is highly suggested to reflect some new diagnostic tools. Therefore, simple measurement of serum urea, creatinine, lipid parameters, and protein may be of good predictive value in toxemia of pregnancy, avoiding the costly endocrinal investigations (45).

So the purpose of this research is to put some diagnostic indications to physicians for the treatment, management, and control of pregnancy-induced hypertension which is the main maternal problem for most mothers during pregnancy. Assessment of the association between RFT, lipid profile, and total protein with PIH has a greater advantage for physicians who care the pregnant women in both management and control of complications due to PIH happened on the pregnant mother.

1.3. Significance of the study

Hypertensive disorders of pregnancy are a global public health concern both in developed and developing countries. However, evidence regarding the risk factors of hypertensive disorders of pregnancy is limited particularly in Ethiopia. As many studies indicated, in Ethiopia the incidence of PIH is rapidly increased from time to time; (48) and increase the medical complication both on the mortality of mother and fetus. But most of the studies done in Africa especially in Ethiopia mainly focuses on the prevalence of pregnancy induced hypertension. Beside indicating the prevalence identification of risk factors and alternation of biomarkers or biochemical tests during PIH are the additional diagnostic tool for management, prevention and control of this complication. But most of the studies especially those done in Ethiopia did not identify the biomarkers associated with PIH. So the present study aims to fill this gap by identification of different biomarkers which are associated with PIH and association of different risk factors with abnormal results of the lipid profile and renal function test results of the study participants.

So the main aim of the present study was to identify the association between plasma lipid profile with PIH, serum creatinine with PIH, serum urea with PIH, and serum total protein with PIH. Assessment of the association between RFT, lipid profile, and total protein with PIH has a greater advantage for physicians who care the pregnant women in both management and control of complications due to PIH happened on the pregnant mother. The physician can simply request analysis of serum lipid profile test, blood urea nitrogen, and serum creatinine. And for policy makers it adds a new diagnostic tool for complication of PIH. This also has a greater advantage for patients in both the availability of the test method in the local area and economical due to the relatively low cost of these analytes.

2. LITERATURE REVIEW

2.1.Pregnancy-induced hypertension

Global studies showed that preeclampsia and eclampsia were associated with higher rates of maternal mortality, prenatal mortality, and morbidity, preterm, and small for gestational age deliveries. Women with PIH are five times more likely to have prenatal death compared with wo men who have no hypertensive disorders of pregnancy as WHO recommendations (32). Most study finding shows that a woman in developing countries is seven times more likely to develop preeclampsia as women of a developed country (48).

In the United States PIH is one of the leading causes of maternal death with an estimated percentage of 7% to 10% of all pregnancies, even if, the mechanisms responsible for the pathogenesis of PIH are unclear (49). Maternal deaths due to PIH accounts approximately one-tenth in Asia and Africa and one-quarter in Latin America; of which preeclampsia and eclampsia have the greatest impact on maternal and newborn morbidity and mortality (32).

Many studies have done both globally and at a national level; and reported that PIH is clinically associated with alternation of different body metabolites. A retrospective cohort study was done in Maternal and Child Health Hospital of Anhui, China from January 2014 and June 2015 on 299 pregnant women by divided into mild and severe Hypoproteinemia (SHP) preeclampsia depend on the value of albumin showed severe hypertension occurred more frequently in severe Hypoproteinemia (SHP) than in mild Hypoproteinemia (MHP) ($P< 0.01$) and concluded that severe hypoproteinemia preeclampsia is associated with a higher risk of adverse pregnancy outcome than mild hypoproteinemia preeclampsia, deserving closer surveillance during pregnancy (33,50). A prospective study conducted in Mahatma Gandhi Mission Hospital, India from October 2012 to 2014 on 200 pregnant women with PIH showed that the level of total cholesterol, triglycerides, LDL Cholesterol, VLDL-Cholesterol increased in both second and third trimester with highly increased in the third trimester and HDL-Cholesterol is decreased compared with normotensive once (46).

Similarly with this study; another case-control study done in India from June 2008 to June 2009, showed that a significant rise of TC, TG, LDL and a significantly fall in HDL was observed with the case compared with normal groups ($P < 0.05$) (45). Also, another case-control study done in India showed that there was significant incensement in the serum TG, TC, LDL levels and decreased level of HDL on pregnant women with PIH as compared to control pregnant women; but these changes were not significant as compared to controls which are in opposite conclusion with the previous two studies in India (51).

A case-control study conducted on 200 pregnant women visiting Jinnah Hospital Lahore, Pakistan from September 2012 to March 2013 to evaluate the alteration of serum lipid profile during normotensives and hypertensive pregnancy participants (50 were normotensives and 150 hypertensive) showed that hypertension was directly associated with increased levels of serum TGs (161.02 ± 3.58 vs. 105.31 ± 8.53), TC (188.90 ± 4.11 vs. 152.45 ± 1.99), LDL (136.50 ± 3.17 vs. 70.48 ± 2.14) and VLDL (117.06 ± 1.05 vs. 41.06 ± 1.70), and fall in HDL (49.41 ± 1.56 vs. 37.16 ± 1.64) as compared to that of normotensives once. The increased levels of TGs, TC, and LDL in hypertensive pregnant women were also correlated with significantly increased values of TC/HDL, TG/HDL, and LDL/HDL in hypertensive pregnant women. Depends on the finding; the study concluded that lipid profile plays a critical role in regulating blood pressure during pregnancy. Increased levels of TC, TG, LDL, and VLDL induced hypertension, whereas, HDL regulated the blood pressure to normal levels. This association may be significant in understanding the development of hypertension during pregnancy and may help in developing the strategies for the prevention and treatment of PIH (52).

A Case-control study conducted at Biochemistry Department of LUMHS Jamshoro, Pakistan from October 2014- November 2015; to evaluate the difference between the lipid profile and serum total proteins between the normotensives and hypertensive persons of the same age showed that serum Cholesterol & LDL were significantly high, HDL and serum total protein significantly reduced in a hypertensive group ($p = 0.001$) and depends on the finding the authors concluded that lipid profile abnormalities and serum total protein abnormalities significantly associated with hypertension (53).

A prospective study done in a national clinic of Nigeria in 2003, showed that the level of serum protein and serum creatinine were significantly higher in the preeclampsia group than the control group; ($p<0.05$) (54). Another study done in South Africa in 2017 showed that a significant alternation of creatinine and protein was found with pregnant women who have PIH compared with pregnant women without PIH (55). On the other hand; a cross-sectional study was done in University of Lagos, Nigeria, in 2018; showed that there was statistically significant reduction in the level of total protein ($p<0.05$) and increase in serum urea and Serum creatinine ($p<0.05$) in preeclampsia when compared with control and concluded that PIH has deleterious effects on renal function (38). Another case-control study carried out in Dhaka Medical College Hospital (DMCH) from September 1999 to June 2000 to observe the serum total protein, albumin and globulin in patients suffering from hypertensive during the first trimester showed that the serum total protein and albumin levels were significantly ($p < 0.001$) higher in hypertensive women's compared to control pregnant women (56).

Hypertensive disorders of pregnancy are also the main cause of poor prenatal outcomes in Ethiopia, even though; there is no study at the national level that shows the prevalence of PIH in Ethiopia (38,48). A systemic review done in 2016 by taking 70 studies showed that the overall prevalence of PIH in Ethiopia was 6.29 %; with a higher prevalence in Southern Nations, Nationalities, and Peoples' Region, and reduction in the rate of HDP is seen from the 1990s to 2010s, (8.54%, 5.71% respectively). This study reported that pregnant women with the age of ≥ 35 years are more likely to develop hypertensive disorders of pregnancy, (OR = 1.64, 95% CI) (48). A health facility-based cross-sectional study carried out in Mizan-Tepi University Teaching Hospital, in 2016 with aim of assessment of pregnancy-induced hypertension and its associated factors among 422 women showed that the prevalence of PIH was 7.9%; of which 15.2% were gestational hypertension, 36.4% were mild preeclampsia, 45.5% were severe preeclampsia and 3% eclampsia (42).

As many studies reported risk factors that have a statistically significant association with the development of PIH/ pre-eclampsia or eclampsia are primigravida, history of preeclampsia on prior pregnancy, multiple pregnancies, receiving nutritional counseling during pregnancy and drinking alcohol during pregnancy (57). Another case-control study done in seven public hospitals of Tigray, Ethiopia indicated that mothers with rural residents and overweight (BMI > 25 Kg/m2) were at greater odds of suffering to hypertensive disorders (OR = 3.7, 95% CI; 1.9, 7.1) and (AOR = 5.5 95% CI; 1.12, 27.6) respectively) and mothers who consume less amount of fruits in their diet had 5 times higher chance to develop hypertensive disorders than those who consume fruits regularly (OR = 5.1, 95% CI; 2.4, 11.15) (58). A comparative cross-sectional study conducted in University of Gondar Hospital, Ethiopia in 2015; reported that pregnant women as compared to non-pregnant had significantly increased total cholesterol (211.9+40.88 and 172.40+29.64 mg/dl) [p<0.05], triglycerides (190.81+81.04 and 107.43+45.80 mg/dl), LDL- cholesterol (116.03+37.26 and 86.12+27.29mg/dl) [p<0.05] and the level of HDL cholesterol was significantly lower in pregnant women (59.58+14.26) than control (63.63+11.4, P <0.05) (59).

3. OBJECTIVES

3.1. General Objective

- ✓ Assessment of renal function, serum total protein, lipid profile and associated risk factors among pregnant women with pregnancy-induced hypertension attending in Asrade Zewudie Memorable hospital (ASZMPH), Gojjam, Ethiopia: Case-control study, 2020.

3.2. Specific objectives

- ✓ To compare serum total protein level between pregnant women with PIH and normotensives pregnant women in Asrade Zewudie Memorable Primary Gojjam, Ethiopia; 2020 G.C.

- ✓ To compare lipid profile (TC, TG, HDL & LDL) level between pregnant women with PIH and normotensives pregnant women in Asrade Zewudie Memorable Primary Gojjam, Ethiopia, 2020 G.C.

- ✓ To compare renal function tests (serum creatinine & blood urea) level between pregnant women with PIH and normotensives pregnant women in Asrade Zewudie Memorable Primary Gojjam, Ethiopia; 2020 G.C.
- ✓ To identify the association of risk factors of PIH with lipid profile, RFT and serum total protein result of pregnant women both in case and control groups, 2020 G.C.

4. MATERIALS AND METHODS

4.1. Study area/ Setting

The study was conducted at Asrade Zewudie Memorable Primary Hospital, the governmental hospital located in Burie town, west Gojjam (Mirab Gojjam), Amhara, Ethiopia. The town is found 400 km North-west of Addis Ababa, the capital city of Ethiopia and 148 km south-west of the Amhara Regional State capital, Bahir Dar. It is located between latitude 10^0 17 -10^0 49 North, and longitude 37^0 00 - 370 11 East. Based on the 2007 national census conducted by the Central Statistical Agency of Ethiopia (CSA), this town has a total population of 11,132. The hospital is established in 2017 and currently has four maternity wards, which are ANC, delivery and labor ward, maternity high-risk ward, and postnatal wards. This hospital gives delivery service additional with other health care services to pregnant mothers come from all health centers in the surrounding and to all health centers in Sekela Woreda, Kuarit Woreda Burie Zuriya Woreda, and Shindi Wonberma Woreda. The hospital (AZMPH) has various professionals that included 22 physicians of which 4 specialists, 123 nurses, 16 midwives, 15 laboratory professionals, 4 emergency surgery specialists, and 65 other health professionals, and 75 administrative staff, making 320 staff.

4.2. Study design and period

The study was a prospective case-control study that was done on pregnant women by classifying them into two groups depending on the blood pressure finding. The data was collected from January 24, 2020, to March 26, 2020, and analysis was done in the period between March 29, 2020 to April 30, 2020. As general data collection and analysis takes a total of five months; three months for data collection and two months for data analysis.

4.3. Population

4.3.1. Source population

The study population was all pregnant mothers attending the maternity centers of Asrade Zewudie Memorable Primary Hospital (AZMPH); Gojjam, Ethiopia, during the study period.

4.3.2. Study Population

The study population was consisting of a total of 200 pregnant women with a gestational period of greater than 20 weeks (100 for each group) divided into two groups. Ages ranged from 18-to-45 years. Obstetricians classify the gestational weeks into three trimesters which were first trimester (<12 weeks), second trimester (12 - 24 weeks), and third trimester (24- 42 weeks). Most Studies in Ethiopia showed that PIH mostly happens after 20 weeks of gestation. So the study participants included in this study were only pregnant women with a gestational age of 20 and above. The two groups divided into case and control groups. A case group was pregnant mothers with pregnancy induced hypertension (> 140/90 mmHg). Hypertensive disorders of pregnancy included gestational hypertension, preeclampsia, eclampsia, and preeclampsia/eclamp sia superimposed on chronic hypertension. A control group was pregnant woman in the maternity wards of the hospital and who did not have a diagnosis of hypertensive disorders (BP < 140/90 mmHg). The two study groups were similar almost in many characteristics with the only difference between those in the presence or absence of pregnancy-induced hypertension.

4.4. **Inclusion and exclusion criteria:**

4.4.1. **Inclusion criteria**

All pregnant women in the maternity ward with a gestational age greater than 20 weeks were prepared for the two groups. Pregnant women with PIH **(**BP >140/90 mmHg**)** who attend maternity care during the study period in AZMPH were included in the case group and those pregnant women without pregnancy-induced hypertension **(**BP <140/90 mmHg**)** and also have not any pregnancy complications was assessed as control groups.

4.4.2. Exclusion criteria

- Pregnant women with a gestational age of fewer than 20 weeks
- Pregnant mothers who have a history of chronic hypertension before pregnancy
- Pregnant mothers with complicated problems and unable to take a blood sample
- Pregnant mothers with previously or currently renal disorders
- Pregnant mothers with the previous history of a liver disorder, cardiac disease
- Pregnant mothers with a history of dyslipidemia
- Pregnant mothers who are on treatment of lipidemic drugs
- Pregnant mothers with history of HIV
- Pregnant mothers who are obesity
- Pregnant mothers below 18 years old and mothers greater than 45 years old
- Pregnant mothers who are not voluntary to give blood samples & sign consent
- Pregnant mothers who have habit of smoking ware excluded from the study.
- Pregnant women's take food within 2hrs.

4.5. Study variables

4.5.1. Dependent variables

- Lipid profile
- Serum total protein
- RFT (Renal function test)
- PIH (Pregnancy-induced hypertension)

4.5.2. Independent variables

- Age
- Drinking alcohol
- Nutritional status
- Residence
- Educational status
- Occupation
- Income status
- Week of gestation
- BMI
- Parity
- Gravidity
- Pregnancy status
- Gestational age
- Trimester

4.6. Sample size calculation and Sampling method

4.6.1. Sample size calculation

The sample size was calculated using a 95% confidence interval with 0.05 precision. Different previous studies which are focusing on the assessment of lipid profile with PIH, serum total protein with PIH and prevalence of PIH in Ethiopia was carefully assessed and a systematic study conducted on the aim of identification risk factor and prevalence of pregnancy-induced hypertension; entitled with a prevalence of hypertensive disorders of pregnancy in Ethiopia was used to calculate the minimum required sample size for this study. The systematic study reported that the overall prevalence of PIH in Ethiopia was 6.29 % (48).

The sample size was calculated based on the comparison of proportions for matched case-control study using

$n = Z^2P(1-P)/d^2$.

Where:

n = sample size,

Z = standard normal deviation at 95% confidence interval which is 1.96,

d = degree of precision (taken as 0.05),

P = proportion of the target population (estimated at 6.29 % which is 6.29/100 = 0.0629)

Q = alternate proportion (1-P) which is 1-0.0629 = 0.9371.

$n = (1.96)^2 (0.0629) (0.9371)/ (0.05)^2$.

$n = 90.58 = 91$

The minimum sample size was estimated to be 91. Even if the study is a prospective study, 10 percent was added by considering possible unforeseen attrition factors, 10% of this value was added to make it up to 100 pregnant women. So that for this study; 200 patients were recruited (100 each for both cases and the control group).

4.6.2. Sampling Method

A prospective convenient sampling method was used for this study. The data was collected until the required sample size is achieved. And all pregnant women with a gestational age of greater than 20 weeks and visiting the four maternity wards of AZMPH in the day time during the study period were participated in the study depend on their willingness and voluntarism.

4.7. Measurement and Data collection

4.7.1. Data collection procedure

The data was collected by using a pre-tested structured questionnaire which is developed following through a review of works of literature from different sources and with included information related to the socio-demographic condition, obstetrics, medical status, lifestyle, and nutritional habits of the participants. The questionnaires were first adapted in English and translate into Amharic by an expert and were translated back to English to see the consistency of the item. The questionnaire contains sections for assessing demographics and associated factors additional with clinical and maternity-related information's.

The questions and statements were grouped and arranged according to the particular objectives that aimed to address. The data was collected by two data collectors who have a BSC degree in midwives to maintain the quality of the data and was supervised by the principal investigator. Data collection was carried out in the maternity ward (antenatal care clinic, labor ward, and delivery ward). Before the actual data collection, a pretest was carried out to evaluate the validity of the format and procedure of the study. In addition to the questionnaire, patient medical records ware reviewed to abstract relevant variables related to laboratory, clinical, and obstetrics data. A detailed history of present pregnancy, history of diabetes, renal disorders, cardiac disorder, thyroid disorders, and family history regarding preeclampsia was taken by the physician before enrolling patients for the study. The BMI was calculated in the maternity ward and those who are obese were excluded from the study.

The actual data was collected by face to face interviews, measurements, and reviewing medical records of the mother using a pretested structured questionnaire by trained data collectors. Data were collected only for those pregnant women who come to the hospital maternity ward only in the day time. Written informed consent was obtained. The participants were interviewed by the data collectors who were occasionally aided by trained assistants and comprising junior resident doctors; with the aid of a semi-structured, pre-tested questionnaire. The participants were allowed to sit and rest for about 5 minutes. The blood pressure reading was taken while the woman seated in the upright position and supine position using a mercury sphygmomanometer apparatus, and for referred women; BP was taken from referral form. Elevated blood pressure was repeated after at least 4 hours.

4.7.2. **General Sample collection procedures**

Following written informed consent, and response to the questionnaire, the participant ware registered on the notebook and a serial number was given for each. Blood sample from pregnant mothers who have eaten food within two hours was collected after a minimum of 3 hours fasting. Then blood sample was collected from each participant based on his or her voluntarism in the laboratory department or the maternity wards depend on the clinical condition by laboratory technologists. 4-5 ml of venous blood was collected in an SST test tube from the medial cubital of the forearm with swabbing by gauze or cotton moistened with 70% alcohol by the principal investigator or trained laboratory professional. The whole blood sample was stored at room temperature for 10-20 minutes until it is coagulated. Then the blood sample was transported to the laboratory department and centrifuged at 3,000 revolutions per minute for 5 minutes to separate the serum from the red cells. After centrifugation, the sample was immediately analyzed by the principal investigator in the laboratory department of the clinical chemistry section at AZMPH.

4.7.3. Principle of the test for biochemical analysis

HDL was measured using the principle apoB containing lipoproteins in the specimen are reacted with a blocking reagent which excluded the apoB containing lipoproteins from the assay and only HDL-chol is detected. The method uses sulfated alpha-cyclodextrin in the presence of Mg+2, which forms complexes with apoB containing lipoproteins. And a two-reagent homogeneous method was used for the measuring of serum LDL-cholesterol. The first detergent (reagent 1) solubilizes only the none LDL-lipoprotein particles and the second detergent (reagent 2) solubilizes the remaining LDL- lipoprotein and chromogenic coupler allow for color formation and the enzyme reaction with LDL-C produce a colored product which is proportional with the concentration of LDL-C in the sample.

Cholesterol was measured using coupled reactions that hydrolyze cholesteryl esters in a peroxidase catalyzed reaction that produces color (proportional to cholesterol concentration) and absorbance was measured at 500 nm. Triglycerides were measured using a series of coupled reactions in which triglycerides are hydrolyzed to produce glycerol which is oxidized to colored products by glycerol oxidase and H_2O_2.

Biuret method which Depends on the presence of peptide bonds was used for the measurement of serum total protein. The biuret reagent contains sodium potassium tartrate to form a complex with cupric acid and maintain its solubility in an alkaline solution. Peptide bonds react with Cu2+ ions in alkaline solutions to form a colored product (proportional to the amount of protein). Then absorbance was measured at 540nm.

Modified Jaffe reaction method with the principle of creatinine reacts with picric acid in alkaline solution (yellow color) to form a red-orange chromogen was used for the measurement of serum creatinine. Serum urea in the sample was measured based on the preliminary hydrolysis of urea with urease (specific enzyme) to liberate ammonium ions, followed by a secondary reaction that measures the amount of ammonium ion spectrophotometrically at 340 nm which is proportional to the amount of urea.

4.7.4. Interpretation of the results

The result was interpreted by using normal reference range specifically for pregnant women; defined by considering different factors like gestational age, hormonal change, trimester, etc which is cited by WHO for each analyte. Therefore, after the test has been analyzed the result for each analyte was interpreted based on this reference range.

Interpretation of results of serum urea, creatinine, lipid profile and serum total protein in pregnant women

(http://perinatology.com/Reference/Reference%20Ranges/Cholesterol,HDL.htm), (60, 61)

Table 1 Normal range of serum total protein, urea, creatinine and lipid profile in both pregnant and non-pregnant adults

Analyte	Units	Non-pregnant Adult	First Trimester	Second Trimester	Third Trimester
Total serum protein	g/dL	6.7 - 8.6	6.2 - 7.6	5.7 - 6.9	5.6 - 6.7
HDL Cholesterol	mg/dL	40 - 60	40 -78	52 - 87	48 - 87
LDL cholesterol	mg/dL	<100	60 -153	77 -184	101- 224
Total cholesterol	mg/dL	<200	141 -210	176 - 299	219 -349
Triglyceride	mg/dL	< 150	40 - 159	75 - 382	131 - 453
Serum urea	mg/dL	7 -20	7 -12	3 - 13	3 -11
Serum creatinine	mg/dL	0.5 - 0.9	0.4 - 0.7	0.4 - 0.8	0.4 - 0.9

4.8. Data Quality Assurance

4.8.1. Data quality control measures:

The quality of the data was assured by using a validated and pretested questionnaire. Before the actual data collection, pre-testing was done on 5% of the total study subjects at Janmeda health center ANC and delivery ward which was not included in the actual study, and based on the findings necessary amendments were made. Data collectors were trained for one day intensively on the study instrument and data collection procedure that included the relevance of the study, the objective of the study, confidentiality of the information, informed consent, and interview technique. The data collectors worked under the close supervision of the supervisors to ensure adherence to correct data collection procedures. The principal investigator reviewed the filled questionnaires at the end of data collection every day for completeness. The principal investigator and the data collectors conducted a morning session to solve the problem if encountered, as early as possible, and to take corrective measures accordingly. Moreover, the data was carefully entered and cleaned before the analysis.

4.8.1.1. Pre-analytical Test

Each activity including blood sample collection, transportation, and storage was based on good laboratory practices (GLP) using standard operating procedures (SOPs) to ensure data quality. The participants also were well prepared and labeling was done from PIH 001 –PIH 100 and NPIH 001 - NPIH 100 on the questionnaire and the SST test tube. The venous blood sample was collected from the antecubital fossa of the forearm by cleaned with 70% alcohol antiseptic and then dispensed to the plane test tube with separator jell. The specimens was stored and transported to the laboratory department. Then the blood sample was centrifuged to separate the serum and the test was analyzed.

4.8.1.2. Analytical Test

The test was analyzed in the AZMPH laboratory department, clinical chemistry section. The equipment had been calibrated monthly by the type-Auto calibrator. Besides, two levels (normal and pathological) of internal quality control (IQC) samples were run along with the serum sample. The control sample results were interpreted using the Westgard multi-rule algorithm. The control sample results have to be within acceptable ranges (± 2SD) before the test is analyzed. The sample was analyzed after well understood the leaflet for each analyte by the principal investigator and senior laboratory technologists.

4.8.1.3. Post Analytical Test

After the test was analyzed by using the selected method, the printed result was checked for all post-analytical factors like a unit of reporting and correctness serial number given by the investigator. And the result was approved by the responsible laboratory technologist in the laboratory. Then the printed result was immediately attached to the questionnaire. In case of the absence of printer paper, the results were recorded carefully on the provided space with its specific ID.

4.9. Data analysis and interpretation:

Data was cleared, edited, checked for completeness manually and entered to Software Package for the Social Science (SPSS) for version 20.0 for Windows® (SPSS Inc., Chicago, IL, the USA) for analysis. After organizing and cleaning the data, frequencies, and percentages were calculated to all variables that are related to the objectives of the study. The extent of serum urea, creatinine, lipid profile, and serum total protein difference between case and control group was checked. Categorical variables were analyzed using the chi-square and continuous variables were analyzed with multiple logistic regression. Pearson's correlation was used to find a correlation between lipid profile with PIH, RFT with PIH, and serum total protein with PIH. The level of statistical significance was set at a 95% confidence interval. A P-value of less than 0.05 was considered statistically clinically significant. Finally, the result was presented using tables and other narrative form

4.9. **Ethical considerations**

The study was conducted after ethical approval was obtained from the Research and Ethics Institutional Review Board of Addis Ababa University College of Health Science, Department of Medical Laboratory Science. An official permission letter was submitted to the Amhara Health bureau and AZMPH. Informed written consent was also obtained from each study participant before the actual data collection. Participants were informed of the risks and benefits of the study, their right to withdraw anytime, how confidentiality is maintained using codes, and their right to get their results for free. Individual's clinically significant laboratory test analysis for tests was linked to the responsible doctor for further diagnosis and treatment accordingly.

4.10. Operational definitions

- **Case groups:** Pregnant women's with pregnancy-induced hypertension
- **Control groups**: Pregnant women's without PIH **(**BP <140/90 mmHg**).**
- **Eclampsia:** a condition that causes a pregnant woman, usually previously diagnosed with preec lampsia (high blood pressure and protein in the urine).
- **Gestational age:** describe how far along with the pregnancy which is measured in weeks, from the first day of the woman's last menstrual cycle to the current date.
- **Gestational hypertension:** a condition characterized by high blood pressure during pregnancy
- **Gravidity:** the number of times that a woman has been pregnant.
- **Lipid profile:** includes HDL cholesterol, LDL cholesterol, triglyceride, total cholesterol.
- **Parity:** the number of times that she has given birth to a fetus with a gestational age of 24 weeks or more, regardless of whether the child was born alive or was stillborn.
- **Preeclampsia:** A condition defined by high blood pressure in women and a high level of protein in their urine and often also has swelling in the feet, legs, and hands which appears late in pregnancy.
- **Pregnancy-induced hypertension:** Pregnancy-induced hypertension (PIH) is defined as systolic blood pressure (SBP) >140 mmHg and diastolic blood pressure (DBP) >90 mmHg.
- **Severe Pre-eclampsia:** defined by blood pressure $\leq$ 110/90 mmHg after 20weeks of gestation with severe headache, blurred vision, Epigastria pain, hyperreflexia, oliguria (urinary output equal or less than 400mls/24hours), proteinuria (protein equal or greater than 5g/24 hours; dipstick +++), increased weight (equal or more than1000g/week and the patient is conscious.

4.11. Dissemination of the result

The result is submitted to Addis Ababa University, College of Health Science, Department of Medical Laboratory Science, Amhara Health Bureau, and AZMPH. Identification of association was one basic thing for physicians who care for pregnant women for the management of PIH easily by using analysis of these analytes rather than requesting other costly and complicated tests. In addition, the result of the study finding may be used as baseline information for further studies. The findings will be presented at national and international scientific conferences. The findings were also be sent/ submitted to different medical journals for publication after a relevant manuscript for publication is prepared.

5. RESULTS

A total of 200 pregnant women were participated in the study. The study participants were classifying into two as case and control groups. Control groups were pregnant women without PIH; whereas case groups were pregnant women with PIH. Each group contains 100 study participants. Out of 200 study participants; 140 (70 %) were from antenatal clinics and the rest 60 (30 %) were from other maternity wards like a delivery ward, postnatal care ward, and high-risk maternity wards. Demographic data related to the studied population was noted and tabulated in table 2.

The age range of total pregnant women who participated in this study was from 18 – 41 years with a mean of 29.07 ± 5.29 years. The mean age of pregnant women with PIH and pregnant women without PIH was 28.23 ± 5.39 years and 29.9 ± 5.07 years respectively. Most of the study participants of both the control and case group found in the age range 25 - 29.9 years with a percentage of 34 % and 43% respectively. The percentage of pregnant women in the age group ≥ 40 years contained the lowest percentage which is 4 % in each group. From a total of 200 pregnant women participated in this study; 142 (71 %) were from urban, 142 (71 %) were literate, 151 (75.5%) husbands were literate, 104 (52%) of them were economically in the middle-income class, 83 (41.5%) were governmental employers (Table 2), most of the study participants were multiparious (55%), 193 (96.5 %) were married, 110 (77.5%) of them were in the third trimester, and 125 (62.5%) were had a gestational week of from 20 to 37 weeks (Table 4).

Both case and control groups had almost similar age distribution which aids the study to compare other factors variations in the two groups by maintaining nearly similar parties between groups (SD 5.07 & 5.4 respectively). Blood pressure is the main variable for this study to classify groups and parameters. In this study the distribution of diastolic and systolic blood pressure in control groups was less disperse than that of the case groups (70.0 ± 8.8 & 115.0 ± 9.03 and 104.7 ± 15.2 & 154.0 ± 15.6 respectively) (Table 2).

Table 2 Blood pressure results & demographic factor distribution of study participants both control& case groups.

Parameters		**Control**	**Cases**	**Total**	**p-valve**
Age range of participants **(**in years**)**	< 25 yrs	22	16		0.068
	25- 29.9 yrs	43	34		
	30- 34.9 yrs	20	28		
	35- 39.9 yrs	11	18		
	> 40 yrs	4	4		
BMI	< 17.3 kg/m^2	9	8		0.001
	18.5- 24.9 kg/m^2	91	72		
	25- 29.9 kg/m^2	0	20		
DBP in mmHg (mean ± SD)		70.0 ± 8.8	104.7 ± 15.2		< .001
SBP in mmHg (mean ± SD)		115.0 ±9.0	154.0 ± 15.6		< .001
Resident	Urban	69 (69 %)	73 (73 %)	142 (71 %)	0.533
	Rural	31 (31 %)	27 (27 %)	58 (29 %)	
Educational status of participants	Illiterate	25 (25%)	33 (33 %)	58 (29 %)	0.009
	Up to 12^{th}	24 (24 %)	7 (7%)	31 (15.5%)	
	Diploma	17 (14 %)	24 (24 %)	41 (20.5%)	
	Degree & above	34 (34 %)	36 (36 %)	70 (35 %)	
Occupational status of mothers	House wife	29 (29 %)	26 (26 %)	55 (22.5 %)	0.68
	Governmental	43 (43 %)	40 (40 %)	83 (41.5 %)	
	NGO	12 (12 %)	18 (18 %)	30 (15 %)	
	Farmer	16 (16 %)	16 (16 %)	32 (16 %)	
Monthly income of study participants	Low level (< 793)	18 (18%)	19 (19%)	37 (37%)	0.010
	Middle (793 -2805)	39 (39%)	20 (20%)	59 (59%)	
	High income (>2805)	43 (43%)	61 (61%)	104 (52%)	

BMI = body mass index**, DBP**= diastolic blood pressure, **NGO**= nongovernmental organization, **PIH**= pregnancy induced hypertension, **SD** = standard deviation, **SBP** = systolic blood pressure

Most of the study participants in both groups were from an urban resident (69 % & 73 %), were degree and above holders (34 % & 26 %), and were governmental employers (43 % & 40 %). The highest percentage of the educational status of the husbands of the pregnant women was degree & above holders in both case and control groups (50 % & 52 %: respectively) and 51% out of the total participants. Changes in demographic factors like income status and educational status in case groups were significant as compared to controls (P^-valve < 0.05); and changes like occupation and residence were not significant as compared to controls (P^-valve >0.05) (Table 2). So depends on this study finding; pregnant women's with higher income level have greater chance to develop PIH (P^-valve = 0.010) (Table 2).

Mean BP (both SBP & DBP) was significantly increased in hypertensive pregnant women as compared to that in normotensive pregnant women (104.70/154.00 mmHg & 74.75/ 112.75 mmHg, respectively). The consistency of high SBP/DBP was maintained throughout the whole pregnancy period in pregnant women with PIH when directly compared to SBP/DBP of normotensive pregnant women. The finding shows that the distribution blood pressure (both DBP & SBP) was more dispersed in the case group than the normal group with a standard deviation of 15.2/8.8 and 15.6/9.03 respectively (table 2). Most of the study participants were within normal weight in both control (91%) and case groups (73%). But the percentage of overweight is higher in the case group than the normal group (19 % & 2 % respectively). In this study BMI was significantly high in case groups relative to control groups (P^- valve < 0.001) and the result indicated that pregnant women's with BMI > 25 Kg/m^2 had high chance to develop PIH than those of with normal (P^- valve < 0.001) (Table 2).

From a total of 200 study participants; 49.5% of the respondents did not have a habit of scheduled physical exercise (39% control & 60% of case groups) and of which only 5.5% (8 % of control & 3% cases) have done always scheduled exercise. Also from a total of pregnant women participated in this study; 47.5% were alcohol drinkers (3.5% drinks always & 44% drinks sometimes), 94 % were have a habit of fruit consumption (3.5% eat fruit always, 26.5 % eat fruit once a week & 64 % eat fruit sometimes) (Table 3).

Table 3 Activity related findings of participants & its association with the dependent variable.

Parameters		**Blood pressure**		**Total**	P-valve
		Control	**Case**		
Have you do scheduled physical exercise?	Yes, always	8 (8%)	3 (3 %)	11(5.5%)	0.005
	Yes 2-3 times a week	26 (26%)	19 (19 %)	45(22.5%)	
	Yes, once a weeks	10 (10%)	4 (4 %)	14(7 %)	
	Yes, irregularly	17 (17%)	14 (14 %)	31(15.5%)	
	Not at all	39 (39%)	60 (60 %)	99(49.5%)	
Are you an alcohol drinker	Yes, always	1 (1%)	6 (6 %)	7 (3.5%)	0.007
	Yes , sometimes	38 (38%)	50 (50 %)	88 (44%)	
	Not at all	61 (61%)	44 (44 %)	105(52.5%)	
Habits of fruit consumption	Always	4 (4%)	3 (3 %)	7 (3.5%)	0.083
	Sometimes	68 (68%)	60 (60 %)	128 (64%)	
	Once a week	25 (25%)	28 (28 %)	53 (26.5%)	
	Never at all	3 (3%)	9 (9 %)	12 (8%)	
P-value ≤ 0.05 considered as statistically significant					

According to the finding of this study change in habit of taking alcohol and doing scheduled exercise was significantly associated with blood pressure (P-valve <0.05); whereas a change in habit of fruit consumption was not significantly associated with blood pressure (P-valve >0.083) (table 3).

In the present study; finding of maternity-related factors were almost similar between the control group and the case group. From a total of 200 pregnant women participated in this study; 68 % 0f the study participants were not have a history of partner change, 96.5% were married once, almost half (51.5 %) of the study participants are multigravidia, 87.5 % were wanted their pregnancy and 55% were with party range of 1-4. The gestational weeks of the pregnant women range from 20 weeks to 42 weeks with a higher percentage in the third trimester (> 24 weeks) both in the control and case groups which accounts 78 % and 77 % respectively, and the rest were in the second trimester (22 % & 23 % respectively). Most of the study participants in both case and control groups are with a gestational week of from 20 to 37 weeks (64 % & 61%; respectively) and this indicates that the gestational weeks of most pregnant women's included in this study are in the range between 20 weeks to 37 weeks (Table 4).

Table 4 Maternity related factors and previous clinical history records distribution and thier association with blood pressure results of the study participants.

Parameter		**Blood Pressure**		**Total**	P-valve
		Control	**Case**		
Partner change	Yes	30(30%)	34 (34%)	64 (32%)	0.544
	No	70 (70%)	66 (66%)	136 (68%)	
Trimester	Second	22 (22%)	23 (23%)	45 (22.5%)	0.866
	Third	78 (78%)	77 (77%)	155 (77.5%)	
Marital status	Married	98 (98%)	95 (95%)	193 (96.5%)	0.381
	Single	2 (2%)	5 (5 %)	7 (3.5 %)	
Gravidity	Primigravida	50 (50%)	47 (47%)	97 (48.5%)	0.68
	Multigravidia	50 (50%)	53 (53%)	103 (51.5%)	
Pregnancy status	Wanted	94 (94%)	94 (94%)	175 (87.5%)	0.987
	Unwanted	6 (6%)	6 (6%)	25 (12.5%)	
Parity	Uniparous	43 (43%)	41 (41%)	84 (42%)	0.604
	Multiparous	57 (57%)	59 (55%)	116 (58%)	
Gestational age	20 -37 weeks	61 (61%)	64 (64%)	125 (62.5%)	0.007
	37- 42 weeks	29 (29%)	14 (22%)	43 (21.5%)	
	> 42 weeks	10 (10%)	22 (22%)	32 (16%)	
Gestational Diabetic Mellitus	Yes	1 (1%)	8 (8%)	9 (4.5%)	NA
	No	99(99%)	92 (92%)	191 (95.5%)	
Family history of chronic hypertension	Yes	6 (6%)	13 (13%)	19 (9.5%)	0.146
	No	94 (94%)	87 (87%)	181 (90.5%)	
Family history of diabetes	Yes	3 (3%)	13 (13%)	16 (7.5%)	0.001
	No	98 (98%)	87 (87%)	185 (92.5%)	
Have you sense of Headache	Yes	14 (14%)	40 (40%)	54 (27%)	0.015*
	No	86 (86%)	60 (60%)	146 (73%)	

As shown in table 4; change in gestational weeks, family history of diabetes and sense of headache were significantly associated with blood pressure results of the study participants ($P < 0.05$) and other maternity factors like partner change, marital status, trimester, party, gravidity, family history of diabetes, gestational diabetes mellitus were not show significant association with blood pressure (P-valve > 0.005).

According to the present study finding; concentration of chemical analytes like blood urea, serum total cholesterol, serum triglyceride, and LDL cholesterol were more dispersed and higher in case pregnant women's relative with control pregnant women (44.86 ± 35.96 for case groups & 29.51 ± 17.79 for control groups, 239.29 ± 65.71 for case groups & 213.30 ± 33.85 for control groups, 196.38 ± 73.25 for case groups & 149.37± 47.49 for control groups, 153.89 ± 56.15 for case groups & 120.65 ± 34.58 for control groups; respectively). But the level of serum creatinine and serum total protein was relatively less dispersed between the two groups; even if some higher results are seen in case groups relative with control groups (1.00 ± 0.45 for case groups & 0.79 ± .196 for control groups, 5.60 ± 1.53 for case groups & 5.47 ± 1.52 for control groups; respectively). Relatively nearly precision measurement and decreased concentration of HDL cholesterol was seen in case groups relative with the control groups (50.94 ± 16.910 for case groups & 76.48 ± 16.24 for control groups; respectively) (Table 5).

Table 5 Comparison of renal function, lipid profile, and total protein concentration between control and case groups of pregnant women.

Parameters	**Group**	**Mean ± 1SD**	**Range**	**Maximum**	**Minimum**
Serum urea (mg/dl)	Control	29.51 ± 17.79	84.64	96.03	11.39
	Case	44.86 ± 35.96	202.90	214.30	11.40
Serum creatinine (mg/dl)	Control	0.79 ± .196	1.32	1.80	0.48
	Case	1.00 ± 0.45	2.22	2.70	0.48
Serum total protein (g/dl)	Control	5.47 ± 1.52	8.39	12.32	3.93
	Case	5.60 ± 1.53	10.88	13.90	3.02
Total cholesterol (mg/dl)	Control	213.30 ± 33.85	165.57	280.10	114.53
	Case	239.29 ± 65.71	317.40	443.20	125.80
Triglyceride (mg/dl)	Control	149.37± 47.49	200.00	275.68	75.68
	Case	196.38 ± 73.25	412.72	496.20	83.48
HDL cholesterol (mg/dl)	Control	76.48 ± 16.24	64.36	103.20	38.84
	Case	50.94 ± 16.910	75.00	97.32	22.32
LDL Cholesterol (mg/dl)	Control	120.65 ± 34.58	212.26	266.98	54.72
	Case	153.89 ± 56.15	266.93	335.80	68.87

HDL= high density lipoprotein, **g/dl**= gram per deciliter, **LDL**= low density lipoprotein, **mg/dl**= milligram per deciliter, **SD**= standard deviation

In the present study the relatively high percentage of abnormal level of TC, TG, LDL, serum creatinine and blood urea level were seen in pregnant women with PIH compared with pregnant women without PIH. The level of serum total protein was nearly similar between the two groups where as the level of HDL-cholesterol was very low in case groups compared with control groups. Abnormal result of RFT were seen in case groups compared with the control groups (serum creatinine level 40 % in case groups & 20% in control groups and blood urea nitrogen level 62 % in case groups & 40% in control groups; respectively). The levels of the serum lipid profile of all participants in the two trimester periods (2^{nd} and 3^{rd}) were also measured and then compared to these serum levels between normotensive and hypertensive pregnant women. The serum levels of TC, TG, and LDL were consistently increased whereas; the serum level of HDL was consistently decreased from 2^{nd} trimester towards 3^{rd} trimester in hypertensive pregnant women (Table 6).

Table 6 Biochemical test finding & its association with the blood pressure of the study participants.

		Blood pressure		Total	P-valve
		Control	Case		
TP	Decreased (< 5.6 g/dl)	69 (69 %)	57 (57 %)	126 (63%)	0.282
	Normal (5.6 - 6.7 g/dl)	17 (17 %)	30 (30 %)	47 (23.5%)	
	Increased (> 6.7 g/dl)	14 (14 %)	13 (13 %)	27 (13.5%)	
Cr	Normal (0.4 - 0.9mg/dl)	80 (80 %)	60 (60 %)	140 (70 %)	0.002
	Increased (> 0.9 mg/dl)	20 (20 %)	40 (40 %)	60 (30 %)	
BUN	Normal (3 -11mg/dl)	60 (60 %)	39 (39 %)	99 (49 %)	0.002
	Increased (> 11mg/dl)	40 (40 %)	62 (62 %)	102 (51 %)	
TC	Hypochelestemia (<219mg/dl)	55 (55 %)	41 (41 %)	96 (43 %)	0.009
	Normal level (219 -349)	45 (45 %)	52 (52 %)	97 (43.5%)	
	Hypercholestemia(> 349 g/dl)	0 (0%)	7 (7 %)	7 (3.5 %)	
TG	Decreased level(< 131mg/dl)	39 (39 %)	17 (17 %)	56 (28 %)	< 0.001
	Normal level (131 - 453 g/dl)	61 (61 %)	81 (81 %)	142 (71 %)	
	Increased level (> 453mg/dl)	0 (0 %)	2 (2 %)	2 (1 %)	
HDL-C	Decreased level(< 48 mg/dl)	3 (3 %)	50 (50 %)	53 (26.5%)	< 0.001
	Normal level (48 - 87 mg/dl)	70 (70 %)	47 (47 %)	117(58.5)	
	Increased level (> 87 mg/dl)	27 (27 %)	3 (3 %)	30 (15 %)	
LDL-C	Decreased level (< 101mg/dl)	33 (33 %)	11 (11 %)	44 (22 %)	< 0.000
	Normal level (101- 224 mg/dl)	66 (66 %)	76 (76 %)	142 (71 %)	
	Increased level (> 224mg/dl)	1 (1 %)	13 (13 %)	14 (7%)	

Cr = Serum creatinine**, HDL-C=** high-density lipoprotein**, LDL-C** = low-density lipoprotein, **TC**= serum total cholesterol, **TG**= serum triglyceride**, TP =** serum total protein, **BUN**= blood urea nitrogen, **g/dl**= gram per deciliter, **mg/dl**= milligram per deciliter

As shown in table 6; the serum levels of TC, TGs, & LDL in hypertensive pregnant women were significantly very high ($P<0.05$). Contrarily, the level of HDL consistently remained very low ($P<0.05$) in case groups in all trimesters compared with control groups. RFTs in case groups were significantly higher than the control groups ($P<0.05$). The ratios of lipid profiles such as values of these ratios (TC/HDL, TG/HDL and LDL/HDL) for case groups were significantly higher ($P<0.05$) as compared to control groups. But the change of serum total protein did not show a significant association with blood pressure ($P^{-valve} = 0.282$) (Table 6).

In the present study; many variables were found to be candidate for binary logistic analysis for the final model. Therefore; a multivariate approach were applied to determine which factor best explained and predict the outcome of lipid profile results of case and control groups. The outcome of the final multiple logistic regression model indicate that factors like BMI, habits of doing scheduled exercise, habit of drinking alcohol, habit of fruit consumption, trimester and gravidity were have significant association with lipid profile tests and was more significant in case groups relative with control groups (Table 7).

According to the present study finding; multivariable analysis revealed that pregnant women incase groups who have BMI > 24.9 kg/m^2 had greater chance to develop abnormally increased level of serum TC (AOR: 6.003, 95% CI: 1.6 - 6.2, P= 004), serum TG (AOR: 4.315, 95% CI: 1.1 -10.1, P= 0.044), LDL cholesterol (AOR: 4.565, 95% CI: 2.5- 46.3, P = 0.012), & abnormally decreased HDL cholesterol (AOR: 2.15 , 95% CI: 1.42 -11.1, P = 0.032). But in control groups the association between lipid profile tests and BMI was not significant ($P-^{valve}>0.05$) (Table 7).

A multiple logistic regression analysis indicates that pregnant women who did not have habits of doing physical exercise have greater chance to have abnormally high level of serum TC (AOR: 3.135, 95% CI: 1.5 - 8.1, $P-^{valve}=0.007$ in case and AOR: 2.50, 95% CI: 1.9 -13.1, $P-^{valve}=0.021$ in control groups), serum TG (AOR: 2.480, 95% CI: 1.6 -11.3, $P-^{valve}=0.046$ in case), serum LDL (AOR: 1.52, 95% CI: 1.5-12.7, $P-^{valve}=0.0101$ in control groups) and abnormally decreased HDL (AOR: 2.907, 95% CI: 1.10 - 7.7, $P-^{valve}=0.032$ in case and AOR: 2.764, 95% CI: 2.61 -12.6, $P^{-valve}=0.018$ in control groups) level compared with those who do schedule physical exercises.

In case groups; pregnant women who drunks alcohol were relatively have almost two times more chance to develop abnormally high TC level (AOR: 2.05, 95% CI: 2.3- 13.4, P-^{valve}=0.05) and nearly 3 times more chance to have abnormally decreased level of serum HDL cholesterol (AOR: 2.047, 95% CI: 1.3 - 8.4, P-^{valve}=0.05) compared with pregnant women with PIH and have no habits of drinking alcohol. Also in control groups increased TC, TG, LDL and decreased HDL were seen in alcohol drunker women, even if the association was not significant (P-^{valve} >0.05). In case groups pregnant mothers who consume less amount of fruits in their diet were have almost 5 times more chance to have abnormally increased level of serum TC (AOR: 4.930, 95% CI: 1.4- 7.46, P-^{valve}=0.027) and nearly two times to have abnormally decreased level of serum HDL cholesterol (AOR: 2.012, 95% CI: 3.1- 28.9, P-^{valve}=0.048) than those who have good habits of fruit consumption. But no significant association was seen with TG, LDL level of case groups and all lipid profile tests of control groups with habits of fruit consumption (P >0.05) (Table 7).

In multiple logistic regressions two maternity related factors have shown significant association with some lipid profile tests of both control and case groups. Pregnant mothers who were in the third trimester were have higher chance to have increased serum TC (AOR: 1.444, 95% CI: 1.3 - 7.5, P-^{valve}=0.004 in case groups and AOR: 1.078, 95% CI: 1.4 - 4.4, P-^{valve}=0.013 in control groups) and increased level of serum TG (AOR: 2.310, 95% CI: 4.5 - 11.0, P-^{valve}=0.022 in case groups and AOR: 1.119, 95% CI: 2.0 - 7.7, P-^{valve}=0.022 in control groups) in both groups. Also in this study pregnant mothers in case group with multigravidia were have a significant greater chance (nearly five times) to have increased serum TC (AOR: 4.915, 95% CI: 3.0 -25.0, P-^{valve}=0.045) and serum LDL cholesterol (AOR: 4.840, 95% CI: 2.6- 42.1, P-^{valve}=0.043) compared with primigravida women's; even if this significant association was not seen in control groups (Table 7).

Table 7 Association between different risk factors of PIH with abnormal lipid profile test results of the study participants

Parameters	**Groups**	**TC**		**TG**		**HDL- C**		**LDL _C**	
		p *valv*	**AOR (95%CI)**	**p *valu***	**AOR (95%CI)**	**p *value***	**AOR (95%CI)**	**p *value***	**AOR (95%CI)**
BMI >25kg/m^2	Case	.004	6.003 (1.6 - 6.2)	.044	4.315 (1.1 -10.1)	.012	4.565 (2.5- 46.3)	.032	2.15 (1.4-11.1)
Not done scheduled exercise	Case	.007	3.135 (1.5 - 8.1)	.046	2.480 (1.6 -11.3)	.032	2.907 (1.10 - 7.7)	.264	5.897 (1.7 - 9.2)
	Control	.021	2.50 (1.9 -13.1)	.239	1.920 (2.3 - 13.1)	.018	2.764 (2.61 -12.6)	.0101	1.52 (1.5-12.7)
Drinking alcohol	Case	.05	2.047 (2.3- 13.4)	.243	5.263 (2.9 - 30.3)	.016	3.23 (1.3 - 8.4)	.458	1.88 (1.4 - 9.9)
Not consume fruit	Case	.027	4.930 (1.4- 7.46)	.495	5.785 (4.4- 95.8)	.048	2.012 (3.1- 28.9)	.933	7.604 (5.4-51.7)
3rd trimester	Case	.004	1.444 (1.3 - 7.5)	.022	2.310 (4.5 - 11.0)	.157	2.425 (2.2 - 11.3)	.233	2.675 (4.8 -22.6)
	Control	.013	1.078 (1.4 - 4.4)	.022	.119 (2.0 - 7.7)	.025	2.06 (1.1 - 5.6)	.067	1.37 (1.7 - 1.9)
Multigravidia	Case	.045	4.915 (3.0 -25.0)	.652	3.310 (1.4 - 9.5)	.869	2.106 (.47 - 9.45)	.043	4.840 (2.6- 42.1)

The reference groups are

For BMI >25kg/m^2 = participants with normal BMI, **Not done scheduled exercise** = who did regular schedule physical exercise, **Drinking alcohol** = those not drunk alcohol, **Not consume fruit**= participants have habits of fruit consumption, **3rd trimester**= participants in the 2nd trimester and **Multigravidia** = participants with prigravidia

A multivariate approach was also applied to determine which factor best explained and predict the outcome of RFT and serum total protein results in both groups. And the multiple logistic regression model indicate that factors like BMI and gravidity were have significant association with blood urea nitrogen and serum creatinine test results of both groups.

A multivariable analysis revealed that pregnant women with BMI greater than 24.9 kg/m^2 were have greater chance to develop abnormally increased level of BUN level (AOR: 2.935, 95% CI: 0.614 - 13.700, P= .027 in case groups & AOR: 1.850, 95% CI: 0.629 - 5.573, P= 0.011 in control groups) and serum creatinine level (not significant in case groups & AOR: 6.507, 95% CI: 1.475 - 28.698, P= 0.013 in control groups) compared with study participants with normal BMI. Pregnant mothers with multigravidia also have high chance to had increased level of serum creatinine (AOR: 2.352, 95% CI: 0.942 - 5.874, P= 0.007, in case groups & AOR: 2.027, 95% CI: 0.702 - 5.858, P= 0.012 in control groups) and blood urea level (AOR: 1.856, 95% CI: 0.754 - 4.566, P= 0.018 in case groups & AOR: 2.288, 95% CI: 0.971 - 5.390, P= 0.045 in control groups) compared with pregnant women's with pri-gravidia (Table 8).

Multiple logistic regression analysis also indicated that pregnant women who were in the third trimester were have significantly more chance to had abnormally low serum total protein result compared with pregnant women in the second trimester in both groups (AOR: 1.254, 95% CI: 0.503 - 3.126, P= 0.028, in case groups & AOR: 1.693, 95% CI: 0.750 - 3.825, P= 0.025 in control groups) (Table 8).

Table 8 Association between different risk factors of PIH with lipid profile, RFT and serum total protein test results of the study participants.

Parameters		Control groups				Case groups			
		P-valve	AOR	95% C.I		P-valve	AOR	95% C.I	
				Lower	Upper			Lower	Upper
BUN	BMI >25kg/m^2	.011	1.850	.629	5.573	.027	2.935	.614	13.700
	Multigravidia	.045	2.288	.971	5.390	.018	1.856	.754	4.566
TP	Not take Fruit	.242	3.711	.412	33.443	.165	2.137	.731	6.249
	3^{rd} Trimester	.025	1.693	.750	3.825	.028	1.254	.503	3.126
CR	BMI>25kg/m^2	.013	6.507	1.475	28.698	.449	1.523	.513	4.526
	Not did Exercise	.018	1.464	.147	1.461	.021	1.825	.706	4.713
	Multigravidia	.012	2.027	.702	5.858	.0067	2.352	.942	5.874

BMI = body mass index, CR=serum creatinine, TP = serum total protein, BUN= blood urea nitrogen

As we have seen in the above table (Table 8), the reference groups for pregnant women with BMI >25 kgm^2, in multigravidia, not consume enough amount of fruit in their diet, in the third trimester, and not did scheduled exercise were pregnant women with normal BMI (BMI 8.5 - 24.9 kg/m2), with a gravid of prigravidia, who consumes enough fruits in their diet and those who have habits of doing scheduled physical exercise. So table 8 focuses on comparison of these contemporary groups.

6. DISCUSSION

Overall in the present study; a significant increase in lipid profile test (serum cholesterol, triglycerides, and LDL-cholesterol) and significant decreased HDL-cholesterol was seen in the case groups compared with the control groups ($P< 0.001$). The findings of the present study were consistent with previously published studies (45,46, 51, 54, 55, 59) all of which reported that there was a significant incensement of TC, TG, and LDL and significant decreased of HDL-cholesterol in case groups relative with normotensive pregnant women. The present finding provides un evidence for physicians and policymakers for control and prevention of complications that happened due to PIH by regularly monitoring abnormal lipid profile tests.

In this study, significant increase results of serum creatinine and blood urea nitrogen were seen in pregnant mothers with PIH compared with pregnant women without PIH ($P< 0.001$). But in the present study incensement of serum total protein in case groups was not significant compared with control groups ($P> 0.05$). The current finding was consistent with different previously published studies (38, 54, 55, 56). But all of these previous studies indicated that the elevation of serum total protein in case groups was significant. Disturbance of Lipid profile tests and renal function tests are the main indicator of the development of pregnancy-induced hypertension and can be used as a diagnostic tool for the management, control, and prevention of pregnancy-induced hypertension.

In the present study pregnant mothers with multigravida, history of preeclampsia on prior pregnancy, multiple pregnancies, not receiving nutritional counseling during pregnancy, drinking alcohol during pregnancy, not consuming fruit, with BMI > 25 Kg/m2, with gestational weeks of greater than 37 weeks, have a family history of diabetes mellitus and age > 35 years were have greater significant chance to develop pregnancy-induced hypertension ($P < 0.05$). These risk factors in our study are in agreement with already published reports [57, 58]. Also in the present study, pregnant mothers from Urban resident have greater chance to develop PIH than pregnant mothers from Rural resident; which is opposite with the previously published study done in Tigray, Ethiopia (58), which reported that mothers with rural residents were at greater odds of suffering to hypertensive disorders (OR = 3.7, 95% CI; 1.9, 7.1); and this variation may be due to lifestyle and nutritional variation between the two study participants.

According to the finding of this study; serum TC, TG & LDL level was increased in preeclampsia when compared to normal pregnancy, and the change was also statistically significant ($P<0.05$). But the serum level of HDL was significantly low in case groups relative to the control groups ($P<0.05$). This finding was similar with the finding of different previous published studies; (45, 46, 52, 53, 59); in which all reported that pregnant women as compared to non-pregnant had significantly increased total cholesterol, triglycerides and LDL- cholesterol ($p<0.05$) and significantly lower level of HDL cholesterol ($P <0.05$). On the contemporarily, the finding this study was not in line with the finding of a case-control study done in India which reported that the changes in lipid profile were not significant as compared to controls (51).

In hypertensive pregnant women, the serum levels of TC, TG & LDL were significantly increased and the serum level of HDL was significantly decreased in the 3rd trimester of pregnancy relative with those in the 2nd trimester. The decreased levels of HDL in the 3rd trimester of hypertensive pregnant women in our study were in agreement with already published reports [52,53,59]. From the results of our study, it can be found that serum levels of TC, TG, HDL, and LDL are more profound in the 3rd trimester of pregnancy in hypertensive pregnant women as compared to that of normotensive pregnant women (Table 7).

According to the present study finding the mean blood urea level in preeclampsia was 44.86 ± 35.96 mg/dl and normal pregnancy was 29.51 ± 17.79 mg/dl and the mean serum creatinine level in pregnant women with PIH was 1.00 ± 0.45 mg/dl and normal pregnancy was 0.79 ± .196 mg/dl. Serum creatinine and blood urea level were increased in preeclampsia when compared to normal pregnancy, and the change was statistically significant ($P<0.05$). And this was similar with the finding of different previously published studies (38, 54, 55); which all reported that a significant alternation of blood urea and serum creatinine was found with pregnant women who have PIH compared with pregnant women without PIH ($P <0.05$).

According to the finding of the present study nearly all of the study participants (in both groups) were has decreased level of serum total protein. The cause for this decreasing value may be due to life style and nutritional based problems. The mean serum total protein level in pregnant women with PIH was 5.60 ± 1.53 g/dl and in women with normal pregnancy was 5.47 ± 1.52 g/dl. The level of serum total protein was somewhat high in case groups relative with control groups but the change was not significantly low in case groups relative with the control groups (P>0.05) which is similar with the finding of different previous studies (54 - 56); which indicated that pregnant women with PIH have increased level compared with pregnant women's without PIH and the change in serum total protein level was significantly ($p < 0.001$); even if; the change in this study was not significant (P>0.05). On the contemporary; the finding of the current study was in opposite with finding of different previously published studies (33, 38, 50, 53); all reported that there was statistically significant decreasing in the level of total protein ($p<0.05$). This difference may be happened due to different variation of study participants like a nutritional habit, ethnicity, and follow up care during ANC, exercise and other factors, which have a greater effect on the protein level of study participants.

A multiple logistic regression analysis indicate that factors like BMI, habits of doing scheduled exercise, habit of drinking alcohol, habit of fruit consumption, trimester and gravidity were have significant association with lipid profile tests, factors like BMI and gravidity were have significant association with RFT and factors like exercise & trimester were have significant association with serum total protein result of the study participants (Table 7).

Pregnant women with BMI > 24.9 kg/m^2 have greater chance to develop abnormally increased level of serum TC (AOR: 3.135, 95% CI: 1.5 - 8.1, P-^{valve}=0.007 in case and AOR: 2.50, 95% CI: 1.9 -13.1, P-^{valve}=0.021 in control groups), serum TG (AOR: 2.480, 95% CI: 1.6 - 11.3, P-^{valve}=0.046 in case), serum LDL (AOR: 1.52, 95% CI: 1.5-12.7, P-^{valve}=0.0101 in control groups), BUN level (AOR: 2.935, 95% CI: 0.614 - 13.700, P= .027 in case groups & AOR: 1.850, 95% CI: 0.629 - 5.573, P= 0.011 in control groups) and serum creatinine level (AOR: 6.507, 95% CI: 1.475 - 28.698, P= 0.013 in control groups) & abnormally decreased HDL-C (AOR: 2.15 , 95% CI: 1.10 - 7.7, P = 0.032). However, in control groups the association between lipid profile tests and serum creatinine with BMI was not significant (P-^{valve}>0.05) (Table 7).

Pregnant women who did not have habits of doing physical exercise have greater chance to have abnormally high level of serum TC (AOR: 3.135, 95% CI: 1.5 - 8.1, P-^{valve}=0.007 in case and AOR: 2.50, 95% CI: 1.9 -13.1, P-^{valve}=0.021 in control groups), serum TG (AOR: 2.480, 95% CI: 1.6 -11.3, P-^{valve}=0.046 in case), serum LDL (AOR: 1.52, 95% CI: 1.5-12.7, P-^{valve}=0.0101 in control groups) and abnormally decreased HDL (AOR: 2.907, 95% CI: 1.10 - 7.7, P-^{valve}=0.032 in case and AOR: 2.764, 95% CI: 2.61 -12.6, P^{-valve}=0.018 in control groups) level compared with those who schedule physical exercises.

In case groups; pregnant women had habit of drinking alcohol were have high chance to have abnormally high cholesterol level (AOR: 2.05, 95% CI: 2.3- 13.4, P-^{valve}=0.05) and nearly 3 times more chance to have abnormally decreased level of serum HDL cholesterol (AOR: 2.047, 95% CI: 1.3 - 8.4, P-^{valve}=0.05). Also in control groups increased TC, TG, LDL and decreased HDL were seen in alcohol drunker women, even if the association was not significant (P-^{valve} >0.05). Not taken fruit were have high chance for abnormally increased level of serum total cholesterol (AOR: 4.930, 95% CI: .40 - 1.46, P-^{valve}=0.027) and decreased level of serum HDL cholesterol (AOR: 2.012, 95% CI: .14 - 28.89, P-^{valve} = 0.048) than those who have good habits of fruit consumption (Table 7).

Pregnant mothers in the third trimester were have higher chance to have increased level of TC (AOR: 1.444, 95% CI: 1.3 - 7.5, P-^{valve}=0.004 in case groups and AOR: 1.078, 95% CI: 1.4 - 4.4, P-^{valve}=0.013 in control groups) and increased level of serum TG (AOR: 2.310, 95% CI: 4.5 - 11.0, P-^{valve}=0.022 in case groups and AOR: 1.119, 95% CI: 2.0 - 7.7, P-^{valve}=0.022 in control groups) and abnormally low serum total protein (AOR: 1.254, 95% CI: 0.503 - 3.126, P = 0.028, in case groups & AOR: 1.693, 95% CI: 0.750 - 3.825, P = 0.025 in control groups) compared to those in the 2nd trimester. Multigravidia was also the other factor to increased level of TC (AOR: 4.915, 95% CI: 3.0 -25.0, P-^{valve}=0.045) and serum LDL cholesterol (AOR: 4.840, 95% CI: 2.6- 42.1, P-^{valve}=0.043), CR (AOR: 2.352, 95% CI: 0.942 - 5.874, P = 0.007, in case groups & AOR: 2.027, 95% CI: 0.702 - 5.858, P= 0.012 in control groups) and BUN (AOR: 1.856, 95% CI: 0.754 - 4.566, P = 0.018 in case groups & AOR: 12.288, 95% CI: 0.971 - 5.390, P= 0.045 in control groups); compared with primigravida women (Table 7 &8).

As general the present study finding indicated that pregnant women with PIH were have significantly increased level of TC, TG, LDL,RFT and significantly decreased level of HDL compared with pregnant women without PIH. The study finding also indicates that pregnant mothers with multigravidia, history of preeclampsia, multiple pregnancies, not receiving nutritional counseling during pregnancy, drinking alcohol during pregnancy, not consuming fruit, with BMI > 25 Kg/m^2, gestational weeks greater than 37 weeks, have a family history of diabetes mellitus and age > 35 years were have greater significant chance to develop pregnancy-induced hypertension ($P < 0.05$). Factors like BMI, schedule physical exercise, drinking alcohol fruit consumption, trimester and gravidity were have significant association with abnormal lipid profile and RFT result of the study participants (Table 7&8).

7. STRENGTH AND LIMITATION OF THE STUDY

7.1. Strength of the Study

- ✓ Cases groups were screened by physician based on their blood pressure
- ✓ Previous medical histories of the study participants were taken from their medical cards
- ✓ The test for both groups was analyzed by same instrument one instrument
- ✓ Data were from more representative demographic population with a broader age distribution localities proportion
- ✓ All gestational weeks greater than 20 weeks were included in the study

7.2. Limitation of the Study

- ✓ The blood specimen was non fasting specimen
- ✓ Limiting resource
- ✓ Limited literature done on the assessment of RFT and serum total protein with PIH pregnant women especially in Ethiopia.

8. CONCLUSION AND RECOMMENDATION

8.1. Conclusion

Elevation of serum lipid profiles and renal function tests were seen among pregnant women with pregnancy-induced hypertension when compared with pregnant women without pregnancy-induced hypertension. To conclude, the findings of the present study suggest that abnormal levels of lipid profile (TGs, TC, LDL, and HDL) and renal function test may contribute to the promotion of hypertension in pregnant women. In the present study the level of TC, TG, LDL, and RFT were significantly higher in case groups relative the control groups. But the level of HDL cholesterol was significantly lower in case groups compared with control groups. This association may help to investigate the underlying pathological process of hypertension in pregnancy. Level of serum total protein was somewhat increased in case groups relative with the control groups; but the change was not significant (P^{valve}= 0.282).

So early detection of altered lipid profile and renal function test in preeclamptics, the incidence of complications can be decreased, which in turn reduces the materno-fetal morbidity and mortality. Therefore; serum lipid profiles and renal function tests must be continuously monitored throughout the whole pregnancy period for early detection and/or developing the strategies to prevent any obstetric-associated complication during PIH and/or at the time of delivery.

8.2. Recommendation

Estimating serum lipid profile and renal function test can improve the feto-maternal outcome by early detection of high-risk patients.

So depending the present study finding the following ways are recommend:

- Different studies have to been done in different area of our country
- Other studies have to been done by including different clusters with consideration lifestyle, attitude and nutritional variation
- Clinicians who follow pregnant women advised to use lipid profiles and RFT tests as screening purposes for PIH before requesting other costly and complicated tests.

REFERENCES

1. van Balen VAL, Spaan JJ, Ghossein-Doha C, van Kuijk SM, Spaanderman ME, Peeters LL. Early pregnancy circulatory adaptation and recurrent hypertensive disease. vascular and renal adjustments. 2018:95.
2. Topel ML, Duncan EM, Krishna I, Badell ML, Vaccarino V, Quyyumi AA. Estimated Impact of the 2017 American College of Cardiology/American Heart Association Blood Pressure Guidelines on Reproductive-Aged Women. Hypertension. 2018;72(4):e39-e42.
3. Brown MA, Magee LA, Kenny LC, Karumanchi SA, McCarthy FP, Saito S, et al. Hypertensive disorders of pregnancy: ISSHP classification, diagnosis, and management recommendations for international practice. Hypertension. 2018;72(1):24-43.
4. Pauli JM, Repke JT. Pitfalls with the New American College of Obstetricians and Gynecologists Task Force on Hypertension in Pregnancy. Clinical obstetrics and gynecology. 2017;60(1):141-52.
5. Mol B. roberts CT, Thangaratinam S., Magee LA, de Groot CJM and Hofmeyr GJ (2016). Pre-eclampsia The Lancet.387:999-1011.
6. Bibbins-Domingo K, Grossman DC, Curry SJ, Barry MJ, Davidson KW, Doubeni CA, et al. Screening for preeclampsia: US preventive services task force recommendation statement. Jama. 2017;317(16):1661-7.
7. Sroussi HY, Epstein JB, Bensadoun RJ, Saunders DP, Lalla RV, Migliorati CA, et al. Common oral complications of head and neck cancer radiation therapy: mucositis, infections, saliva change, fibrosis, sensory dysfunctions, dental caries, periodontal disease, and osteoradionecrosis. Cancer medicine. 2017;6(12):2918-31.
8. Ghodke B, Pusukuru R, Mehta V. Association of lipid profile in pregnancy with preeclampsia, gestational diabetes mellitus, and preterm delivery. Cureus. 2017;9(7).
9. English FA, Kenny LC, McCarthy FP. Risk factors and effective management of preeclampsia. Integrated blood pressure control. 2015;8:7.
10. Kar K, Sinha S. Role of lipids, ionized calcium, and alkaline phosphatase in progress of pregnancy-induced hypertension-A prospective study in Kolkata. International Archives of Integrated Medicine. 2016;3(2):129-35.
11. Soma-Pillay P, Catherine N-P, Tolppanen H, Mebazaa A, Tolppanen H, Mebazaa A. Physiological changes in pregnancy. Cardiovascular journal of Africa. 2016;27(2):89.

12. Gupta S, Agarwal A, Sharma RK. The role of placental oxidative stress and lipid peroxidation in preeclampsia. Obstetrical & gynecological survey. 2005;60(12):807-16.
13. Serrano NC, Guio-Mahecha E, Quintero-Lesmes DC, Becerra-Bayona S, Paez MC, Beltran M, et al. Lipid profile, plasma apolipoproteins, and pre-eclampsia risk in the GenPE case-control study. Atherosclerosis. 2018;276:189-94.
14. Gao Q, Tang J, Li N, Liu B, Zhang M, Sun M, et al. What is precise pathophysiology in the development of hypertension in pregnancy? Precision medicine requires precise physiology and pathophysiology. Drug discovery today. 2018;23(2):286-99.
15. Yadav S, Agrawal M, Hariharan C, Dewani D, Vadera K, Krishna N. A comparative study of the serum lipid profile of women with preeclampsia and normotensive pregnancy. Journal of Datta Meghe Institute of Medical Sciences University. 2018;13(2):83.
16. Singh M, Pathak MS, Paul A. A study on atherogenic indices of pregnancy-induced hypertension patients as compared to normal pregnant women. Journal of clinical and diagnostic research: JCDR. 2015;9(7):BC05.
17. Wild R, Weedin EA, Wilson D. Dyslipidemia in pregnancy. Cardiology clinics. 2015;33(2):209-15.
18. Ngene NC, Moodley J. Role of angiogenic factors in the pathogenesis and management of pre-eclampsia. International Journal of Gynecology & Obstetrics. 2018;141(1):5-13.
19. Al Ghazali B, Al-Taie AA-H, Hameed RJ. Study of the clinical significance of serum albumin level in preeclampsia and in the detection of its severity. Am J BioMed. 2014;2:964-74.
20. Thadhani RI, Maynard SE. Proteinuria in pregnancy: Evaluation and management. Monografía internet) Glassock RJ: UpToDate. 2015:1-150.
21. Phillips JK, McBride CA, Hale SA, Solomon RJ, Badger GJ, Bernstein IM. Examination of prepregnancy and pregnancy urinary protein levels in healthy nulliparous women. Reproductive Sciences. 2017;24(3):407-12.
22. Hong DSC, Oh IH, Park J-S, Lee CH, Kang CM, Kim G-H. Evaluation of urinary indices for albuminuria and proteinuria in patients with chronic kidney disease. Kidney and Blood Pressure Research. 2016;41(3):258-66.

23. Tan EK, Tan EL. Alterations in physiology and anatomy during pregnancy. Best practice & research Clinical obstetrics & gynecology. 2013;27(6):791-802.
24. August P, Sibai BM. Preeclampsia: Clinical features and diagnosis. UpToDate Accessed December. 2017;22.
25. Hladunewich MA, Melamad N, Bramham K. Pregnancy across the spectrum of chronic kidney disease. Kidney international. 2016;89(5):995-1007.
26. Aziz MM, Kulkarni A, Shah L, Lashley S, Oyelese Y. Physiologic proteinuria in labor and postpartum: The results of the postpartum proteinuria trial (PoPPy). Pregnancy hypertension. 2018;13:22-4.
27. Osungbade KO, Ige OK. Public health perspectives of preeclampsia in developing countries: implication for health system strengthening. Journal of pregnancy. 2011;2011.
28. Gaym A, Bailey P, Pearson L, Admasu K, Gebrehiwot Y, Team ENEA. Disease burden due to pre-eclampsia/eclampsia and the Ethiopian health system's response. International Journal of Gynecology & Obstetrics. 2011;115(1):112-6.
29. Abdella A. Maternal mortality trend in Ethiopia. Ethiopian Journal of Health Development. 2010;24(1).
30. Wolde Z, Segni H, Woldie M. Hypertensive disorders of pregnancy in Jimma University specialized hospital. Ethiopian journal of health sciences. 2011;21(3).
31. Nugteren JJ, Snijder CA, Hofman A, Jaddoe VW, Steegers EA, Burdorf A. Work-related maternal risk factors and the risk of pregnancy-induced hypertension and preeclampsia during pregnancy. The Generation R Study. PloS one. 2012;7(6):e39263.
32. Organization WH. WHO recommendation: calcium supplementation during pregnancy for the prevention of pre-eclampsia and its complications. 2018.
33. Villar J, Abdel-Aleem H, Merialdi M, Mathai M, Ali MM, Zavaleta N, et al. World Health Organization randomized trial of calcium supplementation among low calcium intake pregnant women. American journal of obstetrics and gynecology. 2006;194(3):639-49.
34. Ladouceur M, Benoit L, Radojevic J, Basquin A, Dauphin C, Hascoet S, et al. Pregnancy outcomes in patients with pulmonary arterial hypertension associated with congenital heart disease. Heart. 2017;103(4):287-92.

35. Organization WH, UNICEF. Trends in maternal mortality: 1990-2015: estimates from WHO, UNICEF, UNFPA, World Bank Group, and the United Nations Population Division. 2015.
36. Obstetricians ACo, Gynecologists. Hypertension in pregnancy. Report of the American College of Obstetricians and Gynecologists' task force on hypertension in pregnancy. Obstetrics and gynecology. 2013;122(5):1122.
37. Asamoah BO, Moussa KM, Stafström M, Musinguzi G. Distribution of causes of maternal mortality among different socio-demographic groups in Ghana; a descriptive study. BMC public health. 2011;11(1):159.
38. Awoke A, Awoke T, Alemu S, Megabiaw B. Prevalence and associated factors of hypertension among adults in Gondar, Northwest Ethiopia: a community based cross-sectional study. BMC cardiovascular disorders. 2012;12(1):113.
39. Teklu S, Gaym A. Prevalence and clinical correlates of the hypertensive disorders of pregnancy at Tikur Anbessa Hospital, Addis Ababa, Ethiopia. Ethiopian medical journal. 2006;44(1):17-26.
40. Seyom E, Abera M, Tesfaye M, Fentahun N. Maternal and fetal outcome of pregnancy-related hypertension in Mettu Karl Referral Hospital, Ethiopia. Journal of ovarian research. 2015;8(1):10.
41. Berhan Y, Berhan A. Causes of maternal mortality in Ethiopia: a significant decline in abortion related death. Ethiopian journal of health sciences. 2014;24:15-28.
42. Gudeta TA, Regassa TM. Pregnancy Induced Hypertension and Associated Factors among Women Attending Delivery Service at Mizan-Tepi University Teaching Hospital, Tepi General Hospital and Gebretsadik Shawo Hospital, Southwest, Ethiopia. Ethiopian journal of health sciences. 2019;29(1).
43. Tessema GA, Laurence CO, Melaku YA, Misganaw A, Woldie SA, Hiruye A, et al. Trends and causes of maternal mortality in Ethiopia during 1990–2013: findings from the Global Burden of Diseases study 2013. BMC public health. 2017;17(1):160.
44. Seong WJ, Chong GO, Hong DG, Lee TH, Lee YS, Cho YL, et al. Clinical significance of serum albumin level in pregnancy-related hypertension. Journal of Obstetrics and Gynaecology Research. 2010;36(6):1165-73.

45. Roohi S, Khan J, Rosaline M, Anees S, Kazi H, Kaleemullah M, et al. Evaluation of Lipid Profile, Calcium and Alkaline Phosphatase in Pregnancy Induced Hypertension Women. UK J Pharma Biosciences. 2015;3(2):24-8.
46. Pusukuru R, Shenoi AS, Kyada PK, Ghodke B, Mehta V, Bhuta K, et al. Evaluation of lipid profile in the second and third trimester of pregnancy. Journal of clinical and diagnostic research: JCDR. 2016;10(3):QC12.
47. Olalere Fd. A Comparative Study of Serum Lipid Profile in Pre-Eclamptic and Normotensive Pregnant Women at the Lagos University Teaching Hospital (LUTH). faculty of obstetrics and gynaecology. 2016.
48. von Dadelszen P, Ayres de Campos D, Barivalala W. Classification of hypertensive disorders of pregnancy. The FIGO Textbook of Pregnancy Hypertension: an Evidence-Based Guide to Monitoring, Prevention and Management London: Global Library of Women's Medicine. 2016:33-61.
49. Poalelungi C, Ceausu I. Hypertension in pregnancy. Journal of Hypertension Research. 2018;4(2):53-9.
50. Chen H, Tao F, Fang X, Wang X. Association of hypoproteinemia in preeclampsia with maternal and perinatal outcomes: A retrospective analysis of high-risk women. Journal of research in medical sciences: the official journal of Isfahan University of Medical Sciences. 2016;21.
51. Chopra S, Bedi P, Mahajan M. study of maternal serum lipids and lipoproteins in pregnancy induced hypertension. Indian Obstetrics and Gynaecology. 2017;7(3).
52. Rabia A, Nureen Z, Kanwal R, Rabail A, Asia P, Muhammad T, et al. Comparative Analysis of Serum Lipid Profile between Normotensive and Hypertensive Pakistani Pregnant Women. Journal of Molecular and Genetic Medicine. 2013; 7 (2).
53. Ali R, Farheen S, Zaheer A, Adnan B. Assessment of Lipid Profile & Serum Total Protein in Patients with Hypertension.Ann. Pak. Inst. Med. Sci. 2017; 23(8)
54. Ahmad MA, Ellahi E, Taqi-Ul-Jawad SM. Pregnancy Hypertensive Disorders Frequency and Obstetric Outcome. pakistan journal of medical & health sciences. 2018;12(1):85-8.
55. Maputle S, Khoza L, Lebese R. Knowledge towards Pregnancy-induced Hypertension among Pregnant Women in Vhembe District, Limpopo Province. Journal of Human Ecology. 2015;51(1-2):47-54.

56. Begum Ja, Sultana R, Naher S. serum levels of total protein, albumin and globulin in woman with hyperemesis gravidarum.j dhaka med coll. 2010; 19(1) : 58-60.
57. Grum T, Seifu A, Abay M, Angesom T, Tsegay L. Determinants of pre-eclampsia/Eclampsia among women attending delivery Services in Selected Public Hospitals of Addis Ababa, Ethiopia: a case control study. BMC pregnancy and childbirth. 2017;17(1):307.
58. Kahsay HB, Gashe FE, Ayele WM. Risk factors for hypertensive disorders of pregnancy among mothers in Tigray region, Ethiopia: matched case-control study. BMC pregnancy and childbirth. 2018;18(1):482.
59. Alemu A, Abebe M, Biadgo B, Terefe B, Baynes HW. Biochemical profiles of pregnant and non-pregnant women attending at the University of Gondar Hospital, Northwest Ethiopia: a comparative cross-sectional study. Ethiopian journal of health sciences. 2018;28(3):331-40.
60. Ramsay M, James D, Steer PJ, Weiner C, Gonik B. Normal values in pregnancy: WB Saunders London; 2000.
61. Berhe AK, Kassa GM, Fekadu GA, Muche AA. Prevalence of hypertensive disorders of pregnancy in Ethiopia: a systemic review and meta-analysis. BMC pregnancy and childbirth. 2018;18(1):34.
62. Campbell OM, Graham WJ; Lancet Maternal Survival Series steering group. Strategies for reducing maternal mortality: getting on with what works. Lancet, 2006, 368 (9543):1284–1299.

ANNEX

Annex I: Information sheet and consent

English version

Project Title: Assessment of RFT, serum total protein, lipid profile tests and associated risk factors among pregnant women with pregnancy-induced hypertension attending in Asrade Zewudie Memorable hospital (ASZMPH), Gojjam, Ethiopia: Case-control study.

PI: Haymanot Tewabe (BSc, MSc candidate)

Name of the Organization: Addis Ababa University College of Health Sciences; Department of Medical Laboratory science

Introduction:

Hello! My name is Haymanot Tewabe and I am an MSc student at Addis Abeba University College of Health science department of Medical Laboratory Technology. I am doing my final research for graduation on assessment of RFT, serum total protein, lipid profile tests and associated risk factors among pregnant women with pregnancy-induced hypertension attending in Asrade Zewudie Memorable hospital (ASZMPH), Gojjam, Ethiopia: Case-control study.. You are invited to participate as a study subject in a research conducted by Msc candidate, from Addis Ababa University. Your participation is voluntary.

Purpose of the research:

As we know, maternal complication during pregnancy is the major problem in our country. So we invite you to take part in this study because we have try to determine the association between serum creatinine, urea, lipid profile, and serum total protein with pregnancy-induced hypertension which aids physicians in easily manage maternal complications besides its function in cost for women.

Procedures:

After agreeing that you can take part, one or more of our research staff will ask you some questions which will take up to 10 minutes. Your weight, height, and vital signs will be measured. And also You will be asked to provide a blood sample and we will collect 5 ml venous blood from you by sterile-disposable vacutainer tube and needle. Then we will conduct laboratory analysis to determine serum creatinine, urea, lipid profile, and serum total protein status.

Confidentiality:

We respect your privacy and confidentiality. Any information that identifies you will not be shared with anyone else outside the study team. The information we will collect from you as part of the study will be kept in a locked file cabinet, or be protected by a password on the computer only accessible to personnel involved in the study.

Risks and Discomfort:

During a collection of specimens from you, appropriate precaution will be taken and all samples will be collected by trained health professionals. If anything happened, appropriate medical care will be provided to you.

Safety: The venous blood sample will be collected using a sterile vacutainer tube/syringe and needle by an experienced health professional after disinfecting the site of puncture by 70% ethanol.

Benefits: You will not receive any payment for your participation in this research study as compensation. However, based on the diagnosis result you will be treated because of that. Besides, the result of the study will be beneficial for the detection and managing of maternal complications.

Incentives: You will know your lipid profile and serum total protein status for free. However, we will not pay you for taking part in this study as well as for treatment costs. For more, we thank you for your participation.

Right to refuse or withdraw:

We like to freely inform you that the involvement of this study depends only on your voluntarism. So we try to select you as our study participants only when if you agree to take part in the study. You can also know that you are free to withdraw from the study at any time and that you will not be discriminated against in any form of service in the hospital.

Contact information: If you have any questions about this study you can contact the following principal investigators and advisors for further information.

Haymanot Tewabe:- Phone**:** 0911412728
E-mail: Haymanottewabe@gmail.com
Dr. Mistire Woldie (Ph.D., Associate Prof.)
Email: **mistire.wolde@aau.edu.et**

Annex II: Informed consent sheet in the Amharic version

Amharic version የ ተሳታፊዎች ፈቃድና መተማመኛ ቅፅ

ሀይማኖት ተዋበ እባላለሁ።በአዲስ አበባ ዩኒቨርሲቲ ጤና ሳይንስ ኮሌጅ የሕክምና ላቦራቶሪ ሳይንስ ት/ክፍል የማስተርስ ድግሪ ተማሪ ተማሪ ስሆን የመመረቂያ ጥናቴን በዚሁ ሆስፒታል ለመስራት አስቢያለሁ። ስለሆነም እርሰዎ በዚህ ጥናት ላይ እዲሳተፉ ተጋብዘዋል። እባክዎ በዚህ ጥናት ለመሳተፍ ከመስማማትዎ በፊት ከዚህ ቀጥሎ የሚገኘዉን ምንባብ በጥሞና ያንብቡና ግልጽ ያልሆነልዎትን ማንኛዉም ሃሳብ ይጠይቁ።

መግቢያ: የጥናቱ ርዕስ “Assessment of RFT, serum total protein, lipid profile tests and associated risk factors among pregnant women with pregnancy-induced hypertension attending in Asrade Zewudie Memorable hospital (ASZMPH), Gojjam, Ethiopia: Case-control study. ”. የእርስዎ በዚህ ጥናት ላይ የሚኖርዎት ተሳትፎ ሙሉ በሙሉ በበጎ ፈቃደኝነት ላይ የተመሰረተ ነዉ። በዚህ ጥናት ዉስጥ ላለመሳተፍ ወይም ለመሳተፍ ከወሰኑ በኃላ ማቋረጥ ይችላሉ። ይህን በማቋረጥዎም በዚህ ሆስፒታል የሚሰጠዉ ማንኛዉም አገልግሎት የማይቋረጥብዎ መሆኑን በክብሮት እንገልጻለን።። በጥናቱ ለመሳተፍ የሚስማሙ ከሆነ የስምምነት ቅጹ ላይ በጹሁፍ ወይም በጣት ፊርማ በማስቀመጥ እንዲተባበሩን በአክብሮት እንጠይቃለን።

የጥናቱ ተሳታፊ ለመሆን የሚጠበቅበዎት ምንድን ነው?

በዚህ ጥናት ለመሳተፍ የሚስማሙ ከሆነ ደም ናሙናዎ ለጥናቱ እንዲሚዉል እና ከርስዎ የሚወሰደው የደም ናሙና ዉጤትም ከዚህ ጥናት ጋር ግንኙነት ካላቸው ሁለት ወይም ሶስት ሰዎች ውጭ ለማንኛውም ሶስተኛ ወገን የማይሰጥ መሆኑን እንገልጻለን። ነገር ግን ይህ አይነቱ መረጃ የርስዎን ማንነት የሚገልጡ መረጃዎችን ማለትም ስም፤አድራሻና የስልክ ቁጥር የመሳሰሉትን መረጃዎችን የማይምር መሆኑንም በአክብሮት እንገልጻለን። ይልቁንም ለዚህ አገልግሎት ብቻ የሚዉል እርስዎን ለማወቅ የሚያስችል መለያ ቁጥር ጥቅም ላይ እንዲዉል ይደረጋል። በአጠቃላይ እዚህ ቅጽ ላይ የሚሰጡት ማንኛውም አይነት መረጃ በምንም አይነት መልኩ የእርሰዎን ማንነት ለሶስተኛ ወገን የማይሰጥ መሆኑን እንዲገነዘቡ እናሳስባለን።

በዚህ ጥናት መሳተፍ የሚያስከትላቸዉ ችግሮች ምንድን ናቸዉ?

የድም ናሙና በሚሰጡበት ወቅት ምንም አይነት የከፋ ችግር አያጋጥምዎትም። ነገር ግን ደም በሚሰጡት ሰኣት በጣም ትንሽዬ ህመም ሊሰማዎት ይችላል። ሆኖም ግን ናሙናዉን ለመሰብሰብ ልምድ ያለዉ ባለሙያ ስለሚመደብና አስፈላጊዉ የጥንቃቄ እርምጃ ስለሚወሰድ የህመም ስሜት አይኖርም።

የህክምና መረጃ በሚስጥር ተጠብቆ መቆየት የሚችለዉ እንዴት ነዉ?

ስለራስዎ የሰጡት ማንኛዉም መረጃና ከተወሰደዉ ናሙና ላይ የተገኘዉ የላቦራቶሪ ዉጤት የሚዉለዉ ለጥናቱ አላማ ብቻ ነዉ። ይህን ማህደር ሊያገኙ የሚችሉት የተወሰኑ የጥናቱ ተባባሪ ሰዎች ብቻ ናቸዉ። ከዚያም በላይ ስለ እርስዎ ያለውን ማንኛውንም መረጃ የተለየ የይለፍ ቃል ባለው የኮምፒውተር የመረጃ ማህደር ውስጥ እንዲቀመጥ ይደረጋል።

በዚህ ጥናት መሳተፍ የሚያስገኛቸው ጥቅሞች ምንድን ናቸው ?

ይህ ጥናት የማስተርስ ዲግሪ መመረቂያ እንደመሆኑ መጠን በዚህ ጥናት በመካፈልዎ በገንዘብ የሚያገኙት ጥቅም ባይኖርም ከጥናቱ የሚገኘውን ውጤት ግን ያለምንም ክፍያ መውሰድ የሚችሉ መሆኑን እንገልጻለን። የእርሶዎ ተሳትፎ በእርስዎንና በወገንዎ ላይ በርግዝና ወቅት የሚከሰትውን ችግር በቀላሉ ለማወቅና ለማከታተል ከፍተኛ ጥቅም ይኖረዋል።

በዚህ ጥናት ተሳታፊ የመሆንዎ መብቶች ምንድን ናቸው ?

በዚህ ጥናት መሳተፍ ሙሉ በሙሉ በእርስዎ ፈቃደኝነት የተመሰረተ በመሆኑ በማንኛውም ሰዓትና ቦታ የማቋረጥ ሙሉ መብት የተጠበቀ ከመሆኑም በላይ እራስዎን ከጥናቱ በማግለልዎ ምክንያት የሚቀርብዎት ምንም አይነት የሆስፒታል አገልግሎት አይኖርም ።ከዚህም በተጨማሪ ጥናቱን በተመለከተ ማንኛውንም አይነት ጥያቄ የመጠየቅና ገለጻ የማግኘት መብት አለብዎት። የላብራቶሪ ምርመራ ውጤቱንም በነጻ ማግኘት ይችላሉ። ነገር ግን እርስዎ በሚሰጡን መረጃ የችግሩን ስፋት ለመከላከል እና ለመቆጣጠር ጠቃሚ ስለሆነ ለሚቀርብልዎት ጥያቄ ቀጥተኛ መልስ ይሰጡን ዘንድ በታላቅ አክብሮት እንጠይቃለን።

ጥያቄ ካለኝ ወይም ችግር ቢያጋጥመኝ ምን ማድረግ ይገባል?

ይህንን ጥናት በተመለከተ ወይም ከዚህ ጥናት ጋር በተዛመደ መልኩ ስለሚያጋጥሙ ድንገተኛ አደጋዎች ወይም ጥያቄ ካለዎት በሚመለከተው አድራሻ ይጠቀሙ።

ሀይማኖት ተዋበ ሞባይል፡ +251-9-11-41-27-28

Annex III. Informed consent form in English version

Card no / ID No_____

I had been informed that the objective of this study is to assess RFT, serum total protein, lipid profile tests and associated risk factors among pregnant women with pregnancy-induced hypertension attending in Asrade Zewudie Memorable hospital (ASZMPH), Gojjam, Ethiopia: Case-control study. The results of this study have an importance to treat me and other patients, and to be used as an input for diagnosing of pregnancy induced hypertension in Ethiopia. I had been also informed about the confidentiality of this study. The principal investigator requested me to participate in the study that would require my willingness to provide the required data that include blood and filling questionnaire. Therefore, with full understanding of the importance of the study, I agreed voluntarily to provide the requested samples and my benefit will be only from the free laboratory investigation result/s.

I_____ hereby give my consent for providing the requested information and specimens as the doctors find best for me.

Signature: _____ Date_____

Annex IV. Informed consent form in Amharic version

የተሳታፊዎች ስምምነት ማረጋገጫ

የሚስጥር ቁጥር ----------

እኔ ስሜ ከላይ የተጠቀሰው ተሳታፊ “assessment of renal function test, total protein and lipid profile concentrations among pregnancy-induced hypertension women in Asrade Zewudie Memorable hospital Gojjam, Ethiopia.” ጥናት ላይ በቂ ገለጻ ተደርጎልኛል። ለጥናቱም ደምናናሙና እንደሚያስፈልግ ተገልጾልኛል።የጥናቱንም አላማዎችም ተረድቻለሁ።በቃለ መጠይቁ ላይ የገለጽኳቸው መረጃዎች በሙሉ በሚስጥር የተጠበቁ እንደሚሆኑ ተነግሮኛል ።በጥናቱ ላይ ያለመሳተፍና ማንኛውንም መረጃ ያለመስጠት እንዲሁም በማንኛውም ጊዜ ከጥናቱ ራሴን የማግለል መብቴ የተጠበቀ እንደሆነ ተገልጾልኛል።

ስለዚህ ለዚህ ጥናት መረጃና የስምምነት ቃሌን የሰጠሁት በአጠቃላይ ሁኔታውን በመረዳትና በፍጹም ፍቃደኝነት ነው፡ በተጨማሪም ጥያቄ ለመጠየቅ ተፈቅዶልኝ ለማወቅ የፈለኩትን ያህል ማብራሪያ አግኝቻለሁ ።የዚህ ጥናት ተሳታፊ በመሆኔ የማገኘው ጥቅም የሁሉንም ምርመራ ውጤት በነጻ ማግኘት እንደሆነ ተረድቻለሁ።

በአጠቃላይ እኔ ከላይ በመተማመኛ ቅፅ የተጠቀሱትን ሁሉ በሚገባና በተረጋጋ መንፈስ አንብቤዋለሁኝ። ስለዚህ በዚህ ጥናት ለመሳተፍ ፈቃደኛ መሆኔን በፊርማዬ አረጋግጣለሁ።

ፊርማ---------- ቀን ----/---/----------

Annex V. Questionnaire English version

S.No	Variables with the specific possibilities of Characteristics for each variable	
1)	ID No given by PI _____ Date _____ Hospital ID No_____ BMI in kg/m2_____ Age _____ Blood pressure_____	
2)	Address of women	1. Rural 2. Urban
3)	Educational status of mothers	1. Illiterate 2. Up to grade 12th 3. Diploma 4. Degree & above
4)	Educational status of husbands	1. Illiterate 2. Up to grade 12th 3. Diploma 4. Degree & above
5)	Occupational status of mothers	1. Housewife 2. Governmental 3. NGO 4. Farmer 5. Others
6)	Income category	1. ≤ 793 (low income) 2. 793 - 2805 (middle income) 3. >2805 (high income)
Physical and cultural activities		
7)	Have you do scheduled physical exercise during the current pregnancy?	1. Yes, 2. No
8)	If yes to Qs number 9, how often?	1. Yes, Always 2. Yes 2-3 times a week 3. Yes, Once a week 4. Yes, Irregularly 5. Not at all
9)	Are you an alcohol drinker	1. Yes 2. No
10)	Habits of fruit consumption	1) Yes, Always 2) Yes, 2-3 times a week 3) yes, Once a week 4) Never at all
Variable related to maternity case		
11)	Partner change	1. Yes 2. No
12)	Trimester	1. Second 2. Third
13)	Marital status	1. Married 2. Single

		3. Divorced 4. Widowed
14)	Gravidity	1. Primigravida 2. Multigravidia
15)	Pregnancy status	1. Wanted 2. unwanted
16)	Parity	1. 0 2. 1-4 3. ≥5
17)	Gestational age	1. < 20 -37 weeks 2. 37-42 3. > 42
Clinical related cases		
18)	Gestational Diabetic Mellitus	1. Yes 2. No
19)	History of chronic hypertension	1. Yes 2. No
20)	Family history of chronic hypertension	1. Yes 2. No
21)	Family/self history of diabetes	1. Yes 2. No
22)	Have you history of liver failures	1. Yes 2. No
23)	Have you history of Kidney disease	1. Yes 2. No
24)	Have you history of heart failure	1. Yes 2. No
25)	Have you sense of Headache	1. Yes 2. No
26)	Have you Epigastric pain	1. Yes 2. No
27)	Family history of preeclampsia	1. Yes 2. No
28)	Previous history preeclampsia	1. Yes 2. No
29)	Have you history of anemia	1. Yes 2. No
30)	Have you history of stroke	1. Yes 2. No

Thank you for your cooperation!

Interview Date: _____

Interviewer name_____

Annex VI. Questionnaire English version

የትናቱ መለኪያ ተለዋዋጮች		
ተቁ	ተለዋዋጭ	መለኪያዎች
1.	ቀን _____ ለጥናቱ የተሰጠ መ/ቁጥር_____ እድሜ_____	BMI በኪ.ግ_____ ግፊት_____
Demographic characters		
2.	የመጡበት አድራሻ	1, ከተማ 2. ገጠር
3.	የጋብቻ ሁኔታ	1. ያገባች 2. ያላገባች 3. የተፋታች 4. የሞተባት
4.	የእናት የትምህርት ሁኔታ	1. ያልተማረች 2. እስከ12 የተማረች 3. ዲፕሎማ ያላት 4. ዲግሪና ከዚያ በላይ ያላት
5.	የባል የትምህርት ሁኔታ	1. ያልተማረ 2. እስከ12 የተማረ 3. ዲፕሎማ ያለው 1. ዲግሪና ከዚያ በላይ ያለው
6.	የባል የስራ ሁኔታ	1. የቤት እመቤት 2. የመንግስት ሰራተኛ 3. የግል ሰራተኛ 4. ነጋዴ 5. ሌላ
7.	የገቢ መጠን	1. ≤ 793 (ዝቅትኛገቢ) 2. 793 - 2805 (መካከለኛ ደረጃ) 3. >2805 (ከፍተኛ ገቢ)
ከእንቅስቃሴና ኣጠቃቀም ጋ የተያያዙ ጉዳዮች		
8.	ቋሚ የሆነ እስፖርታዊ እንቅስቃሴ ያደርጋሉ?	1.አዉ 2.የለም
9.	አወ ከሆነ መልስዎ ምን ያክል ሰኣት ልዩነት ያደርጋሉ?	1. ሁል ጊዜ 2. 2-3 ጊዜ በሳምንት 3. አንዴ በሳምንት
10.	አልኮል ይጠጣሉ?	1. አንዳንዴ እጠጣለሁ 2. አወ ሁሌም እጠጣለሁ 3. በጭራሽ አልጠጣም
11.	ፍራፍሬዎችን ይጠቀማሉ?	1. አወ ሁሌም 2. አወ አንዳንዴ 3. ቢበዛ በሳምንት አንዴ 4. በጭራሽ አልጠቀምም

ከእርግዝና ሁኔታ ጋር የተያያዙ ሁኔታዎች		
12.	የእርግዝና ሁኔታ	1. አንድ 2. ከአንድ በላይ
13.	የትዳር ጓደኛዎን ይቀያይራሉ?	1. አዎ 2. በጭራሽ
14.	የእርግዝና ጊዜ	1. 2ኛ 2. 3ኛ
15.	የጋብቻ ሁኔታ	1. ያገባች 2. ያላገባች 3. የተፋታች 4. በሞት ያጣች
16.	ስንተኛ እርግዝናዎ ነው?	1. የመጀመሪያ 2. 2ና ከዚያ በላይ
17.	የርግዝና ሁኔታ	1. የተፈለገ 2. ያልተፈለገ
18.	ካሁን ምን ያክል ጊዜ ወልደዋል?	1. 0 2. 1-4 3. ≥5
19.	የእርግዝና ሳምንት	1. <20 -37 2. 37-42 3. > 42
ጤና ነክ ጉዳዮች		
20.	በቤተሰብዎ ውስጥ በደም ግፊት ብዛት ታማሚ አል?	1.አው 2.የለም
21.	የሰኳር በሽታ አጋጥሞዎት ያውቃል?	1.አው 2.የለም
22.	የጉበት አጋጥምዎት ያውቃል?	1.አው 2.የለም
23.	የኩላሊ አጋጥምዎት ያውቃል?	1.አው 2.የለም
24.	የልብ ህመም አጋጥምዎት ያውቃል?	1.አው 2.የለም
25.	የራስ ምታት ችግር አለብዎት?	1.አው 2.የለም
26.	የሆድ ህመም አለዎት?	1.አው 2.የለም
27.	በቤተሰብዎ በርግዝና ምክንያት ችግር አጋጥምዎት ያውቃል?	1.አው 2.የለም
28.	ካሁን በፊት በርግዝና ምክንያት ችግር አጋጥምዎት ያውቃል?	1.አው 2.የለም
29.	የደም ማነስ አጋጥምዎት ያውቃል?	1.አው 2.የለም
30.	የደም መርጋት ችግር አጋጥምዎት ያውቃል?	1.አው 2.የለም

ስለትብብርዎ ከልብ እናመሰግናለን!

ቃለ መጠይቁ የተደረገበት ቀን:_____

የቃለ መጠይቅ አድራጊው ስም_____

Annex VII: Laboratory producers

After the participants have agreed and signed the informed consent and are voluntary to give blood; the whole blood sample was collected and prepared for the following basic procedure for all analytes studied in this study.

- ✓ 3- 5 ml of the venous blood sample was collected and transfer gently to the pale test tube (SST test Tube) from the syringe and then stored at room temperature for 10-15 minutes until coagulated
- ✓ After clotting, the whole blood was centrifuged at centrifugation force of 3000 rpm for 5 minutes to separate the serum from the red cells.
- ✓ Then turn on the clinical chemistry analyzer machine
- ✓ Check the expiry date of all reagents
- ✓ Check the daily, weekly, monthly, quarterly and yearly controls, standards and
- ✓ calibration results of the analyzer
- ✓ Analyze the specimen based on the leaflet procedure for each parameter
- ✓ Finally, the result was printed from the machine

Annex VIII: Principle of each test

1. High-density lipoprotein (HDL cholesterol)

✧ **PRINCIPLE**

HDL is measured directly in serum by enzymatic method using the principle apoB containing lipoproteins in the specimen are reacted with a blocking reagent that renders them non-reactive with the enzymatic cholesterol reagent under conditions of the assay. The apoB containing lipoproteins are thus effectively excluded from the assay and only HDL-chol is detected under the assay conditions. The method uses sulfated alpha-cyclodextrin in the presence of Mg+2, which forms complexes with apoB containing lipoproteins, and polyethylene glycol-coupled cholesteryl esterase and cholesterol oxidase for the HDL-cholesterol measurement.

The reactions are as follows:

(1) ApoB containing lipoproteins + α-cyclodextrin + Mg+2 + dextran SO4 ---> soluble non-reactive complexes with apoB-containing lipoproteins

(2) (2) HDL-cholesteryl esters PEG-cholesteryl esterase > HDL-unesterified cholesterol + FA

(3) (3) Unesterified chol + O2 --PEG-cholesterol oxidase > cholestenone + H2O2

(4) (4) H2O2 + 5-aminophenazone + N-ethyl-N-(3-methyl phenyl)-N'_succinyl ethylene diamine + H2O + H+ peroxidase > quinone imine dye + H2O

Absorbance is measured at 600 nm

2. Low-density lipoprotein (LDL cholesterol)

✧ PRINCIPLE

The LDL-Cholesterol test is a two reagent homogenous system. The assay is comprised of two distinct phases. In phase one a unique detergent solubilizes cholesterol from non-LDL-lipoprotein particles. This cholesterol is consumed by cholesterol esterase, cholesterol oxidase, peroxidase and 4- aminoantipyrine to generate a colorless end product. In phase two a second detergent in reagent 2 releases cholesterol from the LDL – lipoproteins. This cholesterol reacts with cholesterol esterase, cholesterol oxidase and a chromogen system to yield a blue color complex which can be measured bichromatically at 540/660nm. The resulting increase in absorbance is directly proportional to the LDL-C concentration in the sample.

Reaction phase 1

- HDL-C, VLDL-C, LDL-C Chylomicrons CHE and CHO Cholest-4-en-3-one + Fatty acids + H2O2
- H2O2 – 4-AAP Peroxidase LDL-C + Colorless end product

Reaction phase 2

- ✓ LDL-C CHE and CHO Cholest-4-en-3-one + Fatty acids + H2O2
- ✓ H2O2 + DSBmT + 4-AAP Peroxidase Blue color complex

3. **Total cholesterol**

✧ **PRINCIPLE**

✧ Cholesterol is measured enzymatically in serum or plasma in a series of coupled reactions that hydrolyze cholesteryl esters and oxidize the 3-OH group of cholesterol. One of the reaction byproducts, H2O2 is measured quantitatively in a peroxidase catalyzed reaction that produces color. Absorbance is measured at 500 nm. The color intensity is proportional to cholesterol concentration. The reaction sequence is as follows:

Cholesteryl ester + H2O --------cholesteryl ester hydrolase--->cholesterol + fatty acid

Cholesterol + O2 --- cholesterol oxidase----------> cholest-4-en-3-one + H2O2

2H2O2 + 4-aminophenazone + phenol -- peroxidase-> 4-(p-benzoquinonemonoimino)-phenazone + 4 H2O

4. Triglyceride

✧ **Principle**

Triglycerides are measured enzymatically in serum or plasma using a series of coupled reactions in which triglycerides are hydrolyzed to produce glycerol. Glycerol is then oxidized using glycerol oxidase, and H2O2, one of the reaction products, is measured as described above for cholesterol. Absorbance is measured at 500 nm. The reaction sequence is as follows:

Triglycerides + 3H2O---lipase----> glycerol + fatty acids

Glycerol + ATP ------glycerokinase------> glycerol-3-phosphate + ADP

Glycerol-3-phosphate + O2 ----glycerophosphate oxidase---> dihydroxyacetone phosphate + H2O2

H2O2 + 4-aminophenazone + 4-chlorophenol ---peroxidase--> 4-(p-benzoquinone-monoamine)- phenazone + 2H2O + HCl.

5. Serum total protein

✧ Principle

The principle for serum total protein measurement is the Biuret method which Depends on the presence of peptide bonds. The biuret reagent contains sodium potassium tartrate to form a complex with cupric acid and maintain its solubility in an alkaline solution. Peptide bonds react with Cu2+ ions in alkaline solutions to form a colored product. Then absorbance produced by this colored product is measured spectrophotometrically at 540nm. The intensity of the color produced is proportional to the amount of protein present in the reaction system.

6. Serum Creatinine

✧ Principle

For the measurement of serum creatinine I have used the Jaffe reaction method with the principle of creatinine reacts with picric acid in alkaline solution (yellow color) to form a red-orange chromogen. The amount of absorbance of the red-orange colored product is directly proportional to the amount of creatinine in the sample.

7. Serum Urea

✧ Principle

Serum urea in the sample is measured based on the preliminary hydrolysis of urea with **urease** (specific enzyme) to liberate ammonium ions, followed by a secondary reaction that measures the amount of ammonium ion spectrophotometrically or by electrode conductivity Called ‘indirect’ methods because these methods measure the amount of ammonia ‘liberated’ from the urea molecule present in the sample. The amount of ammonium ion produced is directly proportional to the amount of urea Glutamate dehydrogenase Spectrophotometric the conversion absorbance of NADH to NAD is measured at 340 nm. The amount of NAD produced is directly proportional to the amount of ammonium ion which is directly proportional to the amount of urea present.

DECLARATION

I, the undersigned, declare that this M.Sc. thesis is my original work, has not been presented for a degree in this or any other university, and that all sources of materials used for the thesis have been duly acknowledged.

M.Sc. candidate: Haymanot Tewabe (B.Sc.)
Signature: _____
Date of submission: _____

This MSC thesis has been submitted with our approval as advisors.

Advisor: Mistire Woldie (Msc, PhD)
Signature: _____
Date: _____
Place: Addis Ababa, Ethiopia.

Advisor: Abebe Edao (Msc, PhD candidate)
Signature: _____
Date: _____
Place: Addis Ababa, Ethiopia.