ADDIS ABABA UNIVERSITY
COLLEGE OF HEALTH SCIENCES
DEPARTMENT OF MEDICAL LABORATORY SCIENCES

Evaluation of hematological and biochemical profiles among pregnant women with pregnancy induced hypertension attending in St Peter Hospital, Addis Ababa, Ethiopia

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This is to certify that the thesis prepared by Yohannes Kidane entitled "Evaluation of hematological and biochemical profiles among pregnant women with pregnancy induced hypertension attending in St Peter Hospital, Addis Ababa, Ethiopia,2024" and submitted in partial fulfillment of the requirements for a Master of Science degree in clinical laboratory (Hematology and Immunohematology, complies the regulations of the University and meets the accepted standards with respect to originality and quality.

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Chair man of the department or Graduate program coordinator

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Abbreviations

FMoH: Federal Democratic Republic of Ethiopia Ministry of Health

EDTA: Ethylene Di-amine Tetra-acetic Acid

SOP: Standard operating procedure for

CBC=Complete blood count

PIH: Pregnancy Induced Hypertension

mmHg: Mili meter mercury

BP: Blood pressure

IUGR = Intra uterine growth retardation

HELLP:hemolyisis, elevated liver enzymes, low platelets

WHO: World health organization

WBC: White blood cells

ALT : Alanine Amino Transferase

AST: Aspartate Amino Transferase

NCCLS: National Clinical Chemistry Laboratory Standards

Hct: Hematocrit

MCV: Mean Cell volume

MCH: Mean Cell Hemoglobin

MCHC: Mean Cell Hemoglobin Concentration

Hgb: Hemoglobin

HDP: Hypertensive disorder of pregnancy

Abstract

Background: Pregnancy-induced hypertension (PIH) is a major pregnancy condition that raises the mother's and the newborn's risk of illness and death. The aim of this study is to evaluate the hematological and biochemical characteristics of pregnant patients hospitalized to St. Peters hospitals in Ethiopia who have pregnancy-induced hypertension.

Objective: To evaluate hematological and biochemical profiles of pregnant women at St. Peter hospital.

Methods: A comparative case control study was carried out between August and October of 2022 among 125 pregnant women with pregnancy-induced hypertension and 125 normotensive women at St. Peter hospital. Through the use of standardized questionnaires and interviews with pregnant women, sociodemographic data was gathered. The complete blood count and biochemical profiles of women were determined from venous blood using Beckman Culter DXH 800 UniCel hematological analyzer and COBAS C311 analyzer, respectively. SPSS version 26.0 statistical software was used to analyze the data. Clinically relevant variables were those whose P-value was less than 0.05 and whose level of statistical significance was defined at a 95% confidence interval.

Result: Alkaline phosphatase (ALP) (119.34±74.66), Alanine transaminase (ALT) (26.71±42.93), and Aspartate transaminase (AST) (36.8±47.43) values were significantly higher in pregnancy-induced hypertension than in controls (ALP = 90.53±68.32, ALT = 14.94±1.91, and AST = 21.40±12.81, respectively). Hemoglobin, hematocrit, and platelet levels were significantly lower than in controls (Hgb: 13.47±1.6 vs. 13.72±0.81, HCT: 37.87±4.3 vs. 39.23±2.52, and PLT: 187.51±78.69 vs. 222.34±78.44). Potassium levels were significantly lower in pregnancy-induced hypertension patients than in controls (4.18±0.41 vs. 4.65±0.57 mg/dL). White blood cell (WBC) count and neutrophil levels were significantly higher in pregnancy-induced hypertension patients than in controls (WBC: 9.48±4.3 vs. 7.92±2.5 mg/dL).

Conclusion: This study showed that pregnant women with PIH had higher levels of ALP, ALT, and AST. The amount of neutrophil was significantly higher. Pregnant women with PIH had significantly decreased levels of Hgb, HCT, and PLT. So it was recommended clinicians use hematological profiles and biochemical tests along with measuring the blood pressure of pregnant mothers to prevent them from PIH effect.

Key word: Hematological profile, anemia, thrombocytopenia, and pregnant women

1. Introduction

1.1 Background

Pregnancy-induced hypertension (PIH) is the most prevalent medical problem associated with pregnancy, with a reported prevalence ranging from 5 to 10%. These conditions can severely affect both the mother and baby. They are important causes of maternal and perinatal morbidity and mortality, often leading to proteinuria and worsening of the condition. Blood pressure must be ≥140/90 mmHg on two separate occasions for a pregnant woman to be considered hypertensive(1,2).

The underlying pathophysiology of PIH is unknown but is believed to involve decreased placental perfusion leading to systemic vascular endothelial dysfunction. This dysfunction is caused by less successful cytotrophoblastic invasion of the uterine spiral arteries. Placental hypoxia then triggers inflammatory processes, disrupts angiogenic factors, and causes platelet aggregation, resulting in endothelial dysfunction, clinically expressed as preeclampsiaDuring a normal pregnancy, cardiovascular and renal functions change significantly to meet the metabolic needs of the mother and fetus. Total peripheral resistance and arterial blood pressure decrease, while maternal cardiac output and blood volume increase by about 40-50%. Renal plasma flow and glomerular filtration rate also increase by 30-40% (3).

In PIH, levels of angiotensin II, renin activity, and renin concentrations increase, but vascular reactivity to angiotensin II declines. Unlike typical pregnancies, women with PIH do not exhibit clear hemodynamic and renal changes. PIH is characterized by increased total peripheral resistance, enhanced angiotensin II responsiveness, and decreased renal blood flow, glomerular filtration rate, and proteinuria(4).

The pathogenesis of hypertensive disorders of pregnancy (HDP) involves preexisting maternal comorbidities, patient characteristics, reproductive history, genetic and immunological factors. Preeclampsia likely results from interactions between placental and maternal pathways. The diverse manifestations of HDP arise from varying contributions of maternal and placental pathophysiological processes. Severe endovascular damage and dysfunction related to HDP can have long-term and intergenerational effects (5).

Pregnancy induced hypertension is thought to begin in the second trimester when trophoblast cells undergo apoptosis and poorly penetrate the maternal decidua, affecting spiral artery remodeling. Reduced uteroplacental blood flow results in fetal oxygen and nutrient deprivation, leading to placental ischemia and the release of substances crucial to the pathophysiology of Pregnancy induced hypertension (6).

PIH can cause complications like Abruptio Placentae and intrauterine death. Hematological, cytokine, and coagulation changes, including thrombocytopenia and leukocytosis, are observed in PIH. PIH can also lead to maternal complications such as liver hemorrhage, adult respiratory distress syndrome, disseminated intravascular coagulopathy, HELLP syndrome, pulmonary edema, acute organ failure, and sepsis (7).

Pregnant women with PIH have high blood pressure, proteinuria, and pathologic edema; PIH is the second most common cause of maternal death worldwide. Severe hypertension increases the mother's risk of cardiac failure, heart attack, renal failure, and stroke. It also raises the risk of fetal complications like inadequate oxygen transfer, growth restriction, premature birth, placental abruption, stillbirth, and neonatal death (8).

There are three main types of pregnancy-related hypertension: gestational hypertension, chronic hypertension, and preeclampsia. Chronic hypertension occurs before 20 weeks of pregnancy or before conception and persists postpartum. Gestational hypertension develops after 12 weeks of pregnancy without proteinuria or other systemic abnormalities. Preeclampsia, the most dangerous form, typically begins around 20 weeks of gestation and involves high blood pressure and often proteinuria (9-11)

Pregnancy induced hypertension affects hematological parameters, often causing hemodilution and lowering hemoglobin and hematocrit levels. Thrombocytopenia, due to platelet consumption and malfunction, is common in HDP, affecting 7-10% of pregnant women. PIH is the second leading cause of thrombocytopenia after gestational thrombocytopenia (12-14)

Biochemical parameters are also affected by pregnancy-induced hypertension (PIH) or hypertensive disorders of pregnancy. Markers of liver enzyme function, such as Aspartate Aminotransferase (AST) and Alanine Aminotransferase (ALT), become elevated due to hepatic dysfunction, which can occur in severe preeclamptic patients with HELLP syndrome (characterized by low platelet count, hemolysis, and increased liver enzymes). In PIH, hypoxia's impact on the liver leads to hepatocyte necrosis and degeneration, raising AST levels.

Preeclampsia triggers the release of several mediators (fibronectin, thrombomodulin, endothelin-1, and thromboxane) from the liver and blood vessel endothelium, causing vasoconstriction and hepatic hypoxia. ALT levels increase in response to hypoxia. High levels of serum creatinine may indicate compromised renal function due to a decreased glomerular filtration rate (GFR) caused by pregnancy-related hypertension. From 2003 to 2009, hypertensive diseases were responsible for over half of maternal deaths globally, along with hemorrhage, sepsis, and other factors (15,16).

Significant efforts from development partners, governments, the WHO, and other parties have focused on reducing these high maternal and related newborn mortality rates, particularly in South Asia and Sub-Saharan Africa (SSA), where maternal fatalities account for almost 90% of all maternal deaths globally. Despite these efforts, elevated rates of maternal and newborn mortality persist (24).

1.2 Statement of the problem

Problems with hypertension brought on by pregnancy affected numerous pregnancies all over the world. Preeclampsia causes complications in 2-8% of pregnancies globally and accounts for 9% of maternal fatalities in Asia and Africa. Globally, poorer nations account for the majority of mortality linked to hypertensive disorders during pregnancy. Preeclampsia is estimated to occur in 2.8% of live births in less developed nations, seven times more frequently than in more developed countries, where it occurs in 0.4% of live births. (1, 2).

In Ethiopia; PIH is a common maternal medical complication during pregnancy. in A health facility based cross-sectional study was carried out from October 01 to November 30/2016, the prevalence of pregnancy induced hypertension was 7.9%. Hypertensive illnesses, the largest cause of death globally, contributed to approximately 22.1% of all maternal fatalities in Latin America and the Caribbean. Pregnancies complicated by hypertension disorders are connected with poor maternal and birth outcomes, with rural women being more affected. It is important to integrate the best antenatal care services with the best obstetric and neonatal care during delivery for the early detection and management of hypertensive disorders of pregnancy (8, 16).

About 2.3% of pre-eclampsia instances occur in impoverished areas, compared to 0.8% in developed areas (17). Pregnancy-induced hypertension (PIH) is expected to affect 7% to 10% of pregnancies in the United States (18). According to another study, the prevalence of PIH in India ranges from 5% to 15% (19) and significant changes to cardiovascular and renal function occur throughout a typical pregnancy to meet the needs of the mother and the fetus in terms of metabolism. (32).

Hemostatic abnormalities ranging from thrombocytopenia, hemolysis, elevated liver enzymes, and low platelets (HELLP) are superimposed. HELLP syndrome develops in 4-12% of women with pre-eclampsia or eclampsia a significant threat to both mother and fetus (21). The specific pre-eclampsia spectrum disorders are listed like eclampsia and hemolysis, elevated liver enzymes, low platelet counts (HELLP) (22, 23).

In Ethiopia, many pregnant women struggle with PIH, but many are ignorant of it and do not seek treatment or medication when necessary. As a result, when patients attend medical facilities to have hematological and biochemical profiles performed, they do not promptly obtain care or treatment-appropriate medication. In Addis Ababa, Ethiopia and other regions, it is believed that

pregnant women are more likely to experience hematological and particular biochemical abnormalities.

The majority of research conducted in Africa, particularly in Ethiopia, are primarily concerned with the prevalence of pregnancy-induced hypertension. In addition to revealing the frequency, hematological and biochemical parameters alternations during PIH are additional diagnostic tools for managing, preventing, and controlling problem related to pregnancy induced hypertension. However, the biochemical parameter changes due to PIH were not found in the majority of research, particularly those conducted in Ethiopia. Therefore this study intended to help for the fulfillment of this gap by identifying several biomarkers linked with PIH and connecting various risk variables to abnormal results from the study participants' renal and liver function tests and hematological assessments. The purpose of this study was to provide medical professionals with diagnostic tools to help them manage and treat pregnancy-related hypertension. By outlining the particular modifications made to these factors and how they affect treatment practice,

1.3. Significance of the study

Hypertension and its complications associated with pregnancy are an international public health problem that affects both developed and developing countries. Nevertheless, very little is known about the effect of hypertensive disorders during pregnancy on biochemical and hematological parameters, particularly in Ethiopia. Multiple investigations have shown that Ethiopia periodically experiences significant rises in the prevalence of pregnancy Related hypertension. The purpose of this study was to determine whether pregnancy induced hypertension had any appreciable effects on the hematological and biochemical markers of pregnant women who had the condition. The study could provide evidence regarding how pregnant women's hematological profiles are affected by the presence of pregnancy-induced hypertension.

In addition to helping physicians working in hospitals, this study was intended to provide baseline evidence-based information about the use of hematologic parameters as a vital clinical condition for preventing the worsening of patients' circumstances for pregnancy-induced hypertension. Lastly, the findings of this study could significantly reduce mortality caused by severe hematological and biochemical abnormalities in pregnant women with pregnancy-induced hypertension.

1.4 Hypothesis

Hematological and biochemical profiles of pregnant women with Pregnancy induced hypertension is different from normotensive pregnant women.

2. Literature review

Pregnancy induced hypertension

Hypertensive disorders associated with pregnancy (HDP) are a category of illnesses characterized by elevated blood pressure. Problems with pregnancy-related hypertension affect public health around the world. Numerous studies have shown a correlation between preeclampsia and eclampsia and higher risks of maternal death, prenatal mortality, morbidity, and early births. Compared to women who do not have pregnancy-related hypertensive diseases, women with pregnancy-induced hypertensions such eclampsia is five times more likely to have perinatal death (25-27).

Hematological parameter alterations in HDP

In a case control study conducted in 2018 at Jhalawar Medical College in India on 126 patients with the goal of examining and correlating the hemoglobin, haematocrit, white blood cell count, lymphocytes, and platelets among 63 PIH patients (case) and 63 healthy pregnant women (control), it was found that preeclamptic patients' mean hemoglobin, mean lymphocyte, and mean platelet count levels were significantly lower than those of the control group. The case group in the same study had significantly higher WBC and mean hematocrit levels than the control group (2) In the case control, study done National Ribat University, Faculty of Medical Laboratory Sciences, Khartoum, Sudan by Eltayeb Tayrab et al it showed that the mean of PLT and hemoglobin levels in the women with pre-eclampsia was decreased than that of normotensives (29).

In observational analytical case control study done January 2015 to January 2016 by Prathab et al, on 500 pregnant women (Among them 200 patients were healthy normotensive pregnant controls, 180 were patients with mild preeclampsia, 90 were patients with severe preeclampsia, and 30 patients were with eclampsia) to compare the biochemical and hematological markers in pre eclampsia and eclampsia patients with normotensives, in JSS Medical College and Hospital, Mysore, India showed that the platelet count significantly lower and Serum alkaline phosphatase showed statistically higher levels in eclamptic women was very significantly lower in severe preeclampsia ($P<0.01$) than that in normal healthy pregnant controls (31) .

A prospective study was carried out in the Department of Gynecology at the Government Medical College in Anantapur, Andhra Pradesh, India, from April 2014 to December 2015. The

study aimed to assess the severity of hypertensive disorders during pregnancy and compare the coagulation profiles of patients with mild pre-eclampsia, severe pre-eclampsia, and eclampsia. The findings indicated that platelet counts decreased with increasing severity, from mild pre-eclampsia to eclampsia. Prothrombin time, activated partial thromboplastin time, bleeding time, and clotting time were prolonged in severe eclampsia and eclampsia (36).

An analytical, prospective case-control study conducted by Biparnak Haldar et al. between January 2017 and June 2018 on 120 patients at the Department of Gynecology and Obstetrics of R.G. Kar Medical College and Hospital, a tertiary care hospital in eastern India, revealed significant findings. The study showed that the Prothrombin Time in the Severe Preeclampsia and Eclampsia groups was significantly higher than in the pregnant women with normal hypertension status. Additionally, the Mean Platelet Volume in both the Mild and Severe Preeclampsia groups was significantly higher than in the normotensive group. The Platelet Count in the Mild Preeclampsia, Severe Preeclampsia, and Eclampsia groups was significantly lower than in the normotensive group (42).

Biochemical parameter alterations in HDP

In a cross-sectional study carried out on 49 preeclamptic women and 50 normotensive healthy pregnant women in Lagos, Nigeria, It was demonstrated that the plasma total protein was significantly reduced, and albumin among preeclamptic volunteers when compared with the normotensive pregnant women. Plasma urea, creatinine, and uric acid levels were significantly raised ($p<0.05$) among preeclamptic population when compared with normotensive control pregnant women (9).

In the prospective study aiming to evaluate biochemical parameters in serum of women with preeclampsia and IUGR, done by Ramadan Dacaj et al on 120 pregnant women divided in to two groups: non Intrauterine growth restriction (IUGR) group included healthy pregnant women (n=60) and IUGR group included pregnant women with preeclampsia and IUGR (n=60) showed that, women with preeclampsia have statistically significant higher values of AST, ALT, LDH and total cholesterol compared with healthy pregnant women (24)

A tertiary hospital in Ghana conducted a cross-sectional study on 368 women and found that pregnancy-induced hypertension is significantly linked to perinatal illness and death, particularly in developing nations. The major adverse perinatal outcomes and proportion of perinatal deaths

determined among women was highest in preeclampsia than other complications categories with the lowest rate occurring in the chronic hypertensive group (25).

In a hospital based cross sectional study done on 65 women attending in Naipaul, Hyd, India, showed that serum uric acid levels in hypertensive mothers showed a statistically important increase to the control group ($p<0.001$). (28) In the case control, study done National Ribat University, Faculty of Medical Laboratory Sciences, and Khartoum, Sudan by Eltayeb -Tayrab et al showed that liver function (LFT) tests (AST, ALT and ALP); significantly increase in the Sudanese women with preeclampsia, than normotensive pregnant women (29).

In an observational study conducted among 90 patients in department of Obstetrics and Gynecology at Zanana hospital, medical college Jhalawar, 18 (20%) pregnant women showed elevated liver enzymes with mean value of ALT and AST (30).

In case control study done on 60 pregnant women with the aim of comparing the liver function tests in pre-eclampsia with normal pregnancy by Lodhi R et al Department of Biochemistry, Shri Shankracharya Institute of Medical Sciences, Junwani Bhilai, Chhattisgarh, India, showed that increased concentrations of serum bilirubin, total protein, albumin and liver enzymes ALT, AST results were found higher in pre-eclampsia cases compared with controls (Normal pregnant women) (33).

Prathap T. et al. conducted a prospective observational analytical case controlled investigation involving 500 pregnant patients who were seen at JSS Hospital., Mysore, India showed that Serum alkaline phosphatase (ALP) showed statistically higher levels in eclamptic women in comparison to mild PIH, severe PIH and normal pregnant women.

The objective of the quantitative retrospective chart review study conducted in the Obstetrics and Gynecology Department of King Abdul Aziz Medical City between 2013 and 2014 was to compare and correlate plasma urea, creatinine, sodium, potassium, and plasma glucose and urine protein in the pre-eclamptic and normotensive groups. The results indicated a significant positive correlation between urea and protein, urea and creatinine, respectively, and that the elevated values of serum creatinine, urea, urine protein, sodium, potassium, and plasma glucose disqualify them from being considered a reliable predictor for pre-eclampsia or pregnancy-related hypertension. Furthermore, the higher plasma glucose and urine protein readings could be utilized to distinguish between moderate and severe hypotension (34).

In a case-control study done by Chopra S et al in Departments of Biochemistry and Physiology, Medical College & Hospital, Kolkata, India showed that the value of serum triglycerides, total cholesterol, VLDL, LDL levels was significantly high and there was decreased level of HDL on pregnant women with PIH as compared to control pregnant women (36) In a prospective comparative cross-sectional study to determine any significant association between pre-eclampsia and lipid profile changes showed that a significantly higher level of VLDL, LDL and triglyceride among the pre-eclamptic group compared to the normotensive(37).

In Case control study done October 2014- November 2015, at the Department of Biochemistry LUMHS Jamshoro, demonstrated that serum total proteins and HDL have been substantially decreased and serum cholesterol and LDL are significantly ($p < 0.05$) elevated in hypertensive patients, leading to the conclusion that abnormalities in these serum lipid profiles and serum total proteins are significantly linked to hypertension (38). In a case-control study performed on 99 Iranian pregnant females showed ALT, AST, ALP, Direct Bilirubin and Total Bilirubin level in preeclamptic pregnant women was significantly higher than normotensive pregnant women (39).

Sakina Roohi et al. conducted a case control study in the Department of Biochemistry at Deccan College of Medical Sciences from the month of June 2008 to June 2009. The study evaluated the relationship between blood rheology in complicated preeclampsia and compared it to normal pregnancy and non-pregnant women. According to the study, preeclamptic pregnant women had considerably higher blood cholesterol levels than normotensive and non-pregnant pregnant women.In this study it was also showed that there was significantly higher value of Alkaline phosphatase (ALP) and Triglyceride (TG) in PIH women than non pregnant and normotensive women (40).

In Prospective cross sectional study for To compare liver and kidney function tests in pre-eclampsia and in uncomplicated pregnancy and to relate the results to physiological reference values done by Makuyana et al in Antenatal clinic and antenatal labor wards. Harare Hospital, Zimbabwe on, 2002, 110 women, 38 pre-eclamptic and 72 normotensive participants. Enzymes associated with the liver function (ALP, AST, AST), were significantly elevated in pre-eclampsia in the same stud the two indices for assessing renal function, serum creatinine and urea, showed significant elevation in pre-eclampsia(42).

A prospective case-control study conducted by Haymanot et al. from January 24, 2020, to April 30, 2020, found that urea levels were significantly higher in cases of pregnancy-induced

hypertension (PIH), with an average of 44.86 ± 35.96 mg/dL, compared to controls, who had an average of 29.51 ± 17.79 mg/dL. The same study also revealed a notable difference in serum creatinine levels between PIH cases and controls. The PIH group had an average serum creatinine level of 1.00 ± 0.45 mg/dL, with values ranging from 0.48 to 2.22 mg/dL, indicating impaired renal function. In contrast, the control group had an average serum creatinine level of 0.79 ± 0.196 mg/dL, with values ranging from 0.48 to 1.32 mg/dL (32).

3. Objectives

General Objective

To evaluate alterations in hematological and biochemical profiles of pregnancy induced hypertensive pregnant women at St Peter Hospitals, Addis Ababa, Ethiopia from August 2022 to October 2022

Specific objectives

- ✓ To compare the hematologic profiles of pregnancy induced hypertensive pregnant women with the normotensive pregnant women
- ✓ To compare the biochemical profiles of pregnancy-induced hypertensive pregnant women with the normotensive pregnant women
- ✓ To compare the hematological and biochemical abnormalities among different severity level of hypertension

4. Materials and methods

4.1 Study area

St. Peter Hospital was the site of the study. In the Gullele sub city of Addis Ababa, Ethiopia, is the hospital, which is a government facility.

The hospital was founded in 1960. Pregnant women from adjacent and distant medical facilities might receive maternity-related healthcare treatments at St. Peter Hospital. In the Hospital, 1275 employees, 332 administrative, and 925 clinical professionals made up the staff. There were 15 doctors and 41 midwives working in the hospital's maternity unit, including ANC, Delivery and Gynecology wards (60).

4.2 Study Design and Period

Comparative study carried out in St. Peter Hospital in Addis Ababa since August 2022 to October 2022

4.3study population

4.3.1 Source Population

All pregnant women receiving prenatal treatment at St. Peter Hospital during the study period made up the source population.

4.3.2 Study Population

Pregnant women with PIH and without PIH attending maternal care at St Peter hospital during the study period.

Control group: - Pregnant women with blood pressure <140/90mmHg

Case group: - Pregnant women with blood pressure ≥140/90mmHg

Eligibility criteria

Inclusion and exclusion criteria was implemented to include eligible study participants and exclude participants with medical conditions and history from the card.

Inclusion criteria

All women who attended delivery and antenatal care service and had given consent and with gestational age greater than 20 weeks.

Exclusion criteria for cases group

- ✓ Women with known chronic hypertension,, chronic liver and chronic kidney disease.
- ✓ A woman with History of systemic illnesses like diabetes mellitus, renal disease, liver diseases, malignancy

- ✓ Women with history of recent blood transfusion
- ✓ Mothers who were previously diagnosed with persistent hypertension before to becoming pregnant
- ✓ Mothers unable to draw blood due to complex health issues
- ✓ Pregnant women with a previous or current history of HIV, smoker,
- ✓ Pregnant women who are who are expecting yet refuse to donate blood samples

4.4 Study Variables

4.4.1 Dependent Variable

Hematologic parameters (Hct, MCV, MCH,MCH, Hgb, Total WBC count, Absolute Neutrophil , Absolute Lymphocyte count, Platelet count). And

Biochemical parameters (Urea, Creatinine, Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT) Alkaline Phosphatase (ALP) , Bilirubin Direct Bilirubin total , Sodium , Potassium and Chlorine)

4.4.2 Independent Variable

- ✓ Age of patients
- ✓ Alcohol consumption
- ✓ Nutritional condition
- ✓ Place of residence
- ✓ Education level
- ✓ Employment
- ✓ Income level
- ✓ Gestational week
- ✓ Body Mass Index (BMI)
- ✓ Number of pregnancies (Gravidity)
- ✓ Blood type

4.5 Sample size and sampling techniques

4.5.1 Sample size calculation

Based on the variance and mean of serum urea from an Ethiopian study, the sample size was determined using the comparison between two means (double population) formula with a confidence interval (CI) of 95% and power of 90%. (32).

$$n1=n2=\frac{(Z_{\alpha/2}+Z_{\beta})\,2\,(\delta_1 2+\delta_2 2)}{\Delta^2}$$

$$n=\frac{(1.96+1.28)^2(17.79^2+35.96^2)}{(44.86-29.51)^2}=$$

$$n=\frac{(10.4976)\,(316.4841+1293.1216)}{235.6225}$$

n=71.7=72

Where;

The critical values of the normal distribution at α/2 and β are, respectively, Zα/2 and 1.96. For example, at a 95% confidence level, α is 0.05 and the critical value is 1.96, while at β, β is 0. 1 and the critical value is 1.28.

δ_1^2 - Variance for case group

δ_2^2 Variance for control group

For each group, a minimum sample size of 72 was calculated. To account for possible unexpected loss reasons, 10% was included to this amount, bringing the total to 80 pregnant women. But I made a research population of 250 in order to expand the investigation.

4.5.2 Sampling Method

Until the necessary sample sizes were reached during the study period, convinient sampling techniques were employed consecutively.

4.6 Measurement and Data Collection

4.6.1 Data Collection Procedure

The information on the participants' nutritional habits, pregnancy and health situation, and sociodemographic condition was gathered through a pre-tested structured questionnaire that was created after a thorough review of literature from various sources. Sections of the questionnaire are dedicated to evaluating demographics and related variables. The statements and questions are

classified and ordered in accordance with the specific goals that they were intended to address. Two data collectors with BSC degrees in midwifery gathered the data. The lead investigator extensively oversaw the data collection process to ensure the data quality.

The gynecology ward, delivery ward, and ANC were the locations where data collecting was happening. A pretest is conducted before to the actual data collection to assess the validity of the study's protocol and format. Apart from administering the questionnaire, the medical records of the patients were examined in order to extract pertinent variables pertaining to laboratory, clinical, and obstetric data. Before enrolling participants in the trial, the doctor took a thorough history of the patient's current pregnancy, diabetes, renal problems, and preeclampsia in the family. In the maternity ward, the subjects' body mass index (BMI) was also computed, and those who are obese were not included in the study. The real data was gathered through in-person interviews, measurements, and a review of the mother's medical records by skilled data collectors utilizing a pretested, structured questionnaire.

Only pregnant women who visit the hospital maternity ward during the day were the subject of the data collection. Informed written consent was acquired. Using a semi-structured, pre-tested questionnaire, the investigator conducted interviews with those interviewed, who included junior physicians with residency and were occasionally assisted by trained assistants. The participants were given around five minutes to sit and relax. Using a mercury sphygmomanometer instrument, the blood pressure reading was obtained while the woman was seated upright and in the supine position.

4.7 Sample collection procedures

Using aseptic methods, 6 ml of venous blood was extracted from each individual and placed in a standard EDTA test tube. The National Clinical Chemistry Laboratory Standards (NCCLS) and the Clinical and Laboratory Standards Institute provided rules for the collection, handling, and transportation of the samples to the laboratory.

The median vein was used to draw a six milliliter blood sample from each case and control participant. The sample was then divided into two milliliters for the CBC and four milliliter for the chemistry tests. The CBC test required 2 ml of blood, which was put to an ethylene-diamine-tetra acetic acid (EDTA) test tube.

4.8 Laboratory testing

On a Beckman Coulter DXH 800 automated hematology analyzer, the levels of White Blood Cells (WBC) , Red Blood Cells (RBC) , Hematocrit (HCT), MCV (Mean Corpuscular Volume): MCH (Mean Corpuscular Hemoglobin) Platelet count (PLT): were analyzed. Using four milliliter blood which was drawn on serum separator tubes (SST) ,biochemical testing was done using Cobas 311 fully automated clinical chemistry analyzer, the following serum components were measured: creatinine, Urea, ALP, ALT, AST, Bilirubin direct, Bilirubin Total, Sodium (Na+), Potassium (K+), and Chloride (Cl).

Principles of measurement for the Beckman Coulter DXH 800

The DxH 800 CBC analysis based on Coulter principle performs 100 samples per hour of 27 hematological parameters. CBC is essential analytical test that evaluates the three main cellular components; WBC, RBC and platelets. Sample preparation and data collection occurs in SAM and CBC modules on the DxH 800. The data analysis is handled by the system manager. The method of counting and sizing in combination with an automatic diluting and mixing device for sample processing, and a single beam photometer for hemoglobinometry. The WBC differential uses VCS technology. Analysis and classification of WBCs use three simultaneous measurements of individual cell volume (V), high frequency conductivity (C), and laser light scatter (S). The scatter gram plots the cells based upon the measurements of these three parameters.

The Beckman Coulter method accurately counts and sizes cells by detecting and measuring changes in electrical resistance when a particle such as a cell, in a conductive liquid passes through a small aperture. Each cell suspended in a conductive liquid (diluents) acts as an insulator. As each cell goes through the aperture, it momentarily increases the resistance of electrical path between the submerged electrodes on either side of the aperture. This causes a measureable electronic pulse. For counting, the vacuum used to pull the diluted suspension of the cells through the aperture must be at a regulated (reproducible) volume. While the number of the pulses indicates particle count, the size of the electrical pulse is proportional to the cell volume. The hemoglobin is photometrically measured at 525 nm using lysed WBC dilution drains to the cuvette from the WBC analysis (counted). The lytic reagent rapidly and simultaneously destroys the RBC and converts Hgb into stable pigment, which is proportional to the concentration of Hgb

Principles of measurement for the Cobas C311

The Cobas C311 analyzer examines biochemical parameters in clinical samples by applying a variety of measuring techniques. An outline of its basic operating principles is provided below:

- ✓ **Photometry**: The Cobas C311 makes use of photometric measurement techniques to determine the chemical concentration in biological samples. Photometry is the measuring of a sample's light absorbance at specific wavelengths. The intensity of light absorbed is directly proportional to the concentration of the substance under test..
- ✓ **Immunoturbidimetry**: Complement components, and immunoglobulins, are measured using immunoturbidimetry assays. This technique relies on the turbidity of the sample being increased by the formation of immunological complexes between antigens and antibodies. As a result, the degree of turbidity is photometrically assessed and is proportional to the target protein concentration.
- ✓ **Enzymatic Assays:**

  Enzymatic assays are used to determine the amount of enzymes present in clinical samples. These assays use specific enzyme-substrate reactions, where the activity of the enzyme catalyzes the transformation of the substrate into a product.The rate of product synthesis is measured by photometric analysis, and this rate is associated with the amount of enzymes present in the sample.
- ✓ **Ion-Selective Electrode (ISE) Technology:** Ion-selective electrode technology can also be used by the Cobas C311 analyzer to detect electrolytes like ionized calcium, potassium, sodium, and chloride. The ion concentration gradient between the sample and the internal reference solution in the electrode produces an electrical potential difference, which is measured by ISE technology. The ion concentration in the sample determines the potential difference.

4.9 Data Quality Assurance

Through the use of a validated and pretested questionnaire, the data quality was guaranteed. Pre-testing was conducted on 5% of the study participants at the st peter hospital ward prior to the actual data collection. This testing was not included in the study, and appropriate adjustments were made in light of the results. Data collectors received a full day of thorough training on the study instrument and data collection process, covering topics such as informed consent,

interview methodology, confidentiality of the information, and the study's relevance and goal. To guarantee adherence to proper data collection processes, the supervisors closely monitored the data collectors while they worked.

At the end of each day's data collection, the lead researcher checked the completed questionnaires for accuracy. Every morning, the lead investigator had a morning session with the data collectors to address any issues that arose and choose the best course of action for remedial action. Additionally, before the study, the data was meticulously entered and cleansed.

Pre-analytical:

In order to guarantee data quality, all activities, including the gathering, handling, and storing of blood samples, were carried out in accordance with standard operating procedures (SOPs) and acceptable laboratory practices. The research subjects also were well aware about the sample collection procedure, prepared and labeling was done on the questionnaire and on the test tubes. From three in the morning to six in the morning local time, specimen collection was done. After being cleansed with 70% alcohol antiseptic, the venous blood sample was drawn from the median cubital fossa of the forearm and placed into an EDTA and serum separator tube (SST). The specimens were moved to the laboratory department after being stored. Blood in an SST tube was centrifuged to separate the serum, and the test was examined in the chemistry unit after the blood sample in an EDTA test tube was delivered to the hematology laboratory department for hematological examination.

Analytical:

T he St. Peter Hospital laboratory sections that dealt with hematological and clinical chemistry examined the test. Calibrated machines were used to conduct the tests. Furthermore, manufacturer-made quality control (Normal, High, and Low) was available and was tested for hematological analysis. It was also important to have verified the manufacturer's expiration date.. Along with the serum sample, internal quality control (IQC) or manufacturer-made normal and pathological controls were tested for chemistry. Before serum sample testing, the control sample results had to fall within acceptable ranges. Senior and skilled laboratory technologists examined the sample after thoroughly reading the leaflet for each analyte.

Post-analytical:

Biochemical findings for the entire Hematologic profile were acquired to investigate any abnormalities further. The results were documented, handled properly, and safeguarded. The results were analyzed for potential magnitude and unit error. Two individuals were engaged in the data entering procedure, including the lead investigator, following which it was checked for completeness and cleaned. The data collected from laboratory examinations of blood samples and questionnaires was processed and evaluated by coding and entered into the SPSS software version 23 package, where the various variables were tested and analyzed.

4.10 Data Analysis and Interpretation

Biochemical findings for the entire Hematologic profile were acquired to investigate any abnormalities further. The results were documented, handled properly, and safeguarded. The results were analyzed for potential magnitude and unit error. Two individuals were involved in the data entering procedure, including the lead investigator, following which it was checked for completeness and cleaned. The data collected from laboratory examinations of blood samples and questionnaires was processed and evaluated by coding and entered into the SPSS software version 23 package, where the various variables were tested and analyzed.

4.11 Ethical Consideration

The study was carried out with permission from the Department of Research and Ethics Committee (DREC) of Medical Laboratory Sciences, College of Health Sciences, Addis Abeba University. An official letter of request was sent to St. Peter Hospital in order to acquire approval and conduct the study titled "Hematological and Biochemical Profile Alteration in Pregnancy-Induced Hypertensive Pregnant Women Attending St Peter Hospital Addis Abeba, Ethiopia." Participants were informed about the study's benefits and drawbacks, their ability to withdraw at any time, how codes are used to protect confidentiality, and their access to a free copy of their results, as well as the fact that no service from the facility was missed due to refusal.

Dissemination of Results

After conducting the investigation, the findings will be submitted to the department of Medical Laboratory Sciences. Furthermore, a hard copy of the results was handed to St Peter's Hospital. The study's findings will be submitted to peer-reviewed publications for publication. The discovery was shared through presentations at scientific conferences.

4.9 Operational definitions

Pregnancy-induced hypertension:- is defined as systolic blood pressure ≥ 140 mmHg and/or diastolic blood pressure ≥ 90 mmHg, with or without proteinuria and/or convulsions.

Preeclampsia:- is a pregnancy disease characterized by systolic blood pressure ≥ 140 mmHg and/or diastolic blood pressure ≥ 90 mmHg, along with proteinuria (≥0.3 g/dl).

Eclampsia:- is a pregnancy disease characterized by high systolic and/or diastolic blood pressures, often with convulsions.

Mild PIH:- is defined as blood pressure above 140/90 mmHg but below 160/110 mmHg after 20 weeks of gestation, with or without proteinuria.

Severe PIH:- is defined as blood pressure ≥ 160/110 mmHg after 20 weeks of gestation, with proteinuria.

Biochemical parameter:- is a list of tests including, Urea, Creatinine, AST,ALT. ALP, Bilirubin Direct, Bilirubin Indirect and Total protein

Eclampsia: a condition that causes a pregnant woman, usually previously diagnosed with preeclampsia (high blood pressure and protein in the urine), to develop seizures or coma

Gestational age: is calculated from the last normal menstrual period (LNMP) and for those women who didn't recall their last menstrual period, and/or ultrasound result was used.

Gravidity: the total number of pregnancies, including abortion, ectopic pregnancy and any other pregnancies documented on the chart.

HEELP Syndrome:-is a life-threatening liver disorder thought to be a type of severe preeclampsia. It is characterized by Hemolysis, Elevated Liver enzymes (indicating liver damage), and Low Platelet count.

Mild pre-eclampsia:- is defined as two diastolic blood pressure readings of 90-110mmHg taken 4-6 hours apart after 20 weeks of gestation, with proteinuria of >300mg/l in 24 hours or up to 2+, and with or without edema.

Parity: is defined as 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.

Proteinuria in preeclampsia can be defined as any of the following: ≥0.3 g protein in a 24-hour urine specimen. The completeness of the 24-hour urine collection can be estimated from creatinine excretion, which should be 15 to 20 mg/kg (133 to 177 micromol/kg) of lean body weight in women.

5. Result

Demographic and socioeconomic status of participant

The study included 250 pregnant women, 125 of whom had PIH and another 125 who appeared to be healthy. Of the 250 study participants, 150 (60%) were from antenatal care (ANC), and the remaining 100 (40%) were from other maternity units, such as a delivery ward. Population information on the investigated population was collected and compiled in a table. The study included pregnant women aged 18-40, with a mean of 27.14±4.409 years. The average age of pregnant women with and without PIH was 27.2 ± 4.45 years and 27.1 ± 4.3 years, respectively.

From all study participated in this study; 109 (87.2 %), 103 (82.4 %) were from urban on case and control group of participants respectively.

(Table 1).

Table 1 Socio-demographic characteristics of study participants

Variables	Case Group		Control Group		
	Frequency	%	Frequency	%	
Age					
Less than 20 Years	4	3.2	3	2.4	0.89
20-24.9 Years	31	24.8	34	27.2	
25-29.9 Years	54	43.2	52	41.6	
30-34.9 Years	25	20	26	20.8	
35-39.9 Years	10	8	9	7.2	
Mean age in years	27.2±4.5		27.1±4.3		
Address					
Urban	109	87.2	103	82.4	0.29
Rural	16	12.8	22	17.6	
Marital Status					
Married	107	85.6	122	97.6	0.001
Unmarried	18	14.4	3	2.4	
Mother Educational status					
Illiterate	10	8	1	0.8	0.009
Elementary school	8	6.4	18	14.4	
Secondary School	19	15.2	14	11.2	
Preparatory School	31	24.8	14	11.2	
Diploma	31	24.8	23	18.4	
Degree and above	26	20.8	55	44	
Mother Job Status					
Housewife	27	21.6	42	33.6	0.000
Governmental	48	38.4	70	56	
Private job	42	33.6	10	8	
Student	4	3.2	0	0	
Other	4	3.2	3	2.4	
Income category					
<2500	25	20	11	8.8	0.000
2501-4999	28	22.4	114	91.2	
>5000	72	57.6	0	0	

Anthropometric measurement of pregnant women

The height of study subjects were 1.63±0.06 meters ranging between 1.62±0.06meters and the body weight was 65.3±5.8kilogram with a range of 64.5±5.1kilogram. The study participants had a mean ± SD BMI of 24.79±2.55Kg/m, ranging from 24.6±2.4 kg/m, which was identical to the case and control values shown in Table 2. There was no significant difference in weight and height of both case and control groups.

Table 2 Anthropometric measurement and blood pressure status of study participants

Variables	Case group Mean± SD	Control group Mean± SD	Mean difference	P-value Case & Control
Weight (kg)	65.34±5.7	64.46±5.1	0.88	0.203
Height (m)	1.63±0.07	1.62±0.06	0.01	0.444
BMI (kg/m2)	24.79±2.55	24.63±2.36	0.16	0.608
Systolic BP (mmHg)	147±7.09	113.5±8.03	33.47	0.000
Diastolic BP(mmHg)	95.79±10.47	73.61±10.05	22.18	0.000

Health-related status of participant

According to the research findings, the majority of pregnant mothers in the case groups did not exercise at all during their pregnancies. Only 28 (22.4%) of the 125 pregnant women in the case group reported engaging in physical activity during their pregnancy (**Table 3**).

Table 3 Health-related status of participant

Variables	Case group		Control group		P value
	Frequency	Percent	Frequency	Percent	
Physical exercise current pregnancy					
Yes	28	22.4	20	16.0	0.200
No	97	77.6	105	84.0	
If yes How often					
Always	0	0	0	0	0.04
2-3 times a week	07	5.6	3	2.4	
Once a week	21	16.8	17	13.6	
Alcohol drinker					
Yes	17	13.6	15	12	0.325
No	108	86.4	110	88	
If yes How Often					
Sometimes	17	100.0	15	100.0	0.397

Pregnancy related clinical status

The majority of study participants (67.2%) had delivered a baby more than once, 228 (91.2%) were married, 141 (56.4%) were in their third trimester, and 41 (16.4%) had a pregnancy week ranging from 20 to 37 weeks.(Table 4).

Table 4 History of current and previous pregnancy related status of participant

Variables	Case group		Control group		
	Frequency	Percent	Frequency	Percent	
Gestational age					
First Trimester	0	0	0	0	0.844
Second Trimester	110	88	109	87.2	
Third Trimester	15	12	16	12.8	
Gravidity					
First	44	35.2	42	33.6	0.65
2	56	44.8	65	52.0	
3 and above	25	20	18	14.4	
TOTAL	125	100	125	100	
Parity					
0	44	35.2	39	31.2	0.057
1	50	40	35	28.0	
2	24	19.2	41	32.8	
3	7	5.6	10	8.0	
TOTAL	125	100	125	100	

Comparison of hematological parameters between hypertensive and normotensive pregnant women

In this study, we observed that the mean total WBC count was significantly higher in the case groups compared to the controls (9.48 ± 4.3 vs. 7.92 ± 2.5). Similarly, neutrophil levels were elevated in the cases (7.24 ± 3.86) compared to the controls (5.72 ± 1.92). Additionally, the Mean Platelet Volume (MPV) was notably higher in patients with Pregnancy-Induced Hypertension (PIH) compared to those without PIH (9.4 ± 1.56 vs. 8.99 ± 1.15). However, the platelet count was lower in pregnant mothers with PIH (187.51 ± 78.69) than in normotensive mothers (222.34 ± 78.44), as shown in Table 5.Table 5

Comparison of hematological parameters between hypertensive and normotensive pregnant women

Variables	Case Group	Control group	Mean difference	p-value
	Mean± SD	Mean± SD		
WBC (103/µl)	9.48±4.3	7.92±2.5	1.56	0.001*
RBC (106/µl)	4.32±0.6	4.51±0.7	-0.19	0.020*
Hgb (g/dl)	13.47±1.6	13.72±0.81	-0.25	0.125
HCT (%)	37.87±4.3	39.23±2.52	-1.36	0.003*
MCV (fl)	89.09±5.2	89.02±5.22	0.07	0.917
MCH(Pg)	31.66±2.45	31.17±2.16	0.49	0.096
MCHC(g/dl)	35.77±1.55	35.24±0.75	0.53	0.001*
RDW	14.31±1.73	13.83±1.06	0.48	0.008*
RDW-SD (fl)	44.41±5.54	43.47±4.63	0.94	0.149
PLT (103/µl)	187.51±78.69	222.34±78.44	-34.83	0.001*
MPV (fl)	9.4±1.56	8.99±1.15	0.41	0.019
Neutrophil (Absolute)	7.24±3.86	5.72±1.92	1.52	0.000*
Lymphocyte (Absolute)	1.59±0.68	1.55±0.76	0.04	0.623
Monocyte (Absolute)	0.55±0.31	052±0.42	0.03	0.559
Eosinophil (Absolute)	0.13±0.18	0.16±0.37	-0.03	0.398

In-dependent-T test; *p values <0.05: statistically significant different

Comparison of biochemical parameters between hypertensive and normotensive pregnant women

The two groups varied in terms of serum creatinine and urea concentrations. In comparison to control groups, the mean level of creatinine increased in case groups (Mean ±SD, 0.58±0.26 for case groups and 0.460.09 for control groups, respectively). The level of urea likewise higher in case groups (19.73±8.67 for case groups and 19.28±7.64for control groups. Pregnant women with PIH had higher ALP, AST, and ALT levels than normotensive. ALP was 119.34±74.66 for the case groups and 90.53±68.32for the control groups; AST was 36.8±47.43 for the case groups and21.40±12.81 for the control groups; ALT was 26.71±42.93 for the case groups and 14.94±1.91 for the control groups) (Table 6).

Potassium concentration was lower in the pregnant women with hypertension compared to the normotensive pregnant women. (4.18±0.41for case groups and 4.65±0.57 for control groups, respectively)

Table 6 Comparison of biochemical parameters between hypertensive and normotensive pregnant women

Variables	Case Group Mean± SD	Control group Mean± SD	Mean difference	p-value
Creatinine (mg/dl)	0.58±0.26	0.46±0.09	0.12	0.000*
Urea (mg/dl)	19.73±8.67	19.28±7.64	0.45	0.667
ALP (U/l)	119.34±74.66	90.53±68.32	28.81	0.002*
AST(U/l)	36.8±47.43	21.40±12.81	15.40	0.001*
ALT(U/l)	26.71±42.93	14.94±1.91	11.77	0.003*
BILLID (mg/dl)	0.11±.07	0.1±0.08	0.01	0.566
BILLIT (mg/dl)	0.32±0.13	0.32±0.12	0.00	0.786
Sodium	140.62±4.34	140.97±3.89	-0.35	0.500
Potassium	4.18±0.41	4.65±0.57	-0.47	0.000*
Chloride	105.84±2.83	106.5±3.16	-0.21	0.583

In-dependent-T test; ***p values*** <0.05: statistically significant different

Comparison of hematological parameters among different severity levels of hypertension

In this study there was no significant difference among different severity levels of hypertension as shown in the table 7 below.

Table 7 Comparison of hematological parameters among different severity levels of hypertension

Parameters	Participants	Mean ± Std. Deviation	P Value
WBC	Moderate hypertensive	9.89±4.89	0.252
	Mild hypertensive	8.77±2.33	
	Severe hypertensive	8.10±2.82	
RBC	Moderate hypertensive	4.37±0.58	0.146
	Mild hypertensive	4.11±0.57	
	Severe hypertensive	4.35±0.52	
HGB	Moderate hypertensive	13.65±	0.088
	Mild hypertensive	12.85±1.61	
	Severe hypertensive	13.45±1.32	
HCT	Moderate hypertensive	38.30±4.23	0.143
	Mild hypertensive	36.36±4.80	
	Severe hypertensive	37.93±3.75	
MCV	Moderate hypertensive	89.26±5.01	0.532
	Mild hypertensive	89.28±6.38	
	Severe hypertensive	87.54±4.17	
MCH	Moderate hypertensive	31.74±2.16	0.691
	Mild hypertensive	31.67±3.41	
	Severe hypertensive	31.12±2.18	
MCHC	Moderate hypertensive	35.84±1.41	0.769
	Mild hypertensive	35.69±2.19	
	Severe hypertensive	35.53±1.00	

Parameters	Participants	Mean ± Std. Deviation	P Value
RDW	Moderate hypertensive	14.31±1.92	0.818
	Mild hypertensive	14.43±1.46	
	Severe hypertensive	14.05±0.62	
RDWSD	Moderate hypertensive	44.27±6.00	0.614
	Mild hypertensive	45.32±5.11	
	Severe hypertensive	43.63±2.19	
PLT	Moderate hypertensive	186.76±80.00	0.938
	Mild hypertensive	186.29±81.57	
	Severe hypertensive	194.92±68.98	
MPV	Moderate hypertensive	187.51±78.69	0.661
	Mild hypertensive	9.32±1.60	
	Severe hypertensive	9.62±1.61	
NEUTROPHIL	Moderate hypertensive	9.52±1.17	0.257
	Mild hypertensive	7.60±4.34	
	Severe hypertensive	6.69±1.99	
LYMPHO	Moderate hypertensive	5.94±2.72	0.273
	Mild hypertensive	1.65±0.76	
	Severe hypertensive	1.45±0.43	
MONOCYTE	Moderate hypertensive	1.42±0.42	0.878
	Mild hypertensive	0.55±0.35	
	Severe hypertensive	0.52±0.20	
EOSINOPHIL	Moderate hypertensive	0.58±0.16	0.533
	Mild hypertensive	0.15±0.20	
	Severe hypertensive	0.10±0.07	
	Severe hypertensive	0.01±0.02	

One way ANOVA test; *p values <0.05: statistically significant

Comparison of biological parameters among different severity levels of hypertension

In the present study the Urea, Na and Cl levels were significant difference among different severity level of PIH (Table 8).

Table 8 Comparison of biological parameters among different severity levels of hypertension

Parameters	Participants	Mean ± Std. Deviation	P Value
Creatinine	Moderate hypertensive	0.61 ±0.29	0.099
	Mild hypertensive	0.49 ±0.14	
	Severe hypertensive	0.54 ±0.19	
Urea	Moderate hypertensive	20.88 ±8.94	0.032*
	Mild hypertensive	15.76 ±6.71	
	Severe hypertensive	19.67 ±8.45	
ALP	Moderate hypertensive	118.14 ±77.98	0.955
	Mild hypertensive	120.86 ±72.83	
	Severe hypertensive	124.43 ±58.02	
AST	Moderate hypertensive	40.40 ±54.96	0.371
	Mild hypertensive	31.82 ±24.30	
	Severe hypertensive	22.27 ±6.04	
ALT	Moderate hypertensive	30.29 ±50.04	0.340
	Mild hypertensive	20.66 ±19.16	
	Severe hypertensive	14.36 ±5.45	
BILLID	Moderate hypertensive	0.10 ±0.06	0.081
	Mild hypertensive	0.12 ±0.09	
	Severe hypertensive	0.13 ±0.05	
BILLIT	Moderate hypertensive	0.31 ±0.13	0.596
	Mild hypertensive	0.31 ±0.11	
	Severe hypertensive	0.35 ±0.16	
Parameters	Participants	Mean ± Std. Deviation	P Value

Na	Moderate hypertensive	140.28 ±4.73	0.402
	Mild hypertensive	141.40 ±3.29	
	Severe hypertensive	141.46 ±3.18	
K	Moderate hypertensive	4.27 ±0.39	0.001*
	Mild hypertensive	4.04 ±0.44	
	Severe hypertensive	3.89 ± 0.32	
Cl	Moderate hypertensive	105.23 ±2.51	0.001*
	Mild hypertensive	107.33 ±3.16	
	Severe hypertensive	107.07 ±2.93	

One way ANOVA test; *****p *values* <0.05: statistically significant

Comparison between which severity levels of hypertension did parameters show statistical difference?

6. Discussion

Hypertension associated with pregnancy is a major cause of illness and mortality in pregnant women and fetuses. Recent studies suggest that the symptoms of endothelial dysfunction, hypertension, proteinuria, and edema, all of which are used to identify preeclampsia syndrome, may be caused by a number of underlying causes or predispositions. Evaluation of hematological and biochemical markers in pregnancy-induced hypertension was the primary objective of the current study (2).

Pregnancy-induced hypertension was associated with significantly increased levels of Alanine Transaminase, Aspartate Transaminase, and Alkaline Phosphatase, which suggests that these liver enzymes are elevated compared to normal pregnant women. This is due to Liver dysfunction caused by Pregnancy-induced hypertension by Aditi Saha et al (13). The study found that pregnant women with PIH had a lower mean serum creatinine level than normotensives. Serum Creatinine levels in pregnancy induced hypertnsion and normal pregnancy differed significantly. This result was consistent with previous research by Haymanot Tewabe et al (32), who found a significant difference in serum creatinine levels between pregnant women with PIH and pregnant women without PIH. The researchers discovered that pregnant women with PIH had considerably higher levels of blood urea nitrogen and serum creatinine than pregnant women without PIH. Although the mean blood urea level in preeclampsia was greater, the difference was not statistically significant

These results of the current investigation contradicted the studies which found that the level of urea had increased statistically significantly Haymanot Tewabeet al (32).

According to the study's findings, the serum ALP, AST, and ALT levels were higher in preeclampsia than in a normal pregnancy, and the difference was statistically significant. Pregnancy-induced hypertension was associated with significantly increased levels of Alanine Transaminase, Aspartate Transaminase, and Alkaline Phosphatase, which suggests that these liver enzymes are elevated compared to normal pregnant women. This was consistent with study by Aditi Saha et al (13), NahlaHwaitalla Mohammed Kambal et al (47).

In this study Pregnant women with PIH had a considerably lower levels of Potassium (K) count and statistically significant than pregnant women in the control group. This was in line with Aditi Saha et al (13), NahlaHwaitalla Mohammed Kambal et al (47), The case groups had lower serum

Na and Cl levels than the control groups. This was consistent with the findings of Aditi Saha et al. (13) and Ekun OA et al. (48).

When comparing normotensive mothers to those with hypertension, it was observed that hypertensive mothers had a lower hematocrit (HCT) count, a statistically significant difference. Additionally, pregnant women with pregnancy-induced hypertension (PIH) exhibited significantly higher neutrophil counts compared to women with healthy pregnancies. This finding aligns with previous studies by Shariff ME et al (44) and Nahla Hwaitalla Mohammed Kambal et al. (45).

The mean platelet count of pregnant women with PIH was substantially lower than that of pregnant women in the control group, and thrombocytopenia was highlighted in numerous subsequent investigations. This observation was consistent with research by Neelam Jhajharia et al (2), Gupta AD et al (13), Abubakar U et al (44),Shariff ME et al (45),and Mohammed Haseeb Khamees (46)

Results of this study showed that pregnant women with PIH experienced significantly lower hemoglobin levels than pregnant women without PIH. It was also shown in studies done by Neelam Jhajharia et al (2), Aditi Saha et al (13), Abubakar U et al (43), Shariff ME et al (44),and Mohammed Haseeb Khameeset al(45)

Compared to normotensive mothers, the lymphocyte count was considerably lower in hypertension mothers. The finding agreed with other researches. Neelam Jhajharia et al (2), Shariff ME et al (44), Mohammed Haseeb Khameeset al (45)

The current study observed that pregnant women with hypertension exhibited significantly higher white blood cell (WBC) counts compared to those without hypertension, a finding supported by previous research by Neelam Jhajharia et al (2), Abubakar U et al (43), and Shariff ME et al (44). Additionally, the study revealed that pregnant women with pregnancy-induced hypertension (PIH) had notably elevated red cell distribution width (RDW) levels compared to controls, a consistent finding with previous studies. Notably, research by Kurt RK et al also reported significantly higher RDW levels in women with severe preeclampsia compared to normotensive individuals. The elevated WBC counts in pregnant women with hypertension suggest an underlying inflammatory response, which has been documented in hypertensive disorders of pregnancy. This aligns with previous studies by Neelam Jhajharia et al, Abubakar U

et al, and Shariff ME et al, reinforcing the robustness of this association across different populations. (2, 43, 44).

Overall, the results of this study showed that, in comparison to pregnant women without PIH, pregnant women with PIH had considerably higher levels of WBC, neutrophils, ALP, ALT, and AST, and significantly lower levels of potassium and chlorine.

Comparison of hematological and biochemical parameters among the different severity levels of pregnancy induced hypertension

Regarding biochemical markers, Significant variations were seen in electrolyte levels between severity levels, especially in potassium and chloride, where lower amounts were seen in severe instances as opposed to moderate and mild cases. To provide a more definitive explanation of any possible correlations between hematological markers and the severity of hypertension, future study with bigger sample sizes would be required.

7. Limitation and strength of the study

Limitations

- ✓ The case definition is incomplete as it does not include additional parameters such as proteinuria evaluation alongside monitoring pregnant women's blood pressure

Strength

- ✓ Usage of appropriate sample size f

8. Conclusion and recommendation

8.1 Conclusion

The risk of pregnancy-induced hypertension to the health of both mothers and fetuses is great, and it significantly raises the rates of morbidity and mortality among expectant mothers. The results of this investigation highlight the complexity of pregnancy-related hypertension, indicating multiple sources and consequences that extend beyond conventional indicators such as endothelial dysfunction, hypertension, proteinuria, and edema. Hematological and biochemical marker studies between pregnant women with and without hypertension showed some interesting differences. Compared to their normotensive peers, women with pregnancy-induced hypertension consistently showed lower levels of potassium and chloride and higher levels of white blood cells, neutrophils, ALP, AST, and ALT. These results support earlier studies and point to a strong correlation between these indicators and pregnancy-related hypertension.

Additionally, subtle patterns were found in the association between the severity of hypertension and hematological and biochemical markers. Even while there were some differences between severity levels, many of them did not approach statistical significance, which emphasizes the need for future studies with bigger sample sizes to more clearly define any possible relationships. The noted changes in hematological parameters, including platelet count, hemoglobin-producing capacity, and red blood cell count, point to possible consequences for hemoglobin-induced hypertension-related illnesses, bleeding risk, and oxygenation status in pregnant women. Furthermore, changes in biochemical markers such as creatinine, AST, ALT, and electrolytes highlight the various systemic effects of hypertension during pregnancy, including the involvement of the liver and kidneys.

Taking into consideration the wide range of physiological changes and potential consequences connected to this illness, these findings highlight the significance of thorough monitoring and management measures for pregnant women with hypertension. Healthcare providers can more effectively diagnose and treat the medical needs of pregnant women with hypertension by comprehending the complex interactions between hematological and biochemical indicators, which will eventually improve outcomes for both the mother and the fetus.

8.2 Recommendation

Close surveillance of renal function is necessary for guaranteeing an appropriate birth before severe renal impairment occurs. A key component of the pathophysiological mechanism behind pregnancy-induced hypertension is altered renal function. This relationship may be used to study the underlying pathogenic process of pregnancy-related hypertension. Therefore, the probability of problems related to pregnancy-induced hypertension can be decreased by early detection of hematologic profile and organ function tests, which in turn lowers the morbidity and mortality of the mother and baby. Hematological and kidney function tests must be followed throughout the pregnancy in order to allow for prompt identification and/or the development of planss to prevent any pregnancy related complications during PIH and/or delivery.

It is recommended to adopt the following strategies based on findings discovered in the current study.

- ✓ Wide range of studies which will show the effect of pregnancy induced hypertension on alteration of hematological and biochemical (including coagulation profiles) tests carried out over our country. Future research should aim to validate the findings of this study, particularly regarding the association between elevated levels of white blood cells, neutrophils, ALP, AST, ALT, and decreased levels of potassium and chloride with pregnancy-induced hypertension. Replication studies will strengthen the evidence base and provide more robust insights into these associations.
- ✓ Additional research must be conducted by including other parameters, taking lifestyle, attitudinal, and nutritional variation into account.
- ✓ Regular antenatal visits with monitoring of blood pressure, urine protein, and other relevant blood tests are critical.
- ✓ In cases where abnormalities become severe and cannot be managed conservatively, by consulting physicians and early delivery may be the safest option for both mother and baby.
- ✓ Regularly monitor sodium, potassium, and other electrolytes, liver function tests (LFTs), and renal function, promptly correcting any imbalances or abnormalities through dietary adjustments, supplementation, or considering early delivery, while avoiding hepatotoxic medications to prevent exacerbation of liver dysfunction.

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ANNEXES

Annex 1. Information sheet (English Version)

Research Project: Hematological and Biochemical profiles alteration in pregnancy induced hypertensive pregnant women attending in st Peter hospitals Addis Ababa, Ethiopia

Sponsor organization: Addis Ababa University, College of Health Sciences.

Principal Investigator: Yohannes Kidane (B.Sc. in medical laboratory science)

Advisors: Zemenu Tamir and RahelAlemu

Introduction

Dear participants you are kindly requested to take part in this research project as a study participant voluntarily. Read the information provided in this sheet carefully and then respond freely and voluntarily to what the investigator interviews you.

Objective of the research project

This information sheet is prepared by the investigator and the advisors at AAU for a project with the objective of hematologic and biochemical parameters among pregnancy induced hypertensive pregnant women

Procedure

If you agree to take part in the study, the investigator or a health worker will give you verbal and/or written information about the study and you was given the consent form to sign, the physician or health professional will ask you some questions about your general health and perform a complete medical examination and assess whether you qualify to participate in the study. If you are fit for the study about 4 ml of blood and Sputum samples will also be collected for only the laboratory examination of CBC, PICT & Gene Xpert and face to face interview for additional questions.

Discomforts and risks benefits and from participation

The degree of discomfort you may encounter in giving the sample is no more than when one does in his/her routine examination. But, there could be cases in which minor pain and change in color of your skin following the blood drawing occur transiently. The blood was withdrawn by licensed health care professionals in the hospital and appropriate care will also be taken. You will not be provided with any direct incentives for your participation in the research. But the cost for general medical examination was covered by the project. In addition, based on the results obtained from the research you was cared accordingly or the results may serve you as a baseline data. In addition, the result of the study was beneficial for the better prevention and care of pregnancy induced hypertensive pregnant patients than before. Hence, you are indirectly benefiting other patients and the society in this aspect.

Confidentiality

All pieces of information about the patients waskept confidential. Log books used in the laboratory will have no names but codes. The information sheet that links the coded number to patient name was locked inside a box and it will not be revealed to anyone except your physician and the principal investigator. You have full right to withdraw from participating in this study at any time before and after consent even without explaining the reason. Your decision will not affect your right to get health service you are supposed to get otherwise.

Contact information

If you have, any questions contact **Yohannes Kidane:** with **0923026655**

Annex 2: Informed consent (English version)

Department of medical Laboratory science, School of graduate studies, College of Health Sciences, Addis Ababa University, Consent form for the participation of the study participants in the research project

Name of the study participant _____

Code number_____

I have clearly been informed about the research project that it aims to Evaluation of Hematological and Biochemical profiles alteration in pregnancy induced hypertensive pregnant women attending in st Peter hospital Addis Ababa, Ethiopia. The objectives of the research project have clearly been explained to me and I have been told that the results obtained from me will help me as well as the community for better management of the disease. I had been also informed about the confidentiality of this research project. Moreover, I have also been well informed of my right to keep hold of information, decline to cooperate and make myself withdraw from the study. Therefore, with full understanding of the importance of the study, I agreed voluntarily to provide the requested samples and my benefit wasonly from the free laboratory investigation result/s.

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

Signature: _____ **Date**_____

Annex 3: Questionnaire (English version)

Dear respondents, you are kindly requested to give correct information accordingly. Thank you for your time and participation.

Questionnaire code number_____

S.No	Variables with the specific possibilities of Characteristics for each variable	
2)	ID No given by PI _____ Date _____ Hospital ID No_____ BMI in kg/m^2_____ Age _____ Blood pressure_____	
3)	Address of women	1. Rural 2. Urban
4)	Marital status	1,Married 2 Divorced 3 Single
5)	Educational status of mothers	1. Illiterate 2. Primary 3. Secondary 4. Preparatory 5. Diploma 6. Degree & above
6)	Educational status of husbands	1. Illiterate 2. Primary 3. Secondary 4. Preparatory 5. Diploma 6. Degree & above
7)	Occupational status of mothers	1. Housewife 2. Governmental 3. NGO 4. Student 5. Self 6. Others
8)	Husband's occupation	1. unemployed 2. student 3.trader 4. self-employed 5. civil servant 6.Other
9)	Income category	1. ≤ 793 (low income) 2, 793 - 2805 (middle income) 3, >2805 (high income)
Physical and cultural activities		
10)	**Have you do scheduled physical exercise during the current pregnancy?**	1. Yes, 2. No
11)	If yes to Qs number 9, how often?	1, Every day 2, 2-3 times a week 3, once a week
12)	Are you an alcohol drinker	1. Yes, 2. No
13)	Have you smoking a cigarette	1. Yes, 2. No
14)	Pregnancy Status	1, Once 2 More than once

Variable related to maternity case		
15)	Partner change	1. Yes, 2. No
16)	Trimester	1. First 2. Second 3. Third
17)	Gravidity	1. Primigravida 2. Multigravida
18)	Pregnancy status	1. Wanted 2. unwanted
19)	Gestational age	1) <37 2) 37-42 3) > 42
20)	History of delivery	1, 0 2, 1-4 3, . ≥5
Clinical related cases		
21)	History of Gestational Diabetic Mellitus	1. Yes, 2. No
22)	History of chronic hypertension	1. Yes, 2. No
23)	History of anemia	1. Yes, 2. No
24)	Previous history of blood clotting problem	1. Yes, 2. No
25)	Family history of chronic hypertension	1. Yes 2. No
26)	Family history of diabetes	1. Yes 2. No
27)	Have you history of liver failures	1. Yes 2. No
28)	Have you history of Kidney disease	1. Yes 2. No
29)	Have you history of heart failure	1. Yes 2. No
30)	Have you sense of Headache	1. Yes 2. No
31)	Family history of pregnancy induced hypertension	1. Yes 2. No
32)	Previous history preeclampsia	1. Yes 2. No

I thank you for your cooperation!

Interview Date: _____

Interviewer name_____

Annex 4: I–nformation sheet (Amharic version)

የተሳታፊዎች የፈቃደኝነት መተማመኛና መረጃ መስጫ ቅፅ

በአዲስ አበባ ዩኒቨርሲቲ የጤና ሳይንስ ኮሌጅ የሕክምና ላቦራቶሪ ሳይንስ የሄማቶሎጂና የኢሚዩኖሄማቶሎጂ ትምህርት ክፍል

ጥናቱን ስፖንሰር ያደረገው ተቋም፤ የአዲስ አበባ ዩኒቨርሲቲ የሕክምና ጤና ሳይንስ ኮሌጅ ነው፡፡

መረጃ መስጫ ቅፅ

በአዲስ አበባ ዩኒቨርሲቲ የሕክምና ጤና ሳይንስ ኮሌጅ የሕክምና ላቦራቶሪ ሳይንስ የሄማቶሎጂና የኢሚዩኖሄማቶሎጂ ት/ክፍል ለሁለተኛ ዲግሪ የተማሪ የመመረቂያ የጥናት ጽሁፍ ላይ እንዲሳተፉ ተጋብዘዋል፡፡

እባክዎ በዚህ ጥናት ለመሳተፍ ከመስማማትዎ በፊት ከዚህ ቀጥሎ የሚገኘውን ምን ባብ በጥሞ እና ያንብቡ እና ግልፅ ያልሆነሎትን ማንኛውንም ሃሳብ ይጠይቁ፡፡

“Hematological and Biochemical profiles alteration in pregnancy-induced hypertensive pregnant women attending in St. Peter Addis Ababa Ethiopia”

የጥናቱ ርእስ ሲሆን አላማውም በእርግዝና ምክኒያት የሚከሰተው የደም ግፊት ህመም በደም ሴሎች ላይ በበውስጣዊ የሰውነት ክፍሎች ማለትም በኩላሊት እና በጉበት የሚኖረውን ተፅዕኖ ለማጥናት ነው፡፡

የጥናቱ ውጤት ለታካሚው ብሎም ለሌላውም ማህበረሰብ የሚጠቅምና የተሻለ የጤና እንክብካቤ እንዲኖር የሚያደርግ ነው፡፡

ስለሆነም እርስዎ በዚህ ጥናት እንዲሳተፉ ተመርጠዋል፡፡

የእርስዎ በዚህ ጥናት ላይ የሚያደርጉት ተሳትፎ ሙሉበሙሉ በበጎ ፍቃደኝነት ላይ የተመሰረተ ነው፡፡

በጥናቱ ከተሳተፉ ለናሙና ይሆን ዘንድ 6-8 ሚሊሊትር ያህል ደም በሆስፒታሉ ጤና ባለሙያዎች የሚሰጡ ሲሆን የደምና ሙናውን በሚሰጡበት ሰአት ሁልጊዜ ለምርመራ ከሚሰጡበት የተለየ ህመምና አለመመቸት የለውም ለምናልባት ቢኖር ተገቢውን የጤና እንክብካቤ የሚያገኙ ይሆናል፡፡

በዚህ ጥናት ውስጥ ለመሳተፍ ወይም ለመሳተፍ ከወሰኑ በኋላ ለማቋረጥ የሚወስኑ ቢሆንም እንኩዋን በዚህ ሆስፒታል የሚሰጠዎት ማንኛውም አገልግሎት ላይ ተጽዕኖ የለውም፡፡

በጥናቱ ለመሳተፍ የሚስማሙ ከሆነ የስምምነት ቅጹ ላይ በጽሁፍ ወይም በጣት ፊርማ ማስቀመጥ ይጠበቅበዎታል።

ግልፅ ያልሆነሎት ጥያቄ ካሎት

አድራሻዬ ፡ ሞባይል ቁጥር 0923026655 ዮሐንስ ኪዳኔ ብለው በመደወል መጠየቅ ይችላሉ

Annex 5: Informed Consent (Amharic Version)

የተሳታፊዎች ስምምነት ማረጋገጫ ቅፅ

የሚስጥር ቁጥር

የተሳታፊው ስም

እኔ ስሜ

ከላይ የተገለጸው ግለሰብ በዚህ ጥናት ተሳታፊ ስሆን "Hematological and Biochemical profiles alteration in pregnancy-induced hypertensive pregnant women attending in St. Peter Addis Ababa, Ethiopia" በሚል ርእስ የሚደረገው ጥናት አላማውና ጥቅሙ ተገልጾ ልኖል። ስለዚህ ለዚህ ጥናት የስምምነት ቃሌን የምሰጠው የጥናቱን አላማና ጥቅም በመረዳትና በፈቃደኝነት ነው። በመጠይቁ ላይ የምሰጠው የእኔ መረጃ እንደማይባክንና ምስጢራዊ እንደሚደረግ ተገልፆልኛል። በተጨማሪም ጥናቱ ውስጥ ለመሳተፍ ከወሰንኩ መብቴ የተጠበቀ እንደሆነና በማንኛውም ጊዜ ከጥናቱ በራሴ ውሳኔ መውጣት ጭምር እንደምችል መብቴ መሆኑና ከጥናቱ በመውጣቴ ምንም አይነት ችግር እንደማይደርስብኝ በሚገባ የተነገረኝ ስለሆነ ምሁኔታውን በሚገባ በማጸን በፈቃደኝነት በምርምሩ ላይ ለመሳተፍ ፈቃደኝነቴን ሰጥቻለው።

በተጨማሪም የምሰጠው የደምና ሙና ለጠቅላላ የደም ሕዋሶች ቆጠራ ምርመራ፤ ለኬሚስትሪ ምርመራዎች ብቻ እንደሚውል ተነግሮኝ ተስማምቻለው። ማንኛውንም ያልገባኝን ነገር የመጠየቅ እድል ተሰጥቶኝ በሚገባኝ ቋንቋ መልስ አግኝቻለው። በተጨማሪም የሁሉን የላቦራቶሪ ውጤት በጊዜው ለሐኪም እንደሚሰጥ ልኝና ውጤቱን ማወቅ እንደምችል ተነግሮኛል። በአጠቃላይ ከላይ በመተማመኛ ቅፅ የተጠቀሱትን ሁሉ በተረጋጋ መንፈስ አንብቤ ያለው። ስለዚህ በዚህ ጥናት ለመሳተፍ ፈቃደኛ መሆኔን በፊርማዬ አረጋግጣለው።

እኔ _____ የተባልኩት ግለሰብ ይህን ሁሉ በማገናዘብ በምርምሩ ላይ መረጃና የደምና ሙና ለመስጠት ተስማምቻለው።

ፊርማ _____ ቀን _____

የተሳታፊ _____ _____

Annex 6: Questionnaire (Amharic version)

መጠይቅ

ውድ ተሳታፊ ቀጥሎ ያለውን መጠይቅ ለመሙላት ስለተባበሩን እናመሰግናለን፡፡

የጥናቱ መለኪያ ተለዋዋጮች		
ተቁ	**ተለዋዋጭ**	**መለኪያዎች**
1)	ቀን _____ ለጥናቱ የተሰጠ መ/ቁጥር_____ ካርድቁጥር _____ እድሜ_____	BMI በኪ.ግ_____ የደምግፊት_____
Demographic characters		
3)	የመጡበት አድራሻ	1, ከተማ 2. ገጠር
4)	የጋብቻ ሁኔታ	1. ያገባች 2. ያላገባች 3.የተፋታች
5)	የእናት የትምህርት ሁኔታ	1. ያልተማረች 2. አንደኛ ደረጃን የተማረች 3. ሁለተኛ ደረጃን የጨረሰች 4. የመሰናዶት ምህርታን የጨረሰች 5. ዲፕሎማ 6. ዲግሪና ከዚያ በላይ ያላት
6)	የባል የትምህርት ሁኔታ	1. ያልተማረ 2. አንደኛ ደረጃን የተማረ 3. ሁለተኛ ደረጃን የጨረሰ 4. የመሰናዶት ምህርቱን የጨረሰ 5. ዲፕሎማ
7)	የእናት የስራ ሁኔታ	1. የቤት እመቤት 2. የመንግስት ሰራተኛ 3. NGO ሰራተኛ 4. ተማሪ 5. የግል 6. ሌላ
8)	የባልየስራሁኔታ	1. የመንግስት ሰራተኛ 2. የግልሰራተኛ 3. ነጋዴ 4. የቀንሰራተኛ 5. ሌላ

9)	የገቢመጠን	1. ≤2500 2. 2501—4999 3. ≥5000
ከእንቅስቃሴናኣጠቃቀምጋርየተያያዙጉዳዮች		
10)	ቋሚየሆነእስፖርታዊእንቅስቃሴያደርጋሉ?	1.አው 2.የለም
11)	አዎከሆነመልስዎምንያክልሰኣትልዩነትያደርጋሉ?	1, ሁልጊዜ 2, 2-3 ጊዜ በሳምንት 3, አንዴበሳምንት
12)	አልኮልይጠጣሉ?	1. አንዳንዴእጠጣለሁ 2. አዎን ሁሌም እጠጣለሁ 3. በጭራሽ አልጠጣም
13)	ሲጋራያጨሳሉ?	1. አንዳንዴ አጨሳለሁ 2. አወ ሁሌም አጨሳለሁ 3.በጭራሽ አላጨስም
14)	የእርግዝና ሁኔታ	1.አንድ 2. ከአንድ በላይ
15)	የፍቅር አጋርዎን ይቀያይራሉ?	1 .አዎ 2. በጭራሽ
16)	የእርግዝና ጊዜ	1. 1ኛ 2. 2ኛ 3. 3ኛ
17)	ስንተኛ እርግዝናዎ ነው?	1, የመጀመሪያ 2. 2ና ከዚያበላይ
18)	የእርግዝና ሁኔታ	1. የተፈለገ 2. ያልተፈለገ
19)	ካሁንምን ያክልጊዜ ወልደዋል?	1) 0 2) 1-4 3) ≥5
20)	የእርግዝናሳምንት	1) <37 2) 37-42 3) > 42
1. **ጤናነክጉዳዮች**		
21)	ከእርግዝና ጋር የተያያዘ የስኳር ህመም ኖሮቦት ያውቃል?	1.አው 2.የለም
22)	የቆየ የደም ግፊት ህመም አለብዎ?	1.አው 2.የለም
23)	የደም ማነስ አጋጥምዎት ያውቃል?	1.አው 2.የለም
24)	ከዚህ ቀደም የደም መርጋት በሽታ አጋጥሞት ያውቃል?	1.አው 2.የለም
25)	በቤተሰብዎ ውስጥ በደም ግፊት ብዛት ታማሚ አለ?	1.አው 2.የለም
26)	የስኳር በሽታ አጋጥሞዎት ያውቃል?	1.አው 2.የለም

27)	የጉበት አጋጥምዎት ያውቃል?	1.አው 2.የለም
28)	የኩላሊት ህመም አጋጥምዎት ያውቃል?	1.አው 2.የለም
29)	የልብ ህመም አጋጥምዎት ያውቃል?	1.አው 2.የለም
30)	የራስ ምታት ችግር አለብዎት?	1.አው 2.የለም
31)	ካሁን በፊት በእርግዝና ምክንያት ችግር አጋጥምዎት ያውቃል?	1.አው 2.የለም
32)	በቤተሰብዎ በእርግዝና ምክንያት ችግር አጋጥምዎት ያውቃል?	1.አው 2.የለም

አመሰግናለሁ!!!

Annex 7: Standard Operating Procedure (SOP) for blood sample collection

For valid test findings, to prevent sample contamination, and to avoid infection from blood-transmissible pathogens, blood must be obtained carefully and with the necessary safety procedures. When taking and handling blood samples, protective gloves should be worn. To prevent damage from lancets and needles, blood-collecting materials should be disposed of safely along with sterile, dry, and lancets, needles, and syringes.

Technique for collecting venous blood

Laboratory staff must not collect venous blood unless they have been adequately trained in the procedure. Newly qualified staff must be supervised until they have gained sufficient experience. When venous blood is required from infants, this should be collected by a medical officer. Do not collect blood for hematological tests from intravenous lines. Select a sterile, dry, preferably plastic 10 m l syringe of the capacity required, .

> **Note:** When not using a disposable syringe or needle, check the syringe for good suction and the needle for any blockage, directing the syringe and needle safely away from the patient. Ensure all air is expelled from the syringe. Whenever possible use a disposable needle and syringe.

Apply a soft tubing tourniquet or Velcro fastening armband to the upper arm of the patient to enable the veins to be seen and felt. Do not apply the tourniquet too tightly or for longer than 2 minutes. Ask the patient to make a tight fist which will make the veins more prominent.

Using the index finger feel for a suitable vein, selecting a sufficiently large straight vein that does not roll and with a direction that can be felt.

Cleanse the puncture site with 70% ethanol and allow drying. Do not re-touch the cleansed area.

With the thumb of the left hand holding down the skin below the puncture site, make the vein puncture with the bevel of the needle directed upwards in the line of the vein. Steadily withdraw the plunger of the syringe at the speed it is taking the vein to fill*. Avoid moving the needle in the vein.

> *If the plunger is withdrawn too quickly this can cause hemolyisis of the blood and the collapse of a small vein.

When sufficient blood has been collected , release the tourniquet and instruct the patient to open his or her fist. Remove the needle and immediately press on the puncture site with a

piece of dry cotton wool. Remove the tourniquet completely. Instruct the patient to continue pressing on the puncture site until the bleeding has stopped.

Remove the needle from the syringe and carefully fill the container(s) with the required volume of blood. Discard the needle safely. Do not attempt to re-sheath it because this can result in needle-stick injury.

Important: Do not fill a container with the needle attached to the syringe. Forcing the blood through the needle can cause hemolyisis.

Mix immediately the blood in an EDTA coagulated container and allow the blood on serum separator tube to clot for about 10-15 minutes to prepare serum for chemistry analysis. Immediately label carefully all the blood samples.

Check that bleeding from the vein puncture site has stopped. Cover the area with a small dressing.

Avoiding hematoma: when collecting venous blood bleeding from a vein into the surrounding tissue (hematoma) can cause unnecessary distress to a patient and result in subsequent bruising. Hematoma can be avoided by ensuring an appropriate vein is selected and the needle is well positioned in it and not accidentally pulled out of the vein when withdrawing the plunger of the syringe. When removing the needle, always release the tourniquet *first* and apply pressure immediately to the puncture site, maintaining it until the bleeding has stopped completely (always check).

Avoiding hemolysis of blood samples

The hemolysis (rupture) of red cells can be a serious source of unreliable test results. If red cells are hemolyzed, substances from the cells are released into the serum or plasma leading to a false increase in the concentration of analytes, e.g. potassium. Hemolysis also interferes with many chemical reactions. Hemolysis can be avoided by:

- Checking that the syringe and needle are dry and that the barrel and plunger of the syringe fit well.
- Not using a needle with too fine a bore.
- Not withdrawing the blood too rapidly or moving the needle once it is in the vein.
- Removing the needle from the syringe before dispensing the blood into the specimen container and allowing the blood to run gently down the inside wall of the container.

- Adding the correct amount of blood to anticoagulant. Do not shake the blood but gently mix it with the anticoagulant.
- Using clean dry glass tubes or bottles for blood from which serum is required. Allow sufficient time for the blood to clot and clot retraction to take place. Red cells are very easily hemolyzed by the rough use of an applicator stick to dislodge a clot.
- Centrifuging blood samples for a minimum period of time. Centrifuging for 5 minutes at about 1000 g is adequate to obtain serum or plasma. Not storing whole blood samples in, or next to, the freezing compartment of a refrigerator.

Annex 8: Standard operating procedure (SOP) for biochemical tests

1. Complete blood count

Principle

Complete blood count test is to be done using Beckman Coulter UniCel® DxH 800Analyzer. The DxH 800 CBC analysis based on Coulter principle performs 100 samples per hour of 27 hematological parameters. CBC is essential analytical test that evaluates the three main cellular components; WBC, RBC and platelets. Sample preparation and data collection occurs in SAM and CBC modules on the DxH 800. The data analysis is handled by the system manager. The method of counting and sizing in combination with an automatic diluting and mixing device for sample processing, and a single beam photometer for hemoglobinometry. The WBC differential uses VCS technology. Analysis and classification of WBCs use three simultaneous measurements of individual cell volume (V), high frequency conductivity (C), and laser light scatter (S). The scatter gram plots the cells based upon the measurements of these three parameters.

The Beckman Coulter method accurately counts and sizes cells by detecting and measuring changes in electrical resistance when a particle such as a cell, in a conductive liquid passes through a small aperture. Each cell suspended in a conductive liquid (diluent) acts as an insulator. As each cell goes through the aperture, it momentarily increases the resistance of electrical path between the submerged electrodes on either side of the aperture. This causes a measureable electronic pulse. For counting, the vacuum used to pull the diluted suspension of the cells through the aperture must be at a regulated (reproducible) volume. While the number of the pulses indicates particle count, the size of the electrical pulse is proportional to the cell volume. The hemoglobin is photometrically measured at 525 nm using lysed WBC dilution drains to the cuvette from the WBC analysis (counted). The lytic reagent rapidly and simultaneously destroys the RBC and converts Hgb into stable pigment, which is proportional to the concentration of Hgb.

2. Test principle for Serum Urea testing

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 measuresthe amount of ammonium ion spectrophotometrically or by electrode conductivity

Called ‘indirect’ methods because these methods measure the amount of ammonia ‘liberated’ from theurea molecule present in the sample. The amount of ammonium ion produced is directlyproportional to the amount of urea Glutamate dehydrogenase Spectrophotometric the conversionabsorbance of NADH to NAD is measured at 340 nm. The amount of NAD produced is directlyproportional to the amount of ammonium ion which is directly proportional to the amount ofurea present.Sample and addition of Reagent 1, Addition of Reagent 2 and start of reaction urea is hemolyzed by urase to form CO32- and ammonia.

Urea + 2H2O $\xrightarrow{\text{urease}}$ 2NH4+ +CO32-The ammonia formed then reacts with α-ketoglutarate and NADH in the presence of GLDH to yield glutamate and NAD+.

2-oxoglutarate + NH4+ +NADH $\xrightarrow{\text{GLDH}}$ L-glutamete +NAD+ +H2O

The rate of decrease in NADH concentration is directly proportional to the urea concentration in the specimen. It is determined by measuring the absorbance at 340nm..

3. Test principle for Serum Creatinine

For the measurement of serum creatinine I have used Jaffe reaction method with 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

Creatinine + picric acid Alkaline PH yellow red complex

In alkaline solution, creatinine forms a yellow red complex with picrate .The rate of dye formation is proportional to the creatinine concentration in the specimen

4. Principle of serum Test principle for ALT testing

Method according to the recommended reference method of the International Federation of Clinical Chemistry, but without Pyridoxal-5’-phosphate. ALT catalyzes the reaction between L-Alanine and 2-oxoglutarate. The pyruvate formed is reduced by NADH in reaction catalyzed by lactate dehydrogenase (LDH) to form L-lactate and NAD+. The rate of the NADH Oxidation is directly proportional to the catalytic. It is determined by measuring the decrease in absorbance at 340nm

5. Test principle for AST testing

Method according to the recommended reference method of the International Federation of Clinical Chemistry, but without Pyridoxal-5'-phosphate. AST catalyzes the reaction between L-aspartate and 2-oxoglutarate to form oxaloacetate The pyruvate formed is reduced by NADH in reaction catalyz and L-glutamate. The oxaloacetate then reacts with NADH, in the presence of malate dehydrogenase(MDH), to form NAD+. The rate of the NADH Oxidation is directly proportional to the catalytic AST activity. It is determined by measuring the decrease in absorbance at 340nm.

6. Test principle for Direct Bilirubin

Conjugated Bilirubin and direct Bilirubin react directly with diazotized sulfanilic acid in acid buffer to form the red-colored azobilirubin. The color intensity is proportional to the concentration of direct Bilirubin in the sample and is determined by monitoring the increase in absorbance at 552 nm

7. Test principle for Total Bilirubin

Since the absorbance maximum for azobilirubin is PH dependent, an oxalic acid/sulfanilic acid buffer system is used to maintain the PH of the reaction. The color intensity is proportional to the concentration of Total Bilirubin in the sample and is determined by monitoring the increase in absorbance at 552 nm.

$$\text{Sufanilic acid} + NaNO_2 \xrightarrow{HCL} \text{Diazotized sulfanilic acid}$$

$$\text{Bilirubin} + \text{diazotized Sulfanilic acid} \xrightarrow{PH\ 1.4} \text{azobilirubin}$$

8. Test principle for ALT

Method according to the recommended reference method of the International Federation of Clinical Chemistry, but without Pyridoxal-5'-phosphate. ALT catalyzes the reaction between L-Alanine and 2-oxoglutarate. The pyruvate formed is reduced by NADH in reaction catalyzed by lactate dehydrogenase(LDH) to form L-lactate and NAD+. The rate of the NADH Oxidation is

directly proportional to the catalytic. It is determined by measuring the decrease in absorbance at 340nm.

9. Alkaline Phosphatase (ALP)

According to the recommended reference method of the International Federation of Clinical Chemistry. Alkaline Phosphatase hydrolyses the colorless 4-nitrophenylphosphate ester to 4-nitrophenoxide and phosphate. The product of enzyme hydrolysis 4-nitrophenoxide, has a yellow color at the PH of the reaction. 2-Amino-2-methyl -1-propanol (AMP) functions as the phosphate acceptor and buffer. The rate of 4-nitrophenoxide formation is directly proportional to the catalytic ALP activity. It is determined by measuring the increase in absorbance at 409 nm.

Annex 9: Standard operating procedure (SOP) for hematologic CBC test

Sample preparation

The aspiration pump activates and aspirates 165 uL of sample. After the probe is removed from the specimen tube a second pull of the aspiration pump draws the blood through the BSV pathway, verifying a proper aspiration at the blood detectors. With each cycle, the BSV directs the delivery of the sample and DxH diluent to the WBC (approximately 6 mL diluent and 28 uL of sample are combined with 1.08 mL of DxH Cell Lyse for a final dilution of 1:251) and RBC (approximately 10 mL of DxH diluent and 1.6 uL of sample are mixed for a final dilution of 1:6250) triple aperture baths.

Procedure

1. Check to see that the reagents needed for the number of the samples to be processed for the day are available.
2. Turn on the IPU switch and log on screen will appear on the computer. Enter the user name and password.
3. Wake up/ Go online the main unit on the machine. Daily-check, auto rinse, temperature stabilization and background check wasautomatically performed, and the "READY LED turns on (ready for analysis) will appear
4. Perform quality control analysis on 3 levels of control blood material (low, normal and high), Latron and Latron Primer to verify that the instrument is performing within the specified ranges of the quality control material
5. If the result of quality control is inacceptable range, run the blood samples. Samples can be run in Single/manual mode, Sampler mode

Single Tube/Manual mode

- Click the single tube icon at the top of the computer.
- Input the tube position number or use bar code reader.
- Mix gently invert (10x) put the tube appropriately in the position labeled purple for whole blood or light green for the body fluid
- Ask “Add diluent” for whole blood if it is inadequate
- Fill the necessary information like MRN, test items (CDR/CBC/WBC-NE), and click “Submit”.

- Remove the tube when the analysis is over
- Review and print the result.

Sampler mode

- ✓ Click the sampler icon at the top of the computer.
- ✓ Prepare and put the samples on a barcode labeled tube rack.
- ✓ Fill the necessary information like tube position number similarly on the tube rack, MRN.
- ✓ Once again check the rack number and tube position number in the Rack Number/Tube Position Confirmation dialog box.
- ✓ Position the tube rack on the sample station so that the tube rack taken for analysis momentarily by the magnetic interaction and start analysis automatically
- Review and print the results
- Review and print the results.

Quality control procedure

1. Bring all the control materials to room temperature except Latron CP-X (already at room temperature) putting them on sample mixer.
2. Wake up/ Go Online the screen and log on screen will appear on the computer. Enter the user name and password.
3. Turn on the main unit on the machine. Self-check, auto rinse, temperature stabilization and background check was automatically performed, and the "READY LED turns on (ready for analysis) will appear.
4. Click the Controller button on the Menu screen.
5. Double-click the QC Analysis icon on the Controller Menu and select QC File dialog box.
6. Select a QC file and click OK.
7. Gently invert eight times the control tubes
8. Hold the opened control tube under the sample probe and press the start switch button.
9. Accept the control result if are within the range of the target limit or repeat the analysis if control results are out of the target limit.
10. All control data are managed using software that provides graphical reports (Levey-Jennings graphs, and monthly cumulative histograms). **(21,22)**

Declaration

I, the undersigned agree to accept responsibility for the scientific ethical and technical conduct of the research project and for provision of required progress reports as per terms and conditions of the research publications office.

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This thesis has been submitted with our approval as advisors.

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Date: _____

Place: Addis Ababa, Ethiopia.

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Signature: _____

Date: _____

Place: Addis Ababa, Ethiopia