SERUM LEVEL OF LACTATE DEHYDROGENASE AND GAMMA GLUTAMYL TRANSFERASE IN HYPERTENSIVE DISORDERS OF PREGNANCY AND ASSOCIATED FACTORS

BY:

AWGICHEW BEHAILE TEKLEMARIAM

A THESIS PAPER SUBMITTED TO THE DEPARTMENT OF BIOMEDICAL SCIENCES, FACULTY OF MEDICAL SCIENCES, INSTITUTE OF HEALTH, JIMMA UNIVERSITY IN PARTIAL FULFILLMENT FOR THE REQUIREMENT OF THE DEGREE OF MASTER OF SCIENCE IN MEDICAL BIOCHEMISTRY

FEBRUARY, 2021

JIMMA, ETHIOPIA

JIMMA UNIVERSITY, INSTITUTE OF HEALTH, FACULTY OF MEDICAL SCIENCES, DEPARTMENT OF BIOMEDICAL SCIENCES

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BY:

AWGICHEW BEHAILE TEKLEMARIAM (BSc)

ADVISORS:

Dr. ENDRIYAS KELTA (DMD, MSc)

Mr. TESFAYE ADUGNA (BSc, MSc)

Mrs. SEMIRA SHIMELES (BSc, MSc)

FEBRUARY, 2021

JIMMA, ETHIOPIA

DECLARATION FORM

This is to certify that a thesis paper prepared by Awgichew Behaile on **Serum Level of Lactate Dehydrogenase and Gamma Glutamyl Transferase in Hypertensive Disorders of Pregnancy and Associated Factors** and submitted for the requirements in partial fulfilment of the Degree of Master of Science in Medical Biochemistry complies with the regulations of Jimma University and meets the accepted standards concerning originality and quality.

Principal Investigator:

Awgichew Behaile _____ _____

Signature Date

Advisors' Name	Signature	Date
1. Dr. Endriyas Kelta (DMD, MSc)	_____	_____
2. Mr. Tesfaye Adugna (BSc, MSc)	_____	_____
3. Mrs. Semira Shimeles (BSc, MSc)	_____	_____

External Examiner

_____ _____ _____

Name Signature Date

Internal Examiner

_____ _____ _____

Name Signature Date

ABSTRACT

Background: Hypertensive disorders of pregnancy (HDP) are predominant causes of maternal and perinatal morbidity and mortality worldwide. Gestational hypertension, chronic hypertension, preeclampsia and eclampsia are common forms of HDP affecting approximately 10% of pregnancies as medical complications in pregnant women. Lactate dehydrogenase (LDH) and gamma glutamyl transferase (GGT) have been suggested as potential biochemical markers to predict the severity of preeclampsia and gestational hypertension and as indicators of multi-organ involvement.

Objective: The aim of this study was to assess serum level of lactate dehydrogenase and gamma glutamyl transferase, correlate with severity of hypertensive disorders of pregnancy and identify associated factors among pregnant women at Jimma Medical Center.

Methods: A hospital based cross-sectional study was undertaken from August 3 to September 27, 2020 in Jimma Medical Center. A total of 97 study subjects (33 preeclamptics, 32 eclamptics and 32 gestational hypertensives) were recruited based on the eligibility criteria. Data were collected using a structured questionnaire through face to face interview and by reviewing participants' medical record. Serum levels of GGT and LDH were evaluated by a fully automated chemistry analyzer called Roche Cobas 6000. The data were statistically analyzed using SPSS version 25.0. Analysis of Variance, independent sample t-test, receiver operating characteristics curve and bivariate correlation analyses were carried out.

Result: We observed highest mean serum level of LDH (580.9 ±193.8 U/L) and GGT (86.1±29.2 U/L) in eclamptics as compared to their level in gestational hypertensives (276.7± 60.7 and 38.3±16.9 U/L) and preeclamptics (353 ±132.8 and 48.8 ± 29.9 U/L) respectively. Both serum GGT and LDH levels were found to have significant correlation with severity of preeclampsia (p=0.007 and 0.002) respectively. The optimal cut-off point for GGT and LDH, to differentiate complicated HDP from uncomplicated HDP, was found to be 46.5 and 376.5 U/L respectively.

Conclusion: GGT is more reliable biomarker with greater sensitivity than LDH and hence, its use as a novel biomarker for better prediction of the severity and/or complications of HDP has to be pragmatic.

Keywords: Preëclampsia; Eclampsia; Gestational hypertension; GGT; LDH; Jimma Medical Center

ACKNOWLEDGEMENT

First and foremost, my special gratitude goes to my advisors; Dr. Endriyas Kelta, Mr. Tesfaye Adugna and Mrs. Semira Shimeles for their unreserved guidance and constructive comments while going through the whole work of this thesis paper.

Furthermore, I would like to extend my thanks to Jimma University for giving me such a chance to conduct this study, and Debre Tabor University for sponsoring me throughout my master of science education program. My sincere gratitude also goes to Mr. Endeshaw Chekol, whose academic support made me inspired and committed enough in doing this thesis paper.

It too is my pleasure to express my heartfelt thanks to laboratory, and obstetrics and gynecology staffs of Jimma medical center for their willingness and optimistic cooperation via collecting blood sample and all necessary data for the study. Last but not least, my sincere thanks is forwarded to all the study participants.

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ABBREVIATIONS AND ACRONYMS

ACOG	American College of Obstetricians and Gynecologists
ANC	Antenatal Care
ANOVA	Analysis of Variance
AUC	Area Under the Curve
BMI	Body Mass Index
DBP	Diastolic Blood Pressure
GGT	Gamma Glutamyl Transferase
HDP	Hypertensive Disorders of Pregnancy
IFCC	International Federation of Clinical Chemistry
ISSHP	International Society for the Study of Hypertension in Pregnancy
JMC	Jimma Medical Center
JNC-7	Seventh Report of Joint National Committee
LDH	Lactate Dehydrogenase
NHBPEP	National High Blood Pressure Education Program
PIHDs	Pregnancy Induced Hypertensive Disorders
ROC	Receiver Operating Characteristics
SBP	Systolic Blood Pressure
SOP	Standard Operating Procedure
SPSS	Statistical Package for Social Sciences
SST	Serum Separator Tube
U/L	Units per Liter
WHO	World health Organization

1. INTRODUCTION

1.1 Background

Hypertensive disorders of pregnancy (HDP) also known as pregnancy-induced hypertensive disorders (PIHDs) are a group of common medical complications in pregnancy, and are among the leading causes of maternal and perinatal morbidity and mortality worldwide(1,2). According to the recommendation of International Society for the Study of Hypertension in Pregnancy (ISSHP) for international practice, HDP are defined as new onset of hypertension (≥ 140 mmHg systolic blood pressure (SBP) and/ or ≥ 90 mmHg Diastolic Blood Pressure (DBP) measured while the woman is at rest and the arm held at the level of heart) after 20 weeks of gestation(3,4).

In 2000, the National High Blood Pressure Education Program(NHBPEP) Working Group on High Blood Pressure in Pregnancy defined four categories of hypertension in pregnancy, namely, chronic hypertension, gestational hypertension, preeclampsia-eclampsia syndrome and preeclampsia superimposed on chronic hypertension(4–6). Gestational hypertension (Pregnancy-Induced hypertension) is defined as new onset of hypertension after 20 weeks of gestation in pregnant women who were previously normotensive, and is characterized by elevated blood pressure (BP ≥ 140/90), measured on two occasions at least 4-6 hours apart, without proteinuria(1). It is a provisional diagnosis that includes women who will eventually develop preeclampsia, those with unrecognized chronic hypertension (diagnosed by persistently elevated BP after 12 weeks in the postpartum period), and women with transient hypertension of pregnancy(3). Approximately, 50% of women diagnosed with gestational hypertension between 24 and 35 weeks of gestation ultimately develop preeclampsia (7).

Preeclampsia (PE), a pregnancy specific syndrome that occurs after midgestation, is defined as the de novo appearance of hypertension (SBP ≥ 140 mm Hg or DBP ≥ 90 mm Hg) accompanied by new-onset proteinuria defined as ≥ 300 mg per 24 hours, and it may tend to present as late as 4-6 weeks postpartum period (5,8,9). Eclampsia is defined as preeclampsia with sudden development of seizure or coma, non-attributable to other neurological diseases that justify the convulsive state, during gestational or postpartum period(10). It contributes to 2-30% of maternal and perinatal mortality, and occurs in 20% of preeclamptic cases, 50% of which is stillborn in India (11,12).

Chronic hypertension is defined as new-onset of hypertension before 20 weeks of gestation or persistence of hypertension beyond 12 weeks' postpartum period. It is characterized by elevated BP (≥ 140/90 mm Hg) taken on two occasions at least 4-6 hours apart, and is associated with preeclampsia (22–25% of women with chronic hypertension will develop preeclampsia in pregnancy), intrauterine growth restriction, and placental abruption (7,13). Preëclampsia superimposed on chronic hypertension is diagnosed in women with chronic essential hypertension, and new-onset proteinuria in the setting of elevated blood pressure is sufficient to confirm the diagnosis (14).

Serum lactate dehydrogenase (LDH) and gamma glutamyl transferase (GGT) are useful biochemical markers reflecting the occurrence of complications associated with preeclampsia and eclampsia, which are preventable and can be managed adequately if identified at their early stage. Serum LDH and GGT levels have also been suggested as potential biomarkers to predict the severity of preeclampsia and indicator of multiorgan involvement(9,12). Most often, they are measured to evaluate the presence of tissue damage associated with endothelial dysfunction(15,16). High serum level of LDH has been correlated well with the severity of the diseases and poor outcomes in preeclampsia and eclampsia (16,17). Therefore, estimation of serum LDH and GGT level in preeclamptic women may be useful for the proper management of patients to decrease maternal and fetal morbidity and mortality(18). Moreover, serum LDH was found to be a good predictor of severity of PIH and bad fetal outcome (18–20).

Lactate dehydrogenase is an ubiquitous and intracellular(cytoplasmic) enzyme, which catalyzes the interconversion of lactate and pyruvate, and its elevated level in serum indicates cellular death and leakage of the enzyme from the cell(12). Human LDH is a tetramer composed of two types of subunits, either H (heart) or M (muscle), the combination of which gives rise to its five isoenzymes found in mammalian tissues (21,22).

The enzyme GGT, having a molecular weight of 68 kilo Dalton(kDa), is a heterodimeric glycoprotein composed of a heavy chain with a molecular weight of 46 kDa and a light chain, being 22 kDa in weight, linked together by a non-covalent bond (23,24). It is processed from a single chain precursor with an autocatalytic cleavage both in prokaryotes and eukaryotes (25,26). Despite its expression in many organs, the highest activity of GGT is present in the kidney followed by the duodenum, small intestine and gallbladder respectively (26,27). At cellular level, its significant activity occurs both in the endothelium

and epithelium and hence, endothelial cell dysfunction within the uteroplacental circulation associated with HDP leads to systemic release of GGT(28,29). GGT catalyzes the transfer of gamma-glutamyl group from gamma-glutamyl containing peptides such as glutathione to acceptors such as peptides and L-amino acids. Hence, it is involved in the transfer of amino acids across the cellular membrane and in glutathione metabolism (26). It too plays an important role in the homeostasis of glutathione (a major cellular antioxidant) and in the detoxification of xenobiotics in mammals(29).

1.2 Statement of the Problem

The four categories of HDP altogether complicate in 5-10 % of all pregnancies worldwide (11), and 6-8 % of pregnancies in the United States with consequent maternal and perinatal mortality(30). Across the world, around 76,000 pregnant women and 500,000 babies die from preeclampsia and related hypertensive disorders per annum (31). Everyday 830 women die as a result of pregnancy related problems, of which 14% are due to hypertensive disorders complicated by pregnancy. These aforementioned and related bad outcomes of HDP on maternal and fetal health are getting higher since recent years due to lack of biochemical markers to be used as diagnostic tests in clinical areas(4,11,32).

Due to their high maternal and fetal morbidity and mortality rates, it is important to identify HDP and institute clinical interventions timely(33). Ineffective prevention of HDP in general, and severe preeclampsia and eclampsia in particular has become the main problem of complications associated with pregnancy(34). In order to prevent PE, the disease must be diagnosed at its earliest stage but to do so, the triads of high blood pressure, edema and albuminuria is neither specific nor sensitive enough. Hence, reliable biomarkers have to be searched for and evaluated to be used as diagnostic tools (21,35). Several potential biochemical markers have been proposed to predict the severity of preeclampsia. Enzymes known as markers of cellular damage are one among others, of these GGT and LDH are most useful (9,28,32). However, some researchers did not find significant difference of serum GGT and LDH level between preeclamptic women and healthy pregnant women, and stated that the result is conflicting so that the precise nature of relationship between the enzymes' serum level and the disease still remains enigma (22,36,37).

Despite years of research, there are no clinically proven diagnostic tests that perform well in predicting the development of gestational hypertension, preeclampsia and eclampsia (17). In spite of many studies conducted among pregnant women suffering from pregnancy

related hypertensive complications at international level(4,28,38–40), in Ethiopia, no studies aimed at investigating the role of GGT and LDH as potential biomarkers in predicting the occurrence and severity of HDP have not been conducted so far. This study was, therefore, the first of its kind conducted among Ethiopian pregnant women with HDP to examine the diseases' extent of severity according to serum levels of GGT and LDH.

1.3 Significance of the Study

The aim of this study was to ascertain significance of serum LDH and GGT, which are cost effective and easy to test, as supportive biomarkers for evaluating the prognosis of HDP and predicting their severity. The findings of this study might alert health care practitioners to employ serum LDH and GGT as supportive biochemical markers to accurately identify women who are at high-risk for PIHDs, for early diagnosis of the diseases and to initiate prompt and appropriate management, which in turn helps to prevent the development of complications and decrease both maternal and fetal mortality.

Furthermore, this study might help clinicians to give due emphasis on utilization of serum GGT and LDH as independent biomarkers for detecting HDP at an earlier and therefore, potentially more treatable stage for eligible pregnant women who are free of comorbidities, which inevitably lead to fluctuation of the aforementioned biomarkers' level. Finally, the result of this study might serve as a baseline data for researchers who are interested in conducting further related studies.

2. LITERATURE REVIEW

2.1 Epidemiology of Pregnancy Induced Hypertensive Disorders

Hypertensive disorders of pregnancy were found to affect about 5-8 % of all pregnant women worldwide. Any hypertensive disorder of pregnancy can result in preeclampsia. It occurs in up to 35% of women with gestational hypertension and up to 25% of those with chronic hypertension(41,42). Preeclampsia is a global health problem of increasing significance, which complicates 2–8% of all pregnancies, and accounts for 15% of preterm deliveries and 9-26% of maternal deaths worldwide(13). Preeclampsia and eclampsia are multisystem disorders capable of causing enormous cellular death, which altogether complicate in 6–8% of all pregnancies globally, and lead to various maternal and fetal complications (43,44). Each year, an estimated 2.9 million babies die during the neonatal period and 2.6 million babies are stillborn across the globe attributable to PIH (45).

In developing countries, the incidence of HDP was reported to be 4-18% with the diseases being the second most common obstetric cause of stillbirths and early neonatal deaths in these countries(46). In developing countries, severe forms of preeclampsia and eclampsia are more common and occur in 4-18% of all deliveries, which is much higher as compared to high income countries (47). It is estimated that 9.1 % of maternal deaths in Africa are due to HDP(48). Incidence rates of preeclampsia alone in the United States, Canada and Western Europe range from 2-5%, while in Latin America, it is the leading cause of maternal death (31). According to the 2018 report of world health organization (WHO), the rate of stillbirth in China was 21.9 per 1000 births in women with PIH and 8.4 per 1000 births in normotensive women(49).

A retrospective cross-sectional study conducted in 2015 at Debre Berhan Referral Hospital, Ethiopia found the prevalence of HDP to be 3.9%, of which the majority was accounted by preeclampsia followed by eclampsia. Furthermore, the same study reported maternal death in 2.5% of pregnant women with HDP in which majority was seen in women with eclampsia with a case fatality rate of 6.67% (50). Another research conducted in Dilla University Referral Hospital, Ethiopia reported the incidence rate of preeclampsia alone to be 2.23 % (51).

2.2 Classification of Blood Pressure

The Joint National Committee on Prevention, Detection, Evaluation and Treatment of High Blood Pressure, and National High Blood Pressure Education Program Working Group on

Hypertension in Pregnancy classified blood pressure(BP) based on the average of two or more properly measured BP readings as follows **(**

Table *1*).

Table 1: Classification of blood pressure: Joint National Committee -7 (JNC-7) Versus National High Blood Pressure Education Program (NHBPEP)

	Category	SBP (mmHg)	DBP(mmHg)
JNC-7 BP Classification (Non-pregnant adults)	Normal	< 120	< 80
	Pre-hypertension	120-139	80-89
	Stage 1 Hypertension	140-159	90-99
	Stage 2 Hypertension	160-179	100-109
	Stage 3 Hypertension	≥180	≥ 110
NHBPEP BP Classification (Pregnant)	Normal/acceptable in pregnancy	< 140	< 90
	Mild hypertension	140- 159	90- 109
	Severe hypertension	≥ 160	≥ 110
Adopted from(48,52)			

2.3 Classification and Sub-classification of HDP

Hypertensive disorders of pregnancy are characterized by elevated blood pressure where proteinuria is an additional characteristic in pre-eclampsia. Table 2 below describes the diagnostic criteria according to the National High Blood Pressure Education Program Working Group on High Blood Pressure in Pregnancy.

Table 2: Classification of hypertensive disorders of pregnancy

Category	Diagnostic criteria

Chronic hypertension	BP ≥ 140/90 mmHg present before pregnancy or diagnosed before 20th week of gestation or does not resolve until 12-week in the postpartum period
Gestational hypertension	BP ≥ 140/90 mmHg on two occasions at least 4 hours apart after 20th gestational week in previously normotensive women and resolves before 12th week in the postpartum period.
Preeclampsia-eclampsia	BP ≥140/90 mmHg, measured on 2 occasions at least 4-6 hours apart, developed after 20th gestational week in previously normotensive pregnant women, and is accompanied by proteinuria (≥300 mg/ 24 h) or protein to creatinine ratio ≥ 0.3. Eclampsia is convulsive phase of preeclampsia.
Pre-eclampsia superimposed on chronic hypertension	Elevated BP (≥140/90 mmHg) predated pregnancy or diagnosed before 20th week of gestation with new-onset proteinuria (≥ 0.3g/ 24 h)

Adapted from(32,52).

Table 3: Sub-classification of preeclampsia based on clinical stages

Category	Mild preeclampsia	Severe preeclampsia (Two or more of the following should present)
Diagnostic criteria	Elevated SBP (140- 159 mmHg) or DBP (90- 109 mmHg)	Elevated BP (≥ 160 mmHg systolic or ≥ 110 mmHg diastolic)
	Proteinuria (0.3–0.5 g/day on urine specimen or (2^+ to 3^+ on dipstick on two random urine samples collected at least 4 hours apart)	Proteinuria (> 0.5 g/24hr on urine specimen or > 3^+ on dipstick on two random urine samples collected at least 4 hours apart)
	Increased serum creatinine (1.2-2 mg/dl)	Increased serum creatinine (> 2 mg/dl)
	Normal platelet count	Thrombocytopenia (<100,000/ μL)
	Normal liver enzymes (AST and ALT)	Oliguria (< 500 ml/24 hours)

	No maternal symptoms	Cerebral symptoms: persistent headache, visual changes, altered consciousness
		Persistent epigastric or right upper quadrant pain (not accounted by differential diagnoses)
		Intrauterine growth restriction and/or oligohydramnios

Adapted from(52). AST: Aspartate Transaminase; ALT: Alanine Transaminase.

2.4 LDH and GGT in Hypertensive Disorders of Pregnancy

Serum LDH and GGT have no metabolic function in the extracellular space, instead, their elevated level there indicates tissue disturbance as a result of pathological consequences(18). Elevated levels of serum LDH and GGT indicate tissue damage related to endothelial vascular damage and are the main promising biomarkers helpful for predicting the occurrence of preeclampsia and eclampsia(15).

Studies have shown that LDH activity and gene expression are higher in placentae of PE than normal pregnancy(53). Serum LDH is an effective biochemical marker which is useful in the early diagnosis of pre-eclampsia and can reflect the disease's severity so that appropriate measures can be taken to reduce morbidity and mortality associated with the disease. Several studies reported that serum LDH level increases with severity of preeclampsia, and showed significant correlation with high blood pressure and poor maternal and perinatal outcomes (20,21,44,54). In preeclampsia, multiple systems of the body such as renal, cardiovascular, hematological and nervous system are affected leading to cellular death and consequent leakage of LDH from cells and therefore, its raised level. Hence, LDH is rightly termed as the cell death marker(19). Highly significant increase in LDH level was found in women with severe preeclampsia ($P<0.001$) as compared to women mild preeclampsia(9).

GGT level increases not only in hepatic injury but also in endothelial vascular damage associated with tissue damage as a major consequence of preeclampsia(18). Damage to vascular endothelium has an important role in gestational hypertension and fetal growth retardation due to placental failure. Biochemical markers of endothelial damage such as GGT might predict the onset or give guidance on the severity of HDP (2,12). Case-control

studies demonstrated significantly higher serum GGT level in women with gestational hypertension and preeclampsia compared with normotensive controls, and found positive correlation with the diseases' severity(28,55). There was no statistically significant correlation between GGT level and body mass index, height of the participants, systolic or diastolic blood pressure in gestational hypertensive patients(18,56).

2.5 Conceptual Framework of the Study

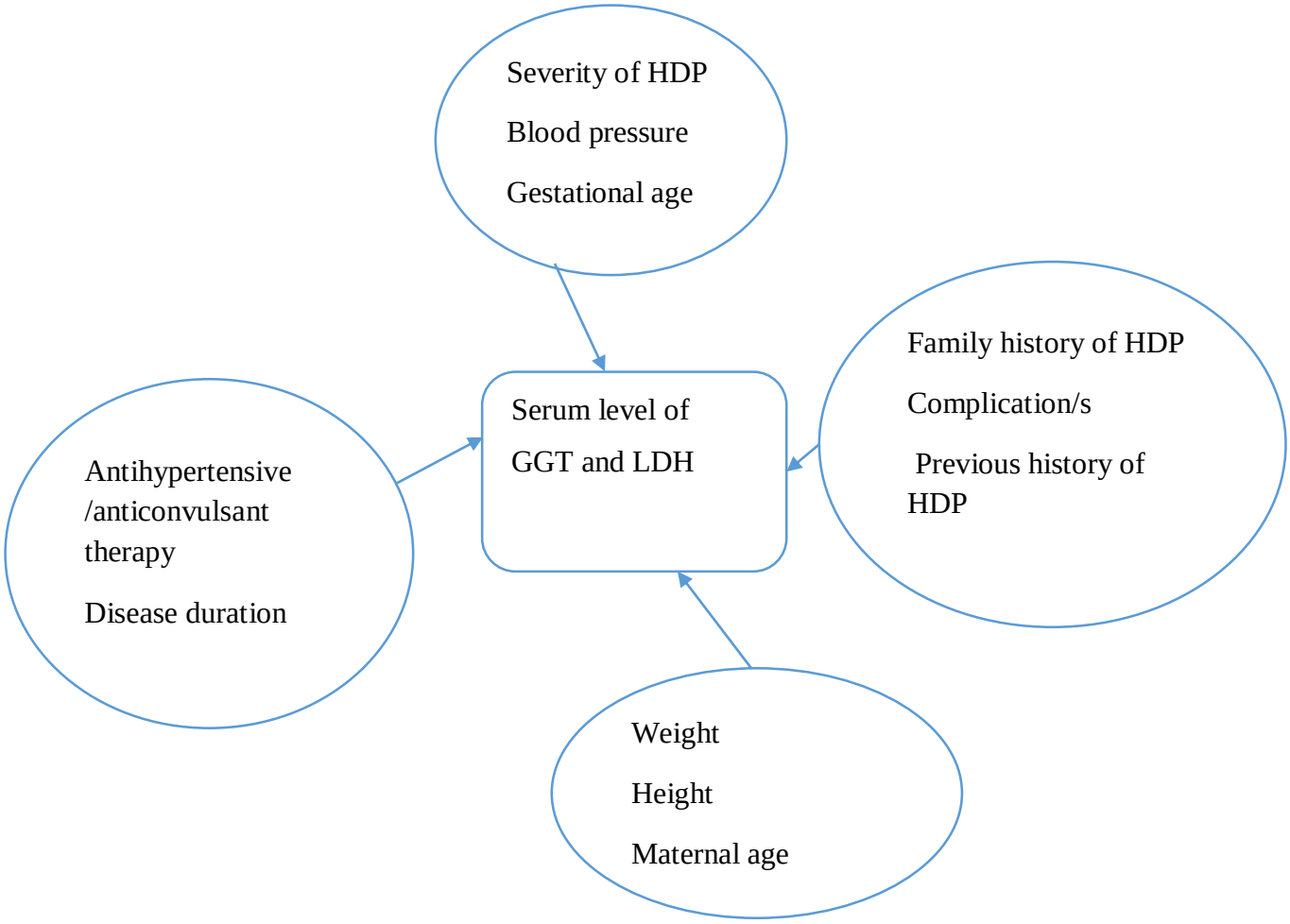


Figure 1: Conceptual framework of the study(32,57,58)

3. OBJECTIVES

3.1 General Objective

- To assess serum levels of LDH and GGT, correlate with severity of diseases and identify factors affecting their level among pregnant women with HDP at Jimma Medical Center (JMC), Jimma, Southwest Ethiopia, 2020.

3.2 Specific Objectives

- To evaluate serum levels of GGT and LDH among pregnant women with HDP
- To correlate serum levels of LDH and GGT with severity of HDP
- To identify factors affecting serum level of GGT among patients with pregnancy-induced hypertensive disorders
- To identify factors affecting serum level of LDH among patients with HDP
- To determine predictive ability of serum LDH and GGT in differentiating complicated and uncomplicated HDP

Hypothesis

Null hypothesis/ H_o: Serum levels of GGT and LDH do not any correlation with the extent of severity of HDP.

Alternative hypothesis/H_A: Serum levels of GGT and LDH do have some predictive correlation with the degree of severity of HDP.

4. METHODS AND MATERIALS

4.1 Study Area and Period

The study was conducted at JMC from August 3 to September 27, 2020. JMC is located in Jimma town, which is 352 km far from the capital city of Ethiopia, Addis Ababa. It is one of the oldest hospitals in Ethiopia having four major departments, namely, medical, surgical, pediatrics and obstetrics and gynecology ward. Of the aforementioned departments, this study was undertaken in obstetrics and gynecology ward. Besides, it is the only teaching and referral hospital in southwest Ethiopia with an approximate capacity of 800 beds and a catchment population of over 15 million people.

4.2 Study Design

A hospital based cross-sectional study design was employed.

4.3 Population

4.3.1 Source Population

All pregnant women with any of the pregnancy-induced hypertensive disorders diagnosed by an obstetrician/ a gynecologist at JMC, obstetrics and gynecology ward.

4.3.2 Study Population

All pregnant women with any one of the pregnancy-induced hypertensive disorders who came for medical and other services at JMC's obstetrics and gynecology ward during the study period.

4.3.3 Eligibility Criteria

4.3.3.1 Inclusion Criteria

All pregnant women with one of the pregnancy-induced hypertensive disorders (preeclampsia, eclampsia or gestational hypertension) diagnosed by obstetricians or gynecologists, and who came for ANC, disease follow up at outpatient department of obstetrics and gynecology ward or who were admitted for better management of a specific HDP at obstetrics and gynecology ward of JMC during the study period were included in this study. Women who were diagnosed for eclampsia in their immediate postpartum period, and those recorded as new cases of gestational hypertension and preeclampsia during the data collection session too were included.

4.3.3.2 Exclusion Criteria

Pregnant women with the following pathological conditions were excluded from the study.

- Pregnant women with pre-existing hypertension or chronic hypertension developed before 20 weeks of gestation.
- Those with gestational or pre-existing diabetes mellitus, thyroid disorder, epilepsy, renal or liver diseases.
- Pregnant women who were found to have history of alcoholism and/ or smoking, and those with hemolytic anemia.

4.4 Sample Size Determination and Sampling Technique

4.4.1 Sample Size Determination

Sample size was calculated using single population proportion formula by considering 25% as the prevalence of elevation of GGT and 22% as the prevalence of LDH elevation in eclampsia from a South African study (34).

$$n = \frac{\left(Z\ 1-\frac{\alpha}{2}\right)^2 p(1-p)}{d^2}$$

Where 'n' is the minimum sample size required for the study; $Z_{1-\alpha/2}$ is a standard normal variable (at 5% type I error or α and hence, at 95% confidence level it is 1.96); P is an estimate of the prevalence of elevated GGT and LDH and 'd' is the margin of sampling error tolerated, and is assumed to be 0.05. Therefore, the calculated sample size became 288 when calculated using prevalence of GGT elevation, and 264 using prevalence of LDH elevation. The greater number of sample size (288) was used in practice for better representativeness of the target population. Since our total target population was less than 10,000, we used finite population correction formula as follows. The total population of our study was 127(taken from nine-month medical database of JMC).

$$nf = \frac{\mathbf{n}}{\mathbf{1+n/N}} = \frac{\mathbf{288}}{\mathbf{1+288/127}} = 88$$

where nf indicates final sample size, n is calculated sample size and N is total population of the study. Adding 10% non-response rate on the final sample size gave 97. Among these, 33 study participants were recruited from pregnant women with preeclampsia, 32 of them from gestational hypertensive patients and the remaining 32 were selected from eclamptic cases by reviewing their medical record and taking the inclusion criteria into account.

The allocation of study participants was made in a one to one proportion because the intension of this study was to compare mean level of serum GGT and LDH among the three study groups, and variation in the number of participants in each group might affect the mean level of the aforementioned parameters and hence, might lead to biased generalization. One extra participant was added there in preeclamptic study group since preeclamptics accounted the highest proportion out of the total target population. Furthermore, obstetricians and gynecologists made the diagnoses preeclamptic study participants as mild and severe preeclampsia at the very beginning and therefore, we took 16 mild preeclamptics and 17 severe preeclamptics from newly diagnosed and admitted preeclamptic cases. On the other hand, in our study gestation al hypertensive study subjects were subcategorized based on the clinical stages of the disease as mild and severe gestational hypertensives depending on the BP reading taken during the data collection session. Accordingly, we took 16 mild and severe gestational hypertensives each for the sake of correlating serum LDH and GGT with the disease's severity.

4.4.2 Sampling Technique

Purposive non-probability sampling technique was used to include all eligible pregnancy induced hypertensive patients who were visiting obstetrics and gynecology ward during the study period.

4.5 Study Variables

4.5.1 Dependent (Criterion) Variables

- ✓ Serum GGT level
- ✓ Serum LDH level

4.5.2 Independent (Explanatory) Variables

- ✓ Family history of HDP and previous history of HDP
- ✓ Gestational age and maternal age
- ✓ Disease duration
- ✓ Anthropometric parameters (weight and height)
- ✓ Clinical parameters (Blood pressure, severity of HDP and antihypertensive therapy)

4.6 Operational Definitions

Gravidity: the number of times a woman has been pregnant throughout her reproductive

age (59).

Parity: the number of times that a woman has given birth to a fetus with a gestational age of 24 weeks or more, regardless of whether the fetus was alive or stillborn (59,60).

Grand multipara: a woman who has already given birth to five or more offsprings at or beyond 24 weeks of gestation (60).

Nulliparous woman: a woman who has never given birth to a child previously regardless of the outcome(61).

Primiparous/Uniparous woman: a woman who has given birth only once (62).

Primigravida woman: a woman who has got pregnant for the first time or who is in her first pregnancy (63).

Mild gestational hypertension: the clinical stage of gestational hypertension characterized by elevated blood pressure(BP), BP ranges from 140/90 to 159/109 mmHg inclusive measured on two separate occasions at least 4-6 hours apart, without significant proteinuria (≥ 0.3g/d) and diagnosed after 20 weeks of gestation(1).

Severe gestational hypertension: the clinical stage of gestational hypertension, which is characterized by elevated blood pressure (≥ 160/110 mmHg) measured on two separate occasions at least 4-6 hours apart without significant proteinuria(1).

Mild preeclampsia: the clinical stage of preeclampsia characterized by elevated blood pressure (SBP 140-159 mmHg and DBP 90-109 mmHg), normal platelet count, normal level of liver transaminases, significant proteinuria (0.3- 0.5 g/24 hour) and no maternal symptoms(7).

Severe preeclampsia: the clinical stage of preeclampsia characterized by elevated blood pressure (≥160/110 mm Hg), thrombocytopenia($<1\times10^5/\mu L$), liver transaminase levels two times the upper normal limit, doubling of serum creatinine level or level greater than 2 mg/dL, proteinuria (>0.5 g/24hr), severe persistent right upper-quadrant pain, pulmonary edema, or new-onset cerebral or visual disturbances(7).

4.7 Data Collection Procedure and Measurement of Parameters

4.7.1 Data Collection Procedure

After written informed consent was obtained from each study subject, all necessary information regarding sociodemographic factors, participants' medical history and related data were collected using a structured questionnaire through face to face interview and by reviewing participants' medical record. Data were collected by three experienced midwife nurses who were working in JMC's obstetrics and gynecology ward.

4.7.2 Physical Measurement of Parameters

4.7.2.1 Blood Pressure(BP) Measurement

BP was measured in the morning, after the woman was made comfortable in a sitting position, her arm set at the level of heart and at least 10 minutes of rest was given, from her right arm in a quiet room with an automatic Omron BP device. Pregnancy induced hypertension (gestational hypertension) was then subcategorized based on its clinical stages as per the guideline of National High Blood Pressure Education Program Working Group on High Blood Pressure in Pregnancy. As per the guideline, patients with pregnancy induced hypertension (PIH) are categorized into two subgroups based on their BP level; mild hypertension if BP ranges from 140/90 to 159/109, and severe hypertension if BP ≥ 160/110 (1).

4.7.2.2 Measurement of Anthropometric Parameters

The weight of all study subjects was measured using a standard weight scale, and their height too was measured using a height measuring scale with light clothing.

4.7.3 Measurement of Biochemical Parameters

4.7.3.1 Blood Sample Collection and Preparation

Blood sample, from each study subject, was collected by three experienced midwife nurses. Once informed consent was obtained, three milliliter(ml) of blood was drawn by venipuncture from medial antecubital vein, and poured into a serum separator tube (SST) which was then taken to the clinical chemistry laboratory unit of JMC, and centrifuged for 10 minutes at 3000 rpm and room temperature, within two hours of withdrawal to obtain the cell free serum.

Materials and equipment: 5cc syringe, SST, centrifuge, micropipette, reaction Cuvettes, refrigerator and Cobas© 6000 (Source: JMC laboratory, clinical chemistry unit SOP for LDH and GGT).

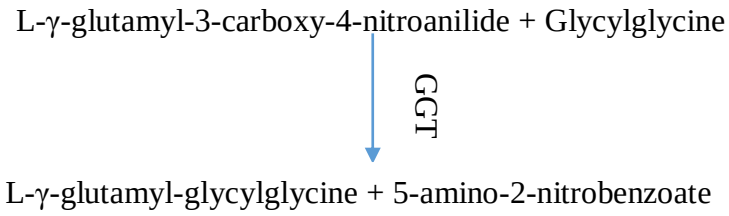
4.7.3.2 Laboratory Analysis

The blood sample collected from study subjects was then analyzed for determination of serum GGT and LDH using a fully automated, highly-sensitive, quantitative chemistry analyzer called Cobas© 6000 at clinical chemistry unit of JMC laboratory. All the activities involving laboratory analysis were undertaken by two experienced laboratory technicians working in clinical chemistry unit of JMC laboratory department.

4.7.3.2.1 Determination of Serum GGT

Measurement of serum GGT level was done by a biochemical technique called enzymatic colorimetric assay.

Principle: GGT catalyzes the transfer of gamma-glutamyl group from the colorless substrate, L-γ-glutamyl-3-carboxy-4-nitroanilide, to an acceptor molecule (glycylglycine) leading to the formation of a colored product 5-amino-2-nitrobenzoate.



The product, 5-amino-2- nitrobenzoate, strongly absorbs light at 410 nm, and an increase in the rate of light absorbance by it at the specified wavelength is directly proportional to the activity of GGT in the patient's sample. Hence, the concentration of GGT is determined by measuring the increase in light absorbance photometrically (64).

Reagents: R1 reagents (TRIS buffer 492 mmol/L, PH 8.25, glycylglycine, preservatives and additives). R2 reagents (L-γ-glutamyl-3-carboxy-4-nitroanilide, acetate, PH 4.5, stabilizer and preservative).

Procedure: Once serum was separated from cells using micropipette, 1ml of serum aliquot was taken and tipped into a reaction cuvette followed by its storage at -80 ^{o}C until assayed for GGT. Serum GGT level was then measured by an enzymatic colorimetric assay, which is an accurate and sensitive *in vitro* quantitative assay approach, using a Roche/Hitachi Cobas© 6000 chemistry analyzer.

4.7.3.2.2 Determination of Serum LDH

Measurement of serum LDH too was done by the same biochemical technique used for serum GGT determination (enzymatic colorimetric assay).

Principle: LDH catalyzes the oxidation of L-lactate to pyruvate with simultaneous reduction of nicotinamide adenine dinucleotide (NAD^+), a hydrogen acceptor, to NADH.

NADH intensely absorbs light at 340 nm unlike NAD^+ which does not do so. The rate of increase in light absorbance by NADH at 340 nm and hence, its rate of formation is directly proportional to the concentration of LDH in a given sample (64). Therefore, serum LDH activity is measured indirectly in terms of NADH concentration, which in turn is determined by measuring photometrically at the specified wavelength.

Reagents: R1 reagents (N-methylglucamine, PH 9.4, L-lactate and stabilizers). R2 reagents (NAD, stabilizers and preservatives).

Procedure: Once serum was separated from cells using micropipette, another serum aliquot of 1ml was tipped into the second reaction cuvette and stored at -80 ^{o}C until assayed for LDH. Serum LDH level was then measured by an enzymatic colorimetric assay using Roche/Hitachi Cobas© 6000 chemistry analyzer.

4.8 Data Quality Management

The questionnaire developed in English language, by reviewing numerous literatures, was translated into local languages (Amharic and Afaan-Oromo language), and then back to English to keep it consistent. Pretest was done on 10% of the study subjects at Shenen Gibe hospital of Jimma zone. Furthermore, training was given for the data collectors before commencement of data collection concerning objective/s of the study, the data collection process and inclusion and exclusion criteria of study participants to prevent the data's quality from being hampered.

In addition, standard aseptic operational procedures were strictly followed by data collectors during blood sample collection. The kits were kept free from contamination, and laboratory analysis of the blood sample was done in accordance with the right procedures stated on the manufacturer's instruction. All laboratory procedures were undertaken by experienced laboratory technicians and results were checked daily for completeness by the supervisor.

4.9 Data Processing and Statistical Analysis

After the data were collected, coded and checked for completeness, they were entered into EPI Info version 7.2.0.1, exported to and went through statistical analysis by Statistical Package for Social Sciences (SPSS) software version 25.0. The data were presented using tables and graphs. Proportions and summary statistics such as mean and standard deviation were calculated for variables as appropriate. Independent sample t-test was used to see the

difference in the mean values of continuous variables, namely, serum LDH and GGT between gestational hypertensive (mild and severe gestational hypertensive) and preeclamptic (mild and severe preeclamptic) subgroups. One-way analysis of variance (ANOVA) with Games-Howell post hoc test was used to see the difference in the mean levels of serum GGT and LDH among the three study groups comprising gestational hypertension, preeclampsia and eclampsia.

The extent of association between the dependent variables and severity of HDP was analyzed using Spearman's rank correlation, a non-parametric test used to assess the correlation between ordinal and continuous variables. On the contrary, the degree of correlation of serum LDH and GGT with continuous explanatory variables was evaluated using Pearson's correlation. Point biserial correlation was also used to assess the correlation between dependent variables (LDH and GGT) and dichotomous variables. Variables with P-value less than 0.05 were considered to be statistically significant.

4.10 Ethical Consideration

Ethical clearance and approval letter for data collection was received from Institutional Review Board (IRB) of Jimma University. Written informed consent was obtained from all the study subjects once they were provided with short and brief information about objective/s of the study prior to data and blood sample collection. Moreover, the study participants were informed as they had the right to terminate their participation at any time during the data and blood sample collection session regardless of the data's exhaustiveness. All the information provided by participants of the study was kept confidential and anonymous too. Corona virus disease-19(Covid-19) spread prevention protocols were strictly made pragmatic throughout the data and blood sample collection period.

4.11 Dissemination Plan

The result of this study will be submitted to Jimma University, Department of Biomedical Science. It will also be submitted to JMC, obstetrics and gynecology department. Presentation of this study's result will be held on annual scientific conferences in case chances are available. Besides, unreserved efforts will be made to disseminate the result through publication on reputable national or international journals.

5. RESULT

5.1 Sociodemographic Characteristics

A total of 97 study participants with hypertensive disorders of pregnancy took part in this study making the response rate 100%. Out of 97 study subjects, 33(34%) were preeclamptics, 32(33%) were gestational hypertensives and the remaining 32(33%) participants were eclamptics. From a total of 33 preeclamptic study participants, 16(48.5%) were mild preeclamptics and 17(51.5%) were severe preeclamptics. Likewise, out of 32 study subjects with gestational hypertension, half (50%) were mild gestational hypertensives and the remaining half were severe gestational hypertensives.

The age range of study subjects was 16 to 40 years. The mean age (in years) of gestational hypertensive and preeclamptic groups was 28.2 ± 5.6 and 26.8 ± 6.7 respectively. Similarly, mean age of participants in eclamptic group was 26.7 ± 5.3 (

Table 4). Regarding educational status of the study participants, 29 (29.9%) of the total study subjects each completed primary and secondary school education while 25 (25.8%) of them were at college or university educational level. Concerning occupation, more than one third, 38 (39.2%), of the total study participants were housewives followed by government employee which accounted for 26 (26.8%). Regarding the place of residence of the study participants, 72 (74.2%) of the total study subjects were urban dwellers and the remaining 25(25.8%) were rural residents.

Table 4: Sociodemographic profile of participants at JMC, November 2020

Variable		Study group with total number(N)		
		Gestational hypertensive (32)	Preeclamptic (33)	Eclamptic (32)
[a] Age (years)		28.2 ± 5.6	26.8 ± 6.7	26.7 ± 5.3
[b] Educational status	Primary school	8 (25)	11(33.3)	10 (31.3)
	Secondary school	10 (31.3)	12 (36.4)	7 (21.9)
	College/university	9 (28.1)	7 (21.2)	9 (28.1)
	Illiterate	5 (15.6)	3 (9.1)	6 (18.8)
Occupation	Government employee	8 (25)	10 (30.3)	8 (25)
	Self-employed	9 (28.1)	3 (9.1)	8 (25)

	Housewife	13 (40.6)	16 (48.5)	9 (28.1)
	Merchant	2 (6.3)	3 (9.1)	3 (9.4)
	Student	0	1 (3 %)	4 (12.5)
Place of residence	Urban	25(78.1)	23(69.7)	24(75%)
	Rural	7(21.9)	10(30.3)	8(25)

[a] Continuous variable expressed in mean ± standard deviation.

[b] Categorical variables represented in frequency and percentage (in parenthesis)

5.2 Obstetric History and Risk Factor Related Profiles of Participants

Concerning parity status of the study participants under gestational hypertensive group, 16(50%) of them were multiparous followed by nulliparous constituting a total of 14 (43.8%) cases. Similarly, 15(46.9%) of eclamptic study subjects were multiparous followed by 13(40.6%) being nulliparous. On the contrary, nulliparity being 16 (48.1%) of the preeclamptic cases was the leading in terms of parity status followed by multiparity being 13 (39.4%) of them under preeclamptic study group. Regarding the gravidity status, more than half of the total study participants were multigravidas with the vast majority accounted by eclamptic group, 21(65.6%), followed by preeclamptic group constituting 20(60.6%) cases, and gestational hypertensive group accounted the least number of multigravidas that is only 17(53.1%) study participants. Approximately, two third of the study participants under gestational hypertensive group, and one half of the eclamptics reported as they did not come across with previous history of HDP. Family history of HDP was predominantly experienced by an eclamptic study group followed by gestational hypertensive group **(Table 5**).

Table 5: Obstetric history and risk factor related profiles of study participants at JMC, November 2020

Variables		Study group with total number (N)		
		Gestational hypertensive (32)	Preeclamptic (33)	Eclamptic (32)
[a] Gestational age (weeks)		34.4 ± 3.5	34.2 ± 4.6	33.6 ± 4.5
[b] Parity	Nulliparous	14 (43.8)	16 (48.1)	13 (40.6)
	Primiparous	1 (3.1)	3 (9.1)	3 (9.4)

	Multipara	16 (50)	13 (39.4)	15 (46.9)
	Grand multipara	1(3.1)	1 (3)	1 (3.1)
Gravidity	Primigravida	15 (46.9)	13(39.4)	11(34.4)
	Multigravida	17 (53.1)	20(60.6)	21(65.6)
Previous history of HDP	Yes	6 (18.7)	5(15.2)	16(50)
	No	26(81.3)	28(84.8)	16 (50)
Family history of HDP	Yes	11(34.4)	8(24.2)	14(43.7)
	No	21(65.6)	25(75.8)	18(56.3)

[a] Continuous variable/s expressed in mean ± standard deviation.

[b] Categorical variable/s expressed in frequency and percentage (represented by numbers in parenthesis)

5.3 Clinical and Anthropometrical Profiles of Study Participants

Most of the preeclamptic patients, 22(66.7%), were found to have one or more complication/s attributed to HDP followed by gestational hypertensives. Eclamptic study subjects were the least, 13(40.6%), in terms of their affliction by complications as compared to gestational hypertensive and preecalmptic groups. Fifty-one (52.7%) participants out of the total study subjects were not using any antihypertensive/anticonvulsant medication and the majority, 22 (43.1%), was accounted by eclamptic group. With Regard to the number of drug therapy, 8 (25%) of eclamptics, 16(48.5%) of preeclamptics and 20 (62.5%) of gestational hypertensives were on monotherapy. Concerning anthropometric parameters, mean values of both weight and height were highest in gestational hypertensive group (68.9 ± 8.6 and 1.66 ± 0.08) respectively **(**

Table 6).

Table 6: Clinical and anthropometrical profiles of study participants at JMC, November 2020.

			Study group with total number(N)		
Variables			Eclampsia (32)	Gestational hypertension (32)	Preeclampsia (33)
[a] SBP (mmHg)			150.0 ± 11.6	150.6 ± 10.4	149.9 ± 10.3
DBP (mmHg)			100.7± 8.8	102.5 ± 9.3	101.0 ± 7.1
Disease duration (Days)			19.2± 12.8	38.0 ± 21.7	17.9 ± 4.8
Weight (Kg)			68.1±7.4	68.9 ± 8.6	65.8 ±9.5
Height (m)			1.63 ± 0.08	1.66 ± 0.08	1.64 ±0.11
[b] Presence of complication	Yes	HELLP syndrome	0	0	13(39.4)
		ARF	5(15.6)	1(3.1)	5(15.2)
		DIC	3(9.4)	7(21.9)	4(12.1)
		Abruptio placenta	5(15.6)	6(18.7)	0
	No		19 (59.4)	18 (56.3)	11 (33.3)
	Yes		10 (31.2)	18 (56.3)	18 (54.5)

Antihypertensive/ anticonvulsant drug use	No		22 (68.8)	14 (43.7)	15 (45.5)
Number of drug therapy	Monotherapy	Methyldopa	1(3.1)	13(40.6)	11(33.3)
		Nifedipine	1(3.1)	4(12.5)	5(15.2)
		Magnesium sulphate	4(12.5)	0	0
		Diazepam	2(6.3)	0	0
	Dual therapy		2 (6.2)	1 (3.1)	2 (6.1)

[a] Continuous variable/s expressed in mean ± standard deviation. [b] indicates categorical variable/s expressed in frequency and percentage (in parenthesis). HELLP: Hemolysis, Elevated Liver enzymes Low Platelet count; ARF: Acute Renal Failure; DIC: Disseminated Intravascular Coagulation

5.4 Assessment of Biochemical Parameters

5.4.1 Assessment of Serum GGT and LDH

Serum levels of both GGT and LDH were examined in all the three study groups and showed elevation from their acceptable upper limit (214 U/L and 36 U/L for LDH and GGT respectively) in majority of the study participants. More specifically, serum level of LDH was elevated in 86 (88.7%) of the total study subjects whilst serum level of GGT did show an elevation in 60 (61.9%) of them. The remaining study participants' serum LDH and GGT levels were in normal range (5-36 U/L for GGT and 135-214 U/L for LDH).

This study showed statistically significant difference ($p < 0.05$) in the mean levels of serum LDH between each pair of the study groups; namely, gestational hypertensive and preeclamptic, gestational hypertensive and eclamptic, and preeclamptic and eclamptic. The mean levels of both LDH and GGT in eclamptic group (580.9 ±193.8 and 86.1 ± 29.2 respectively) were found to be the highest as compared to their mean levels in preeclamptic and gestational hypertensive groups. Similarly, mean level of serum GGT and LDH in the preeclamptic group (48.8 ± 29.9 and 353 ±132.8 respectively) were higher than gestational hypertensive group's mean level **(**Error! Not a valid bookmark self-reference.).

Table 7: Serum LDH and GGT level among different groups of hypertensive disorders of pregnancy at JMC, November 2020.

	Study Group with total number(N)		
Parameter	Gestational hypertensive(32)	Preeclamptic (33)	Eclamptic (32)
[a] GGT (U/L)	38.3 ± 16.9	48.8 ± 29.9	86.1 ± 29.2
LDH (U/L)	276.7 ± 60.7	353.0 ±132.8	580.9 ±193.8

[a] Values of parameters are expressed in mean ± standard deviation

The result of multiple pairwise comparison from Games-Howell post hoc test revealed statistically significant LDH and GGT mean differences (P= 0.000) between eclamptic group and each of gestational hypertensive and preeclamptic group **(Table 8**Error! Reference source not found.**)**. Likewise, significant mean difference for LDH was noted, from multiple pairwise comparison, between gestational hypertensive and preeclamptic group (P =0.012) **(Table 8**).

Table 8: Multiple pairwise comparison of LDH level among the three groups of HDP using Games-Howell post Hoc test at JMC, November 2020.

					95% CI	
Outcome variable	Group (I)	Group (J)	Mean difference (I-J)	P-value	Lower bound	Upper bound
Serum LDH (U/L)	Eclampsia	Gestational hypertension	304.19*	0.000	216.53	391.85
		Preeclampsia	227. 88*	0.000	128.3	327.45
	Gestational hypertension	Eclampsia	-304.19*	0.000	-391.85	-216.53
		Preeclampsia	-76.31*	0.012	-138.08	-14.54
	Preeclampsia	Eclampsia	-227.88*	0.000	-327.45	-128.3
		Gestational hypertension	76.31*	0.012	14.54	138.08

**Mean difference is significant at 0.05 level. CI: Confidence interval*

However, the mean difference of serum GGT between gestational hypertensive and preeclamptic groups was found to be statistically insignificant (P =0.20) **(**

Table 9).

Table 9: Multiple pairwise comparison of GGT level among the three groups of HDP using Games-Howell post Hoc test at JMC, November 2020.

					95% CI	
Outcome variable	Group (I)	Group (J)	Mean difference (I-J)	P-value	Lower bound	Upper bound
Serum GGT(U/L)	Eclampsia	Gestational hypertension	47.84*	0.000	33.44	62.25
		Preeclampsia	37.37*	0.000	19.48	54.96
	Gestational hypertension	Eclampsia	- 47.84*	0.000	-62.25	-33.44
		Preeclampsia	-10.48	0.20	-25.00	4.04
	Preeclampsia	Eclampsia	- 37.37*	0.000	- 54.96	- 19.78
		Gestational hypertension	10.48	0.2	- 4.04	25.0

**Mean difference is significant at the level of 0.05. CI: Confidence Interval*

5.4.2 Serum LDH and GGT Level Between Subgroups of Gestational Hypertension and Preeclampsia

Serum levels of LDH and GGT were compared between gestational hypertensive subgroups (mild and severe gestational hypertensive) through independent samples t-test. Our study came up with a statistically insignificant mean difference ($p>0.05$) for both LDH and GGT between severe and mild gestational hypertensive subgroups. Despite the mean difference being statistically insignificant (P= 0.492), the mean level of LDH was higher in severe gestational hypertensive group (284.3 ± 45.9 U/L) than it was in mild gestational hypertensive group (269.2 ± 73.4 U/L). Alike LDH level, mean level of GGT too did show higher level in severe gestational hypertensive group (44.3 ± 20.4 U/L) than its level in mild gestational hypertensive group (34.7 ± 6.8 U/L) although the difference was found to be statistically insignificant (P =0.092) (

Table 10).

Table 10: Comparison of mean serum GGT and LDH level between subgroups of gestational hypertension using independent samples t-test at JMC, November 2020

	Gestational hypertensive subgroup		
Parameter	Mild gestational hypertensive	Severe gestational hypertensive	p-value

[a] Serum GGT (U/L)	34.7 ± 6.8	44.3 ± 20.4	0.092
[a] Serum LDH (U/L)	269.2 ± 73.4	284.3 ± 45.9	0.492
[a] indicates values of parameters expressed in mean ± standard deviation			

Independent samples t-test was also used to compare mean serum level of LDH and GGT between subgroups of preeclampsia. Our study revealed mean differences of both parameters between the two preeclamptic subgroups (mild and severe preeclamptics) to be statistically significant (P <0.05) (

Table 11).

Table 11: Comparison of mean serum GGT and LDH level between preeclamptic subgroups using independent samples t-test at JMC, November 2020.

	Preeclamptic subgroup		
Parameter	Mild preeclamptic	Severe preeclamptic	P-value
[a] Serum LDH (U/L)	292.3 ± 105.9	457.4 ± 132.6	0.008*
[a] Serum GGT (U/L)	37.4± 14.6	62.5± 32.8	0.009*
**Significant in t-test (two-tailed). [a] Values of parameters are expressed in mean ± standard deviation*			

In general, mean levels of GGT and LDH were found to be the highest in an eclamptic group as compared to subgroups of preeclampsia and gestational hypertension. Furthermore, the mean level of serum GGT and LDH was higher in severe preeclamptics followed by mild preeclamptic subgroup, and then severe gestational hypertensive subgroup. The lowest mean value of GGT and LDH was observed in mild gestational hypertensive subgroup **(Figure 2**).

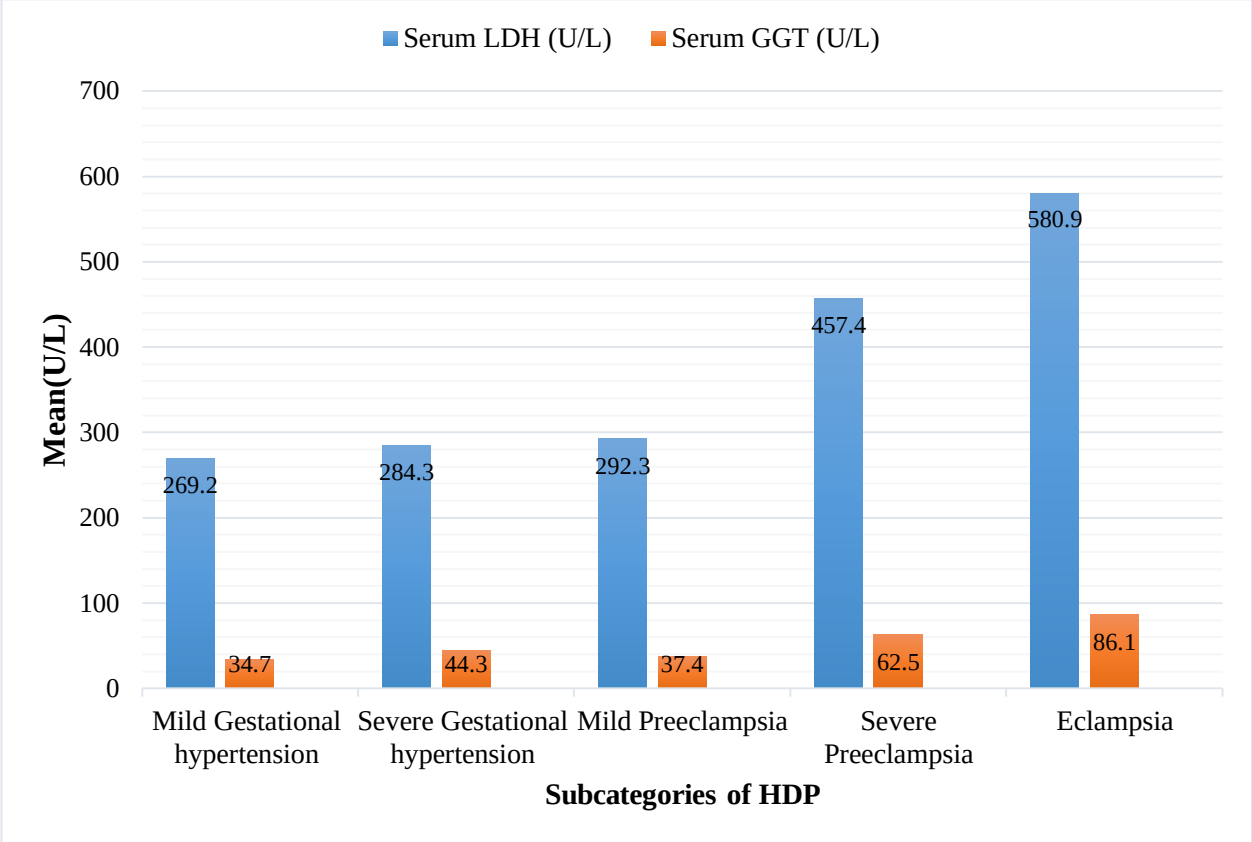


Figure 2: Mean level of GGT and LDH across subgroups of both preeclampsia and gestational hypertension ,and eclampsia at JMC, November 2020.

5.5 Correlation of Serum LDH and GGT Level with Severity of Preeclampsia and Gestational Hypertension

Bivariate correlation analysis, using Spearman's rank correlation, was carried out to assess the degree of association between severity of preeclampsia and serum levels of GGT and LDH. Spearman's correlation analysis showed statistically significant positive association between serum GGT and severity of preeclampsia (ρ =0.462; p= 0.007). As the severity of preeclampsia progressed from its mild clinical stage to severe preeclampsia, the level of serum GGT too did show an increment (**Table 12, Figure 3**). Serum LDH level too was observed to have statistically significant positive correlation with the degree of severity of preeclampsia (ρ =0.519; p =0.002). The more severe the disease, the higher the level of serum LDH **(Table 12, Figure 4**).

Table 12 : Spearman's rank correlation depicting association of serum GGT and LDH with severity of preëclampsia and gestational hypertension at JMC, November 2020.

	Serum LDH	Serum GGT

Severity of preeclampsia	Spearman's rho correlation coefficient (ρ)	0.519*	0.462**
	P value	0.002	0.007
Severity of gestational hypertension	Spearman's rho correlation coefficient	0.112	0.359*
	P value	0.543	0.043

*** Correlation is significant at the level of 0.01 (two-tailed). * Correlation is significant at 0.05 level (two-tailed).*

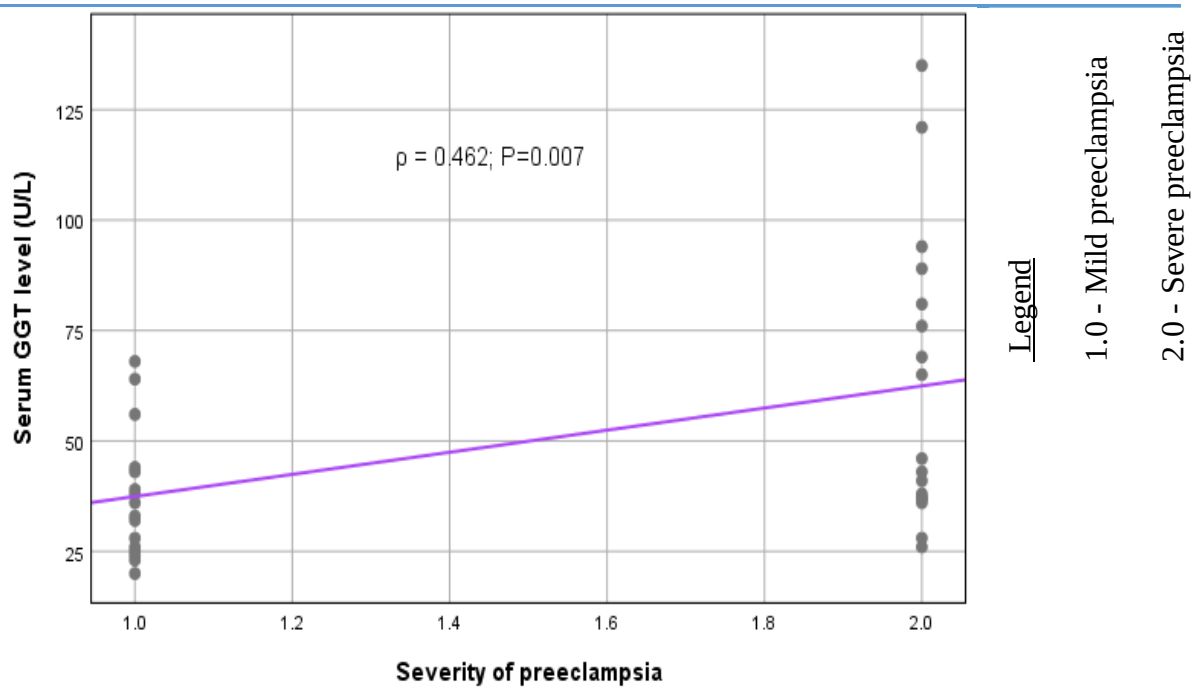


Figure 3: Scatter plot depicting the trend of association between serum GGT and severity of disease in preeclampsia at JMC, November 2020.

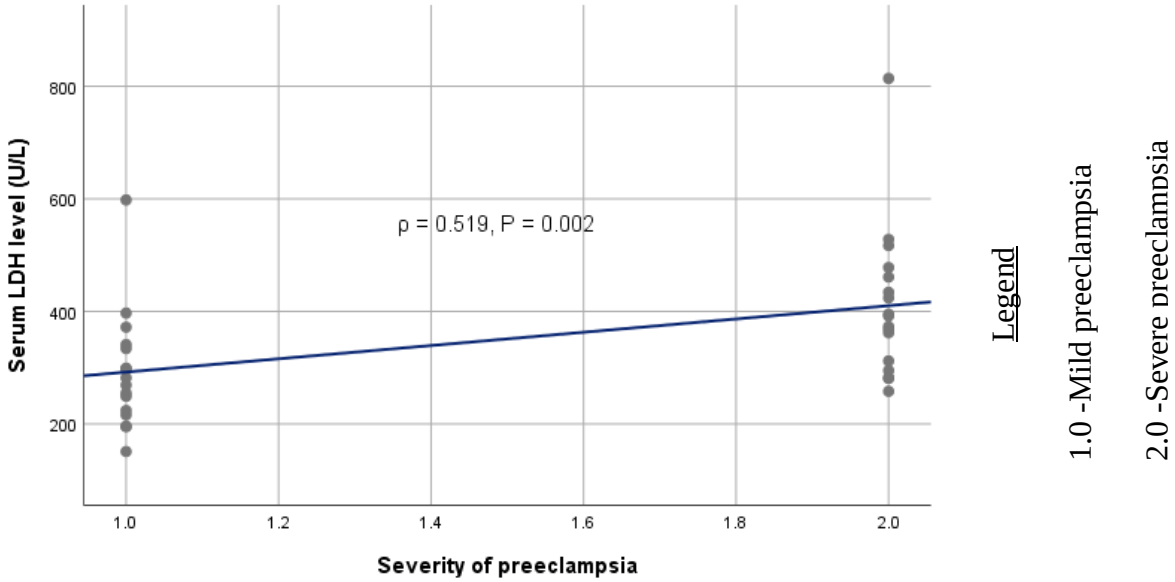
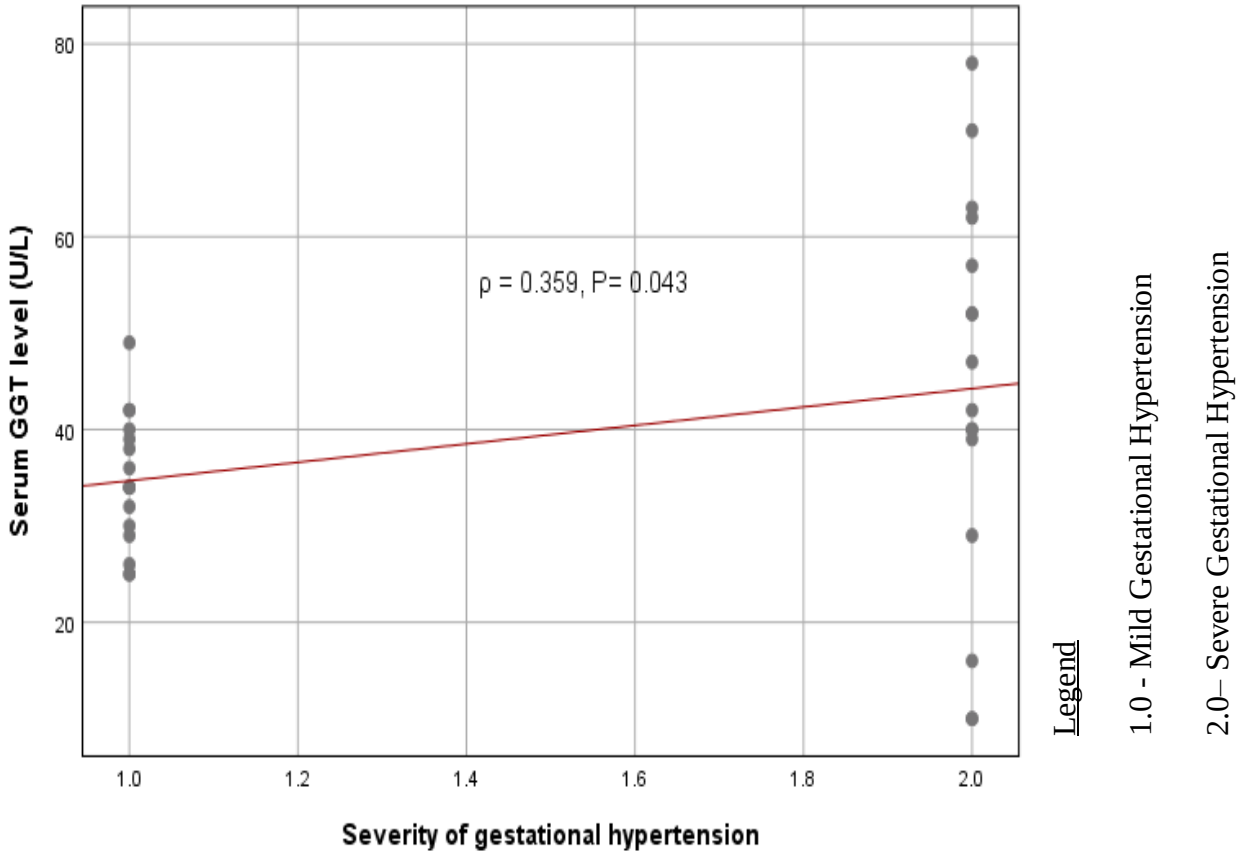


Figure 4: Scatter plot showing the trend of correlation between serum LDH and severity of preeclampsia at JMC, November 2020.

On the contrary, the extent of correlation between serum LDH and severity of gestational hypertension was found to be weak positive and statistically insignificant (ρ= 0.112, p= 0.543) **(Table 12, Figure 5)**.

Error! Reference source not found. Unlike the state of correlation between I severity of gestational hypertension, correlation between serum GGT and se gestational hypertension was found to be statistically significant (ρ= 0.359, p (**Table 12, Figure 5)**. As the disease (gestational hypertension) progressed fron clinical stage to a severe form, the level of serum GGT too did show an incremen

Legend
1.0 - Mild Gestational Hypertension
2.0 – Severe Gestational Hypertension



ρ: Spearman's rho correlation coefficient

Figure 5: Scatterplot showing the trend of correlation between serum GGT level and severity of gestational hypertension at JMC, November 2020.

5.6 Association of GGT and LDH with Explanatory Variables

The extent of correlation of both serum GGT and LDH with each of the explanatory variables, other than the severity of HDP (preeclampsia and gestational hypertension), was assessed using bivariate correlation. Depending on the type of independent variables, point biserial correlation and Pearson's correlation were used to figure out their degree of correlation with GGT and LDH.

In our study, systolic blood pressure (r =0.238, p =0.019), diastolic blood pressure (r =0.110, p = 0.035), previous history of HDP (rpb = 0.475, p = 0.000) and weight (r= 0.204, p=0.045) were found to have statistically significant positive correlation with serum GGT level. Among all other independent variables, correlations of which are revealed in the table below, serum LDH was found to have statistically significant positive correlation exclusively with previous history of HDP (rpb = 0.255, p = 0.012) and disease duration (r=

0.312, p= 0.002), and no significant association with the remaining explanatory variables (**Table 13**).

Table 13: Association of serum GGT and LDH with explanatory variables other than severity of HDP at JMC, November 2020.

	Correlation coefficient		P-value	
Correlation	GGT	LDH	GGT	LDH
Age	[r] 0.017	-0.057	0.869	0.581
Systolic blood pressure	[r] 0.238*	0.145	0.019	0.157
Diastolic blood pressure	[r] 0.110*	0.077	0.035	0.456
Disease duration	[r] -0.129	0.312**	0.208	0.002
Number of drugs	-0.014 [r]	-0.152	0.889	0.136
Previous history of HDP	[rpb] 0.475**	0.255*	0.000	0.012
Family history of HDP	[rpb] 0.177	0.010	0.084	0.923
Presence of complication	[rpb] 0.051	-0.022	0.620	0.830
Current history of drug use	[rpb] -0.019	-0.098	0.856	0.339
Weight	[r] 0.204*	-0.004	0.045	0.967
Height	[r] 0.061	-0.019	0.555	0.851
Gestational age	[r] -0.132	-0.130	0.196	0.203

*** Correlation is significant at 0.001 level. * correlation is significant at 0.05 level*

[r] Pearson's correlation coefficient, [rpb] Point biserial correlation coefficient

5.7 Diagnostic Performance of LDH and GGT

A receiver operating characteristic (ROC) curve was generated so as to evaluate the diagnostic performance or accuracy of serum LDH and GGT in distinguishing cases with and without complications attributed to HDP by considering all subjects of the study. We found the area under the curve (AUC) of LDH and GGT, measuring their overall performance, to be 0.895 (95% CI: 0.816,0.974; p = 0.000) and 0.905 (95% CI: 0.844,0.965: p = 0.000) respectively. As it can be understood from the ROC curve, AUC of both parameters was significantly different from 0.5 and hence, they did show good overall performance in distinguishing complications attributed to HDP. More remarkably, serum GGT was found to have greater diagnostic performance than serum LDH for screening complications (**Figure 6**).

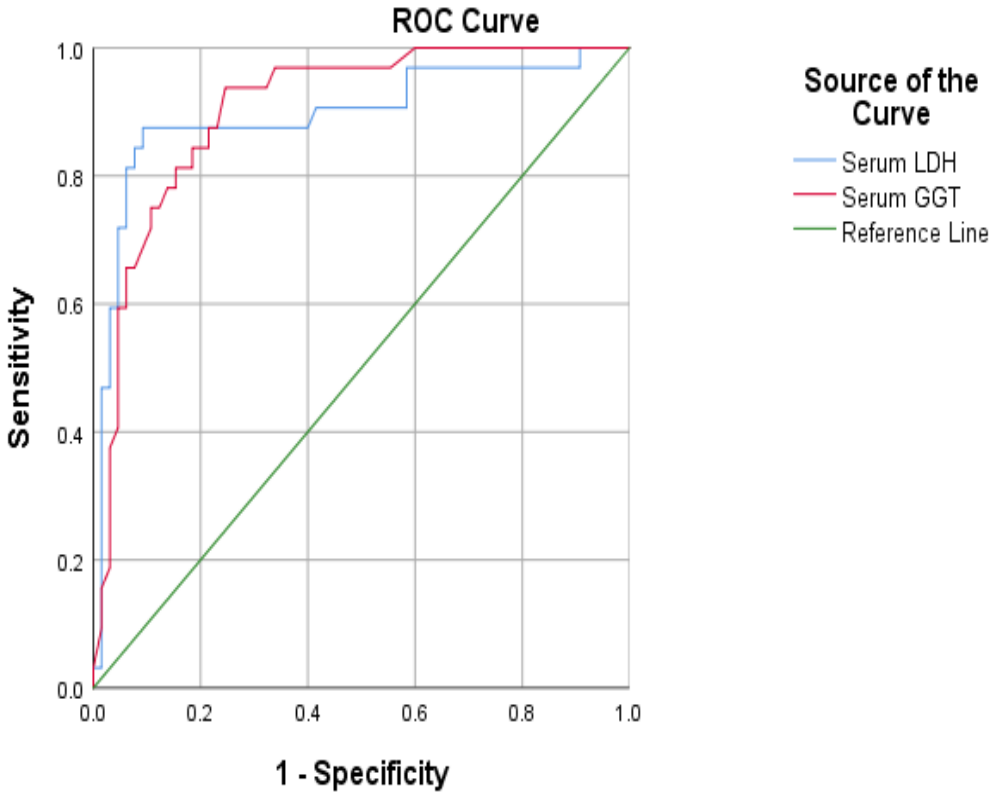


Figure 6: ROC curve revealing diagnostic performance of GGT and LDH at JMC, November 2020.

From the above ROC curve analysis, the optimal cut-off point for GGT was found to be 46.5 U/L with 93.8% sensitivity and a specificity of 75.4%. On the other hand, the optimal cut-off point for LDH was found to be 376.5 U/L, the sensitivity and specificity of which were 87.5 % and 90.8 % respectively.

6. DISCUSSION

In the present study, significantly higher mean serum LDH level was observed in severe preeclamptic women than in mild preeclamptic pregnant women. This result is supported by a number of similar studies (20,21,31,54,65–67). Possible explanations for the observed increment could be; progressively increased LDH level in severe preeclampsia indicates progression of cellular injury with the severity of this disorder. Moreover, multiorgan dysfunction including maternal liver, kidney, lung, nervous system, hematological and coagulation system in severe preeclampsia caused by vascular endothelial damage may lead to excessive LDH leakage and its elevation in serum(68).

In our study, mean level of serum LDH in mild and severe preeclamptic study subjects was 292.3 ± 105.9 U/L and 457.4 ± 132.6 U/L respectively. This finding was lower as compared to other studies done in Iran (mean level of serum LDH was 337.89 ± 173.15 U/L and 556.41±193.02 U/L in mild and severe preeclamptic groups respectively), and in Visakhapatnam mean level of which was 323.3 ± 77.4 U/L in mild preeclamptic and 636.2± 132.29 U/L in severe preeclamptic groups(21,54). This discrepancy might likely be due to shorter duration of the disease in our study subjects in contrast to longer disease duration for subjects in the latter studies.

Regarding mean level of serum LDH in eclamptic subjects, our study came up with a relatively lower result (580.9 ± 193.8 U/L) as compared to findings of studies done in India, mean level of which was 854.05 ± 47.5 U/L(65), and Visakhapatnam where it was 649.32 ± 153.53 U/L(21). This observed discrepancy might possibly be due to the lowering effects of anticonvulsants such as magnesium sulphate and diazepam, which were being taken by some of our study subjects. Besides, comorbidities particularly anemia and diabetes mellitus were recorded in the latter studies' study subjects and hence, might significantly account for raised serum LDH level(9,20).

Serum level of GGT did show significant positive correlation with severity of gestational hypertension in our study, and this finding was in line with the findings of other studies (28,54). Our study found mean level of GGT to be 48.8 ± 29.9 U/L in preeclampic subjects, and was much higher as compared to finding of a study done in India on similar subjects where it was 22.5 ± 14.1U/L(18). Concerning mean level of GGT in preeclamptic subgroups (mild and severe preeclamptics), our study too did show more elevated level, which was found to be 37.4 ± 14.6 and 62.5 ± 32.8 U/L in mild and severe preeclamptic

groups respectively, than studies undertaken in India, mean level of which was 18.5 ± 5.9 in mild preeclamptics and 23.9 ± 5.8 in severe preeclamptics, and in Sudan(14.5 ±7.8 and16.3±8.3 U/L in mild and severe preeclamptics) respectively(15,55).These discrepancies might be owing to differences in chemistry analyzers and substrates used. In the latter studies, L-γ-glutamyl-p-nitroanilide, which is less soluble and less stable than L-γ-glutamyl-3-carboxy-4-nitroanilide (used in our study), as a substrate and semi-automated chemistry analyzer were used(9).

In this study, significantly higher levels of serum LDH and GGT were noted in eclamptic group than preeclamptic and gestational hypertensive groups (P=0.000), and was found to be in line with several studies(17,43,44,53). A more plausible explanation could be; in HDP especially in eclampsia, local platelet-endothelial interaction is postulated to happen secondary to abnormal placentation. Therefore, it is possible that endothelial cell destruction within the uteroplacental circulation leads to systemic release of GGT and LDH. Our study found mean level of serum LDH to be significantly higher in preeclamptics as compared to gestational hypertensive study groups (p=0.012), and was supported by a study(31).This might be owing to progressive endothelial dysfunction in maternal vascular system induced by toxins released from hypoxic placenta of preeclamptic patients, which in turn tends to cause profound vasoconstriction affecting all organ systems including liver. This hypoperfusion induces ischemic injury to hepatic cells and other organs leading to increased release of intracellular LDH to the circulation(18,20).

Our study revealed AUC for LDH, from ROC curve analysis, to be more than 0.80 with moderate sensitivity and high specificity, and thereby declared LDH to have an overall good screening efficacy for complicated preeclampsia and uncomplicated preeclampsia. This finding was found to be in line with few studies conducted on similar subjects(43,54). On the other hand, the AUC for GGT showing its screening efficacy was found to be less than 0.7 as evidenced by a study conducted in China(24), and was contradictory to our finding (we found an AUC of 0.905 corresponding to GGT). This difference might be attributed to variations in specimen storage conditions before assay and delay in transportation of blood sample as reported in Wu *et al*.'s study.

In this study, serum level of LDH was not found to have significant association (p >0.05) with systolic and diastolic blood pressure. On the contrary, studies conducted in Sudan (55), and Iran (66) found significant positive moderate correlation between LDH and

diastolic and systolic blood pressure in severe preeclampsia. This difference in finding might be attributed to genetic /familial variation among the study subjects, effects of antihypertensive medications which were being taken by some of our study participants, and differences in BP apparatus used.

Limitations of the Study

Despite unreserved efforts made for the success of this thesis work, it was not without some limitations as described below.

- Since the study was conducted exclusively in one hospital and the sample size was relatively small, our findings might not well represent all cases and be sufficient for generalization.
- Besides, since the study was cross sectional, there might be the possibility of residual confounding variables as in observational studies, and because the exposure and outcome were assessed concomitantly, and thereby it did not show causal association.

7. CONCLUSION AND RECOMMENDATION

7.1 Conclusion

- GGT had statistically significant positive correlation with severity of both preeclampsia and gestational hypertension while LDH showed statistically significant association exclusively with severity of preeclampsia.
- GGT had shown significant positive correlation with weight, systolic blood pressure, diastolic blood pressure and previous history of hypertensive disorders of pregnancy.
- LDH, on the other hand, was found to have statistically significant positive correlation with disease duration and previous history of HDP.
- GGT is more reliable biomarker, having a greater sensitivity and larger area under the curve, than LDH and hence, its use as a robust diagnostic biomarker for better prediction of the severity and/or complication/s of HDP has to be pragmatic.

7.2 Recommendation

We would like to forward our recommendations for researchers as follows.

- Our study was exclusively descriptive and hence, we recommend the study to be undertaken comparatively (with healthy pregnant women as controls) to establish more reliable baseline data on clinical utility of GGT and LDH as biomarkers for early prediction, and thereby judicious management of HDP.

- The number of sample size in our study was relatively small and therefore, we recommend further studies to be conducted on larger sample size for better generalization.

Furthermore, this study was cross sectional and therefore, we strongly suggest further studies to be done by researchers on more robust cohort basis.

- For more accurate evaluation of serum LDH and GGT level in different stages of gestational hypertension and preeclampsia.

- To establish the causality of association between LDH and GGT level and severity of HDP, and their diagnostic implication on prediction of complications associated with HDP.

- To see how GGT and LDH can predict complications more accurately in early stages of HDP as well as to compare their accuracy with other diagnostic modalities,

biochemical and imaging tests that are being used to specifically detect different types of complications associated with preeclampsia, eclampsia and gestational hypertension.

Pregnant women with HDP (gestational hypertension, preeclampsia and eclampsia) should be screened out as early as possible, by health care professionals using reliable diagnostic tools, and provided with appropriate management to reduce double burden of the diseases.

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ANNEXES

Participant's code _____

Annex I: English Consent Form

I, the undersigned participant, am volunteer to take part in this research project aimed to investigate plasma levels of gamma glutamyl transferase and lactate dehydrogenase among pregnancy-induced hypertensive patients in Jimma University Medical Center. I was clearly informed about the objective of the study by the investigator and then I agreed to provide relevant information for the study. The investigator also informed me as I have full right to discontinue my participation at any time regardless of the data's completeness. Finally, I have understood that the researcher will collect the data he requires anonymously and my confidentiality as a study participant will be kept.

Participant's Signature _____

Data collector's name _____

Signature _____

Date _____

Thank you!!

Annex II: English version questionnaires

Participant's code _____ Participant's Medical record number_____

Instruction: choose from the given alternatives and encircle the participant's response for closed ended questions and fill in the provided blank space with appropriate response/measurement results for open ended questions.

PART I: SOCIODEMOGRAPHIC VARIABLES		
S.no	Questions	Response
101	What is your age?	_____ year
102	Educational level	1.Illiterate 2. Primary school 3. Secondary school 4. College/ university
103	What is your occupation?	1. Government employee 2. Self-employed 3. House wife 4. Merchant 5. Student 6. Others(specify)_____
104	How much is your monthly income ?	_____ ETB
105	Where are you currently living?	1. Urban 2. Rural
PART II CLINICAL PARAMETERS		
201	Medical diagnosis(to be taken from participant's medical record)	1. Gestational hypertension 2. Mild Pre-eclampsia 3. Severe preeclampsia 4. Eclampsia 5. Superimposed preeclampsia on chronic hypertension
202	Blood pressure	_____ mmHg

203	If '1'is the answer for Q_{201}; which subcategory does the disease belong? (To be filled by PI)	1. Mild 2. Severe
204	Duration of disease since diagnosis	_____ days
205	Does the patient have complication/s attributable to HDP?	1.Yes 2. No
206	If 'yes' for Q205, what is/are the complication/s?	1. HELLP syndrome 2. Acute renal failure/ARF 3. DIC/Disseminated Intravascular Coagulation 4. Abruptio placenta 5. Others _____
207	Does the patient use antihypertensive/anticonvulsant medication/s?	1. Yes 2. No
208	If 'yes' for question 207, what is/ are the medication/s the patient is currently using?	_____ _____
209	If yes for Q207, how many drugs does the patient use?	_____
PART III: OBSTETRIC HISTORY AND RISK FACTOR ASSESSMENT		
301	Gestational age (from medical record or by physical examination)	_____ weeks
302	Parity	1. Nulliparous 2. Primiparous 3. Multipara 4. Grand multipara
303	Gravidity	1. Primigravida 2. Multigravida

304	Have you ever had previous history of one or more of the pregnancy-induced hypertensive disorders? (only for multigravidas)	1. Yes 2. No
305	Do you have family history of pregnancy-induced hypertensive diseases?	1. Yes 2. No
PART IV: ANTHROPOMETRIC VARIABLES		
401	Weight of participant	_____ Kg
402	Height of participant	_____ meter
PART V: BIOCHEMICAL PARAMETERS		
501	Lactate dehydrogenase level	_____ U/L
502	Gamma glutamyl transferase level	_____ U/L

ቅጥያ III: **አማርኛ የስምምነት ማረጋገጫ ቅጽ**

እኔ ከዚህ በታች የፈረምኩት ተሳታፊ የጥናቱን መሰረታዊ ዓላማ እና ሌሎች መረጃዎችን በሚገባ ተገንዝቤያለሁ። ጥናቱ በጅማ ዩኒቨርሲቲ የህክምና ማዕከል በሚገኙ የደም ግፊት ያለባቸው ነፍሰጡር እናቶች ላይ የፕላዝማ ጋማግሉታማይል ትራንስፈሬስ እና ላክቴት ዲሃይድሮጅኔስ መጠንን ለመለካት እንደሆነ በሚገባ ተገንዝቤያለሁ። ተሳትፎዬ በፍቃደኝነት ላይ ብቻ የተመረኮዘ እንደሆነም ተረድቻለሁ። ማንኛዉም ሰብዓዊም ሆነ ህጋዊ መብቴ ሳይነካ ከጥናቱ ራሴን ማግለል እንደምችልም በሚገባ ተነግሮኛል። ስለ ጥናቱ ዝርዝር ጉዳይ በግልፅ ከተረዳሁት በተጨማሪ ማብራሪያ ብፈልግ መጠየቅ እንደምችልም አዉቄያለሁ። በመጨረሻም የጥናቱ ባለቤት የዚህ ጥናት መረጃ ውጤት ይፋ የሚሆነው ለእኔ ብቻ እንደሆነ ፣ ስሜ እንደማይጠቀስ እና ከእኔ የሚወስዳቸው ማንኛውም መረጃዎች ሚስጥራዊነት የተጠበቀ እንደሚሆንም ተረድቻለሁ።

የተሳታፊዋ ኮድ _____ የመረጃ ሰብሳቢ ስም _____

ቀን _____ ቀን _____

ፊርማ _____ ፊርማ _____

አመሰግናለሁ ።

ቅጥያ IV ፡ አማርኛ መጠይቅ

የሚስጥር ቁጥር _____ የካርድ ቁጥር _____

<u>መመሪያ</u>፡ ለምርጫ ጥያቄዎች ተሳታፊዋ የምትሰጠውን መልስ ይክበቡ ፣ ለአጭር መልስ ጥያቄዎች ደግሞ ፊት ለፊት ባለው ክፍት ቦታ መልስዎን ይጻፉ።

ክፍል 1፡ ማህበረዲሞግራፊያዊ መጠይቆች		
ተ ቁ	ጥያቄ	የተሰጠ መልስ
101	ዕድሜዎ ስንት ነው?	_____ ዓመት
102	የትምህርት ደረጃ	1. ያልተማረች 2. የመጀመሪያ ደረጃ 3. ሁለተኛ ደረጃ 4. ኮሌጅ ወይም ዩኒቨርሲቲ
103	ስራዎ ምንድን ነው?	1. የመንግስት ሰራተኛ 2. የግል ስራ 3. የቤት እመቤት 4. ነጋዴ 5. ሌላ(ይጥቅሱ)_____
105	በአሁኑ ሰዓት የሚኖሩት የት ነው ?	1. ከተማ 2. ገጠር
ክፍል 2: ከህክምናው ምርመራ ጋር ተያያዥ ጥያቄዎች		
201	የምርመራ ውጤት ወይም የበሽታው ዓይነት (ከተሳታፊዋ የህክምና መዝገብ ላይ የሚወሰድ)	1. ገስቴሽናል ሃይፐርቴንሽን 2. ፕሪኢክላምሺያ 3. ኢክላምሺያ 4. ሱፐርኢምፖዝድ ፕሪኢክላምሺያ
202	የደም ግፊት መጠን	_____ ሚሜ ሜርኩሪ

203	ለጥያቄ ቁጥር 06 መልስዎ ‘1’ ከሆነ በሽታው ከየትኛው ደረጃ ይመደባል?	1. ማይልድ / መካከለኛ 2. ሰቨር / በጣም ሃይለኛ
204	ለጥያቄ ቁጥር 06 መልስዎ ‘2’ ከሆነ የፕሪኢክላምሺያ በሽታው ከየትኛው ደረጃ ይመደባል ?	1. ማይልድ/ መካከለኛ 2. ሰቨር / በጣም ሀይለኛ
205	በአሁኑ ሰዓት የደም ግፊት መድኃኒት እየተጠቀሙ ነው ?	1. አዎ 2. አይደለም
206	ለጥያቄ ቁጥር 11 መልስዎ አዎ ከሆነ እየተጠቀሙ ያሉትን የመድኃኒት ብዛት ይግለጹ	_____
207	ለጥያቄ ቁጥር 11 መልስዎ አዎ ከሆነ የሚጠቀሙትን የመድኃኒት ዓይነት ይጥቀሱ`	_____ _____ _____
208	በሽታው በምርመራ ከታወቀ በሗላ ምን ያክል ጊዜ ሆነው ?	
ክፍል 3፡ ከአብስቴትሪክስ እና ለበሽታው አጋላጭ ከሆኑ ምክንያቶች ጋር ተያያዥ ጥያቄዎች		
301	የፅንሱ የዕርግዝና ዕድሜ	_____ ሳምንት
302	የአሁኑ ስንተኛ ዕርግዝናዎ ነው ?	1. የመጀመሪያ 2. ሁለተኛ እና ከዚያ በላይ
303	ከዚህ በፊት ስንት ልጆች ወልደዋል ?	1. ምንም 3. ከሁለት እስከ አምስት 2. አንድ 4. ከአምስት በላይ
304	ከዚህ በፊት በዕርግዝና ጊዜ በሚከሰት የደም ግፊት በሽታ ታመው ያውቃሉ ?	1. አዎ 2. አያውቅም
305	ከቤተሰብዎ ወይም ከዘመዶችዎ ውስጥ በዕርግዝና ጊዜ በሚከሰት የደም ግፊት በሽታ የተያዘ ሰው አለ?	1. አዎ 2. የለም

ክፍል 4፡ በመለካት የሚታወቁ ቫሪያብሎች		
401	የተሳታፊዋ ክብደት	_____ ኪሎ ግራም
402	የተሳታፊዋ ቁመት	_____ ሜትር
ክፍል 5፡ በላብራቶሪ ምርመራ የሚታወቁ ፓራሜትሮች		
501	የላክቴት ዲሃይድሮጂኔስ መጠን	_____ ዩኒት/ ሊትር
502	የፓማግሉታማይል ትራንስፈሬስ መጠን	_____ ዩኒት/ ሊትር

Annexii V: Oromifaa Waliigallatti Unkaa

Ani armaan olllitti kan farame offi kiyya eyyemme qo'anna kanna irrati hirmachuf dhigaa kiyya irraa gammaa glutamayl transferasii and laactate dehydrogenasii

Annexii VI: Gaffillewan Afaan Oromoo

Codii hirmaachaa _____ lakkofsaa cardii _____

Kuttaa tokkoffaa -waa'ee sociodemographic		
No	Gaaffi	Deebbi
1	Umriin kee meqaa	_____
2	Sadarka barnotta	A. Dubbisu fi barressu hin danda'uu B. 1-8ffaa C. High school D. Collage/Yuniversiry
3	Hojjin kee maal dha?	A. Hojjatta Mottuma B. Hojjatta dhufaa C. Hadha mana D. Dinagde E. Kan birra
4	Jiaa dhan gaalli kee meqaa dha	_____
5	Essaa jirraatta	1. Badaa 2. Maggala
	Kutaa lammaffaa	
6	Gossaa dhukkubaa	6. Gestational hypertension 7. Pre-eclampsia

		8. Eclampsia
7	Dhiibba dhigaa	_____ mmHg
8	Protinni finca irraa	_____ mg/sa'aatti24
9	Gaaffi lakkofsaa 06ffaf deebiinn kee 1 erga ta'ee kam keessatti ramadamma	1. Salpha 2. Chimma
10	Gaaffi lakkofsaa 06ffaf deebiinn kee 2 erga ta'ee kam keessatti ramadamma	1. Salpha 2. Chimma
11	Qorricha dhiga denfaa fudhacha jirra?	1. Eyyen 2. Mitti
12	Gaffii 11ffaaf debbin issa eyyen irga ta'e ammaas fudhacha jirti?	1. Eyyen 2. Mitti
13	Gaffii 11ffaaf debbin issa eyyen irga ta'e qoricha meqaa fudhatti?	
Gaddammessafi issaa wallin qabatu		
14	Ulfaa ishee torbe meqaa irraati jira	_____ torbe
15	Ulfaan kun meqaffaa dha	1. Kan jalqaba 2. Tokko ol
16	Ijjolle meqaa nagadhaan dhalatte	1. Konkumma 2. Tokko 3. Lama ol 4. Shanifi ol
17	Kannaan duraa ulfaa irraa kan ka'ee dhiga danfaa si dhukkube bekka?	1. Eyyen 2. Mitti
18	Sanyiin dhaan ulfaa irraa kan ka'ee dhukkuba dhiga danfaa jirraa?	1. Eyyen 2. Mitti
Qaama saafaru		
19	ulfatinnaa	_____ kg
20	dheerinna	_____ m
21	Ulfatinnaa irraa dherinna	_____ kg/m^2

	Biochemicalii Parameterisii	
22	Lactate dehydrogenasii plasma level	_____ U/L
23	Gamma glutamyl transferasii plasma leveli	_____ U/L