PATTERN OF PLATELET INDICES AS A POTENTIAL MARKER FOR PREDICTION OF PREECLAMPSIA AMONG PREGNANT WOMEN ATTENDING AT WACHEMO UNIVERSITY NIGIST ELENI MOHAMMED MEMORIAL REFERRAL HOSPITAL, HOSANNA, SOUTHERN ETHIOPIA

A RESEARCH PAPER SUBMITTED TO THE SCHOOL OF MEDICAL LABORATORY SCIENCES, FACULTY OF HEALTH SCIENCES, JIMMA UNIVERSITY, FOR THE PARTIAL FULFILLMENT OF THE DEGREE OF MASTER OF SCIENCE IN CLINICAL LABORATORY SCIENCES (HEMATOLOGY AND IMMUNOHEMATOLOGY SPECIALITY TRACK)

BY: SOLOMON GEBRE

JUNE, 2019

JIMMA, ETHIOPIA

JIMMA UNIVERSITY INSTITUTE OF HEALTH

FACULTY OF HEALTH SCIENCES

SCHOOL OF MEDICAL LABORATORY SCIENCES

PATTERN OF PLATELET INDICES AS A POTENTIAL MARKER FOR PREDICTION OF PREECLAMPSIA AMONG PREGNANT WOMEN ATTENDING AT WACHEMO UNIVERSITY NIGIST ELENI MOHAMMED MEMORIAL REFERRAL HOSPITAL, HOSANNA, SOUTHERN ETHIOPIA

ADVISORS: LEALEM GEDEFAW (MSc, Assoc.Prof)

WONDIMAGEGN ADDISU (BSc, MSc)

DR. BIRHANU NIGUSE (MD, Gynecologist)

JUNE, 2019

JIMMA, ETHIOPIA

ABSTRACT

Background: Preeclampsia is the most significant health risk for the pregnant women and the fetus. Inconsistency in reports and shortage of literature in Ethiopia regarding platelet pattern among preeclampsia group and normal pregnancy necessitates the current study.

Objective: To evaluate the pattern of platelet indices for prediction of preeclampsia among pregnant women attending at Wachemo University Nigest Eleni Mohammed Memorial Referral Hospital, Hosanna, from January to April 2019.

Method: A comparative cross-sectional study was conducted on a total of 180 pregnant women who attended anti- natal care follow up from January to April 2019. EDTA anti coagulated venous blood sample was analyzed usingMind ray BC-3000Plus, Shenzhen, china. The Kolmogorov-Smirnov normality test, Kruskal-Wallis H test in conjunction with the Mann Whitney U test, Post-hock test supplemented with Benforeni, receiver operating characteristics curve, Spear Man rank order correlation was done using SPSS version21 software. A P-value of <0.05 was considered as statistically significant.

Result:The platelet and platelatecrit tends to decrease with severity of preeclampsia. While mean platelet volume and platelet distribution width were significantly, increased with severity of preeclampsia (P<0.001). Mean arterial pressure showed statistically significant positive correlations with platelet distribution width (rho =0.731, p<0.001), and mean platelet volume (rho=0.674, p<0.001). Platelet distribution width was found to be the best parameter for predicting preeclampsia (AUC=0.986; 95%CI (0.970, 1).

Conclusion: Platelet indices such as platelet count, mean platelet volume, platelet distribution width and platelatecrit were identified as good candidate markers for prediction of preeclampsia in pregnant women. Serial analysis of these indices at different trimesters of pregnancy should be carried out in future research to evaluate whether it is possible to predict preeclampsia before second half of pregnancy.

Key words: Platelet indices, pregnancy, preeclampsia, predictive values, Hosanna, southern Ethiopia.

ACKNOWLEDGEMENTS

First of all,my heartily thank goes to my advisors, Mr. Lealem Gedefaw (MSc, Assoc.Prof), Mr. Wondimagegn Addisu (BSc, MSc) for their untiring advice as well as Dr. Birhanu Niguse (MD, Gynecologist) for his facilitation of this work by giving clinical advice. I would like to express my appreciation to Jimma University School of Medical Laboratory Sciences for its provision of the research work.I am grateful to administrative bodies of Nigest Eleni Mohammed Memorial Referral Hospital for acceptance and permission as well as giving necessary background information of hospital relevant to my work. I am thankful to all staffs of Obs &Gyn especially Mr. Robel Girma who is a matron nurse of the hospital, for his coordination and assigning data collectors from the site. My gratitude also goes to the study participants for their voluntarily participation. The last but not the least my gratitude goes to the laboratory staff who participated by performing laboratory analysis for my data.

TABLE OF CONTENTS

CONTENTS PAGE

ABSTRACT.....I

ACKNOWLEDGEMENTS.....II

TABLE OF CONTENTS.....III

LIST OF FIGURES.....V

LIST OF TABLES.....VI

ABBREVIATIONS/ACRONYM.....VII

OPERATIONAL DEFINITION.....VIIVIII

CHAPTER ONE: INTRODUCTION.....1

1.1. Back ground.....1

1.2. Statement of the problem.....4

1.3. Significance of the Study.....6

CHAPTER TWO: -LITERATURE REVIEW.....7

CHAPTER THREE:-OBJECTIVES.....13

3.1. General objective.....13

3.2. Specific objectives.....13

CHAPTER FOUR: MATERIALSAND METHODS.....14

4.1. Study area and period.....14

4.2. Study design.....15

4.3 Population.....15

4.3.1. Source population.....15

4.3.2. Study Population.....15

4.4. Sample size determination and sampling technique.....15

4.4.1 Sample size determination.....15

4.4.2. Sampling method.....16

4.5. Variables.....16

4.5.1. Dependent variable.....16

4.5.2. Independent variables.....16

4.6. Inclusion and exclusion criteria.....17

4.6.1. Inclusion criteria.....17

4.6.2. Exclusion Criteria.....17

4.7. Data collection.....17

4.7.1. Socio-demographic and related clinical data 17
4.7.2. Laboratory data 18
4.8. Quality assurance 19
4.9. Data processing and analysis. 19
4.10. Ethical clearance 20
4.11. Dissemination of the result 20
5.RESULT 21
5.1. Characteristics of study participants 21
5.2. Platelet Indices among pregnant mothers 23
5.3. Comparison of platelet indices across preeclamptic and normotensive pregnant women 24
5.4. Comparing platelet indices between normotensive, non- severe and sever preeclamptic pregnant women. 25
5.5. Inter group analysis of platelet indices across normotensive, non-severe and severe preeclamptic pregnant women. 26
5.6. Co -relational analysis of platelet indices with mean arterial pressure in study participants 27
5.7. The diagnostic role of platelet indices for preeclampsia 28
6. DISCUSSION 31
7. CONCLUSION AND RECOMMENDATION 39
7.1. CONCLUSION 39
7.2. RECOMMENDATION 39
REFRENCES 41
ANNEXES 50

LIST OF FIGURES

Figure1: -Location map of WUNEMMRH Study Site in Hadiya zone, Hosanna southern Ethiopia 2019…….14

Figure2: Frequency of thrombocytopenia among study participants in WUNEMMRH from January to April 2019……25

Figure3: The ROC curve analysis of PLT indices among study participants in WUNEMMRH from January to April 2019…….....32

LIST OF TABLES

Table1: Socio-demographic and clinical characteristics of study participants inWachemo University Nigest Eleni Mohammed Memorial Referral Hospital fromJanuary toApril 2019……24

Table2: - Comparisons of platelet indices between normotensive and preeclamptic pregnant women inWachemo University Nigest Eleni Mohammed Memorial Referral HospitalfromJanuary toApril 2019……26

Table3: Comparisons of platelet indices between normotensive, non- severe and severe preeclamptic pregnant mothers inWachemo University Nigest Eleni Mohammed Memorial Referral Hospital, fromJanuary toApril 2019…….27

Table4: Pair wise comparisons of platelet indices among normotensive, non-severe and severe preeclamptic pregnant women in Wachemo University NigestEleni Mohammed Memorial Referral Hospital, fromJanuary to April in 2019…….....28

Table5: Correlation between platelet indices with mean arterial pressurefor the study participants in Wachemo University Nigest Eleni Mohammed Memorial Referral HospitalfromJanuary to April in 2019……29

Table6. The diagnostic role of platelet indices among study participants in Wachemo University NigestEleniMohammed memorial referral hospital in 2019…….31

ABBREVIATIONS/ACRONYM

ANC……Antenatal Care

AUC……Area under the Curve

BP……Blood Pressure

BMI……. Body Mass Index

CBC……. Complete Blood Count

DBP……...Diastolic Blood Pressure

MAP……. Mean Arterial Pressure

MPV ……Mean Platelet Volume

MD……Medical Doctor

NEMMRH……. NigestEleniMohammed Memorial Referral hospital

PLT……...Platelet Count

PCT……Platelet -Crit

PDW……. Platelet Distribution Width

PPV……...Positive Predictive Value

NPV……...Negative Predictive Value

ROC……...Receiver Operating Characteristics

SOP……...Standard Operating Procedure

SBP……Systolic Blood pressure

WUNEMMRH……...Wachemo University Nigest Eleni Mohammed Memorial Referral Hospital

WHO……. World Health Organization

OPERATIONAL DEFINITION

Preeclampsia: - Was pregnancy specific hypertensive disease occur after 20 weeks of pregnancy and characterized by systolic blood pressure ≥140 and/or a diastolic blood pressure ≥90 mmHg on two measurements together with new onset proteinuria of ≥1+ by dipstick in a random urine sample.

Non-severe preeclampsia: - If systolic blood pressure ≤160mmHg and/or a diastolic blood pressure ≤110 mmHg and proteinuria of ≥1+ or more using dipstick.

Severe: Preeclampsia - If it presented with one or more of the following criteria: systolic blood pressure ≥160 mmHg or diastolic blood pressure ≥110 mmHg or both, new-onset cerebral or visual disturbance, epigastric or right upper quadrant pain and pulmonary edema. **Definition and classification of preeclampsia** is based on the recommendation of the American College of Obstetricians and Gynecologists task force on hypertension in Pregnancy(1).

Normal pregnancy: - All healthy women with gestational age ≥20week, normal blood pressure, and without proteinuria

Pattern of platelet indices: The variation of platelet indices such as PLT, MPV, PDW and PCT between preeclamptic and normotensive pregnant women as well as with severity of preeclampsia.

Thrombocytopenia: Was defined as a drop in platelet count below 150,000 PLT /µl blood.

CHAPTER ONE: INTRODUCTION

1.1. Back ground

Normal pregnancy causes significant metabolic and hemodynamic changes in a woman's physiology to allow for fetal growth, but inability to adapt to these changes might result in the development of pregnancy related complications(2) The most significant complication is pregnancy induced hypertension (3). It is the second leading cause of maternal death next to hemorrhagic disorders worldwide (4). It is responsible for complication of about 7–10% of all pregnancies which result in significant morbidity and mortality of the mother and fetus(5).

Preeclampsia is specific form of pregnancy induced hypertensions, with new-onset hypertension and proteinuria at ≥20 weeks of gestation and it can be non-severe or severe based on its presentation(1). It complicates 5-8% of pregnancies(6) and the cause of 9%–26% of global maternal mortality, significant proportion of preterm delivery, and maternal and neonatal morbidity(7). The authors in Ethiopia carried out its trend analysis reported increasing trend and associated maternal complications from year to year(8).

Pregnancy has remarkable changes in hematological variables(9). But several hematological abnormalities are also associated with the preeclampsia. Decreasing of some plasma clotting factors(10), abnormal morphology , rapid hemolysis in red cell and thrombocytopenia are among identified changes. But thrombocytopenia is most commonly identified at a times and may become severe enough to be life threatening(11). The exact pathogenic aspect of preeclampsia is not completely known despite attempts(12) but impaired placentation due to influence of various genetic and epigenetic factors govern current understanding of preeclampsia (13). Following bad placental perfusion and oxidative stress, various vasoactive agents released into the maternal circulation that initiate production of inflammatory cytokines, disturb the balance between vasoconstriction and vasodilatation, in turn result in vascular endothelial dysfunction of different organs and organ systems of pregnant woman and the fetus(12).Since

the integrity of endothelium is kept with blood Platelet(PLT) endothelial dysfunction in preeclampsia may end up with haemostatic alterations (14). This in a broader sense necessitates, exploring relation between PLT with preeclampsia.

The blood PLTis multi-functional cell play an essential role in inflammatory process, microbial host defense, wound healing, angiogenesis, and remodeling(14). They kept in quiescent state under physiological conditions, due to presence of mediators such as nitric oxide and prostacyclin secreted by healthy endothelium which inhibit Plt activation as well as adhesion to the endothelium. However, the diminished production of these protective mediators in preeclampsia due to inflammation of endothelium induces vasoconstriction, adhesion and aggregation of PLT(15). More over activation of coagulation continued and generates further hypoxic damage to the endothelium. Therefore, preeclampsia may aggravate Plt activation, reduced lifespan and enhance thrombi generation in the microcirculation of several target organs which may end up with declining of PLT count. Therefore, those pregnant women with preeclampsia may have different pattern in level PLT activation markers such as Mean Platelet Volume (MPV), Platelet Distribution width (PDW), Platelatecrit (PCT) than normotensive women. Furthermore, preeclamptic women experience increased expression of PLT markers such as CD31, CD40L, CD42a, CD61, CD62 and CD63 than normotensive pregnant women(16). This is the indicative of the inflammatory function of PLT is more role player in the pathogenesis of preeclampsia.

Regarding the trend of management, well established and full proof methods have not been developed till date for the prevention of preeclampsia(17, 18). World Health Organization (WHO) puts direction as retrieval of up-to-date research-based evidence to equip clinicians with additional information regarding this complex disease(19).In spite of much researches to develop a reliable test in the past decade, and several biochemical markers described recently for predicting preeclampsia such as angiogenic/antiangiogenic factors, placental proteins, their role in resource poor hospitals is doubtful and some them are still at the stage of research(20).

Recently, identification of pregnant women with risks of preeclampsia is the most important goals in obstetrics(18). Therefore, obstetricians nowadays rely increasingly on laboratory test for the management of pregnant women (21) . The detection, close surveillance, a precise diagnosis and a timely pregnancy intervention are achieved through use of sensitive and specific biomarkers. Good candidate marker on this regard is evaluating Plt indices, since it is a simple and habitually done method, with lower cost and greater accessibility in the clinical laboratory(22)

In light of technology, Plt indices such as Plt, MPV, PDW PCT and PLCR are derived from routine blood counts in hematological analyzers though all analyzers may not perform all of them(23). They have key role in different disease diagnosis including preeclampsia now days(24). Though their values are not fully substantiated in our country(25), some of them have been the most studied Plt activation markers associated with preeclampsia such as Plt and MPV ,but some of them have been added recently as novel indices to find out whether they have predictive and/or prognostic value for preeclampsia(26). The aim of this study was to evaluate pattern of PLT indices during pregnancy as potential marker for prediction of preeclampsia among pregnant women attending WUNEMMRH, Hosanna, Southern Ethiopia.

1.2. Statement of the Problem

Preeclampsia is a potentially serious complication of pregnancy. Globally over half a million women die each year from pregnancy related causes, out of this 99% is in low and middle income countries(27). Preeclampsia accounts 9%–26% of global maternal mortality and a significant proportion of preterm delivery(7). In Africa out of 77,884 total deliveries, it accounts 1,804 (2.32%) as well as it is accused for 25 of maternal death, 296 prenatal death, 469 preterm birth and 400 low birth weight(28). In Ethiopia, its prevalence reaches 5.47% (29) . Approximately 1% of all deliveries, 5% of all women with complications were documented as having severe preeclampsia, nearly 16% of direct maternal deaths and relatively high case fatality rate of 3.6% was also reported among all pre-eclamptic women in Ethiopia(30).

Preeclampsia can pose short term as well as long term impact on life of mother and baby. Short term impacts on baby include:- poor intrauterine fetal growth and preterm birth also in the mothers' it can cause stroke, hemolysis, renal failure, low PLT and disseminated intravascular coagulation (2). As the long term impact, it triggered metabolic stress which causes vascular injury, thus contributing to the development of cardiovascular disease and/or chronic kidney disease in future. The risk of developing cardio vascular disease in later life is 1.6 times higher in women with preeclampsia compared with uncomplicated pregnancies(31). This risk appears to be increased especially in women with a history of recurrent preeclampsia. Despite its drawback, inducing delivery irrespective of gestational age is the only effective treatment currently for preeclampsia(3) . Therefore, by considering the burden of the disease in the context of social and economic impact in addition to the evident clinical repercussions, it is important to foresee the condition using reliable marker(32). This can help to design a better tracking system for antenatal (ANC) care programs and for early prevention and intervention. Furthermore, utilizing simple markers play role as prognostic tool in management without extra cost(10).

Even though PLTs are the major element in pathogenesis of preeclampsia(15)and its parameters are among proposed biomarkers for the prediction(33),the trend of using PLT indices in disease diagnosis including preeclampsia is not growing in Ethiopia. A hospital-based questionnaire survey in Addis Ababa revealed only less than 10% of the clinicians used the MPV or PDW in

medical practice in spite of the clinical benefits(25). Even though the inflammatory role PLT and preeclampsia as inflammatory disease relate PLT and preeclampsia recently (14), results from different studies comparing PLT pattern between preeclamptic and normotensive pregnant women is not consistent. Therefore, endothelial damage assumed to be equal criteria for diagnosis of preeclampsia currently, furthermore, proteinuria as diagnostic criteria of the disease is expected to be replaced by other specific biomarkers in future(13),controversial reports regarding significant difference of PLT indices with preeclampsia and normal pregnancy, needs further research to obtain concrete evidence. Even though studies has been conducted on the prevalence of preeclampsia among pregnant women in Ethiopia(8)(30), few literatures are available in comparing the PLT indices in preeclampsia and normal pregnancy and no study has been done in the study area. Therefore, the aim of this study was to evaluate pattern of platelet indices during pregnancy as potential marker for prediction of preeclampsia among pregnant women attending Wachemo University Nigest Eleni Mohammed Memorial Referral Hospital (WUNEMMRH).

1.3. Significance of the Study

Preeclampsia is the big threat in developing countries including Ethiopia and PLT activation is indicator of the disease, but there is the limitation in the data and the controversies between studies on whether pattern of PLT indices can predict presence or absence of preeclampsia. Therefore, this study believed to persuade weather it is used as clinically useful biomarkers as well as it is diagnostic or supportive for the management of preeclampsia. The information of the study expected to enrich clinicians with knowledge for early identification of preeclampsia as well as have role in incorporating these indices as part of routine antenatal investigation, which can improve the potential for preventing bleeding problems in patient cases with platelet disorders in preeclampsia. Based on the finding, the study is expected to be the gate way to explore future area of research on hematological aspect of preeclampsia regarding PLT pattern. Furthermore, it is expected to influence strategies on anti-platelet therapy during preeclampsia as well as it will have contribution in decreasing morbidity and mortality of pregnant women. In addition to being reference for diagnosis of preeclampsia it can be base line for related studies in the area.

CHAPTER TWO: -LITERATURE REVIEW

The significant difference in pattern of PLT indices between pregnant women with preeclampsia and normotensive pregnant women as well as with severity of the disease were documented by many studies. The retrospective case-control study conducted in Turkey revealed significantly higher value of MPV and PDW in pre-eclamptic pregnant women as compared to the normotensive pregnant women. The study documented sensitivity of 64%, specificity of 50%, PPV of 65.8%, NPV of 48.1 % and AUC of 0.611 for PDW, whereas the cut off value of MPV documented as 8.35 fl with sensitivity of 68%, specificity of 52%, PPV of 68%, NPV of 52% and with AUC of 0.574 for prediction of preeclampsia. But the study didn't found significant difference with regard to PLT and PCT when preeclampsia group compared with normotensive group as well as the significant difference was not observed in terms of severity of disease regarding to PLT, MPV and PCT(22).

Another retrospective case-control study in similar study area turkey revealed significantly lower PLT in preeclampsia patients than in normotensive group. In sub group analysis, PLT showed significant difference, between non-severe preeclampsia group and normotensive group ($p=0.0008$, $p<0.01$) but not between severe preeclampsia group and normotensive pregnant women ($p = 0.086$; $p>0.05$) as well as no significant difference was observed between non severe and severe preeclampsia group. Whereas MPV showed a significant difference between the severe preeclampsia group and control group ($p = 0.009$; $p<0.01$) but not between non severe preeclampsia group and control group ($p = 0.135$; $p>0.05$). The cut of value for PLT for diagnosis of preeclampsia was $\leq 190\times10^9$/l with AUC of 59.3%. Whereas cutoff value of MPV was ≥9fl for diagnosis of preeclampsia ($p<0.01$) the study but not found statistically significant differences in PDW across groups(34).

According to longitudinal comparative study conducted in Korea, the PLT and PCT decreased, however MPV and PDW increased as the disease progressed from normotensive, non-severe and severe preeclampsia. In this study, all participants had a normal PLT but PLT decreased as the disease progressed. Moreover, PDW showed significant elevation in the severe preeclampsia group as compared to the levels of other platelet indices and showed statistically significant positive correlation with MAP with the ($r = 0.231$, $p < 0.05$), with an AUCof 0.74. Therefore, following their finding the authors concluded, PDW as a candidate marker for development of preeclampsia and predicting the severity of hypertension(35). comparative study done in India, revealed lower PLT while increased level of MPV and PDW in pre-eclampsia as compared to control group and found the relationship between PLT indices and severity of pre-eclampsia. Following their finding the authors also stated that estimation of PLT indices as early, simple and rapid procedure in the assessment of severity of pre-eclampsia(36). This indicates that pattern of PLT indices can be the marker to predict the development of preeclampsia.

Based on the finding of comparative study done in India, mean PLT count was significantly lower in preeclampsia groups than that of in the normal pregnancy. The study also found a total of 30 thrombocytopenia cases among preeclamptic groups and out of which 9 cases in non-severe preeclampsia patients and 21 cases in severe preeclampsia. Therefore, the study directly related the frequency of thrombocytopenia with the severity of disease, and found PLT as a simple and cost effective tool to monitor the progression of preeclampsia(37). Another prospective observational study conducted in similar study area notedsignificantly lower PLT in severe preeclampsia than non-severe preeclampsia andcontrols. Thrombocytopenia was seen in total of 33 cases of severe pre-eclampsia. Based on their finding the authors concluded PLT count as simple and routine tests which is highly helpful in suspecting a deranged coagulationstatus early in the course of the disease(38). Other observational prospective study from India revealed severe preeclampsia in 66% of cases and 56% of thrombocytopenia. The study noted high significant relationship between the degree of thrombocytopenia with the severity of the hypertension ($p < 0.001$) and stated thrombocytopenia as the most common complications of preeclampsia(39)

The case control study conducted in Brazil documented lower PLT and PCT in severely preeclamptic pregnant women as compared to normotensive pregnant (P = 0.031 and 0.035), for PLT and PCT respectively. Despite decreased level of PLT with elevated MPV, it has been found within normal range, as stated by the study. According to this study, the cut of value of $\leq 221 \times 10^3/\mu l$ for PLT with sensitivity 68.97%, specificity of 70.69% and with an AUC of 0.73 can differentiate normotensive pregnant women from preeclamptic pregnant women.Whereas PCT accounts a cut of value of ≤0.179% with sensitivity of 55.17%, specificity of 84.48% and AUC of 0.69 (p =0.0007) for predicting preeclampsia but, it is not the good candidate marker for prediction of preeclampsia as concluded by the authors. The study also revealed cut off value of ≥18.3, for PDW can predict preeclampsia with sensitivity of 55.17%, specificity of 86.21% and with an AUC of 0.77, (p <0.001) whereas cut off value of ≥9.6fl for MPV can predict development of preeclampsia with sensitivity of 51.72%, specificity of 82.76% and AUC of 0.72(40)

Cross-sectional prospective study conducted in Lahore revealed decreased PLT in subjects with preeclampsia as compared to controls and a statistically significant difference was observed with a (P-value of < 0.001) in pregnant females having preeclampsia than normotensive subjects(41). In accordance with this finding study conducted in Bangalore noted lower PLT and increased MPV and PDW in preeclampsia and compared to control group. They also noted severity of disease and thrombocytopenia are closely correlated. Following their finding authors stated as changes in PLT indices can be associated with severity of preeclampsia and the estimation of PLT indices can be considered as an early, simple and rapid procedure in the assessment of severity of preeclampsia and which can be used as a prognostic marker(42).

On the other hand,case control study done in Saudi Arabiadidn't found significant difference in PDW and MPV between the preeclamptic and control women. But according to this study PLT was lower in the women with preeclampsia compared with the controls. This study didn't document significant difference in the PLT, PDW and MPV, when women with non-severe preeclampsia compared with severe preeclampsia. According to this similar study, the PLT cutoff was ≤248.0×103/μL for diagnosis of preeclampsia (P=0.019) with an AUC of 62.4%(43). Another comparative study conducted in Istanbul, turkey, also didn't found statistically

significant differences between patients with preeclampsia and normal pregnancy with regard to PLT and MPV(44). Another case control study done in similar study area didn't document statistically significant difference regarding PLT and MPV when pre-eclamptic patients were compared with controls. Therefore, the authors didn't observed prognostic significance of PLT count and MPV on the presence and/or severity of pre-eclamptic condition. The authors explained the cause for conflicting reports from different studies regarding preeclampsia and PLT Pattern could be the difference in methods and/or equipment used for automated blood count(45).

The prospective study conducted in south Asia, noted significantly decreased PLT with preeclampsia as compared to normal pregnant patients (19.4%vs 7.4%) but MPV increased in preeclampsia (44.5%vs 9.2%) and increase in PDW was also observed significantly in patients with preeclampsia (47.19% vs.29.4%). The authors concluded that patients with preeclampsia are more likely to have significant decrease in PLT, increase in MPV and PDW and these changes can be observed at earlier gestational age than significant rise in Blood Pressure (BP) and can be observed and are directly proportional to progressive rise in hypertension. So, estimation of PLT indices can be considered as early, simple and cost effective procedure in assessment of severity of preeclampsia(46). The prospective case control study done in Datta Meghe, also explained role of PLT for prediction ofincreasing grade of pregnancy induced hypertension and showed inverse relationshipwith severity of pregnancy induced hypertension. Whereas MPV and PDW showed consistent relationship with pregnancy induced hypertension(47).

According to the recent cohort study conducted in India, PLT decreased while MPV and PDW increased as pregnancy advances, and these changes are more pronounced in preeclampsia than normotensive pregnancy. Among preeclamptic patients,16 (32%) of them had thrombocytopenia , where as in normotensive group none of the patients had lower PLT count(48). From the report of case control study conducted at India Karnataka district comprising of 50 preeclamptic females casesand 50 normotensive pregnant females, significant difference in PLT and PDW in patients with preeclampsia were found as compared to normotensive healthy females but ,this

study document no significant differences in MPV in normal and preeclamptic women and between severities of preeclampsia(49).

From the case control study in India which aimed to evaluate PLT counts at frequent intervals in pre -eclampsia and to assess its role as a prognostic tool in management, 11 cases (22%) of preeclamptic women had thrombocytopenia but in the control group there was no significant thrombocytopenia(50). According to cross sectional study conducted in Egypt, PLT wassignificantly lower in women with severe preeclampsia where as MPV andPDW were significantly higher in women with severe preeclampsia as compared to women with non-severe preeclampsia and normal pregnant women. From ROC curve analysis, cut off value of ≤ 198,000/µl for PLT can differentiate normotensive pregnant women from non-severe preeclamptic patients with a sensitivity of 90% and specificity of 92% PPV of 91.8%, NPV of 90.2% with an AUC of 0.866. Whereas MPV at cut off value of ≥ 9.3 can differentiate normotensive pregnant women from non-severepreeclampsia group with sensitivity of 90.0% specificity of 92%, PPV of 91.8%, NPV of 90.2% and with an AUC of 0.885. The cut off value for PDW was ≥ 12.6fl, with sensitivity of90.0%,, specificity of92%,ppv of 91.8%,npv of 90.2% and AUC of 0.886 to differentiate normotensive pregnant women from non-severe preeclampsia group(51).

Another longitudinal study conducted in similar study area Egypt revealed decreasing of PLT while increasing of MPV and PDW as preeclampsia progressed. From the ROC curveanalysis, it accounts $\leq 233\times10^3$/µl with sensitivity of81.5%, specificity of78.3% and with an AUC of 0.171. Whereas MPV had the cut off value≥9.5fl, sensitivity of 92.6%, specificity of 87.0% and an AUC of 0.940 .to discriminate the presence or absence of preeclampsia. According to this similar study, PDW had the cut off value of ≥19.9fl, with sensitivity of 96.3%, specificity of 91.3% and with an AUC of 0.980. According this study, PDW had the largest area under curve (AUC) [0.980 (95% CI: 0.964 - 1.000)],making it the best marker for predicting development of preeclampsia. It showed the most statistically significant correlation with mean arterial pressure (MAP) (r = 0.902, p = 0.000), making it thebest marker for predicting severity of hypertension. Following the finding the authors concluded that PLT decreases while MPV and PDW increase as pregnancy advances, and these changes are morepronounced in Preeclampsia than

normotensive pregnancy and these changes predate development of preeclampsia by 2 -8 weeks and are proportional to the progress of this disorder. More over according to conclusion of authors, PDW, have the potential to be utilized as markers for not only prediction of preeclampsia developmentbut also severity of hypertension(52).

The prospective case control study conducted in Sudan found significantly higher MPV and PDW in preeclampsia than normal group with p-value of (P; 0.02) and (P; 0.03) for MPV and PDW respectively. According to this study PLT and PCT were lower in Preeclampsia group but didn't show significant variation. Which accounts (P; 0.1) and (P; 0.64) for PLT and PCT respectively(53). A cross-sectional study conducted in Gondar revealed increased level MPV and PDW and while decreasing of PLT in preeclampsia groups, moreover MPV and, PDW showed statistically significant positive correlations with MAP with ($r=,0.37$ $p<0.001$ $r=0.43$, $p<0.001$) for MPV and PDW respectively. Whereas PLT displayed a statistically significant negative correlation with a MAP($r=.-0.33, p<0.001$) .The study was concluded as MPV and PDW were increased as preeclampsia advanced and PLT decreased with the severity of the disease as well as evaluation of these parameters can be supportive clinical marker in the assessment of severity of preeclampsia and may assist the management(54). But this study did not include ROC curve analysis to obtain cut off points for PLT indices in discriminating presence or absence of preeclampsia, thus this gap was filled with current study since we included ROC curve analysis to determine cut off of values as well as AUC for each PLT indices. In turn it can help us to know the sensitivity, specificity and predictive values of this indices in predicting development of preeclampsia and to reach on conclusion of wether PLT indices are prognostic or supportive in diagnosis of preeclampsia.

Therefore, going through all the literatures reviewed, there were controversies for significance difference of pattern of PLT indices in pregnancy with preeclampsia and its severity. There are also few literatures on this area in our country Ethiopia. As my literature search, there is only one published report from our country and no research is conducted in study area. Hence, this study believed to fills this gap by providing additional information to widen the existing scientific knowledge on PLT pattern and preeclampsia and could make the reports on this field more conclusive.

CHAPTER THREE:-OBJECTIVES

3.1. General objective

To evaluate pattern of platelet indices for prediction of preeclampsia among pregnant women attending at Wachemo university Nigest Eleni Mohammed Memorial Referral Hospital from January to April, 2019

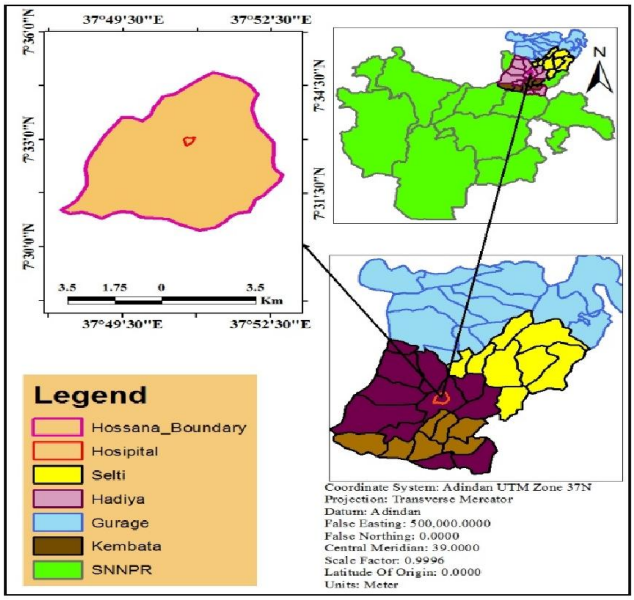
3.2. Specific objectives

- To compare the platelet indices of pregnant women with and without preeclampsia
- To determine difference between platelet indices in normotensive, non-severe and severe stages of preeclampsia.
- To determine correlation of platelet indiceswith severity of preeclampsia.
- To determine sensitivity, specificity and predictive value of platelet indices for the diagnosis of preeclampsia**.**

CHAPTER FOUR: MATERIALSAND METHODS

4.1. Study area and period

The study was conducted at Wachemo University Nigest Eleni Mohammed Memorial Referral Hospital which is found in Hadiya Zone, Hosanna from January to April 2019. Hadiya Zone is located 232 km South West of Addis Ababa, the capital of Ethiopia and 194 km west of Hawassa (regional city). The total population of the zone is estimated to be 1,506,733 among whom 745,381 (49.47%) are males and 761,352 (50.53%) are females. The NEMMRH which is established in 1976 E.C provides its referral and non-referral services for around 3,200,000 populations in its catchment areas. On average, a total of 1979 pregnant women are visiting the hospital annually as per the 2010 E.C Hospital report.



SOURCE. ETHIO -GIS.

Figure1: -Location Map of WUNEMMRH Study Site in Hadya zone, HosannaSouthern Ethiopia 2019

4.2. Study design

Comparative cross-sectional study was conducted.

4.3 Population

4.3.1. Source population

All pregnant women who visit Wachemo University Nigest Eleni Mohammed Memorial Referral Hospital(WUNEMMRH)

4.3.2. Study Population

A pregnant woman who fulfills the inclusion criteria during the study period and volunteers to take part in the study was the study population.

4.4. Sample size determination and sampling technique.

4.4.1 Sample size determination

The sample size for this study was calculated using the G-power statistical software version 3.1. Level of significance is set to 0.05. Power of the test: 100(1-β) %, is 80%, which is equal to 0.8 and effect size of 0.468. The mean of PDW for normal pregnancy was 12.15 and preeclamptic women was13.37 and with a standard deviation for both normal pregnancy and preeclampsia were 2.35and2.84, respectively. The values were taken from the study conducted in Sudan(53). The sample size obtained was 164. By adding 10% non-response rate a final sample size was180. To increase the accuracy, we used 1=2 allocation ratios; the number control was twice number of cases. Therefore, a 60 pregnant women with preeclampsia (30 non-sever preeclamptic and 30 sever preeclamptic pregnant women) and 120 with healthy pregnancy as a control, a total of 180 study subjects were recruited.

4.4.2. Sampling method

All preeclamptic and normal pregnant mothers who attending ANC follow up and admitted in WUNEMMRH during study period were included consecutively.

4.5. Variables

4.5.1. Dependent variable

- Platelet indices
- Sensitivity, specificity and predictive values of platelet indices

4.5.2. Independent variables

- Preeclampsia
- Gestational age
- Residence
- Maternal age
- Body mass index
- Number of delivery
- Number of pregnancy

4.6. Inclusion and exclusion criteria

4.6.1. Inclusion criteria

Pre-eclamptic pregnant women and healthy normotensive pregnant women, who were volunteer to participate and at ≥20-week gestation, were, eligible and approached for recruitment.

4.6.2. Exclusion Criteria

The pregnant women with Poor past obstetric history (recurrent miscarriage, pre-term labor, intrauterine growth restriction), Gestational or insulin-dependent diabetes and known previous hypertension, history of preeclampsia, renal or hepatic dysfunction, Disseminated intravascular coagulation, symptomatic infectious disease (bacterial, parasitic, viral and etc.), autoimmune conditions such as lupus, took drugs which alter PLT count such as heparin, corticosteroid were excluded by Medical record review, interviewing study participants to manage missing information, communicating with attending physician (for those clinically diagnosed cases).

4.7. Data collection

4.7.1. Socio-demographic and related clinical Data

Those volunteer pregnant women of ≥20 weeks of gestation after getting an informed consent were clinically examined by the physicians. The Blood Pressure were measured (BP) and recorded using mercury sphygmomanometer according to the recommendation of Guideline for management of Hypertensive disorders(55) and as a marker of preeclampsia severity, Mean Arterial Pressure was calculated as Diastolic Blood Pressure plus 1/3 of the difference between Systolic Blood Pressure and Diastolic Blood Pressure(56). Then after those pregnant women with systolic blood pressure of ≥ 140 mm Hg or diastolic blood pressure of ≥ 90 mmHg recorded twice 4 hours apart or a single measurement of ≥ 160/110 mmHg, accompanied by significant proteinuria was considered as preeclamptic(cases) and out of this pregnant women, those with blood pressure of ≥ 160 mmHg (systolic) or 110 mmHg (diastolic) and associated proteinuria of ≥ 0.3 grams(+1 on dipstick) and with severity signs in clinical examination such as new-onset cerebral or visual disturbance, epigastric or right upper quadrant pain and pulmonary edema

considered as severe preeclamptic and those whose blood pressure less than 160 mmHg (systolic) or 110 mmHg (diastolic) with proteinuria greater than ≥ 0.3, grams(+1 on dipstick) ,was considered as non- severe preeclampsia but those pregnant women without this feature of hypertension and proteinuria was considered as normotensive(controls). Then, sample of both preeclamptic and normotensive pregnant women that fulfils the inclusion criteria was recruited consecutively. The socio demographic data such as maternal age, gestational age, gravidity, parity, residence was collected using structured questionnaire from both pregnant women with pre-eclampsia (cases) and normal pregnant women (control) and BMI was computed as the ratio of maternal weight in kilograms and the square of maternal height in meters the result can be validated using four ranges for BMI recommended by BilanoVL.(28).

4.7.2. Laboratory data

The three (3 ml) of venous blood was collected once from both preeclamptic and normal pregnant women by clean venipuncture, using vacutainer tube method, into commercially prepared concentration of EDTA containers following SOP. Blood sample was gently mixed to prevent clump and clot formation for PLT indices. Samples were measured in MINDRAY-BC-300Plus, Nanshan, shenzen518057 P.R, China within 1 hours of blood collection to determine the value of the PLT indices. The MINDRAY-BC-300Plus performs speedy and accurate analysis of 19 parameters using impedance principle for counting and cyanide free testing for hemoglobin. After the analysis the results obtained was print out and registered on registration books. The sample of thrombocytopenia (PLT<150, 0000/µl normal PLT (PLT 150,000/µL-450,000/µL) was rechecked by examining Wright stained blood film on microscope to exclude the error of machine.The result with abnormal PLT was reported to physicians.

4.8. Quality assurance

The Personal protective equipment were used appropriately, while performing the procedure as well as the SOP was followed while collecting the sample and the blood sample was collected by trained phlebotomist ,checked for criteria's like; hemolysis, clotting, volume and collection time and labeling after collection as well as homogenized by inverting 5-6 times prior to analysis according to the recommendation of guide lines on the laboratory aspects of assays used in haemostatic and thrombosis(57). The quality of sample and reagents was assured based on SOP and performance of the hematological analyzer was maintained by running three levels hematology cell controls (Normal, Low and High) based on protocol of laboratory. All the result of PLT below and above reference limit as well as sample of normal PLT count was rechecked by examining Wright stained blood film. The completeness of each data was checked on daily base. The result of PLT indices was printed and registered on request prepared for this purpose

4.9. Data processing and analysis.

The socio-demographic and laboratory data were entered in epidata version 3, and then the data were exported and analyzed using SPSS version 21. The Kolmogorov-Smirnov normality test was run for checking the distribution of PLT indices. Kruskal-Wallis H test in conjunction with the Mann Whitney U test was used for comparison of non-normally distributed parameters and the results was presented as median and minimum and maximum value. Benforeni, post-hoc test was done for comparison of PLT indices across the three groups of women (severe preeclampsia, non-severe preeclampsia and normal pregnant women). ROC curve was done to determine sensitivity, specificity; AUC and cut of value for a given PLT indices (PLT, MPV, PDW and PCT) in discriminating the presence or the absence of preeclampsia. Based on estimations of sensitivity and specificity for cut-off value of the PLT indices, PPV and NPV for each of them were determined using MedCalc©. Statistical software version19.0.4 where, sensitivity and specificity were obtained from ROC curve and known case with disease was60 and known case without disease was 120. Spearman rank order test was used to evaluate correlation between PLT indices with MAP. A P-value of 0.05 was considered as statistically significant and the data was described by figures and tables

4.10. Ethical clearance

The study was conducted after it was ethically reviewed and approved by Institute of Health Ethical Review board (IRB) Jimma University. By getting permission from Hadiya zone health office and WUNEMMRH hospital. The study's' aims, risks, benefits and right for withdrawal anytime from the study was explained for the study participants and informed consent was obtained. Samples were coded and confidentiality of patient data was maintained throughout the study by locking hard copies and password protecting electronic files.

4.11. Dissemination of the result

The finding of this study was presented and submitted to Jimma University, Institute of Health, School of Medical Laboratory Science and WUNEMMRH so as to encourage for using PLT indices in medical practice for diagnosing preeclampsia in pregnant woman. The study abstract will be submitted to local associations like Ethiopian Medical Laboratory Association to present the results of the survey during continuous medical education events organized through this association. Findings also will be presented in relevant workshops, seminars and scientific conferences. The manuscript will be submitted to national or international journal for publication.

5.RESULT

5.1. Characteristics of study participants

A total of 180 study participants from two groups were recruited in the study. The first group included 120 normotensive pregnant women and the second group 60 pregnant women with preeclampsia. Out of 60 cases, 30 of them were non-severely pre-eclamptic and the remaining 30 cases were severely preeclamptic. The median (min-max) age of the normotensive, non-severe and severe preeclampsia group was 25.00(20-36), 28.00(18-37) and 28.50(18-39) years, respectively. In this study, no statistically significant differences were observed between the three groups in age, residence, number of pregnancies (gravidity), number of deliveries (parity), gestational age and BMI, but there was a significant difference between the three studied groups with regards to SBP, DBP and MAP which increased with severity of preeclampsia (P<0.001) (Table1).

Table 1: -Socio-demographic and Clinical Characteristics of Study Participants in Wachemo University Nigest Eleni Mohammed Memorial Referral Hospital from January to April 2019

CHARACTERISTICS	NORMOTENSIVE (N=120)	NON-SEVER PRECLAMPTIC(N=30)	SEVERE-PRECLAMPTIC(N=30)	P-VALUE
AGE (full year)	25(20-36)	28(18-37)	29(18-39)	0 .088
Urban	64(69.5%)	16 (17.3%)	12(13%)	
RESIDENCE				0.413
Rural	56(63.6%)	14(15.9%)	18(20.4%)	
GRAVIDITY	1(1-5)	2(1-6)	2(1-6)	0.896
PARITY	1(0-4)	1(0-5)	0(0-5)	0.690
BMI(KG/M^2)	24.60(21-31)	25.9(20.2-30.7)	25.1(20.6-30.8)	0.84
SBP(MM/HG)	114(93-137)	143(130-159)	160.50(160-170)	<0.001
DBP(MM/HG)	72(50-93)	100 (90-119)	111.50(100-129)	<0.001
MAP(MM/HG)	85.16(53-119)	113.83(103.3-131)	128.00(120.33-141.33)	<0.001
PROTENUIRIA	0	2(1-3)	3(1-3)	<0.001

"P-value is significant at level of 0.05, "The result is expressed with median (min-max) and number (%)"

5.2. Platelet Indices among pregnant mothers

Level of thrombocytopenia among study participants

According to this study, twenty-eight (28) of the study participants were thrombocytopenic (PLT<$150\times10^3/\mu l$) which accounts 15.6%. Whereas 152 of the total study participants appeared with normal PLT count ($150\times10^3/\mu l$-$450\times10^3/\mu l$) and accounts 84.4%. The level of thrombocytopenia in sever preeclampsia case, cases with non- severe feature and normotensive pregnant women accounts 6/120, 8/30 and 14/30 for normotensive, non-severe preeclampsia and sever preeclampsia respectively(Figure2).

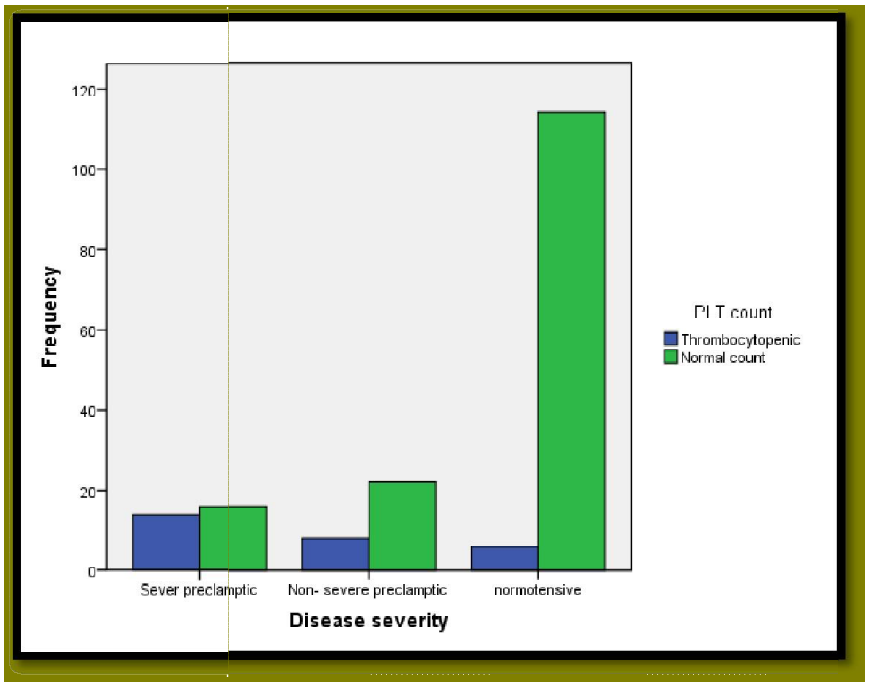


Figure2: Frequency of thrombocytopenia among study participants in WUNEMMRH from January to April 2019

5.3. Comparison of platelet indices across preeclamptic and normotensive pregnant women

The median (min-max) value of PLT and PCT were significantly lower in preeclamptic pregnant women as compared with normotensive women. The value of PLT accounts 170(97-352) $\times 10^3/\mu l$ for preeclamptic women and 251(139-445) $\times 10^3/\mu l$ for normotensive pregnant women ($p<0.001$). The value of PCT for the two groups of pregnant women was 0.1530(0.016-0.292) % for preeclamptic women and 0.1975(0.098-0.398) % for normotensive pregnant women ($p<0.001$). Whereas MPV and PDW were significantly higher in preeclampsia group than control group. The value of MPV among preeclamptic women was 9.25 (8-12.5) and its value among normotensive pregnant women was 8(6.9-9.3) fl. The level of PDW among preeclamptic women was 16.250(15.5-18) and for normotensive pregnant women, its value was 15(14-16.1) fl with ($p<0.001$) in Mann-Whitney U test (Table2).

Table2: - Comparisons of platelet indices between normotensive and preeclamptic pregnant women in Wachemo University Nigest Eleni Mohammed Memorial Referral Hospital from January to April 2019

Platelet indices	Normotensive pregnant women	Preeclamptic pregnant women	P-value
PLT$\times 10^3/\mu l$	251(139-445)	170 (97-352)	<0.001
MPV(fl)	8(6-9.3)	9.25(8-12.5)	<0.001
PDW(fl)	15(14-16.1)	16.2(15-18)	<0.001
PCT (%)	0.1975(0.098-0.398)	0.153(0.016-0.292))	<0.001

"P-value is significant at level of 0.05. The result is expressed with median (min-max) value.

5.4. Comparing platelet indices between normotensive, non- severe and sever preeclamptic pregnant women.

In Kruskal-Wallis H test, MPV and PDW have shown significant differences among the three groups. The values were significantly elevated as the disease severity advances ($p<0.001$). In this study, the PLT count was significantly decreased as disease progressed from normal, non-sever to severe stage with the values 251(139-445), 196.50(110-352) and 155(97-230) for normotensive, non-sever and severe preeclamptic pregnant women, respectively ($p<0.001$) (Table3).

Table 3: _ Comparisons of platelet indices among normotensive, non- severe and severe preeclamptic pregnant mothers in Wachemo University Nigest Eleni Mohammed Memorial Referral Hospital(WUNEMMRH) from January to April 2019

PLATELET INDICES	NORMOTENSIVE PREGNANT WOMEN	NON-SEVERE PREECLAMPSIA GROUP	SEVERE PREECLAMPSIA GROUP	P-VALUE
PLT×10^3/µl	251(139-445)	196.5(110-352)	155(97-230)	<0.001
MPV(fl)	8(6-9.3)	9(8-10.4)	9.6(8-12.5)	< 0.001
PDW(fl)	15(14-16.1)	16.0(15-17.1)	16.5(15.9-18)	<0.001
PCT(%)	0.1975(0.098-0.398)	0.166(0.111-0.292)	0.146(0.016-0.207)	<0.001

"The result is expressed with median (min-max) value", "P-value is significant at level of 0.05 level.

5.5. Inter group analysis of platelet indices across normotensive, non-severe and severe preeclamptic pregnant women.

In Bonferroni pair wise comparison tests between groups, there were statistically significant differences among the normotensive and non- severe preeclampsia group, normotensive and severe preeclampsia groups as well as non-severe preeclampsia group and severe with regard to PLT and PCT which showed significantly declining value with the severity of disease, whereas MPV and PDW significantly increased with the severity of preeclampsia (Table4)

Table4: _ Pair wise comparisons of plateletindices among normotensive, non-severe and severe preeclamptic pregnant women in Wachemo University Nigest Eleni Mohammed Memorial Referral Hospital from January to April 2019 (post hock)

PLATELET INDICES	NORMOTENSIVE	NON-SEVERE	SEVERE	*P-VALUE	**PVALUE	***P-VALUE
PLT×10^3/µl	251(139-445)	196.5(110-352)	155(97-230)	0.019	<0.001	<0.001
MPV(fl)	8(6-9.3)	9 (8-10.4)	9.6(8-12.5)	< 0.001	< 0.001	< 0.001
PDW(fl)	15(14-16.1)	16.0((15-17.1)	16.5(15.9-18)	0.018	< 0.001	< 0.001
PCT(%)	0.1975(0.098-0.398)	0.166(0.111-0.292)	0.146(0.016-0.207)	0.045	< 0.001	0.004

*P- comparison of non- severe and severe preeclampsia, p-value was significant at 0.05.

**p- comparison of severe preeclampsia group and normotensive pregnancy.

***p- comparison of non- severe preeclampsia group and normotensive pregnancy

5.6. Co -relational analysis of platelet indices with mean arterial pressure in study participants

In this study, the spear man rank order correlation of PLT indices with the MAP was computed to evaluate their association with severity of disease. In correlation analysis, a MAP showed statistically significant positive correlations with PDW (rho =0.731, p <0.001), and MPV (rho=0.674, p<0.001). Moreover, MAP showed significant negative correlation with PLT (rho= -0.503 and PCT (rho=-0.369, p<0.001) (Table5)

Table5: - Correlation between Platelets indices with Mean Arterial Pressure for the study participants in Wachemo University Nigest Eleni Mohammed Memorial Referral Hospital from January to April 2019

	PLATELET INDICES			
	PLT	MPV	PDW	PCT
MEAN ARTERIAL PRESSURE(MAP)	**rho** -0.503	0.674	0.731	-0.369
	<0.001	<0.001	<0.001	<0.001

Correlation is significant at the 0.01 level (2-tailed)

5.7. The diagnostic role of platelet indices for preeclampsia

The ROC curve analysis was used to determine the optimal cut-off values of PLT indices for prediction of preeclampsia. The analysis showed that PLT can differentiate normotensive pregnant women from preeclamptic pregnant women at a cut off value ≤224x10 $^{3/}$µl with sensitivity of 88.3%, specificity of 64.2%, PPV of 71.1% and NPV of 84.5% with an AUC of 0.858. Whereas MPV can differentiate normotensive pregnant women from preeclamptic pregnant women at a cut off value ≥8.55fl with sensitivity of 86.6 %, specificity of 89.2%, PPV of 88.9%, NPV of 86.94% while PDW can differentiate normotensive pregnant women from preeclamptic pregnant women at a cut off value of ≥15.45 with sensitivity of 98.3 %, specificity of 91.7%, PPV of 67.42%, and NPV of 96.86%. The PCT at cut off value of ≤0.1915% with sensitivity of 83.3%, specificity 52.5%, PPV of 63.68% and NPV of 75.86% can differentiate normotensive pregnant women from preeclamptic women. PDW has largest area under the curve (AUC=0.986; 95%CI (0.970, 1), indicating as it is the best parameter for predicting preeclampsia. The second most important predictor identified was MPV (AUC=0.954; 95%CI (0.925, 0.984) followed by PLT (AUC=0.858; 95%CI (0.801, 0.916) (Figure 3 and table6).

Table6: -The Diagnostic values of plateletindices for preeclampsia among study participants in Wachemo University Nigest Eleni Mohammed Memorial Referral Hospital from January to April 2019.

Platelet indices	Sensitivity(%	Specificity (%)	PPV (%)	NPV (%)	Cut-off value	AUC	(95%CI)	P-Value
PLT	88.3	64.2	71.1	84.5	≤224	0.858	(0.801,0.916)	<0.001
MPV	86.6	89.2	88.9	86.94	≥8.55	0.954	(0.925,0.984)	<0.001
PDW	98.3	91.7	67.42	96.86	≥15.45	0.986	(0.984, 1.000)	<0.001
PCT	83.3	52.5	63.68	75.86	≤0.1915	0.779	(0.707,0.851)	<0.001

P-value is significant at level0.05.

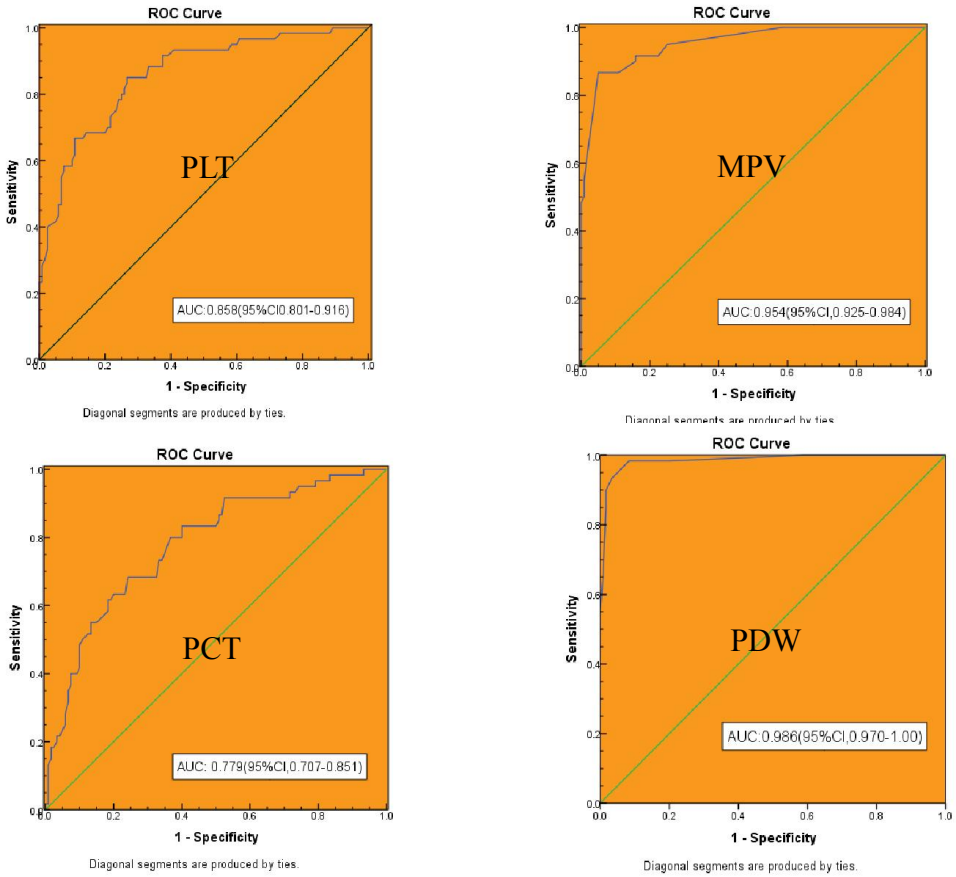


Figure3:The ROC curve analysis of PLT indices for study participants in WUNEMMRH from January to April 2019.

6. DISCUSSION

The current study noted significantly low level of PLT and PCT while higher MPV and PDW among preeclampsia group than normal pregnancy as well as this value was more pronounced with severity of the disease. As the severity indicators, PDW and MPV showed positive correlation with MAP, where as PLT and PCT showed negative correlation. From ROC curve analysis, PDW has highest AUC, which was followed by MPV, PLT and PCT respectively. Among the total study participants, twenty-eight (28) of them appear with thrombocytopenia and level of thrombocytopenia increased with severity of hypertension. That means 14 cases of thrombocytopenia occurred among severe preeclampsia group ,8 cases in non-severe preeclampsia group and 6 cases was observed among normotensive pregnant women. This can persuade scenario of absence of proteinuria may not exclude preeclampsia as stated by revised guide line of study of hypertension in pregnancy(58).

The median of 170 (97-352) $\times 10^3/\mu l$ versus 251(139-445) $\times 10^3/\mu l$ represented PLT among preeclamptic pregnant women and normotensive pregnant women respectively ($p<0.001$) and 251(139-445), 196.5(110-352) and 155(97-230) $\times 10^{3/}\mu$ of PLT among normotensive pregnant women, non-severe preeclampsia and severe preeclampsia group respectively ($p<0.001$). This is because of increased burden on placental endothelium following severity of disease, result in increased vascular reactivity and PLT activation in turn give rise to consumption of PLT(14).

Concordant finding with our study was documented from study done in Korea which revealed lower PLT in preeclamptic pregnant women than normotensive pregnant women, but unlike our finding, the study didn't found significant difference between normotensive and non- severe preeclamptic pregnant women(35). Similar finding with our study was documented from study done in Brazil(40). Another studies done in India reported similar finding(36) and the study in similar area India(37) reported also concordant finding with our data . Our result was also in agreement with similar study done in Egypt(51) and the finding was also confirmed by study from similar area which relate severity of preeclampsia with decreasing level of PLT (52). The study from Ethiopia also noted the depletion of PLT with severity of preeclampsia(54).

On the other hand, in harmony withour result, the study done in turkey revealed significantly lower PLT among non-severe preeclampsia group than normotensive group with ($p=0.0008$, $p<0.01$) study, but in contrary to our finding, this study was not observed significant difference between severe preeclampsia group and normotensive pregnant women regarding this parameter ($p = 0.086$; $p>0.05$) as well as between non -severe and severe preeclampsia group(34).Similar with this study, but different from our result, the study done in turkey didn't found significant difference with regard to PLT between preeclamptic and normotensive group as well as the significant difference was not observed in terms of severity of disease(22). Another study also didn't observed significant difference with regard to PLT among preeclampsia group and normotensive controls(45). The more likely cause for conflicting result might be the variation in sample collection time, the time dependent PLT activation due to maximal delay between sample collection and analysis which not concern our study, because all the samples were collected and analyzed within not more than one hour. The effect of anticoagulant used and the variation in hematological analyzer and small sample size which may affect evaluating power of real difference.

In accordance with our study, the study done in Sudan documented lower PLT among preeclamptic pregnant women than normotensive pregnant women, but in contrary to our finding, the values were not statistically significant(53).The cause for inconsistent finding might be the presence of confounders in their study that have similar effect on PLT as preeclampsia even in normotensive pregnant women, so it is difficult to discriminate change in PLT indices between the groups. Another likely cause might be the small sample size that they compared PLT indices between 37 preeclamptic and 50 normotensives on a total of 87 study participants. This is in contrary to understanding power and rules of thumbfor determining sample sizes recommended by VanVoorhis CW, Morgan BL(59).

The current study revealed 28 thrombocytopenia cases 6 in normotensive group, 8 in non-severe preeclampsia group and 14 among severe preeclampsia groups. This was comparable with the finding of study documented 30 cases, 9 in non-severe preeclampsia patients and 21 cases in severe preeclampsia, but unlike our finding this study not found thrombocytopenia in normotensive group(37). But slightly larger value of 33 case among severe preeclampsia group

was documented from another study(38). But smaller value of 16 thrombocytopenia cases among study subjects was documented in another study(48). While lower value of 11 case was documented from another study(50). But larger report of 56% thrombocytopenia case was documented from study done in India among preeclampsia cases(39). In contrary to our finding studies such as (40, 43) noted normal range of PLT count among all study participants. Furthermore, studies not found thrombocytopenia among normotensive pregnant women, unlike our study. But our study document 6 cases of thrombocytopenia among normotensive pregnant women which is 5%. This might be due to gestational thrombocytopenia because it can occur in approximately 8%-10% of all pregnancies due to hemo-dilution, aggregation and consumption of PLT but in preeclampsia it is more correlated with severity of hypertension. It is a common clinical manifestation of decreased bone marrow production, increased spleen sequestration and accelerated destruction of PLT, but in preeclampsia, the third cause is suspected due to placental-vascular endothelial activation, but unknown mechanism also can lead to the phenomena(60).

In our study, the PLT cutoff value was $\leq 224\times10^3/\mu l$ to discriminate preeclamptic women from normotensive pregnant women with sensitivity, specificity, PPV, NPV of 88.3%, 64.2%, 71.15% and 84.5% respectively with an AUC of 0.858. The finding was inconsonance with the study done in Brazil which documented cutoff value $\leq 221\times10^3/\mu l$ with sensitivity of 68.97% , specificity of 70.69% and AUC of 0.73 with ($p < 0.001$)(40). Whereas slightly larger cut off value of $\leq 248\times10^3/\mu l$ was documented from study done in Saud Arabia(43) ,but smaller cut off value of $\leq 198{,}000/\mu l$ was reported by study done in Egypt with a sensitivity of 90% and specificity of 92% to differentiate normotensive pregnant women from non-severe-preeclamptic patients (51). Concordant finding with our result was documented from another similar study done in Egypt, which documented cut off value of $\leq 233\times10^3/\mu l$ with sensitivity of 81.5 7,specificity of 8.3%(52) but unlike our study it documented lower AUC of 0.17. The cause might be difference in hematological analyzer. Because some analyzers can over estimate some parameters and under estimate than others(23) Similar to this finding but slightly smaller cut off value of $\leq 190\times10^9/l$ than our data, with AUC of 59.3% , was reported from study done in turkey(34). Therefore the current study noted PLT has significant role in discriminating presence

or absence of preeclampsia despite few studies in literature didn't found significant difference(53).

The current study documented lower PCT value of 0.153(0.016-0.292) % ($p<0.001$) among preeclamptic pregnant women than 0.1975(0.098-0.398) % for normotensive pregnant women. Whereas the values 0.1975(0.098-0.398) %, 0.166(0.111-0.292(%, 0.146(0.016 0.207) % represent normotensive, non-severe preeclampsia group and severe preeclampsia group respectively. In agreement with this finding, study done in Korea revealed decreased level of PCT among preeclamptic than normotensive pregnant women (35) but unlike our study significant difference was not observed between normotensive and non-sever preeclampsia group. Similar finding with our study was documented from Brazil(40). But in contrary to current study, significant difference between preeclamptic and normotensive pregnant women was not observed from the study done in turkey(22) and similar to this finding but different from our result, study from Sudan didn't observe significant difference between preeclamptic women than normotensive pregnant women (53).

Based on our finding at cut of value of ≤0.1915% with sensitivity of 68.3% and specificity of 69.2%, PCT can differentiate normotensive pregnant women from preeclamptic pregnant women and it has AUC of 0.776, ($p <0.001$). This makes it as good marker to predict preeclampsia. Comparable cut off value of ≤0.179% was documented from Brazil with sensitivity of 55.17% ,specificity of 84.48% and AUC of 0.69 ,($p =0.0007$) but unlike our study the authors concluded it was not the good candidate marker for prediction of preeclampsia as compared to other PLT indices(40). Similar to this finding, but unlike our result, AUC of 0.37 was documented from study done in Korea(35). In agreement with our finding another study documented PCT as best marker for prediction of preeclampsia(61). Therefore, our result persuade, PCT tends to decrease with severity of preeclampsia. Because rate of PCT under 0.1% is an indirect sign of PLT transfusion and it is a more specific determinant than PLT number in thrombocytopenic patients including preeclampsia(62).But inconsistencies between researches need further scientific study on this field.

According to the finding of current study, the value of MPV was higher in preeclamptic women as compared to normotensive women. It accounts, 9.25(8-12.5) fl and 8(6-9.3) fl ($p<0.001$) for preeclamptic women and normotensive women respectively. This value was also increased with severity of hypertension and accounts 8(6-9.3) fl, 9(8-10.4) fl, 9.6(8-12.5) fl with ($p<0.001$) for normotensive, non –severely preeclamptic and sever preeclampsia group respectively. This is due to marrow production of young PLT with large volume as compensatory mechanism for consumption and destruction of PLT in preeclampsia (43).Our Result is in agreement with finding from Turkey which documented significantly higher value of MPV in preeclamptic pregnant women as compared to the normotensive pregnant women(22).In consonance with our result, MPV increased as the disease severity increased from study done in Korea(35). Similar finding was documented from India(36). In accordance with our result study from done in turkey documented significantly higher MPV between the severe preeclampsia group and control group ($p = 0.009$; $p<0.01$) but in contrary to our study, significant difference was not observed between non severe preeclampsia group and control group(34).Though the current finding was supported by similar studies (40),(46), (47), (48), (51),(52),(53),(54),inconsistent result was documented from(43),(44),(45),(49). The inconsistency between results of different studies might be due to difference in analyzer used and effect of anticoagulant. Because derived parameters of PLT are highly dependent upon the individual technology and are influenced by the anticoagulant and delay time from sampling to analysis. That means in impedance counting the MPV rises over time as the PLT swell in EDTA, can increase of 7.9% within 30 minute(23).

From ROC analysis, at cut off value ≥8.55fl, MPV can differentiate preeclamptic pregnant women from normotensive pregnant women with a sensitivity of 86.6%, specificity of 89.2%, PPV of 88.9% and NPV of 8.9%. As noted by the study, this value has AUC of 0.954. Comparable result ≥8.35 fl was documented from study conducted in turkey with sensitivity of 68% and specificity of 52% and AUC of 0.603 for prediction of preeclampsia(22). But slightly larger value of ≥9.3fl, was reported from Egypt with sensitivity of 90.0% and specificity of 92%, and with AUC of 0.885(51). While another study in Egypt document value of ≥9.5fl with sensitivity of 92.6%, specificity of 87.0% and with AUC of 0.940 to predict the development of preeclampsia(52). Comparable cut off value of >9.6fl with this study, but larger than our study was documented from study done in Brazil. This value has sensitivity of 51.72%, specificity of

82.76% and AUC of 0.72(40). Therefore, in view of this finding, MPV can be the good candidate marker to discriminate the presence or absence of preeclampsia though conflicting report for disease prediction along with PDW in literature(63). In response to PLT activation and consumption in preeclampsia, immature PLT which inter circulation have functional modification than mature PLT, because they are active metabolically as well as enzymatically(64). Furthermore, as concluded by systematic review conducted in Italy, the function and role in thrombosis related condition such as hypertension in pregnancy must be well understood for PLT as well as periodic monitoring of markers such as MPV play great role in controlling the incidence of complications(65). So not only the PLT count but also its function should be carefully assessed in diagnosis of preeclampsia for future.

The current study documented significantly increased value of PDW in preeclamptic pregnant women as compared to normotensive pregnant women and showed gradual increase as disease severity increased. In harmony with our study, similar study done in Brazil documented increased level of MPV and PDW in pre-eclampsia as compared to control group (40) and the same finding was documented from study done in India(36). Concordant finding was documented from similar study done in Korea which revealed increased level of PDW as the disease severity progressed(35).But unlike our finding, similar study conducted in Turkey didn't found significant difference between preeclamptic women and normotensive women(34) ,but recent cohort study done in India, revealed increasing level of PDW and MPV along with decreasing of PLT as pregnancy advances, and these changes are more pronounced in preeclampsia(48). In contrary to our finding another study from Saud Arabia didn't document significant difference regarding this parameter between preeclamptic and normotensive women(43), but authors in another study revealed PDW as simple, specific and practical marker of activation of coagulation(66). Other studies which documented similar finding with our study were (42), (44),(46),(47),(51),(52),(53),(54).

Therefore, PLT activation in preeclampsia might be the cause for increased level of PDW. Because it gives rise to size anisocytosis due production of large PLT in response to consumption. Another is the heterogeneity in morphology of PLT upon activation, due to development of pseudopodia(67). This in turn can increase the distribution. This is why PDW

was higher in preeclampsia group than normotensive pregnant women. The conflict in report between studies might be due difference in hematological analyzers used.

Based on the finding of current study, the cutoff value of ≥15.4fl for PDW can discriminate preeclamptic pregnant women from normotensive pregnant women with sensitivity of 98.35%, specificity 91.7%, PPV 67.42% and NPV 96.86%($p<0.001$). This value has AUC of 0.986. In harmony with our finding, study done in Korea documented comparable cutoff value of >13.5fl as the optimal cut-off level to the predict severity of preeclampsia with AUC of 0.74(35).But unlike or finding, smaller AUC 0.611 for PDW was documented from study done in turkey which accounts the sensitivity of 64%, specificity of 50%, PPV of 65.8% and NPV of 48.1 % for prediction of preeclampsia(22).Unlike this finding, but comparable with our result, cutoff value of ≥18.3fl,was documented from study done in Brazil as optimal cut-off point for prediction of preeclampsia with sensitivity of 55.17%, specificity of 86.21% and AUC of 0.77 (40). Our study documented slightly larger cut off value than study done in Egypt, which revealed cut of value of ≥ 12.6fl with sensitivity 90%, specificity 92%, PPV 91.8% NPV 90.2% and AUC of 0.886 to discriminate normotensive women from non-severe-preeclamptic women(51). Another similar study done in Egypt revealed the cut off value of ≥19.9fl, with sensitivity of 96.3, specificity of 91.3 and AUC of 0.980(52), which confirmed finding of our data with regard to AUC, that due to its large AUC this parameter was found as the most important predictor of preeclampsia as compared to other indices.

Therefore, despite conflicting reports regarding PDW in the literature, most studies explained its relation with preeclampsia. Our study also found it was the outstanding parameter for prediction of preeclampsia due to its large AUC. Because as the general rule of thumb for interpreting AUC, for evaluating the diagnostic ability of a test in discriminating the true disease status of a patient, AUC of 0.5 indicates no discrimination, 0.6≥AUC>0.5 indicates poor discrimination, 0.7≥AUC>0.6 indicates acceptable discrimination whereas 0.8≥AUC>0.7 indicates excellent discrimination but AUC>0.9 indicates outstanding discriminationas recommended by Yang S, Berdine G(68).In view of this information, PDW and MPV were the outstanding discriminators of presence or absence of preeclampsia whereas PLT and PCT were excellent discriminators of presence or absence of preeclampsia. So, attention should be given to these parameters during

diagnosis of preeclampsia because the indices especially PDW and PCT were the newly added PLT markers for the diagnosis of an adverse outcome in preeclampsia even in women presenting with normal PLT counts(26).

Regarding correlation of PLT indices with MAP, our study noted significant positive correlation between PDW and MPV with MAP with ($r = 0.731$, $p < 0.001$) and ($r = 0.674$; $p < 0.001$) for PDW and MPV respectively. According to our data, significant negative correlation was observed between MAP with PLT ($r = -0.503$, $p < 0.001$) and PCT ($r = -0.369$, $p < 0.001$). In consonance with our result, study done in Korea revealed statistically significant positive correlation PDW with MAP with ($r = 0.231$, $p < 0.05$) but in contrary to our result, other PLT indices were not showed significant correlation(35) . But another study documented, MPV as the severity marker of preeclampsia(69). Concordant finding with our study was documented from study done in Egypt, which found statistically significant positive correlation of PDW ($r = 0.902$, $p = 0.000$) and MPV ($r = 0.475$; $p = 0.000$) with MAP and noted MPV as possible modest marker and PDW as thebest marker for predicting severity of hypertension(52). Similar with current study, finding from study done in Asia noted negative correlation of MAP with PLT, while positive correlation was observed in MPV and PDW(46).Another study done in Datta Meghe confirmed this finding(47).In harmony with our finding study done in Gondar also revealed the positive correlation between PDW and MPV with MAP ($r = 0.43$, $p < 0.001$) and ($r = 0.37$; $p < 0.001$ for PDW and MPV respectively(54).

Therefore, in view of our result, the inflammatory role PLT in preeclampsia can be the likely cause for making these indices as indicator and predictor of severity of preeclampsia. Furthermore, rather than relying on PLT count alone, using all the PLT indices in combination while diagnosing preeclampsia may guarantee reliable diagnosis, since they compensated limitation of one another. In light of current study, likely cause for thrombocytopenia observed among the study participants, might be consumption of PLT as well as hemo- dilution, however, for the sake of the scope, the study didn't assessed another contributing factors for occurrences of thrombocytopenia, so this area and applying PLT functional test on preeclamptic patients in comparison with normotensive pregnant women need additional study in feature especially to

quantify the level of markers such as CD31, CD40L, CD42a, CD61, CD62 and CD63 using flow cytometry principle to substantiate their role in the diagnosis of preeclampsia.

Even though, examining the samples on microscope to exclude the error of machine and to appreciate morphological changes of PLT especially on preeclamptic patients, analyzing sample with minimum delay to reduce likelihood of time dependent PLT activation as well as incorporating somePLT indices such as PCT which is not studied as many times as other indices in preeclampsia were among the strength of current study, the study was not free from some limitations. Since all hematological analyzers didn't include all PLT parameters, this study was not included platelet- large cell ratio as the parameters. The study was not longitudinal to include serial analysis of PLT indices throughout different trimesters to evaluate whether it is possible to predict preeclampsia before second half of the pregnancy and small sample size for this cross-sectional time.

7. CONCLUSION AND RECOMMENDATION

7.1. CONCLUSION

In summary, PLT indices such as PLT, MPV, PDW and PCT were identified as good candidate markers for prediction of preeclampsia and can be the criteria to diagnose the disease. Increasing of MPV and PDW, decreasing of PLT and PCT showed significant change with severity of preeclampsia. Their pattern not only predicts development of preeclampsia but also show severity of hypertension. The PDW with largest AUC was the main parameter in predicting preeclampsia and the MPV found to be the next.

7.2. RECOMMENDATION

- Large scale longitudinal study should be conducted in study area for serial analysis of PLT indices throughout different trimesters to evaluate whether it is possible to predict preeclampsia before second half of the pregnancy

- The PLT indices such as PLT, MPV, PDW and PCT should be part of routine antenatal investigation, since it can play its role in combating bleeding problem as well as help to induce delivery irrespective of gestational age due to thrombocytopenia in preeclampsia.
- Comparative scientific study should be conducted on quantifying the selected PLT markers such as CD31, CD40L, CD42a, CD61, CD62 and CD63 inpreeclamptic women as compared to normotensive pregnant women to substantiate their role for diagnosis of preeclampsia

REFRENCES

1. American College of Obstetricians and Gynecologists. Hypertension in pregnancy. Report of the American College of Obstetricians and Gynecologists' task force on hypertension in pregnancy. Obstetrics and gynecology. 2013;122(5):1122.

2. Gongora MC, Wenger NK. Cardiovascular Complications of Pregnancy. Int J Mol Sci. 2015;16(10):23905-28.

3. Leveno K, Bloom S, Spong C, Dashe J. Williams Obstetrics 24th edition McGraw-Hill Education. Medical; 2014.

4. Say L, Chou D, Gemmill A, Tunçalp Ö, Moller A-B, Daniels J, *et al*. Global causes of maternal death: a WHO systematic analysis. The Lancet Global Health. 2014;2(6): e323-e33.

5. Ahmad AS, Samuelsen SO. Hypertensive disorders in pregnancy and fetal death at different gestational lengths: a population study of 2 121 371 pregnancies. BJOG. 2012;119(12):1521-8.

6. Eiland E, Nzerue C, Faulkner M. Preeclampsia 2012. Journal of pregnancy. 2012;2012.

7. Townsend R, O'Brien P, Khalil A. Current best practice in the management of hypertensive disorders in pregnancy. Integr Blood Press Control. 2016; 9:79-94.

8. Wagnew M, Dessalegn M, Worku A, Nyagero J. Trends of preeclampsia/eclampsia and maternal and neonatal outcomes among women delivering in addis ababa selected government hospitals, Ethiopia: a retrospective cross-sectional study. The Pan African medical journal. 2016;25(Suppl 2).

9. Kaur S, Khan S, Nigam A. Hematological profile and pregnancy: a review. International Journal of Advances in Medicine. 2014;1(2):1.

10. Han L, Liu X, Li H, Zou J, Yang Z, Han J, *et al.* Blood coagulation parameters and platelet indices: changes in normal and preeclamptic pregnancies and predictive values for preeclampsia. PloS one. 2014;9(12): e114488.

11. Habas E, Rayani A, Ganterie R. Thrombocytopenia in hypertensive disease of pregnancy. J Obstet Gynaecol India. 2013;63(2):96-100.

12. Chaiworapongsa T, Chaemsaithong P, Yeo L, Romero R. Pre-eclampsia part 1: current understanding of its pathophysiology. Nat Rev Nephrol. 2014;10(8):466-80.

13. Mirkovic L, Nejkovic L, Micic J. A new pathophysiological concept and new classification of pre-eclampsia. Vojnosanitetski pregled. 2018;75(1):83-94.

14. ONISAI M, VASILACHE V. The endothelial-platelet dysfunction in preeclampsia. Mædica A Journal of Clinical Medicine. 2007;2(3):214.

15. Sahin S, Ozakpinar O, Eroglu M, Tetik S. Platelets in Preeclampsia: Function and Role in the Inflammation. Journal of Marmara University Institute of Health Sciences. 2014:1.

16. Jakobsen C, Larsen JB, Fuglsang J, Hvas A-M. Platelet function in preeclampsia–a systematic review and meta-analysis. Platelets. 2019:1-14.

17. Jena M, Mishra S, Jena S, Pradhan S, Das S, Jena J, et al. Pregnancy induced hypertension & pre eclampsia: Pathophysiology & recent management trends: A review. International Journal of Pharmaceutical Research & Allied Sciences. 2016;5(3).

18. Meads C, Cnossen J, Meher S, Juarez-Garcia A, Ter Riet G, Duley L, *et al.* Methods of prediction and prevention of pre-eclampsia: systematic reviews of accuracy and effectiveness literature with economic modelling. 2008.

19. World Health Organization. WHO recommendations for prevention and treatment of pre-eclampsia and eclampsia. 2011.

20. Rana S, Powe CE, Salahuddin S, Verlohren S, Perschel FH, Levine RJ, *et al*. Angiogenic factors and the risk of adverse outcomes in women with suspected preeclampsia. Circulation. 2012;125(7):911-9.

21. Abbassi-Ghanavati M, Greer LG, Cunningham FG. Pregnancy and laboratory studies: a reference table for clinicians. Obstetrics & Gynecology. 2009;114(6):1326-31.

22. Kurtoglu E, Kokcu A, Celik H, Bildircin FD, Tosun M, Alper T, *et al*. Validity of platelet indices in predicting the risk of developing preeclampsia. Clinical Research. 2016;33(2):57-61.

23. Briggs C, Harrison P, Machin S. Continuing developments with the automated platelet count 1. International journal of laboratory hematology. 2007;29(2):77-91.

24. Budak YU, Polat M, Huysal K. The use of platelet indices, plateletcrit, mean platelet volume and platelet distribution width in emergency non-traumatic abdominal surgery: a systematic review. Biochem Med (Zagreb). 2016;26(2):178-93.

25. Birhaneselassie M, Birhanu A, Gebremedhin A, Tsegaye A. How useful are complete blood count and reticulocyte reports to clinicians in Addis Ababa hospitals, Ethiopia? BMC Blood Disorders. 2013;13(1):11.

26. Singh A, Varma R. Role of Platelet Distribution Width (PDW) and Plateletcrit in the Assessment of Nonthrombocytopenic Preeclampsia and Eclampsia. The Journal of Obstetrics and Gynecology of India. 2018;68(4):289-93.

27. Dolea C, AbouZahr C. Global burden of hypertensive disorders of pregnancy in the year 2000. GBD 2000 Working Paper, World Health Organization, Geneva. http://www. who …, 2003.

28. Bilano VL, Ota E, Ganchimeg T, Mori R, Souza JP. Risk factors of pre-eclampsia/eclampsia and its adverse outcomes in low- and middle-income countries: a WHO secondary analysis. PloS one. 2014;9(3):e91198.

29. Berhe AK, Kassa GM, Fekadu GA, Muche AA. Prevalence of hypertensive disorders of pregnancy in Ethiopia: a systemic review and meta-analysis. BMC Pregnancy Childbirth. 2018;18(1):34.

30. Gaym A, Bailey P, Pearson L, Admasu K, Gebrehiwot Y. Disease burden due to pre-eclampsia/eclampsia and the Ethiopian health system's response. Int J Gynaecol Obstet. 2011;115(1):112-6.

31. Brouwers L, van der Meiden-van Roest A, Savelkoul C, Vogelvang TE, Lely AT, Franx A, *et al.* Recurrence of pre-eclampsia and the risk of future hypertension and cardiovascular disease: a systematic review and meta-analysis. BJOG: An International Journal of Obstetrics & Gynaecology. 2018;125(13):1642-54.

32. Snydal S. Major changes in diagnosis and management of preeclampsia. J Midwifery Womens Health. 2014;59(6):596-605.

33. Poon LC, Nicolaides KH. Early prediction of preeclampsia. Obstet Gynecol Int. 2014; 2014:297397.

34. Doğan K, Guraslan H, Senturk MB, Helvacioglu C, İdil S, Ekin M. Can platelet count and platelet indices predict the risk and the prognosis of preeclampsia? Hypertension in pregnancy. 2015;34(4):434-42.

35. Yang SW, Cho SH, Kwon HS, Sohn IS, Hwang HS. Significance of the platelet distribution width as a severity marker for the development of preeclampsia. European Journal of Obstetrics & Gynecology and Reproductive Biology. 2014;175:107-11.

36. Annam V, Srinivasa K, Yatnatti SK. Evaluation of platelet indices and platelet counts and their significance in pre-eclampsia and eclampsia. Int J Biol Med Res. 2011;2(1):425-8.

37. Gupta A, Gaur BS, Mishra KB, Dubey I. A comparison of platelet count in severe preeclampsia, mild preeclampsia and normal pregnancy. International Journal of Research in Medical Sciences. 2018;6(2):671.

38. Sameer M, Meshram D, Deshpande S, Sadhu D, Pandit S. Role of platelet count as important prognostic marker in pregnancy induced hypertension. IOSR J Dent Med Sci. 2014;13:39-43.

39. Donimath K, Sambrani A, Rathod P. A study on association of thrombocytopenia with pregnancy induced hypertension. Int J Reprod Contracept Obstet Gynecol. 2016;5(3):808-12.

40. Freitas LG, Alpoim PN, Komatsuzaki F, Carvalho M, Dusse LM. Preeclampsia: are platelet count and indices useful for its prognostic? Hematology. 2013;18(6):360-4. Epub 2013/05/17.

41. Myatt L. 784: Platelet count and mean platelet volume in prediction of preeclampsia in a low risk population. American Journal of Obstetrics & Gynecology. 2009;201(6):S282.

42. Vijaya C, Lekha M, Shetty A, Geethamani V. Evaluation of platelet counts and platelet indices and their significant role in pre-eclampsia and eclampsia. Journal of Evolution of Medical and Dental Sciences. 2014;3(12):3216-20.

43. AlSheeha MA, Alaboudi RS, Alghasham MA, Iqbal J, Adam I. Platelet count and platelet indices in women with preeclampsia. Vasc Health Risk Manag. 2016;12:477-80. Epub 2016/12/07.

44. Toptas M, Asik H, Kalyoncuoglu M, Can E, Can MM. Are Neutrophil/Lymphocyte Ratio and Platelet/Lymphocyte Ratio Predictors for Severity of Preeclampsia? Journal of Clinical Gynecology and Obstetrics. 2016;5(1):27-31.

45. Ceyhan T, Beyan C, Baser I, Kaptan K, Gungor S, Ifran A. The effect of pre-eclampsia on complete blood count, platelet count and mean platelet volume. Ann Hematol. 2006;85(5):320-2.

46. Dadhich S, Agrawal S, Soni M, Choudhary R, Jain R, Sharma S, et al. Predictive value of platelet indices in development of preeclampsia. J SAFOG. 2012;4(1):17-21.

47. Bhavana T, Vishal K, Prashant T. Platelet indices in pregnancy induced hypertension. J Cont Med A Dent. 2016;4(3):20-6.

48. Dhakre R, Nandmer GK, Sapkal R. Correlation of platelet indices with severity of pre-eclampsia: a prospective study from central India. International Journal of Reproduction, Contraception, Obstetrics and Gynecology.2018;7(4):1417.

49. Amita K, Kumar HN, Shobha S, Shankar V. The role of platelet parameters as a biomarker in the diagnosis and in predicting the severity of preeclampsia. Indian Journal of Pathology and Oncology. 2015;2(2):57-60.

50. Revs IJMR. Evaluation of platelet count as a prognostic index in eclampsia and pre eclampsia. Int J Modn Res Revs. 2014;2(10):447-52.

51. Alkholy[1] Ea-M, Farag Ea, Behery Ma, Ibrahim[1] Mm. The Significance Of Platelet Count, Mean Platelet Volume And Platelet Width Distribution Inpreeclampsia. Aamj. 2013;11(1).

52. Nooh AM, Abdeldayem HM. Changes in Platelet Indices during Pregnancy as Potential Markers for Prediction of Preeclampsia Development. Open Journal of Obstetrics and Gynecology. 2015;05(12):703-12.

53. Abass A-E, Abdalla R, Omer I, Ahmed S, Khalid A, Elzein H. Evaluation of Platelets Count and Indices in Pre-Eclampsia Compared to Normal Pregnancies. IOSR Journal of Dental and Medical Sciences. 2016;15(07):05-8.

54. Sitotaw C, Asrie F, Melku M. Evaluation of platelet and white cell parameters among pregnant women with Preeclampsia in Gondar, Northwest Ethiopia: A comparative cross-sectional study. Pregnancy Hypertension. 2018; 13:242-7.

55. Lowe SA, Bowyer L, Lust K, McMahon LP, Morton M, North RA, et al. SOMANZ guidelines for the management of hypertensive disorders of pregnancy 2014. Australian and New Zealand Journal of Obstetrics and Gynecology. 2015;55(5).

56. Kundu R, Biswas S, Das M. Mean Arterial Pressure Classification: A Better Tool for Statistical Interpretation of Blood Pressure Related Risk Covariates. Cardiology and Angiology: An International Journal. 2017;6(1):1-7.

57. Mackie I, Cooper P, Lawrie A, Kitchen S, Gray E, Laffan M, *et al.* Guidelines on the laboratory aspects of assays used in haemostasis and thrombosis. International journal of laboratory hematology. 2013;35(1):1-13.

58. Tranquilli AL, Dekker G, Magee L, Roberts J, Sibai BM, Steyn W, *et al*. The classification, diagnosis and management of the hypertensive disorders of pregnancy: A revised statement from the ISSHP. Pregnancy Hypertens. 2014;4(2):97-104.

59. VanVoorhis CW, Morgan BL. Understanding power and rules of thumb for determining sample sizes. Tutorials in quantitative methods for psychology. 2007;3(2):43-50.

60. Jodkowska A, Martynowicz H, Kaczmarek-Wdowiak B, Mazur G. Thrombocytopenia in pregnancy–pathogenesis and diagnostic approach. Advances in Hygiene & Experimental Medicine/Postepy Higieny i Medycyny Doswiadczalnej. 2015;69.

61. Karateke A, Kurt RK, Baloğlu A. Relation of platelet distribution width (PDW) and platelet crit (PCT) to preeclampsia. Ginekologia polska. 2015;86(5).

62. Mohr R, Martinowitz U, Golan M, Ayala L, Goor D, Ramot B. Platelet size and mass as an indicator for platelet transfusion after cardiopulmonary bypass. Circulation. 1986;74(5 Pt 2):III153-8.

63. Xu RL, Zheng ZJ, Ma YJ, Hu YP, Zhuang SH. Platelet volume indices have low diagnostic efficiency for predicting bone marrow failure in thrombocytopenic patients. Exp Ther Med. 2013;5(1):209-14.

64. Ibrahim H, Schutt RC, Hannawi B, DeLao T, Barker CM, Kleiman NS. Association of immature platelets with adverse cardiovascular outcomes. Journal of the American College of Cardiology. 2014;64(20):2122-9.

65. Juan P, Stefano G, Antonella S, Albana C. Platelets in pregnancy. Journal of prenatal medicine. 2011;5(4):90.

66. Vagdatli E, Gounari E, Lazaridou E, Katsibourlia E, Tsikopoulou F, Labrianou I. Platelet distribution width: a simple, practical and specific marker of activation of coagulation. Hippokratia. 2010;14(1):28.

67. Golebiewska EM, Poole AW. Platelet secretion: From haemostasis to wound healing and beyond. Blood Rev. 2015;29(3):153-62.

68. Yang S, Berdine G. The receiver operating characteristic (ROC) curve. The Southwest Respiratory and Critical Care Chronicles. 2017;5(19):34-6.

69. Gameti DPV. Role of Mean Platelet Volume in Diagnosing Severity of Preeclampsia. Journal of Medical Science And clinical Research. 2018;6(6).

ANNEXES

Annex 1: Questioner in English Version

Serial number……. ID. No…….. Date……

No	Questions	Category/response	Skip to
	Part-I: Socio Demographic and clinical data		
1	What is your age in full year?	_____year	
2	Where do you live? (Residence)	0. Urban 1. Rural	
3	What is your number of pregnancy including current pregnancy?	_____	
4	BMI	_____KG/M^2	
5.	Number of previous delivery?	_____	
6	What is your Gestational age in full week?	_____	
7	Systolic blood pressure(SBP)	_____mm/Hg	
8	Diastolic blood pressure(DBP)	_____mm/hg	
9	Mean arterial pressure(MAP)	_____mm/hg	
10	Disease severity	0. Normotensive 1. Non-severe preeclamptic 2. Severe-preeclamptic	

Laboratory data			
1	Proteinuria	_____ (0, +1, +2, +3…..)	
2	PLT	_____$\times 10^3/\mu l$	
3	MPV	_____fl	
4	PDW	_____fl	
5	PCT	_____%	

Annex2. Questioner in Amharic Version

ተራ ቁጥር ……..**መለያ ቁጥር**…… **ቀን**……..

ቁጥር	**ጥያቄ**	**መልስ**	**ዝለል**
1	**እድሜዎ(በሙሉ ዐመት) ስንት ነዉ**✍	_____	
2	**የመኖርያ ቦታዎ የት ነው**	0. **ከተማ** 1. **ገጠር**	
3	**ክብድት መጠኝ**	_____	
4	**ለስንተኛ ግዜ አርግዘዋል**?	_____	

5	**ስንት ግዜ ሞልደዋል?**	_____	
6	**ካረገዙ ስንተኛ ወር /ሳምንት ነው?**	_____	
7	**የደም ግፊት መጠን**	_____mm/Hg	
	የላቦራቶሪ ናሙና		
1	**የውሃ ሽንት ናሙና ፕሮትን መጠን**	_____	
2	PLT	_____$\times 10^3/\mu l$	
3	MPV	_____fl	
4	PDW	_____fl	
5	PCT	_____%	

Anex3: Questionnaire Hadiyisa version

Annanaaxi xigo…… Summa…… Balla……

xigo	Xamicha	**Dabacha**	**hige**
1	Umur mee'o?	_____	
2	Heech beyy hanno?	0. Beero'o 1. Hax uulla	
3	Lanfoolanina meekore?	_____	
4	Guurat	_____	
4	Meekore qacho qattakka;a?	_____	
5	Kado lanfoolania mee saanta?	_____	
6	Xiiqq gafech	_____mm/hg	
	Laaboraatoore'I xambo		
1	Shuim prootiin qaxoom	_____	
2	PLT	_____$\times 10^3/\mu l$	
3	MPV	_____fl	

4	PDW	_____fl	
5	PCT	_____%	

Annex-4. Procedure for Venous blood collection

Supplies

- Disposable glove
- BD Vacutainer tube (EDTA anticoagulant tube)
- Dry gauze
- Cotton Swab
- Vacutainer tube holder
- Needle with holder
- 70% Ethanol alcohol
- Tourniquet
- Band aid
- Safety box
- Waste disposal bag

Method (procedure)

1. Assemble the necessary materials and equipment
2. Thread the short end of the double-pointed needle into the holder and push the tube forward until the top of the stopper meets the guide mark on the holder
3. Identify the right patientand allow her to sit comfortably preferably in an armchair stretching her arm
4. Reassure the patient
5. Apply the tourniquet

6. Prepare the arm by swabbing the antecubital fossa with a gauze pad or cotton moistened with 70% alcohol.
7. Grasp the back of the patient's arm at the elbow and anchor the selected vein by drawing the skin slightly taut over the vein
8. Insert the needle properly into the vein;
9. Then the point of the needle is advanced 0.5-1.0cm into the subcutaneous tissue (at an angle of 45^0) and is pushed forwardat a lesser angle to pierce the vein wall
10. When the needle is properly in the vein, the vacuum tube is pushed into the needle holder all the way so that the blood flows into the tube under vacuum.
11. The tourniquet should be released the moment blood starts entering the vacuum tube
12. After drawing the required blood sample, apply a ball of cotton to the puncture site and gently withdraw the needle.
13. Instruct the patient to press on the cotton
14. Remove the tube from the vacutainer holder and gently invert several times. Invert 5-6 times for EDTA tube.
15. Label the tubes with patient's name, hospital number and other information required by the hospital (before the patient leaves the collection area)
16. Re-inspect the venipuncture site to ascertain that the bleeding has stopped.
17. Do not let the patient go until the bleeding stops. If bleeding does not stop apply band aid
18. Farewell to the patient with smile
19. Clean up supplies from the work area, remove gloves, and wash hands.
20. Discard all contaminated supplies in a biohazard disposal bag.

Annex 5. SOPS

1.1. SOP for MINDRAY-BC3000-Hematology Analyzer

Specimen Requirement

1. Whole blood specimen collected in K2EDTA anticoagulant tube.
2. The instrument aspirates approximately 13µl of whole blood and 20µl for pre-diluted mode
3. Transport and Storage:2-8 ^{0}C

Cause for rejection

- Hemolysis
- clotted specimen
- Tube not filled with minimum volume
- Improperly labeled specimen.

Reagents

- Diluent
- Lysing reagent
- Cleaner
- Hematology Control

Reagents preparation:

Reagents are commercially prepared.

Reagents stability and storage: All reagents are stable at room temperature up to their expiry date.

Principle

It uses electrical impedance method for counting and cyanide free method for hemoglobin. The Mind ray BC-300 performs speedy and accurate analysis of 19 parameters including a 3-part WBC differential plus histograms for RBC, PLT and WBC in blood (WBC, LYM%, MXD%, NEUT%, LYM#, MXD#, NEUT#, RBC, HGB, HCT, MCV, MCH, MCHC, RDW-CV, RDWSD, PLT, PDW, MPV, and PCT) and employs three detector blocks and two kinds of reagents for blood analysis. The WBC count is measured by the WBC detector block using the Direct current (DC) detection method. The RBC count and platelets (PLT) are taken by the RBC detector block, also using the DC detection method. The HGB detector block measures the hemoglobin concentration using the non-cyanide hemoglobin method

Acceptable background count

WBC, RBC and HGB ≤0.5% but PLT≤1%

Background Abnormal

When testing background， one or some of the test results are out of the reference range.

1. Contaminated diluents, diluents lines or bath (s);
2. Expired diluents;
3. The tubes at the back of the analyzer are pressed.

Recommended Action

1. Check if the diluents are contaminated or expired;
2. Check if the tubes connected at the back of the analyzer is pressed;
3. Enter the “Count” screen and press [STARTUP] (or [F3] of the external keyboard) todo the startup procedure;
4. If the problem remains, enter the “Service → Maintenance” screen and do the probe cleanser cleaning procedure as instructed in operation manual. When the procedure is finished, return to the “Count” screen and do the backgrounds check again.

Quality control

Quality control checks (low, high normal) were performed according to the laboratory's protocol before running patient sample. Commercial control materials were properly warmed and mixed according to the manufacturers' recommendations.

Procedure to perform patient testing

- Mix the sample sufficiently
- Remove the plug while taking care not to allow blood scatter
- Set the tube to the sample probe and in that condition, press the start switch
- The buzzer sounds two times - "beep, beep" - and when the LCD screen displays "Analyzing, "remove the tube. After that, the unit executes automatic analysis and displays the result on the LCD screen. Then the unit turns to the Ready status, becoming ready for analysis of the next samples.
- When the LCD screen displays "Ready," prepare the next samples and repeat the above procedures.

Description of Platelet (PLT) indices analyzed with MINDRAY-BC 3000-plus hematology analyzer.

Parameter	Description	Calculation	Unit
Platelet count(PLT)	Total amount of PLT in a micro litter of blood	Directly measured	Micro litter($\times 10^3$ µl
Mean plate late volume(MPV)	Analyzer-calculated measure of thrombocyte volume	$MPV(fl) = \frac{PCT(\%)X1}{PLT} \times 10^3/\mu l$	Femtoliter(fl
Platelet distribution width(PDW)	Indicator of volume variability in PLT size	PDW= Width of the size distribution curve in fl at the 20% level when the peak distribution curve is taken as 100%	Femtoliter(fl)
Platelatecrit(PCT)	Volume occupied by platelets in the blood	$PCT = \frac{MPV(fl) \times PLT}{10,000} \times 10^3/\mu l$	Percentage (%)

1.2.SOP FOR WRIGHT STAINING

Reagents

Wright's stain Reagent: Wright's stain deteriorate rapidly when the stain absorbs moisture or is stored at high temperatures or in bright sunlight. wright's stain should also be renewed every 3 months and left 3–5 days before being used.

PH 6.8 buffered water Reagent:Some users of Wright stain prefer to use 6.4 buffered water

Method

1. Place the air-dried smear film side up on a staining rack (two parallel glass rods kept 5cm apart).
2. Cover the smear with undiluted filtered stain and leave for 1 minute
3. Add equal the volume of pH 6.8-buffered water (i.e., the same number of drops as the stain)
4. Mix by blowing until a metallic sheen appears.
5. Allow the diluted stain to act for 3-5 minutes
6. Wash off the stain with running tap water/wash bottle
    - Don't tip off the stain, because this will leave a fine deposit covering the film.
7. Wipe the back of the slide clean and stand it in a draining rack for the smear to dry (head part down).
8. The blood film should appear neither too pink nor too blue
9. Then after examine the blood film microscopically by 100x oil immersion for platelet clump and the number, platelet count 8-20 called adequate.

    Note; if platelet clump is seen and pseudo thrombocytopenia is confirmed, replace EDTA and repeat the test with sodium citrate

Annex 6: - Information sheet in (English Version)

Good morning/good afternoon. My name is……. I am working for investigators of study conducted on pattern of platelet indices during pregnancy as potential markers for prediction of preeclampsia among pregnant women attending WUNEMMRH. The purpose of this study is to evaluate the pattern of platelet indices in preeclampsia compared to normal pregnancy. You are invited to participate in the study after giving your consent by giving blood samples of 3mlwhich is collected from your arm. There will be some pain during blood collection but this is the normal fate of the procedure it is not serious and not harmful to your health. There is no additional time required from you to stay during study.

There is no any financial benefit to you. But the result of the study will be used for your clinical care as well as plays a role if platelet indices could use for diagnosis and prediction of preeclampsia and will play a role in minimizing maternal mortality and morbidity rate. There is no compensation for using your blood sample. The results of the laboratory findings will be kept confidential and could only be accessed by the researcher and the responsible physician. There will be no personal information to be attached to your data.

You have full right to refuse, withdraw or completely reject part or all of yourparticipation in the study. But we encourage your full participation as your taking part in this study is very important and helps for planning preeclampsia control and prevention measures. Your withdrawal of consent will not affect your right to receive medication. If you have questions, you can ask at any time and also I will provide you the answers. You may contact me at e-mail address

solomongebre16@gmail.com or mobile +251 916538444

Are you voluntary to participate? 1. Yes 2. No

Annex 7: - Information sheet (Amharic version)

ጤና ይስጥልኝ! እኔ አቶ/ዎ/ሮ/ዎ/ሪት……አባላለሁ፡፡ እዚህ ሆስፕታል ፕለትሌት የሚባሉ የደም ዓይነቶች ለውጥ በነብሰጡር ሴቶች በደም ብዛት ምክንያት የሚመጣውን በሽታ ለማወቅ ይረዳናል ወይ በምለው ጥናትና ምርምር ለማያካሄዱ በመስራት ላይ ኢገኛለሁ፡፡

የጥናቱም ርእስ ከጅማ ዩኒቨርስቲ የላቦራቶሪ ሳይንስ ትምህርት ቤት የምርምር ሥራ ሥነ-ምግባር ኮሚቴ ፈቃድ ያገኘ ሲሆን እርሶም ፈካደኛ ከሆኑ የጥናቱ ተሳታፊ እንድሆኑ እጋብዞታለሁ፡፡ የጥናቱ ኣላማ ፕላትሌት የሚባሉ የደም ዓይነቶች ለውጥ ኣንዱት ነብሰጡር ሴት በደም ብዛት ምክንያት የሚመጣውን በሽታ ለማወቅ ይረዳናል የሚለውን ለማወቅ ነው፡፡ በዚህ የጥናት ሥራ ለመሳተፍ ፈቃደኛ ከሆኑ 3 ሚሊሊትር (አንድ የሻይ ማንኪያ የሚሆን) የደም ናሙና ከክንዶ ለይ ይሰጣሉ፡፡ ይህ የደም ናሙና በደምዎ ውስጥ በዚህ ጥናት በመሳተፍዎ ሙሉ የደም ምርመራ ውጤትዎን ለማወቅ ዕድል ይሰጠዎታል፡፡ጥናቱ በርስዎ ላይ የሚያመጣው ጉዳት የሌለ ሆኖ ለጥናቱ የሚያጠፉት ተጨማሪ ጊዜም አይኖርም፡፡ ከጥናቱ የሚያገኙት ምንም ዓይነት የገንዘብ ጥቅም ባይኖረውም ከጥናቱ በሚገኘው ውጤት በእርግዝና ወቅት በምከሰተው የደም ግፊት የእናቶችን ሞትና ተጋላጭነት ለመቀነስ ይጠቅማል፡፡ ከርስዎ ምናገኘው መረጃ እና፣ በሰጡትደም ላይ የሚደረገው የምርመራ ውጤት ሙሉ በሙሉ ሚስጥራዊነቱ እንደተጠበቀ ሆኖ ለጥናቱ ዓላማ ብቻ ጥቅም ላይ እንደሚውል ላረጋግጥልዎ እወዳለሁ፡፡በዚህ ጥናት ላይ ያለዎትን ጥያቄ በማንኛውም ሰዓት ሊጠይቁና ምላሽ ሊያገኙ ይችላሉ፡፡በጥናቱ ላይ ያለመሳተፍም ሆነ በመሀል የማቋረጥ ሙሉ መብት አልዎት፡፡ በማንኛውም ሰዓት በጥናቱ ላይ ያለዎትን ጥያቄ ለመመለስ ደስተኛ ነኝ! በሚቀጥለው አድራሻ ሊያገኙኝ ይችላሉ፡፡

ስልክ፡- 091653 8444

Email: solomongebre16@gmail.com

ለመሳተፍፈቃደኛኖት? 1. ኣዎ 2. አይደለሁም

Anex8: Information sheet (Hadiyisa Version)

Xumma gattakka'a/hossakka'a? abbaachi/aayyich…….yamamoommo. Ka hospitaalanne lanfoollanone qooccamoo xiqqi gafech jabbone saarayyoo saarayyaanina baxummuuyyi siidamoommo.. Ka saarayyim baxikim horoor woshi jimm unversiteiinse eeyite sidaakkohan ihukkuuyi Eebikkina ki'nnem kasarayyim baxonne baxxantakona eeyyite xaiminoommo. Ka saarayyim baxikim horoor woshi plaatileetaa yakkam xiiqqi baxanch annanaat hinkid lanfoollanone qooccamoo xiqqi gafech jabbo laimina awwaadooko yoohan ihukkuuyi kinem baxantakken eeyyite uwwitakolas sas militer 3ml teim mat qash qax qaxoom xiga uwwitakamo. Uwwtakkam xiigim lulei xiiqqi woro'l xisso laqqakkona hara'mmookko.ku saarayyim baxim kinenne afisoo hawoj beee'an ihukkuuyi, Ayyi ki'n uwwitakkam xambim daphitenne te'immoqqo'onne amadamoohan ihookko. Ka sarayimanne baxamanch kinena maham bi'l haramat hee'ubeeaarem ka saarayyimiins siidamoo mish lanfoolanone qoocamoo xiiqqi gafech jabbo hooo'lamiminaa ege'liminaa awaadookko. xamboomannem summa ayyoommanoo cakkisooluch kitaabamooyyo. Ka saarayyim baxonne hamaaramimmina(baxxamimina) doo'lbeelas hink ammanem uullisimma xantakkamo. Woshsh ihukkarem ki'n uwwitakkam sawwit ka jabbo hoo'illamimmina aege'illimmina odim ka jab mashkainne waroo ciiluwikaa amo'i leho hoo'llamimina issamoo yakitina lobakata awwaadohan ihubikkina baxxantakkamisina qoxxinsoomo. xa'mmich yoolash hinkammanem xa'immimma xantakkamo.

Baxxantakeena iittantaka'a? 1. Eeyya. 2. Aa'e

Silka 0916538444

Iimeela/solomongebre16@gmail.com

Annex9: - Informed consent form English version

I have read or understood the information sheet above and clearly understood the purpose and anticipated benefit of the research. I hereby need to assure with my signature below that I, without any coercion or forceful act by the research team, have decided to voluntarily participate in the study.

Study subject'sSupervisor's

Code number..... Name.....

Signature..... Signature.....

Date..... Date.....

Annex 10: - Informed consent form (Amharic version)

ከላይያየ ተገ ለጸጣንየ ጥናቱንዓለማእናጥቅምከአግባበበመሪዳትጥናቱላይለመሳተፍመኝምክያስገ ድያኝበራሰፍቃደየ ወስንከመ ሆኑንበፍርሜአረጋግጣለሁ ፡

የተሳታፊወየ ተቆጣጣሪው.

መለያቁጥር---------- ስም---------- ፊርማ----------

ፊርማቹቀን---------- ቀን----------

Annex 11: - Informed consent form (Hadiyisa version)

Hanaan caakkukki saarayyim bax horoor wosha aawwaadoo siyya'aamisinne qoossaa ka saarayyim baxonne daayyameena ayyim giddisoo'in doo'illinne qoodumman ihukkisa furma'inne naqqasoommo.

Baxxamaanch Seeregessiisanch

Annanaax xigo…… Summa……

Furma…… Furma…….

Balla…… Balla……

DECLARATION

I, the under signed, declare that this research is my own work and it has not been presented in other universities, colleges or other institutions for similar degree or other purpose. Where other peoples work has been used, it has been carefully acknowledged and referenced in accordance with the requirements.

Principal investigator

SOLOMON GEBRE (BSc) Signature_____ Date _____

Approval of Examiners

External Examiner: BEMLAKU ENAWGAW (Msc, Assoc.Prof) from university of Gondar

Internal Examiner: DR. TILAHUN YEMANE (MD, Msc)Signature_____Date_____

Approval of the first Advisor

LEALEM GEDEFAW (MSc, Assoc.Prof) Signature_____ Date_____

Approval of the Second Advisor

WONDIMAGEGN ADDISU (MSc) Signature_____ Date_____

Approval of the third Advisor

DR. BIRHANU NIGUSE **(**MD. Gynecologist**)**Signature_____ Date_____

School Head_____ _____ _____ Signature Date