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Prediction of Preeclampsia during Pregnancy Using Platelet Parameters at Ayder Comprehensive Specialized and Mekelle General Hospitals in Mekelle, Tigray, Ethiopia.

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This is to certify that this thesis prepared by Feven Tesfay, entitled: ***Prediction of Preeclampsia during Pregnancy Using Platelet Parameters at Ayder comprehensive specialized and Mekelle General Hospitals in Mekelle, Tigray, Ethiopia***and submitted in partial fulfillment of the requirements for Master of Science degree in Clinical Laboratory Sciences (Hematology and Immunohematology specialty track) complies with the regulations of the University and meets the accepted standards with respect to originality and quality.

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Abbreviations

ACSH	Ayder comprehensive Specialized Hospital
AUC	Area under curve
ANC	Antenatal care
BP	Blood pressure
CBC	Complete blood count
DRERC	Departmental research and ethical review committee
EDTA	Ethylene diamine tetra acetate
FL	Femto liter
G/dl	Gram per deciliter
Gyn-Obs	Gynecology and Obstetric
HELP	Hemolysis elevated liver enzyme and low platelet count
IUFD	Intra-uterine fetal death
MGH	Mekelle General Hospital
Ml	Milliliter
mmHg	Millimeter mercury
mPE	Mild preeclampsia
MPV	Mean platelet value
NPV	Negative predictive value
PC	Platelet count
PDW	Platelet distribution width
PE	Preeclampsia
PLCR	Platelet large cell ratio
PLT	Platelet
PC T	Plateletcrit
PPV	Positive predictive value
ROC	Receiver operating characteristic
SD	Standard deviation
Sn	Sensitivity
SOP	Standard operating procedure

Sp	Specificity
SPSS	Statistical package for social science
sPE	Severe preeclampsia
WHO	World health organization

Operational definitions

Preeclampsia:- Is a disorder of pregnancy characterized by high blood pressure(blood pressure (BP) ≥140/90 mmHg ,repeated two times four hrs. apart), proteinuria (≥0.3 g/dl), edema and other major symptoms like head ache, blurred vision, right upper quadrant pain may begin as early as the 20th gestational week and last for 6 weeks after delivery.

Mild Preeclampsia:-If BP is between 140/90 and 160/110 with proteinuria ≥0.3g/dl without the major symptoms.

Severe Preeclampsia:-If BP is ≥160/110 and proteinuria ≥0.3, BP 140/90-160/110 with the major symptomsand BP ≥160/110 with or without the major symptoms.

Thrombocytopenia (reduced platelet value):-Refers to a disorder in which there is a relative decrease of thrombocytes, commonly known as platelets, present in the blood.A normal human platelet count ranges from 150,000 to 450,000 platelets per microliter of blood.One common definition of thrombocytopenia that requires emergency treatment is a platelet count below 50,000 per microliter.

Normal pregnancy:-A uni-foetal pregnancy that occurs in a normotensive woman without proteinuria, who remained normotensive and delivers after 36 weeks of pregnancy a healthy neonate with a weight adequate for gestational age.

Mean platelet volume (MPV):-Is the average platelet size in an individual's blood sample.

Platelet distribution width (PDW):- Uniformity of platelets in terms of size.

Platelet large cell ratio (PLCR):-Is the ratio of large platelets from the 12fldiscriminator or larger to total platelet count.

Abstract

Background: Platelet abnormality is one of the most commonly identified hematological abnormality in preeclampsia.Preeclampsia is a major obstetric problem and cause of maternal mortality especially in developing countries. However, platelet parameter of preeclamptic women is not well recognized as a tool for prediction and prognosis of preeclampsia in Ethiopia.

Objectives: To evaluate the role of platelet parameters in prediction of preeclampsia at Ayder comprehensive specialized and Mekelle General Hospitals.

Methods:A cross sectional comparative study was conducted on 219 pregnant women at ACSH and MGH in Mekelle city from January to March 2017.Using convenient sampling method,79 diagnosed cases of preeclampsia and 140 healthy pregnant women were selected. Platelet parameters were analyzed from EDTA venous blood sample by automated hematology analyzer (SYSMEX-XT 4000i).One-way ANOVAsupplemented with post-hoc test was done to compare the mean platelet parameters difference across the three groups of women. Receiver Operating Characteristics (ROC) curve was used to calculate sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), for a given platelet parameters in discriminating the presence or the absence of preeclampsia. Pearson correlation test was used to see the relationship between continuous variables. The data was cleaned, entered and analyzed using SPSS version 20 software. A P-value of <0.05 was considered as statistically significant

Result: The platelet count in severe preeclampsia was significantly lower than in mild pre eclampsia andcontrols while all platelet indices were increased with severity of preeclampsia with a statistical significant difference P<0.05. Results showed a negative correlation between platelet count and strong positive correlation between platelet indices .ROC analysis showed that MPV was seen to have largest area under the curve (AUC=0.85; 95%CI (0.79, 0.89)) with cutoff value >9.45fl , sensitivity of 83.5% , specificity of 86.4%, positive predictive value of 77.6% and negative predictive value of 90.3%, indicating as it is the best parameter for predicting preeclampsia. The second most important predictor parameter identified was platelet count.

Conclusion: The estimation of platelet parameters may be considered as reliable, economic and rapid method for prediction of preeclampsia and assessment of its severity.

Keywords: platelet indices, platelet count, preeclampsia, Predictive value

1. Introduction

1.1 Back ground

Platelets (PLT) are small anucleated cell which are cytoplasmatic fragments of bone marrow megakaryocytes, with a diameter of 3-5 μm and a volume of 4.5–11 Femto liter (Fl). A single megakaryocyte can give rise to 1500–2000 of them to the bloodstream, where they circulate for 7–10 days [1]. Platelets are dynamic blood particles whose primary function, along with the coagulation factors, is hemostasis, or the prevention of bleeding. Platelets interact with each other, as well as with leukocytes and endothelial cells, searching the vascular bed for sites of injury. There, they become activated and when stimulated, platelets undergo a shape change and their surface area increases. Platelets play a pivotal role in the first steps of clot formation by adhering to damaged blood vessel as well as donating their membrane phospholipids for the activation of coagulation factors [2, 3].

In addition to their important role in hemostasis and thrombosis, accumulating evidence demonstrates that platelets contribute to the inflammatory process, microbial host defense, wound healing, angiogenesis, and remodeling [4, 5]. Platelet indices, such as mean platelet volume (MPV) and platelet distribution width (PDW), platelet large cell ratio (PLCR) are a group of derived platelet parameters obtained as a part of the automatic complete blood count. Emerging evidence suggests that platelet indices may have diagnostic and prognostic value in certain diseases [5]. Platelets indices have been recently used in the prediction, diagnosis, and prognosis of many diseases being reported as clinically useful biomarkers; one of the diseases is preeclampsia [6,7].Thrombocytopenia is attributed to two main causes. Failure of platelet production and early excessive platelet consumption, where the second cause is observed in preeclampsia [6].

Preeclampsia (PE) is a serious multi-systemic pregnancy complication affecting between (5-8 % of pregnant women) worldwide [7, 8]. Therefore it is considered as one of the major health

problems associated with pregnancy and one of the causes of maternal mortality [9, 10]. The prevalence of PE in developing countries ranges from 1.8% to 16.7% [11]. Generally the diagnosis depends mainly on finding of hypertension and proteinuria after 20 weeks of pregnancy [12]. PE is characterized by hypertension (blood pressure >140/90 mmHg), proteinuria (>0.3 g/d), edema and other symptoms and may begin as early as the 20th gestational week and last for 6 weeks after delivery [13].

Preeclampsia has been a major cause of poor result in pregnancy and the category “hypertensive diseases of pregnancy” and is a leading cause of maternity death in Africa [14]. The pathogenesis of PE remains unknown, and the many theories related to the etiology of PE pose great challenges for future investigation. The abnormal invasion of placenta and the release of placenta-derived adverse factors during the first trimester are thought to be the main cause of the extensive damage to the maternal endothelium and systemic inflammatory response involving many systems and organs in late pregnancy [15].Although the causes of PE are completely unknown, one of the responsible mechanisms is thought to be activation of inflammatory systems with predominant involvement of cytokines and chemokines. However, there is an ongoing debate about whether inflammatory system hyperactivity indeed exists during PE, and if available data are sufficient for justification of broad anti-immune system treatment strategies [16, 17]. PE can be classified into two degrees, mild PE (mPE) and severe PE(sPE) [18].

Platelet indices are biomarkers of platelet activation. They allow extensive clinical investigations focusing on the diagnostic and prognostic values in a variety of settings without bringing extra costs. Among these platelet indices, mean platelet volume (MPV), and platelet distribution width (PDW), platelet large cell ratio (PLCR) are a group of platelet parameters determined together in automatic CBC profiles; they are related to platelets’ morphology and proliferation kinetics. The volume of platelets in the bloodstream is heterogeneous, and their structures and metabolic functions differs [2, 3, 19].

The platelet count and mean platelet volume (MPV) are measured during a routine automatic whole blood count and hence are commonly available parameters nowadays. Mean platelet volume (MPV), which is commonly used as a measure of platelet size, indicates the rate of

platelet production and platelet activation [12, 19]. The MPV has been shown to correlate with the function and activation of platelets. The importance of MPV has been emphasized as an inflammation marker in some diseases such as preeclampsia [20].

Platelet activation begins in the first month of pregnancy in women with risk for preeclampsia [21].Several studies suggested that when platelets are activated they become larger in size which causes increased platelet indices such as MPV, PDW and PLCR [22]. So, platelet indices can give an idea of platelet activation. Recent advances in automated blood cell analyzers have made it possible to measure MPV, PDW and PLCR. The normal values for MPV are 8.4-12.0 Fl, PDW 8.0-14.0 Fl and P-LCR 10-30% [23].

Even though the exact cause of elevated blood pressure in preeclampsia is obscure, platelets get activated and attach to the endothelium at the site of injury. In the early stages of hypertension in pregnancy, platelet aggregation is increased.A typical case picture is one of a vasoconstrictive state with low plasma volume and cardiac output, high blood pressure and systemic vascular resistance in combination with signs of organ damage or HELP syndrome [proteinuria, hemolysis, elevated liver enzyme, low platelets]. As a result bone marrow releases young platelets which are large, resulting in increased Mean platelet volume (MPV) [24]. Automated analyzers are widely distributed in Ethiopia and the platelet parameters are part of the routine complete blood count (CBC).

1.2 Statement of the problem

Preeclampsia is a serious multi-systemic pregnancy complication affecting between (5-8 % pregnant women) worldwide. As stated by world health organization itis a major public health concern in developing countries like Ethiopia; it is one of the causes of maternal death [7]. A study in Ethiopia reported that majority of the hematological parameters including platelet indices are underutilized in clinical patient management [25]. Whereas these are being reported as clinically useful biomarkers in many diseases including preeclampsia [6,7].

Early detection of platelet count abnormalities in preeclampsia is a sign of worsening disease. Thus, utilization of these simple markers could have facilitated early detection of maternal and fetal complications thereby play a role as prognostic tool in management. To date, there is no effective treatment for PE in addition to the termination of pregnancy. Therefore, a reliable predictor for PE would play an important role in early prevention and intervention [26].

Platelets are activated early in pregnancy in women who have preeclampsia; the platelets may be used up prior to delivery, leading to an insufficient amount of platelets remaining to allow clotting and haemostasis to occur. This could be the reason why some preeclamptic women have bleeding problems at delivery. Data regarding the association of platelet parameters with preeclampsia are conflicting. There are studies globally which show platelet parameters having an association with preeclampsia [6,7,23,24,27-34, 36-38].While others demonstrated no significant relationship between platelet parameters and preeclampsia[35,39-41].

As to my knowledge though many studies have been published in Ethiopia describing the prevalence of preeclampsia in pregnant women, no research has been published in comparing the platelet parameters in preeclampsia and normal pregnancy.

1.3 Significance of the study

This study was carried out to see if there is a significant relationship between platelet count and platelet indices with preeclampsia. It could help to suggest that platelet indices have diagnostic and prognostic value in certain diseases like preeclampsia.

This study will also contribute to the conflicting body of evidence whether platelet count and Platelet indices can be used as clinically useful biomarkers. Currently there is no test for early detection of preeclampsia, so the disorder is commonly not discovered until the third trimester, when the three main symptoms, hypertension, edema, and proteinuria, are commonly picked up [13].If preeclampsia can be detected at an earlier stage by looking at the platelets, the potential for preventing bleeding problems can exist. The information of the present study might enrich the knowledge of clinicians for early identification of preeclampsia. In preeclampsia, increased platelet indices may occur earlier than development of other recognized manifestations like hypertension and proteinuria when the vascular changes occur. On the other hand, lowered platelet count and increased platelet indices in pregnant women during antenatal check may be considered as a risk factor for development of preeclampsia. The finding will help to suggest that this group of pregnant women may be considered for special attention to controlthe development of preeclampsia.

This study will also contribute in proving evidence based information of using platelet parameters as useful biomarkers for early identification of preeclampsia for clinicians working in hospitals as well as other health care facilities which provide antenatal care. By doing so, the findings of this study could play an important role in decreasing morbidity and mortality of pregnant women as a result of preeclampsia. Furthermore, this study could be used as a reference or a bench mark study for related studies.

2. Literature review

There are studies which establish significant difference in platelet count and platelet parameters in preeclampsia compared to normal pregnant women, thus suggesting the usefulness of these tests for diagnosis and predicting the severity of preeclampsia [27].

Howarth S *et al* (1999) conducted a study in US involving 349 normal pregnancies at various gestational stages, and in 30 cases of preeclampsia.Platelet count and mean platelet volume (MPV) were estimated.A probability plot was constructed from these data using discriminant analysis of MPV versus platelet count for the preeclamptic versus normal pregnancies. The study found that the sensitivity of MPV was 90%and specificity 83.3% for the prediction of preeclampsia development [28].

A study done in Stanford University northern California stated that platelets appear to play an important role in prediction of preeclampsia. Enhanced platelet activation, as determined by whole blood analyzed using flow cytometry and increased levels of platelet endothelial cell adhesion molecule-1 (PCAM-1) also occur in women who develop preeclampsia as demonstrated by Taylor *et al*, (1999) [29] and Roberts's *et al*, (1989) [30].

Freitas L *et al*.2013 in Brazil stated that Lower PLT count and PCT were observed in sPE comparing to normal pregnant and to non-pregnant women with P-Value<0.001. PDW was higher in sPE comparing to normotensive pregnant and to non-pregnant women.MPV was higher in sPE comparing to normotensive pregnant and non-pregnant women.Analysis from the ROC curve and its areas for each variable showed that the parameters have regular diagnostic significance, except for PCT, which is considered as not good for this purpose. The study claimed that sPE females had a significantly higher MPV (cutoff: 9.6 FL) with a sensitivity of 51.72% and a specificity of 82.76%. However, only a few patients developed thrombocytopenia [31].

Kaito K *et al*. (2005) in Britain also explained that hyper destruction of platelet increases MPV, PDW and PLCR. They also stated that increased PLCR indicate increased bone marrow activity [23]. Earlier, Neiger *et al(*1992) in United Kingdom have reported that the platelet counts in 67 preeclamptic women were lower when compared with 71 healthy pregnant controlsno difference

was observed in terms of platelet count between mild and severe preeclamptic cases. However, in this study, healthy pregnant women were studied as control group, and it has been stated that even in normal pregnant women compared to non-pregnant control the platelet numbers have decreased in the third trimester [32].

The platelet count decreased significantly with the severity of preeclampsia as demonstrated by Dadhich S *et al*. (2012) in southern Asia,they also noted that the decrease in platelet count was antedating significant increase in blood pressure by 4 to 6 weeks. As a result, the authors concluded that this Platelet parameter can be used to predict development of progressive hypertension in at risk patients [27].

A cross sectional study was conducted in Dhaka the capital city of Bangladesh by Sultana R *et al* (2012) in a total number of 100 pregnant women in their third trimester of pregnancy attendingin Obstetrics and Gynecology Department of Dhaka Medical Hospital. Among them 50 diagnosed cases of preeclampsia were selected as casesand 50 normal healthy pregnant women as controls. Platelet count was measured in allstudy participants and the mean (±SD) platelet count in cases and controls were 144,260±96,472 and 198,100±51,219, respectively. There was statistically significant difference of mean platelet count between cases and controls. The lower platelet count in preeclampsia is associated with abnormal activation of the coagulation system and is believed to reflect increased platelet consumption. Distinguishing preeclampsia from other causes of abnormal screening results would aid doctors in the diagnosis and prompt treatment of their patients. The authors, therefore, concluded that Platelet count may be used as simplest, cheapest and earliest indicator of preeclampsia [33].

A Case control study done by Amita *et al.(*2015) in India comprising 50 preeclamptic cases between 20 to 24 weeks of gestation and 50 normotensive pregnant females matched for age and gestation as controls. Preeclamptic patients were separated intothree groups as mild =25, moderate=13 and severe preeclampsia=12. Platelet count, mean platelet volume and plateletdistribution width measured by automated hematology analyzer Sysmex KX 21 wererecorded in all the groups. Comparison of the three platelet parameters i.e., Platelet count, MPV and PDW among the preeclampsia and normotensive pregnant control group was done by using independent t test. Platelet count was lower in the preeclampsia group as compared to control group and this was statistically significant. Though MPV was higher in preeclampsia

group as compared to control group, the finding was not statistically significant; However, PDW was significantly higher in preeclampsia group as compared to the control group [34]. There was also another study done by Mohapatra S *et al*(2007) in India on pregnancy induced hypertension. They observed that platelet count was inversely related to the severity of pregnancy induced hypertension [35].

A study in Turkey by Sontas *et al.* 2016 revealed that PLCR was found to be associated with the severity of PE when it was compared with mild stage. This finding may be related to cytokine-dependent defective maternal immune activation in PE pathogenesis. In addition, PLCR may also be an indirectly available and simple reflector for degree of immune activation in PE [36].

A case control study conducted by Ahmed *et al.* (1993) in Egypt revealed that platelet numbers were decreased by 50×10^9/l in 12 of the 15 patients with preeclampsia. They also revealed persistent increase of 0.8 fl in MPV in 14 out of 15 preeclamptic patients between 24 weeks and 38 weeks of gestation; however, MPV remained constant in normal pregnancies between the first trimester and the end of pregnancy [37].

On the other hand, an observational longitudinal study was done by Nooh .A *et al*. in 2015 in Egypt in women attending antenatal clinic (ANC) and/or admitted to maternity ward atZagazig hospital, Egypt over the period from 2nd June 2014 to 28th May 2015. By usinga total of 2813 pregnant women the study provides evidence that PLT count decreases while MPV and PDW increase as pregnancy advances, and these changes are more pronounced in PE than normotensive pregnancy. Changes in platelet indices predict development of PE by 2 - 8 weeks and are proportional to the progress of this disorder. The selected platelet indices, especially PDW, have the potential to be utilized as markers for not only prediction of PE development but also severity of hypertension [24].

To evaluate the platelet indices and their significance in assessment of severity of preeclampsia and their correlation with pregnancy outcome, a prospective study was conducted by Ahmed W, *et al* from January 2012 to March 2013 in EL-Galaa Teaching Hospital in Cairo Egypt. Blood samples were collected from200 pregnant women, 100 normotensive and 68 cases mild and 32 cases sever preeclampsia), and analyzed for platelet indices. The pregnant women were followed up for both maternal and fetal outcome. The study revealed that the platelet count was decreased,

while MPV, PDW and PLCR increased with severity of preeclampsia, poor maternal prognosis and poor fetal prognosis. They found a relationship between platelet indices and severity of preeclampsia and pregnancy outcome [38].

A study done in Sudan by Elkareem A et *al* (2016) compared the platelet count and platelet indices in preeclampsia and normal pregnancy. The result revealed that among 87 total pregnant women, 37 were preeclamptic pregnant women, and there were significant differences in the platelets indices of MPV, PDW, and P-LCR among the study groups [39].

On the other hand there are studies which came up with contradicting findings. For example, a study conducted by Yayuzcan A *et.al,* in Turkey in 2014 compared Mean Platelet Volume, Neutrophil-LymphocyteRatio and Platelet-Lymphocyte Ratio in SeverePreeclampsia in healthy pregnant and non-pregnant women. The study showed that when the PLCR value of the three groups namely severe preeclampsia, healthy pregnant and non-pregnant women were compared, there was no statistically significant difference between the three groups. The situation was the same for the paired comparison; there were no significant difference between patients with severe preeclampsia and healthy pregnant women or between patients with severe preeclampsia and controls or between healthy pregnant women and controls [40].

Similarly, Santos et al, in Turkey found no difference in platelet count in normotensive and preeclampsia women. However, all other platelet indices were significantly higher in the preeclampsia [36]. Elkareem *et al*(2016) in Sudan also studied the platelets count and plateletcrit (PCT) and demonstrated that these parameters did not vary significantly between PE and normal pregnancy group [39]. Kulkarini and Sutaria also did not observe any significant difference in respect to platelet count in their study [41].

Regarding the correlation of platelet count and platelet induces a study done in Ethiopia by Negash M *et al* (2016) in Diagnostic predictive value of platelet indices for discriminating hypo productive versus immune thrombocytopenia purpura due hyper destruction of Platelets which is also thought to be the main cause for reduced platelet count in preeclampsia.They found statistically significant negative correlationbetween platelet count and the platelet indices in immune thrombocytopenia purpura patientswhich was PLT Vs MPV **(**r (correlation

coefficient)=-0.5 P=0.004**)**, PLT Vs PDW **(**r=-0.6 P<0.001**)** and correlation between PLT Vs PLCR **(**r=-0.5 P=0.001**)**[42].

Taken together, the studies reviewed above provide conflicting evidences as to the association of platelet count and indices with preeclampsia and its severity though mounting body of data favors the presence of association and clinical utility of these markers. As far as my literature search goes there is no published report from Ethiopia. Hence, this study tried to fill this gap and lend additional data to broaden the existing scientific knowledge globally. Since CBC is the most frequently requested test in general and during pregnancy in particular, there is a need forgeneration of more data since introduction of these simple markers improve the clinical management of pregnant women at risk of preeclampsia with no additional cost.

3. Objectives

3.1. General objective

To evaluate the role of platelet parameters in prediction of preeclampsia at Ayder comprehensive specialized and Mekelle General Hospitals.

3.2. Specific objectives

- To compare the platelet count, MPV, PDW, and PLCRin mild and severe stages of preeclampsia and normal pregnancy.
- To determine correlation between platelet count and platelet indices in mild and severe stages of preeclampsia and normal pregnancy.
- To determine sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), for PLT Count, MPV, PDW and P-LCR in discriminating the presence orthe absence of preeclampsia.

4. Hypothesis (Ho)

There is no difference in platelet parameters of preeclamptic pregnant women compared to normal pregnant women.

5. Materials and methods

5.1. Study area

This study was conducted at Ayder comprehensive specialized (ACSH) and Mekelle General Hospital (MGH) , which are found in Mekelle City. Mekelle is capital city of Tigray Regional State and is located in the Northern part of Ethiopia, at 783 km from the capital city, Addis Ababa.The Ayder Referral Hospital commenced rendering its referral and non-referral services for around 8 million population in its catchment areas of the Tigray, Afar and South-eastern parts of the AmharaRegional States with total patient flow of above 100,000 per year.It has a total capacity of 500 beds in four major departments, with ANC attending women of >150 per month and other specialty units along with six other affiliated hospitals in the Tigray regional state. It is also used as a teaching hospital for the College of Health Sciences, Mekelle University.The hospital has more than 45 specialists in the various areas of medical specializations and adequate number of other health professionals which constitute the health care team[43].

Mekelle Hospital is the largest regional hospital serving about 6 million people residing in Tigray, Northern Amhara and Western Afar regions, with ANC patient flow above 600 per month.

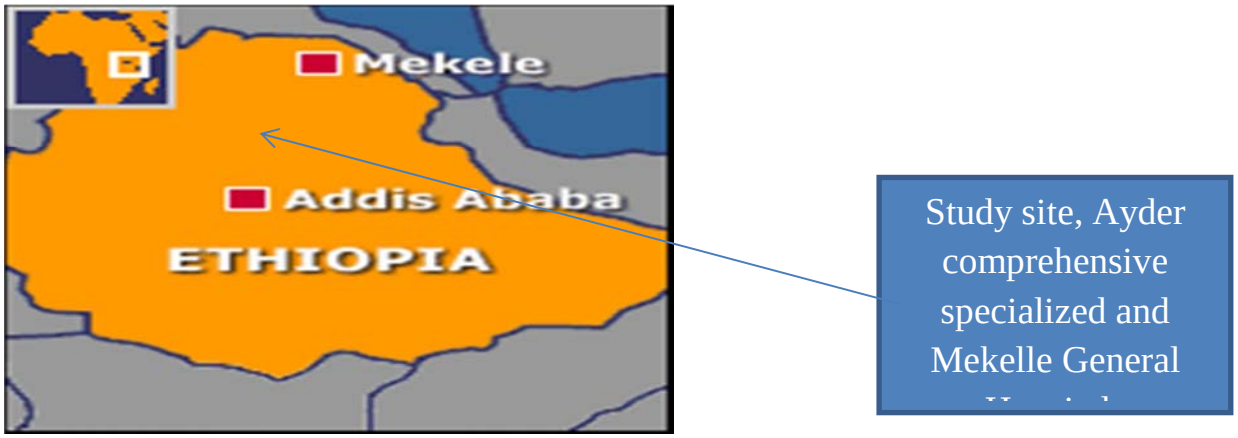

Figure.1 Mekelle study site: ACSH and MGH.

5.2. Study design and period

A cross-sectional comparative study was conducted from January to March 2017.

Study Design FlowChart

Pregnant women

If the pregnant women fulfills the inclusion criteria
And obtain consent paper

Looking at her chart and conducting her physician
labeling by code if she is preeclamptic or normal pregnant

Sample collection, labeling with code No.

Testing the sample with SYSMEX- XT 4000i automated hematology analyzer

Registering the value of Platelet count, MPV, PDW and PLCR

Data entry and Sample analysis

Interpretation

5.3. Population

5.3.1. Source population

All pregnant women who visit ACSH and MGH.

5.3.2. Study Population

Pregnant women that fulfill the inclusion criteria during the study period and volunteer to take part in the study were the study population.

5.4. Eligibility criteria

5.4.1 Inclusion Criteria

- Volunteer Preeclamptic pregnant women (cases)
- Volunteer Healthy normotensive pregnant women at ≥20 weeks gestation with a live singleton fetus were eligible and approached for recruitment.

5.4.2. Exclusion Criteria

- Intra-uterine fetal death (IUFD)
- Poor past obstetric history [recurrent miscarriage, pre-term labor, intrauterine growth restriction]
- Currently suffering from a systemic disease, gestational or insulin-dependent diabetes, heart disease, renal or hepatic dysfunction) and inflammatory diseases.
- Any hematological diseases which can affect the platelet parameters and bone marrow were excluded by looking in to their charts and consulting their physician's.

5.5. Study variables

5.5.1. Dependent variable

- Platelet parameters: PLT count, MPV, PDW and PLCR
- Sensitivity, specificity, NPV, PPV of Platelet parameters: PLT count, MPV, PDW, PLCR

5.5.2. Independent variables

- Preeclampsia (mild and severe)
- Gestational age
- Age

5.6. Measurement and Data collection

5.6.1. Sample size determination

For this study the sample size was calculated using the formula of hypothesis testing for two population means.

Required information:

Standard deviation S_1, S_2

Level of significance which is usually set to a level of 0.05; respective Z value is 1.96

Power of the test : 100(1-β)%,which is usually set to 80%, which is equal to 0.84

Sample size can be estimated using the formula

$$n = \frac{2\sigma^2[z_1 - \frac{\alpha}{2} + z_1 - \beta]^2}{(\mu 1 - \mu 2)^2}$$

Where (σ^2) is pooled variance = $\frac{S_1{}^2+S_2{}^2}{2}$

The anticipated mean of platelet indices (MPV) for preeclampsia is 10.15 and for normal pregnancy is 9.48 with a standard deviation of 1.10 and 0.87 respectively. Since no studies were conducted in our country previously, the values are taken from studies conducted in Sudan [39].

i.e.σ^2 = $\frac{0.87^2+1.10^2}{2}$=0.983

$$n = \frac{2(0.983)[1.96 + 0.84]^2}{(10.15 - 9.48)^2}$$

n = 35

35+ (15% for contingency) = 40

The minimum sample size required for group 1, n1=40

To evaluate the clinical accuracy of platelet parameters in preeclampsia, it is possible to make the number of controls (normotensive pregnant women) double of the cases (preeclamptic pregnant women). The sample required for group 2 n2=2(n1) =80

N =n1+n2 = 40+80

N(total sample size) = 120 This was taken as minimum sample size and, thus, the study included79 cases and 140 controls total 219 study participants were involved.

5.6.2. Sampling method

Convenient sampling method was used to collect data.

5.6.3. Data collection procedure

Volunteering pregnant women after getting an informed consent wereclinically examined by the doctors whether she is preeclamptic or normal by making diagnosis based on brief clinical history, B.P. urine examination for protein and checking for not taking any therapy for preeclampsia. Those preeclamptic and normotensive pregnant women attending the antenatal care clinic of the two public hospitals and those who fulfills the inclusion criteria were recruited, consecutively. Blood sample was collected from the study participants following standard operating procedures (SOPs) by a qualified laboratory thnologist. About 3-4 ml blood sample from each patient was collected into a vacutainer tube (purple cap) containing 2.0 mg/ml

ethylenediamine tetraacetic acid (EDTA-K2) and preserved at room temperature for platelet analysis for ≥2 hrs..

5.6.4. Hematological analysis

Blood sample collected in EDTA tubes was mixed well to prevent clump and clot formation for platelet count and platelet indices. Samples were measured in Hematology auto analyzer (SYSMEX-XT 4000i, Sysmex Corporation, Kobe, Japan) within 2 hours of blood collection to determine the exact value of the platelet parameters. SYSMEX-XT 4000i provides a 27-parameter CBC, including a 6-part WBC Differential with reportable Immature Granulocytes. Reticulocyte Hemoglobin (RET-He) is standard on the fluorescent reticulocyte panel. The XT-4000*i* also performs body fluid analysis with seven parameters.Hematology analyses is performed according to the Hydro Dynamic Focusing (DC Detection), flow cytometer method (using a semiconductor laser), SLS-hemoglobin method and traditional impedance method for platelet count when Blood cells suspended in the diluted sample pass through an aperture causing a change in the direct current resistance between electrodes. The size of the blood cell is detected as electric pulses and the number of blood cells is calculated by counting the pulses.And the three discriminators the upper (UD),lower (LD) and the fixed discriminator used for discriminating platelet sizes. The results obtained were registered on registration books prepared for this purpose.

5.7. Data Quality Assurance

Blood sample quality was ensured by collecting and processing according to the standard operating procedures.

Pre-analytic

- Samples were checked whether they are in the acceptable criteria like; hemolysis, clotting, volume and collection time, were checked for the correct labeling.
- Safety procedure and specimen handling procedure were strictly followed
- Manufacturer procedures and SOP were strictly followed.
- Prior to analysis samples were checked for time of collection

Analytical

- Prior to analysis, samples were homogenized by inverting 10-15 times.
- The performance of automated hematology analyzer was checked by running three levels hematology cell controls (Normal, Low and High).

Post analytical

- The result of platelet counts and platelet indices were registered as the exact number (value)

5.8. Data analysis and interpretation

The data obtained from the automated CBC machine was entered and analyzed by using Statistical Package for Social Science (SPSS) version 20 (SPSS INC, Chicago, IL, USA). The mean and standard deviation were used to summarize platelet parameters. One-way ANOVA (analysis of variance) supplemented with post-hoc test was done to compare the mean platelet parameters difference across the three groups of women (normal, mild or severe preeclampsia). Receiver Operating Characteristics (ROC) curve was used to calculate sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), for a given platelet parameter and indices (PLT, MPV, PDW and P-LCR) in discriminating the presence or the absence of preeclampsia. Based on estimations of sensitivity and specificity for cut-off value of the platelet parameters, the Area under Curve (AUC) for each of them was determined. The largest AUC is associated with the best parameter for development of preeclampsia. Pearson correlation test was used to see the relationship between continuous variables. A P-value of <0.05 was considered as statistically significant.Tables and figures and were used for the description of the data.

5.9. Ethical considerations

The study was conducted after it was ethically reviewed and approved by the Department of Medical Laboratory Science research and ethical review committee (DRERC), College of Health Science, Addis Ababa. By getting permission from Tigray health bureau, ethical clearance was also obtained from Ayder and Mekelle hospitals. Then permission was obtained from the

hospitals' administration office and a letter informing each departmentswas written. The study 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.

5.10. Dissemination of the result

The finding of this study will be presented and submitted to Addis Ababa University, College of Health Science Medical Laboratory Science Department, to Ayder referral hospital and Mekelle hospital so as to influence for using platelet parameters in predicting preeclampsia in pregnant woman. Findings will be presented in relevant workshops, seminars and scientific conferences and manuscript will be submitted to peer reviewed national or international journal.

6. Results

This study included 2 groups of pregnant women: first group included 140 pregnant women with normal pregnancy and second group included 79 pregnant women with preeclampsia, 35 cases of second group were mild preeclampsia and 44 cases of second group were sever preeclampsia.139 study participants were from ACSH and the rest 90 study participants were from MGH.

6.1 Baseline Characteristics of Pregnant Women with and without Preeclampsia

There was a significant difference between the three studied groups with regards to blood pressure both systolic and diastolic which increased with severity of preeclampsia (P<0.05).Duration of pregnancy was significantly shorter in patients with preeclampsia than normal pregnant women groups (P-value < 0.05) (Table 1).While there was no significant difference between the three studied groups with regard to age with mean age of 25.64±3.9 in normal pregnant, 25.20±3.5 in mild preeclampsia and 25.64±5.26 in severe preeclampsia.

Table 1. Characteristics of Pregnant Women with and without Preeclampsia at ACSH and MGH, Mekelle, Ethiopia, from January –March 2017 (n=219)

		Preeclampsia		
	Normal(n=140)	**Mild(n=35)**	**Severe (n=44)**	**P-value**
Age	25.64±3.9	25.20±3.5	25.64±5.26	0.85
Gestational age (in weeks)	35.99±3.58	34.51±4.48	34.61±3.99	0.031
Systolic BP(mmHg)	102.50±10.6	143.14±5.29	157.95±16.07	<0.001
Diastolic BP(mmHg)	66.93±8.03	97.71±6.17	103.86±14.3	<0.001

6.2 Comparison of platelet parameters across severity of preeclampsia

Changes in platelet parameters in the three groups of women are described in Table 2. The mean±SD platelet counts were291.6±58.4x10^9/L, 226.42±56.52x10^9/L and185.28±60.22x10^9/L,in normal pregnancy, patients with mild preeclampsia and severe preeclampsia, respectively. The platelet count decreased across the severity of preeclampsia, while MPV,PDW and PLCR increased with severity of preeclampsia (*P* <0.05). The average platelet parameters were significantly different among the categories of women (P=0.000). Post-hoc tests also showed significant mean platelet parameter variation among each pairs of severity of preeclampsia (**Table 2**).

Table 2: Comparison of platelet parameters by severity of preeclampsia among pregnant women attendingACSH and MGH, Mekelle, Ethiopia, January-March 2017 (n=219).

Platelet parameters	**Preeclampsia**			**P-value*** **
	Normal(n=140) Mean±SD	**Mild(n=35) Mean±SD**	**Severe (n=44) Mean±SD**	
PC(10^9L)	291.6±58.4	226±56.5	185.3±60.2	<0.001
MPV(fl)	8.4±0.9	11.5±2.1	12.3±1.7	<0.001
PDW(fl)	10.8±1.8	11.1±1.6	14.3±3.4	<0.001
PLCR(%)	29.1±6.8	30.8±6.6	35.3±8.9	<0.001

Mean (Standard Deviation); F-test (One way-Anova) for mean difference across severity of preeclampsia; PC-Platelet Count; MPV-Mean Platelet Volume; PDW-Platelet Distribution Width; PLCR-platelet large cell ratio. ***(P-value is<0.001).

6.3 Correlation between the platelet parameters by severity of preeclampsia

In normal pregnant women, platelet count (PC) was negatively correlated with MPV, PDW and PLCR. For example, decrease in PC was proportional with increase in MPV (r=-0.35; p=0.000). While, the other parameters had strong positive relationship. In women who had mild preeclampsia none of the platelet parameters had significant correlation. In women with severe preeclampsia, PC was negatively correlated with PDW and PLCR (p<0.05) (**Table 3**).

Table 3: Correlation between platelet parameters in normal, mild and severe preeclampsia among pregnant women visiting ACSH and MGH, Mekelle EthiopiaJanuary –March 2017(n=219)

Platelet parameters	**Preeclampsia**		
	Normal (r)	Mild (r)	Severe (r)
PC vs. MPV	-0.35***	0.12	-0.16
PC vs. PDW	-0.36**	0.22	-0.32*
PC vs. PLCR	-0.38**	-0.1	-0.39**

r: Pearson correlation ,*** (P-value is <0.001),**(P-value is <0.01),*(P-value is <0.05)

6.3.1 Correlation between platelet count (PC) and platelet indices in women with preeclampsia

In women who had preeclampsia the platelet count had significant negative correlation with PDW and PLCR ($p<0.05$). While, PC was not significantly correlated with MPV with correlation coefficient ($r=-0.99$ and $p=0.385$ (Figure 2).

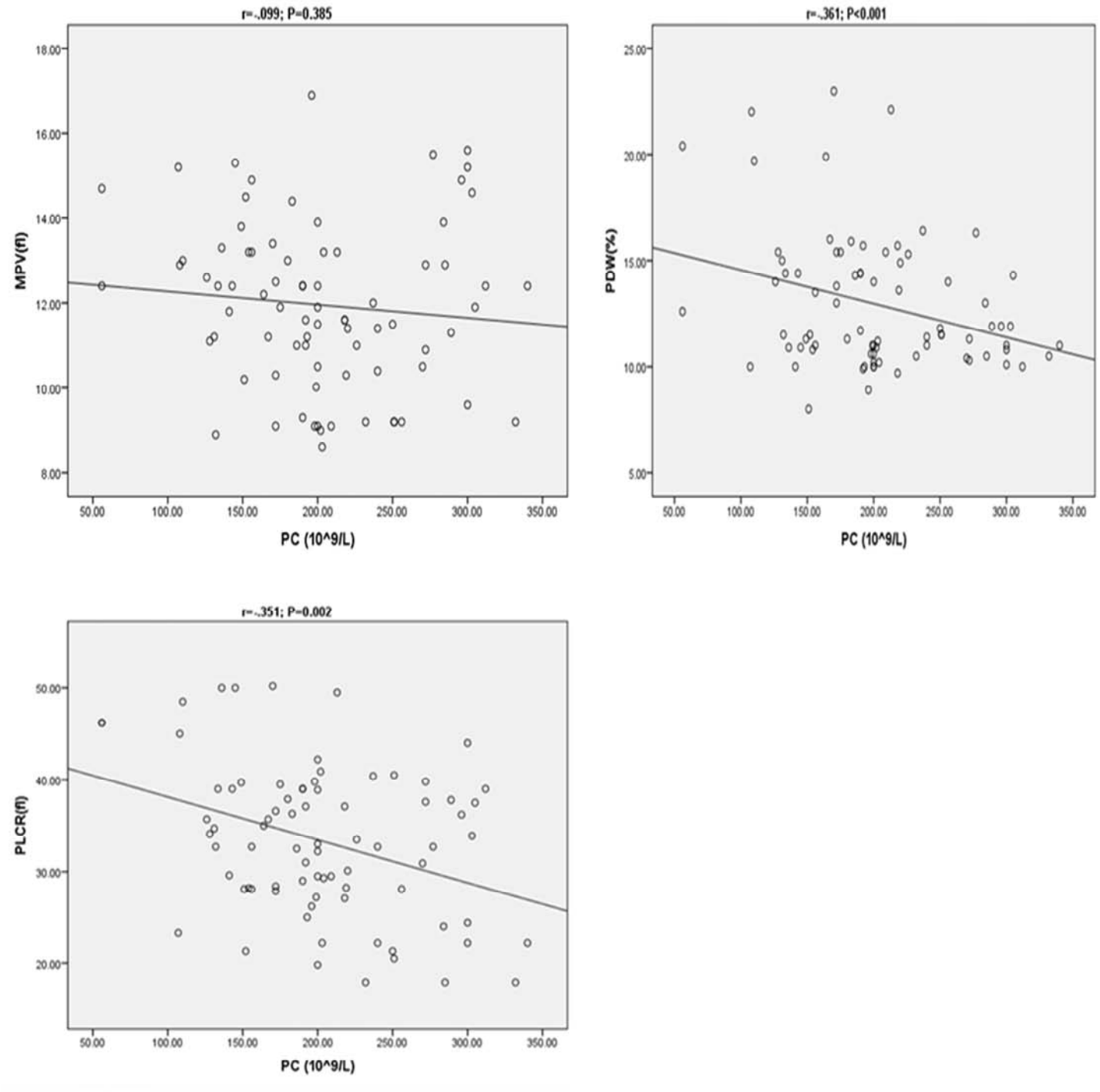

Figure 2: Correlation between platelet count (PC) and mean platelet volume (MPV), platelet distribution width (PDW), platelet large cell ratio (PLCR) in women with preeclampsia (where; r = correlation coefficient).

6.4 Comparison between the diagnostic values of platelet parameters

In this study, the optimal cut-off levels of platelet parameters for prediction of development of preeclampsia were identified using ROC analysis. ROC curve analysis showed that platelet count can differentiate normotensive pregnant women from preeclamptic pregnant women at a cut off value <233x10 ^{9}L with sensitivity of 70.9 % and specificity of 83.9 %.MPV can differentiate normotensive pregnant women from preeclamptic pregnant women at a cut off value >9.45fl with sensitivity of 83.5 % and specificity of 86.4%.PDW and PLCR have a sensitivity of 72.2%, 52.1% and 81.0%, 35.0% at a cut off values >10.85fl and >26.2% respectively. MPV has largest area under the curve (AUC=0.85; 95%CI (0.79, 0.89)), indicating as it is the best parameter for predicting preeclampsia (Figure 3 and Table 4). The second most important predictor parameter identified was PC.

Table 4: Comparison between the diagnostic values of platelet parameters in the study participants visiting ACSH and MGH, Mekelle, Ethiopia, January-March2017 (n=219).

Platelet indices	**Sensitivity (%)**	**Specificity (%)**	**PPV (%)**	**NPV (%)**	**Cut-off**	**AUC(95% CI)**
PC(10^9l)	70.9	83.9	70.9	83.6	<233	0.77(0.71, 0.83)
MPV(fl)	83.5	86.4	77.6	90.3	>9.45	0.85(0.79, 0.89)
PDW (fl)	72.2	52.1	46.0	76.8	>10.85	0.625(0.557, 0.686)
PLCR(%)	81.0	35.0	41.3	76.6	>26.2	0.58(0.52, 0.639)

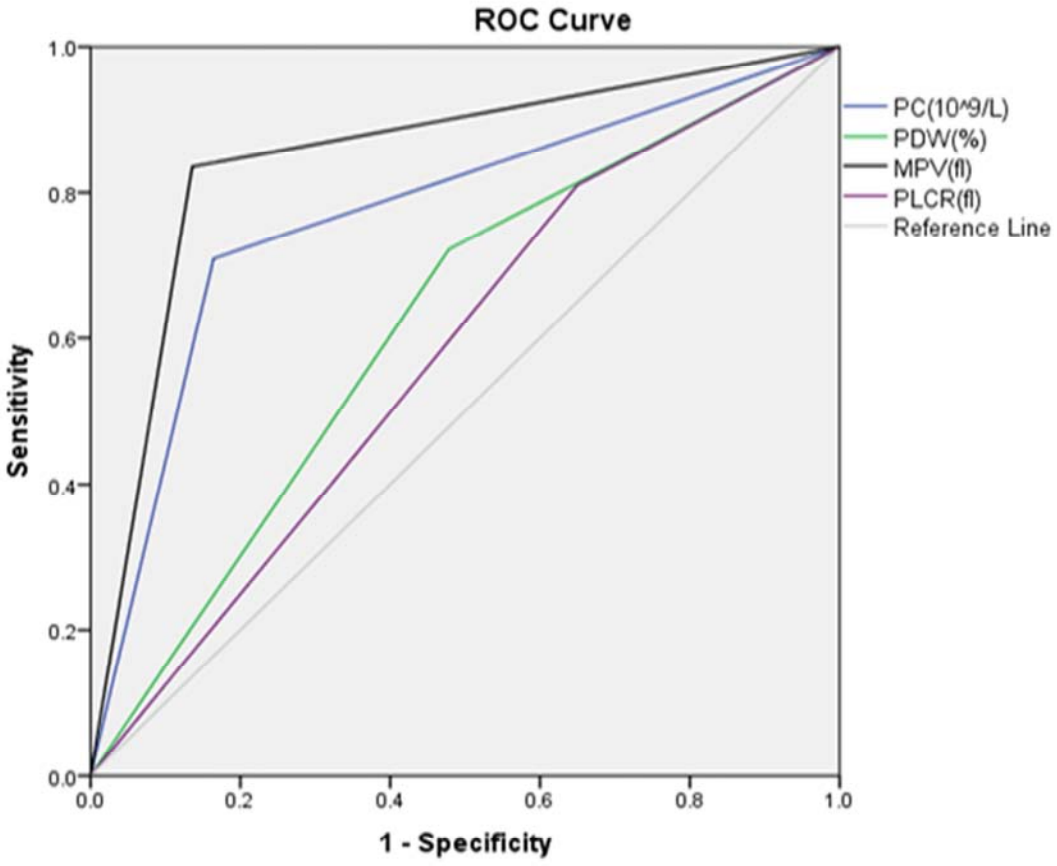

Figure 3: Receiver Operating Characteristics (ROC) curve of platelet parameters in pregnant women with preeclampsia (mild or severe) visiting ACSH and MGH , Mekelle, Ethiopia, January-March 2017 (n=219).

7. Discussion

CBC parameters including platelet count and platelet indices such as MPV, PDW and PLCR are widely available and are cost-effective. However, in daily practice, most physicians consider only CBC parameters .thus neglecting other parameters like platelet indices [25].PE remains a significant cause of maternal and fetal mortality and morbidity. [44]

In the present study, which aimed to evaluate the role of platelet parameters in prediction of preeclampsia, the three groups were comparable as regard to maternal age.According to the pathophysiology of preeclampsia, endothelial activation leads to increased platelet aggregation which in turn is responsible for decrease in the platelet count. In the current study, there is a significant gradual decrease in platelet count from normotensive pregnant women 291.6±58.4 mm^3) to mild PE group (226±56.5mm^3) and severe PE group (185.3±60.2mm^3).

Similar findings were reported by Dadhich *et al* [27]who observed declining platelet count with severity of preeclampsia. The authors noted that the decrease in platelet count was antedating significant increase in blood pressure by 4 to 6 weeks of pregnancy and concluded that the Platelet parameter can be used to predict development of progressive hypertension in patientsat risk. Accordingly similar inverse relation between platelet count and severity of preeclampsia was alsodocumented byseveral researchers including Mohamed*et al* [24].Freitas *et al* [31], Kaito K*et al* [23], and similar studies also by Neiger *et al* [32], Sultana R *et al* [33], and Amita *et al*describe that platelet consumption and aggregation increases as the disease gets more severe [34].

In the current study the PC cutoff value was <233x10^9/L in preeclamptic females (sensitivity 70.97%; specificity 89.3%) which was in agreement with Eman *et al* [45] which showed that platelet count at cut off value < 198,000 can differentiate normotensive pregnant women from mild PE patients with a sensitivity of 90% and specificity of 92% and at a cut off value of < 149,000 can differentiate mild PE from severe PE patients with sensitivity of 84% and specificity of 92 %. On the other hand there are studies which didn't found a significant difference in Platelet count of the groups such as Santos *et al* in Turkey [36], Elkareem *et al* [39], and studies done by Kulkarini and Sutaria *et al* [41].

Due to increased consumption and destruction of platelets, bone marrow producesand release large platelets leading to increase MPV in preeclampsia [23].However literature reveals conflicting results regarding the relation between MPV and preeclampsia. Dadhich et al, in their study described MPV as a good marker of platelet dysfunction in preeclampsia [27].
Moreover therewas a gradual increase in MPV of the current study from normotensive pregnant women [8.4±0.9fl] to mild preeclampsia [11.5±2.1fl] and sever preeclampsia patients [12.3±1.7] with P-value < 0.001.Kaito k *et al* in 2005 in Britain also explained that hyper destruction of platelet results in an in Increases MPV, PDW, and PLCR [23] similar findings were found by Hutt *et al* [46] reported an increase in MPV which was noted about two weeks before the increase in blood pressure and other clinical features of pre-eclampsia developed.Freitas [31], Nooh *et al* [35], Ahmed W [38] *et al*, Elkareem *et al* [39] and Santos *et al* [35].

On the other hand, Cyehan *et al* [47]and Amita *et al* [34]did not find a significant difference in the MPV between preeclampsia and normal pregnant group.Some researchers have suggested that Platelet count and MPV cannot predict the risk of PE. Ceyhan et al claimed that MPV used to predict PE: however it may be unreliable, because of differences in the methods and/or equipment used to obtain CBCs [47]. For example, addition of EDTA increases the MPV and various hematological counters yield different MPV data. In the present study, the time between sample collection and CBC measurements was less than 2 hrs. perhaps minimizing such concerns.

By ROC curve analysis we found that MPV at cut off value >9.45 can differentiate preeclamptic pregnant women from normal women with a sensitivity of 83.5% and specificity of 86.4%.Dundar *et al* [48] stated that an MPV cutoff of 8.5 fl (sensitivity 78%; specificity 86%) was indicative of a risk of PE. Freitas *et al* [31] considered a slight higher that a cutoff of 10 fl (sensitivity 51.72%; specificity 82.76%). Howarth S *et al* [28]on the other hand suggested that a combination of reduced platelet count and elevated MPV afforded a sensitivity of 90% and a specificity of 83.3% when used to predict PE. These results are in agreement with Dadhich *et al*. and Yin *et al* [27, 19] as they described MPV as a good marker of platelet dysfunction in preeclampsia. On the other hand, Kashanian *et al* [49] observed that MPV changes did not

predict preeclampsia or preterm labor. Moreover Altibas *et al* [22] reported that MPV is not a significant predictor of severity of preeclampsia [22].

In the present study there was significant increase in PWD from normotensive pregnant women (10.8±1.8fl) to mild PE (11.1±1.6 fl) and severe PE groups (14.3±3.4 fl) with P-value < 0.001. These results are supported by Dadhich *et al* [27] who demonstrated a month wise increase in PDW in preeclampsia group as compared to those in normal pregnant group. These results were in agreement with that reported by Santos and Nooh *et al* [35, 24], Ahmed W *et al* [38], Freitas *et al* [31] and Song Y*et al* [18]. The increased PDW is explained by increased platelet turnover which would support the idea that platelet survival time is decreased resulting in increased destruction of platelets. This may be also because of increased bone marrow activity of unknown stimulus. Similarly rise in PDW serves as an important indicator of disease severity [22].A previous study in Ethiopia by the supervisors of this research have demonstrated the predictive value of platelet indices for discriminating the two causes of thrombocytopenia hyper-destruction versus hypo-production where higher values of the indices were found in patients with hyper-destruction thrombocytopenia [42].

In our study ROC curve analysis showed that PWD can differentiate preeclamptic pregnant women from normal pregnant women at a cut off value ≥ 10.8.fl with sensitivity of 72% and specificity of 52 % with AUC of 0.625.Nooh AM *et al* (2015)in Egypt foundROC curve analysis of PDWwith a little higher AUC (0.980) PDW>19.9 as optimal cut-off for the prediction of PE development. This cut-off level had a sensitivity of 96.3% and a specificity of 91.3% for prediction of PE development [24]. Consistent with Eman et al(2005) ROC curve analysis showed that PWD can differentiate normotensive pregnant womenfrom mild PE at a cut off value ≥12.6fl with sensitivity of 90 % and specificity of 92 % and can differentiate mild from severe PE at a cut off value ≥ 16.4fl with sensitivity of 84 % and specificity of 92 % with AUC of 0.886 and 0.862 respectively [45].

There was also significant increase in PLCR from normotensive pregnant women (29.1±6.8%) to mild PE (30.8±6.6%) and severe PE groups (35.3±8.9fl) with P-value < 0.001.This finding is consistent with findings of Kaito *et al* [23] also stated that increased PLCR indicate increased bone marrow activity,Santos *et al* [36], Ahmed W *et al* [38] and Elkareem*et al* [39]. Contradicting result was found by Yayuzcan *et al* [40].

ROC curve analysis showed that PLCR can differentiate preeclamptic pregnant women from normal pregnant women at a cut off value >26.2% with sensitivity of 81% and specificity of 35% with AUC of 0.58. Formerly similar study was done by Han L et al (2014) found AUC of PLCR to predict preeclampsia very close with our study which was 0.587[50].

The final findings regarding correlation between platelet parameters were, in normal pregnant women, PC was negatively correlated with MPV, PDW and PLCR. For example, decrease in platelet count was proportional to an increase in MPV (r=-0.35; p=0.000).While, the other parameters had strong positive relationship. The highest negative correlation index was found betweenplatelet count and platelet distribution width in preeclampsia patients. In women who had mild preeclampsia none of the platelet parameters had significant correlation. In women with preeclampsia, platelet count was negatively correlated with PDW and PLCR ($p<0.05$).

Eventhough no study has been done regarding correlation of platelet count and platelet induces in preeclampsia patients there was a study done in our country Ethiopiaregarding correlation of platelet count and platelet induces in hyper destruction thrombocytopenia which is the main cause for reduced platelet count during preeclampsia by Negash N *et al* (2016) found statistically significant negative correlation between platelet count and the platelet indices in immune thrombocytopenia purpura patients due hyper destruction of platelets which was PLT Vs MPV **(**r (correlation coefficient)=-0.5 P=0.004**)**, PLT Vs PDW **(**r=-0.6 P<0.001**)** and correlation between PLT Vs PLCR **(**r=-0.5 P=0.001**)** [42].

8. Strength and limitation

8.1. Strength of the study

- ✓ All blood samples for CBC assessment were processed within two hours after vein puncture.
- ✓ Assessments were carried out using the same anticoagulant and the same automated counter.
- ✓ The study is the first of its kind in our country to assess the role of platelet parametrs in predicting PE.

8.2. Limitation of the study

- ✓ As the study was designed to be cross-sectional, to establish the role of platelet parameters in prediction and assessing the severity of preeclampsia in the course of the pregnancy period was not possible.

9. Conclusion and recommendation

9.1 Conclusion

Our work showed that platelet count, MPV, PDW and PLCR were useful to detect the risk of PE, due to increased platelet destruction and platelet turnover in patient with preeclampsia, decreasing platelet count and increasing MPV, PWD and PLCR may play a role in predicting preeclampsia although our sensitivities and specificities with regard to PDW and were a little less than those of other studies. In addition the estimation of platelet indices may be considered as an easy, reliable, economic and rapid method for detection of preeclampsia and assessment of its severity.

9.2 Recommendations

- ✓ Platelet parametrs are easily obtained together with the CBC report; thus, clinicians should evaluate platelet count and indices when assessing the risk of PE.
- ✓ Further research including more study participants would further help us to assess whether or not preeclampsia can be predicted in the early second trimester in a similar way. Women who have their blood drawn early, mid, and late second trimester could be followed for the development of preeclampsia and predict the prognosis of the mother and the fetus.

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11. Annexes

Annex I Participant information sheet

Principal Investigator: Feven Tesfay Kinfe

Addis Ababa University College of Health Sciences

Introduction: You are being asked to take part in research study on Changes in Platelet parameters duringPregnancy as Potential Markers forPrediction of Preeclampsia Development. The study has been approved by Addis Ababa University Medical Laboratory Science department research ethics committee.

Purpose: The purpose of this study is to evaluate the changes in platelet count and platelet indices in preeclampsia compared to normal pregnancy at Aydercomprehensive specialized and Mekelle General Hospitals.

Procedures to be carried on: you are invited to participate in the study after giving your consent by giving blood samples if CBC is not ordered by your doctor.

Risks associated with the study: There is no risk and serious invasive procedure at the beginning as well as at the end of the study and there is no additional time required from you to stay during study.

Benefits of the 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 parameters 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.

Confidentiality of your information: 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.

Termination of the study: We will respect your decision if you later on change your mind.

Your withdrawal of consent will not affect your right to receive medication. . Also you have the right to have question about the study. I will be glad to answer your questions about this study at any time.

You may contact me at e-mail address *feva.tesfay@gmail.com* or mobile *+251 912 10 2948* Department of Medical Laboratory Science research ethics office +251 11 275 5170

Participants information sheet (Amharic version)

ስለ ጥናቱ ለተሳታፊዎች መረጃ የሚሰጥ ቅጽ

ስሜ- ፌቨን ተስፋይ ክንፈ እባላልሁ

እርስዎ በዚህ የምርምር ሥራ ለመሳተፍ ፍቃደኛ ኖት? ከአዲስአበባ ዩኒቨርስቲ የላቦራቶሪ ሳይንስ ትምህርት ቤት የምርምር ሥራ ሥነ-ምግባር ኮሚቴ ፈቃድ ያገኘ ሲሆን ከጥናቱ የሚገኘዉ ዉጤት ለማወቅ ይጠቅማል፡፡

የጥናቱ ኣላማ ፡ ፕላትሌት በሚባሉ የደም ዓይነቶች የሚመጣ ለዉጥ ኣንዴት ነብሰፀር ሴት በደም ብዛት መከንያት የሚመጣን በሽታ ለማወቅ ይረዳናል ወይ እሚለዉን ለማወቅ ነው። በዚህ የጥናት ሥራ ለመሳተፍ ፈቃደኛ ከሆኑ ከ3-4 ሚ (አንድ የሻይ ማንኪያ የሚሆን) የደም ናሙና ይሰጣሉ፡፡

ይህ የደም ናሙና በደምዎ ውስጥ በዚህ ጥናት መሳተፍዎ ሙሉ የደም ምርመራ ውጤትዎን ለማወቅ ዕድል ይሰጠዎታል፡፡

ከጥናቱ ጋር ተያይዞ ሚመጣ ጉዳት: ጥናቱ በርሶ ላይ ምያመጣዉ ጉዳት የሌለ ሆኖ ለጥናቱ ምያጠፉተ ጨማሪ ጊዜ ኣይኖርም።

ከጥናቱ የምያገኘት ጥቅም ፡ ምንም ኣይነት የገንዝብ ጥቅም ባይኖረውም ከጥናቱ በሚገኘዉ ዉጤት ለማወቅ እና ተጠ ቃሚ እንዲሆኑ ይጠቅማል ላላማው የእናቶቸ ሞትና ተጋላችነት ለመቀነስ እንደመሆኑ፡፡

ከርስዎ ምናገኘዉ መረጃ እና ሚስጥራዊነቱ:

በሰጡትደም ላይ የሚደረገው የምርመራ ውጤት ሙሉበሙሉ ሚስጥራዊነቱ እንደተጠበቀ ሆኖ ናለጥናቱ ዓላማ ብቻ ጥቅም ላይ እንደሚውል ላረጋግጥልዎ እወዳለሁ፡፡በዚህ ጥናት ላይ ያለዎትን ጥያቄ በማንኛውም ሰዓት ሊጠይቁና ምላሽሊ ያገኙ ይችላሉ፡፡በጥናቱ ላይ ያለመሳተፍም ሆነ በመሀል የማቋረጥ ሙሉመብት አለዎት፡፡

በማንኛውም ሰዓት በጥናቱ ላይ ያለዎትን ጥያቄ ለመመለስ ደስተኛ ነኝ!

በሚቀጥለው አድራሻ ሊያገኙን ይችላሉ

ስልክ፡- 0912102948

Email :feva.tesfay@gmail.com

ህክምና ላቦራቶሪ ትምህርት ክፍል የምርምር ሥነምግባር ቢሮ ስልክቁጥር +251 11 275 5170

Participants information sheet (Tigrigna version)

ስለ እቲ ፅንዓት ንተሳታፍቲ ሓበሬታ ዝህብ ዓንቀፅ

ስመይ፡ ፌቨን ተስፋይ ክንፈ ይብሃል።

ንሰን በዚ ናይ ምርምር ስራሕ ፍቃደኛ ድየን? ካብ አዲስአበባ ዩኒቨርስቲ ናይ ላቦራቶሪ ሳይንስ ቤትትምህርቲ ናይምርምር ስራሕ ሥነ፡ምግባር ኮሚቴ ፈቓድ ዘግነየ ኮይኑ ካብቲፅንዓት ዝግነይ ዉፅኢት ንምፍላጥ ይጠቅም፡፡

በዚ ፅንዓት ንምስታ ፍፈቃደኛ ተኮይነን ካብ 3-4ሚሊ (ሐደ ናይ ሻሂ ማንካ ዝኸዉን) ናይ ደም ናሙና ይህባ፡፡እዚ ናይ ደም ናሙና አብ ደመን ውሽጢ በዚ ምርምር ብምስታፈን ሙሉእ ናይ ደም ምርመራ ውፅኢተን ንምፍላጥ ዕድል ይወሃበን፡ ፡አብ ዝሃበአ ደም ዝግበር ናይ ምርመራ ውፅኢት ሙሉእ በሙሉእ ሚሸጥራዊነቱ ዝተሓለወ ከምዝከዉን እና ነቲ ምርምር ዓላማ ብቻ አብ ጥቅሚ ከምዝዉዕል ከረጋግፀለን ይፈቱ፡፡

አብዚ መፅናዕቲ ዘለወን ጥያቄ በማንኛውም ሰዓት ከጥይቃ ከምኡ እዉን መልሲ ክረክባ ይክእላ እየን፡፡አብቲ መፅናዕቲ ንምስታፍ ኮነ አብ ማእከል ናይ ምቑራፅ ሙሉእ መሰል አለወን፡፡

አብ ማነኛውም ሰዓት አብቲ መፅናዕቲ ዘለወን ሕቶ ንምምላ ሀጉስቲ እየ፡-

ብዝቅፅል አድራሻ ከግንያኒ ይክእላ እየን።

ስልኪ፡፡ 0912102948

Email :feva.tesfay@gmail.com

ሕክምና ላቦራቶሪ ክፍሊ ትምህርቲ ናይ ምርምር ሥነምግባር ቢሮ ስልኪ ቁፅሪ: +251 11 275 5170

Annex II Informed consent form

By signing below, you are agreeing that: (1) you have read and understood the participant information sheet, (2) questions about your participation in this study have been answered satisfactory, (3) you are taking part in this research study voluntarily (without any coercion).

Participant's ID

---------- ----------

Participant's signature Date

---------- ----------

Name of person obtaining consent Signature of person obtaining consent and date

Informed consent form (Amharic version)

እርስዎ በጥናቱ ለመሳተፍ ሙሉ ፈቃደኛ መሆኖን የሚገልጽበት ቅጽ

ከዚህ በታች ያለውን በጥናቱ ለማሳተፍ ፈቃደኛ መሆኑዎን እንዲያረጋግጡ በሚጠይቀው ቅጽ ላይ ሲፈርሙ ነው::

1. **ከላይ የተሰጠዎትን ስለጥናቱ መረጃ የሚሰጠውን ጽሑፍ ማንበብዎን ና መረዳትዎን**
2. **በጥናቱ ላይ ላነሱት ጥያቄ አጥጋቢ ምላሽ እንዳገኙ**
3. **በጥናቱ ለመሳተፍ የወሰኑት በራስዎ ሙሉ ፈቃደኝነት ና ምንምዓይነት ተጽዕኖ ወይም ግፊት ሳይደረግብዎ መሆኑን ያረጋግጣሉ::**

……..
የተሳታፊው መለያቁጥር

……. …….
የተሳታፊው ፊርማ ቀን

……. ……..
ስምምቱን የተቀበለው ሰው ስም ፊርማ እና ቀን

Informed consent form (Tigrigna version)

እተን ተሳተፍቲ አብቲ መፅናዕቲ ንምስታፍ ሙሉእ ፈቃደኛ ሙኹዋነን ዝገልፅ ዓንቀፅ
ካብዚ ንታሕቲ ዘሎ አብቲ መፅናዕቲ ንምስታፍ ፈቃደኛ ሙኻነን ንምርግጋፅ አብዝጥይቅ ዓንቀፅ እንትፍርማ

1.አብ ላዕሊ ዝተወሃበን ስለእቲመፅናዕቲ ሓበሬታ ዝህብ ፅሑፍ ምንባበን ንምርድአንን
2.እቲ መፅናዕቲ መሰረት ጌረን ዘልዐልኦ ሕቶ ኣፅጋቢ መልሲ ከምዝረከባ
3.ኣብቲ መፅናዕቲ ንምስታፍ ዝወሰና ብባዕለን ሙሉእ ፈቃደኝነትን ምንምዓይነት ጸቅጥ ወይ ድፍኢት ዘይተገበረለን ምኻኑ የረጋግፃ::

…….። ……::
ናይ ተሳታፊት መለለይ ቁፅሪ ፈርማ እና ዕለት

…….:: ……::
ሀበሬታ ዝኣክበ በዓል ሞያ ሸም ፈርማ እና ዕለት

Annex III. Standard Operating procedures

SOP for Blood collection

In order to draw blood and collect to perform a variety of laboratory tests there are equipments necessary.

Equipment

- 21 gauge needle for each participant with closed vacutainer system
- Blood collection tubes for each participant
- Tourniquet
- Box of nitrile /vinyl gloves (Do not use latex gloves due to allergies/sensitivities.)
- Alcohol wipes
- Cotton balls/swabs
- Bandages
- Pillow/pad for raising arm to comfortable elevation
- Apple/orange juice and snacks for fasting participants
- Disposable, single use materials or equipment are to be used whenever possible
- Any reusable materials or equipment must be cleaned and disinfected with Alcohol-based sanitizers before use with another participant

Safeguards /safety procedures

- A new pair of disposable latex/vinyl gloves is used with each participant. Gloves are to be for single-procedure use only. Gloves should always be removed Using a glove-to-glove or skin-to-skin technique which will prevent Contaminating the hands.
- The use of gloves does not replace the need for hand hygiene. Hands should be Properly washed before the gloves are put on and after the gloves are removed. Hand hygiene is also needed before and after the replacement of gloves during a Procedure or in between tasks.
- Participants are reminded to do no heavy lifting for 24 hours.

Procedure for drawing blood

Step 1 – Assemble equipment

Collect all the equipment needed for the procedure and place it within safe and easy reach on a tray or trolley, ensuring that all the items are clearly visible.

Step 2 – Identify and prepare the patient

Step 3 – Select the site

Step 4 – Perform hand hygiene and put on gloves

Step 5 – Disinfect the entry site

Step 6 – Take blood

Step 7 – Fill the laboratory sample tubes

- When obtaining multiple tubes of blood, use evacuated tubes with a needle and tube holder. This system allows the tubes to be filled directly. If this system is not available, use a syringe or winged needle set instead.
- If a syringe or winged needle set is used, best practice is to place the tube into a rack before filling the tube. To prevent needle-sticks, use one hand to fill the tube or use a needle shield between the needle and the hand holding the tube.
- Pierce the stopper on the tube with the needle directly above the tube using slow, steady pressure. Do not press the syringe plunger because additional pressure increases the risk of hemolysis.
- Where possible, keep the tubes in a rack and move the rack towards you. Inject downwards into the appropriate colored stopper. DO NOT remove the stopper because it will release the vacuum.
- If the sample tube does not have a rubber stopper, inject extremely slowly into the tube as minimizing the pressure and velocity used to transfer the specimen reduces the risk of hemolysis. DO NOT recap and remove the needle.
- Before dispatch, invert the tubes containing additives for the required number of times (as specified by the local laboratory).

Step 8 – Draw samples in the correct order

Step 9 – Clean contaminated surfaces and complete patient procedure

Step 10 – Prepare samples for transportation
Step 11 – Clean up spills of blood or body fluids

Quality control

Quality assurance is an essential part of best practice in infection prevention and control (1). In phlebotomy, it helps to minimize the chance of a mishap.

SOP for haematology analyser SYSMEX –XT 4000i

Sysmex XE -4000i

Provides a 27-parameter CBC, including a 6-part WBC Differential with reportable Immature Granulocytes. Reticulocyte Hemoglobin (RET-He) is standard on the fluorescent reticulocyte panel. The XT-4000*i* provides you with body fluid analysis with seven parameters including a 2-part WBC Differential.

Principle

TheSysmexXT-4000i Automated Hematology System utilizes the power of fluorescent flowcytometry and hydrodynamic focusing technologies. Using a unique, state-of-the art, diodelaserbench, Sysmex fluorescent flowcytometry provides the sensitivity needed for measuring and differentiating cell types in whole blood and body fluid samples. Fluorescent technology and hydrodynamic focusing enable theXT-4000i to consistently classify normal WBC,RBC and PLT populations from abnormal populations, thereby decreasing the number of manual interventions. Performs hematology analyses according to the Hydro Dynamic Focusing (DC Detection), flow cytometry method (using a semiconductor laser), and SLS-hemoglobin method.

= Platelet counts by a fluorescent optical method and by traditional impedance technology to improve accuracy of very low and very high PLT counts

- Obtain accurate counts when interferences are present
- Offers automated judgment for reporting PLT-O or PLT-I through instrument settings

ReagentsCELLPACTM (Diluent)

STROMATOLYSER-FBTM (Lyse)

STROMATOLYSER-4DLTM (Lyse)

STROMATOLYSER-4DS TM (Stain)

SULFOLYSER (Lyse)

RET-SEARCH II (Diluent)

RET-SEARCH II (Stain)

Throughput Approximately 80-100 whole blood specimens/hr depending on mode used. Approximately 38 body fluid specimens/hr.

Quality Control e-Check (XE) – 3 Levels

Comprehensive QC files including (Total QC Management) "current"and"new" lotfeature Levey-Jennings controlchart X-barMfile Online QualityAssurance Program–InsightTm

IVD Parameters WBC, RBC, HGB, HCT, MCV, MCH, MCHC, PLT, NEUT%/#, LYMPH%/#, MONO%/#, EO%/#, BASO%/#, RDW-CV, RDW-SD,PDW ,MPV, RET%/#, IRF, IG%/#, RET-He# WBC-BF, RBC-BF, MN%/#, PMN%/#, TC-BF

Specimen

Specimen Type/Stability/Storage

Some anticoagulants will alter test results due to their effects on hemolysis and blood platelet agglutination. Therefore, use EDTA-2K, 3K or 2Na as the anticoagulant.

Specimens should be stored at room temperature of 18 - 26ºC or in the refrigerator of 2 - 8ºC . If stored in a refrigerator, samples should be returned to room temperature, for approximately 30 minutes, before analysis. Otherwise correct results may not be obtained.

Equipment and apparatus

1. Sysmex XT-4000i

Procedure

Instrument Start-Up

01.8.2 Running Controls

01.8.3 Running Patient Samples

Internal Quality Control

Control samples are analyzed by the X bar or the L-J control programs, and the data is stored in the quality control file. At least two levels of controls should be run before analyzing the patient samples. X bar program analyzes the control twice in succession and the average data is used. L-J program on the other hand, uses the results from one analysis as one control data. In this lab we use the L-J program.

HAEMATOLOGY ADULT NORMAL RANGES

INSTRUMENT USED: SYSMEX XT 4000i

PARAMETER	REFERENCE RANGE
Red Blood Cell Count (RBC)	
Men	4.5 – 5.5 x 10^{12}/l
Women	3.8 – 4.8 x 10^{12}/l
Haemoglobin (Hb)	
Men	13 – 17 g/dl
Women	12 – 15 g/dl
Haematocrit (HCT)	
Men	40 – 50 %
Women	36 – 45 %
Mean Cell Volume (MCV)	
Men	83 – 99 fl
Women	83 – 99 fl
Mean Cell Haemoglobin (MCH)	
Men	27 – 32 pg
Women	27 – 32 pg
Mean Cell Haemoglobin Concentration (MCHC)	
Men	32 – 34 g/dl
Women	32 – 34 g/dl
Red Cell Distribution Width (RDW)	11.6 – 14.0 %
White Blood Cell Count (WBC)	4-10 x 10^{9}/l
Differential White Cell Count (Diff)	
Neutrophils	40 – 80 % (2 - 7 x 10^{9}/l)
Lymphocytes	20 – 40 % (1 – 3 x 10^{9}/l)
Monocytes	2- 10 % (0.2 – 1.0 x 10^{9}/l)
Eosinophils	1 – 6 % (0.02 – 0.5 x 10^{9}/l)
Basophils	< 1- 2 % (0.02 – 0.1 10^{9}/l)
Platelet Count	150 – 400 x 10^{9}/l
MPV	
F-	7.2-10.4 FL
M-	7.5-11.5 FL
PDW	9-14 FL

PLT Particle Size Distribution

Platelet particle size distributions are analyzed using three discriminators: a lower discriminator (LD) and upper discriminator (UD), which are automatically set up between 2 - 6 fL and 12 - 30 fL, respectively; and a fixed discriminator, which is set at 12 fL. PLT particle size distributions are checked for abnormalities, including abnormal relative frequencies at the lower discriminator, abnormal distribution widths, and the existence of more than one peak.

1. PDW (PLT Distribution Width) With the peak height assumed to be 100%, the distribution width at the 20% frequency level is PDW. Units are expressed in fL , with 1 fL equal to 10^{-15}L.
2. P-LCR (Platelet Large Cell Ratio) The P-LCR is the ratio of large platelets from the 12 fL discriminator or larger. It is calculated as a ratio comparing the number of particles between the fixed discriminator and UD, to the number of particles between LD and UD.
3. MPV (Mean Platelet Volume) The MPV is calculated from the following equation: $MPV(FL)=\frac{PCT(\%)X1}{PCTX10^{3}/UL}$

Interpretation of platelet indices results

Platelet Indices

- Platelet indices are potentially useful markers for the early diagnosis of thromboembolic diseases.
- An increase in both mean platelet volume (MPV) and platelet distribution width (PDW) due to platelet activation, was suggested due to :
    - Platelet swelling.
    - Pseudopodia formation.
    - MPV and PDW are simple platelet indices, which increase during platelet activation.

Mean Platelet Volume (MPV)

- Definition : MPV is a machine-calculated measurement of the average size of platelets found in blood and is typically included in blood tests as part of the CBC.
    1. **MPV is higher** when there is destruction of platelets.
        1. This may be seen as in inflammatory bowel disease.
        2. In immune thrombocytopenic purpura (ITP).
        3. In myeloproliferative diseases and Bernard-Soulier syndrome.
        4. It may also be related to pre-eclampsia, and recovery from transient hypoplasia.
    2. **Low MPV** values seen in :
    3. Primarily with thrombocytopenia when it is due to :
        1. Impaired production as in aplastic anemia.

Platelet distribution width (PDW)

- PDW is simple, practical and specific marker of activation of coagulation.
- PDW is a more specific marker of platelet activation, since it does not increase during simple platelet swelling.
- PDW has been receiving attention due to its usefulness for distinguishing between reactive thrombocytosis and thrombocytosis associated with myeloproliferative disorder.
- Determination of the PDW reference range is fundamental.
- Mean platelet volume (MPV) and platelet distribution width (PDW) are useful in the differential diagnosis of aplastic anemia and idiopathic thrombocytopenic purpura.
- An increased PDW is an indication for the anisocytosis of platelets.
- Standard PDW ranges from 9 to 14 fL.

Declaration

Assurance of Principal Investigator

The undersigned agrees to accept responsibility for the scientific ethical and technical conduct of the research project and for provision of required progress reports as per terms and conditions of the research publications office in effect at the time of grant is forwarded as the result of this application.

Name of the student: Feven Tesfay Kinfe

Date _____ Signature _____

Approval of Advisors:

Aster Tsegaye, MSc, PhD

Date_____ Signature _____

Jemal Alemu, MSc, PhD Candidate

Date_____ Signature _____

Mikias Negash, MSc

Date _____ Signature _____