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The diagnostic predictive value of platelet count and platelet indices in patients with acute coronary syndrome: A comparative cross sectional study at TikurAnebesa Specialized Hospital, Addis Ababa, Ethiopia.

By: KalkidanGera (BSc)

Advisors: FekaduUrgessa(Msc,PhD candidate)

MelatworkTibebu(MSc, PhD candidate)

Collaborator: TigistSeleshi(MD, Cardiologist)

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This is to certify that this thesis prepared by Kalkidan Gera, entitled; **The diagnostic predictive value of platelet count and platelet indices in patient with acute coronary syndrome: A comparative cross sectional study at TikurAnebesa Specialized Hospital, Addis Ababa, 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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Advisor_____ Signature _____Date

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Contents

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Abbreviations

ACS··· Acute Coronary syndrome

ASMR...Age-standardized mortality rates

AUC – Area under curve

CBC··· Complete Blood cell count

CHD··· Coronary Heart Disease

CKMB···Creatine Kinase Myoglobin binding

ECG ... Electrocardiogram

EDTA···Ethylene diamine tetra acetate

FL·····.Femto liter

HGB··· Hemoglobin

MPV··· Mean Platelet Volume

NPV… Negative predictive value

MI··· myocardial infarction

NSTEMI··· Non segment elevation myocardial

PDW··· Platelet distribution width

PLCR··· Platelet large cell ratio

PLT··· Platelet

PCT··· Plateletcrite

PPV···Positive predictive value

RBC··· Red blood cells

ROC···. Receiver operating characteristic

STEMI ··· Segment elevation myocardial infarction

SOP ··· Standard operating procedure

SPSS··· Statistical package for social science

TASH ··· TikurAnebesa Specialized Hospital

UA···.. Unstable angina

WBC··· White blood Cells

WHO… World health organization

Abstract

Background: Acute coronary syndrome(ACS) is the leading cause of death worldwide . The abnormality of Platelet and platelet indices are one of the most commonly identified hematological changes in ACS.

Objective: To determine the diagnostic predictive value of platelet count and platelet indices in patients with ACS at TikurAnbesa Specialized Hospital, Addis Ababa, Ethiopia from April 2021-March2023.

Methods: Hospital based comparative cross-sectional study was conducted among 76 ACS patients of cases and 152 controls (apparently healthy individuals) in TikurAnbesa specialized hospital(TASH)in Addis Ababa conducted from April 2021 to March 2023. Convenient sampling technique was used. Independent t testwas used for comparison betweenthe twogroups.ROC(receiver operating characteristic) curve test used to calculate positive and negative predictive values, sensitivity and specificity for a given platelet indices. Data was entered and analyzed using SPSS version 25 and level of significance was determined when P value was < 0.05.

Result: The mean age of ACS group(50.91±11.33) and control group (51.85±11.7) was matched. Males study participants predominated females in all groups.The mean of Mean Platelet Volume(MPV) (11.66±.1.19)fl, Platelet Large Cell Ratio(PLCR) (37.35 ± 9.58)% and Platelet Distribution Width(PDW) (15.10 ±3.53)fl of ACS group was significantly higher than the control group (10.17±1.24)fl, (26.55 ±11.20)%,(11.52 ±2.88)fl, respectively with (p value <0.001) . In Roc analysis MPV ,PDW, PLCR had cut off value of >10.9fl, >12.7fl, >33.2%, value, respectively and can differentiate ACS from healthy individuals with Sensitivity of 84.2%,78.9%76.3%, Specificity of 75%,67.1%74.3%,Postive Predictive value(PPV)of 66%,54.1%,61.1% and Negative predictive value (NPV) of 90.4%,86.3%,86.3% ,respectively. The Area under Curve (AUC) of MPV, PDW and PLCR was 0.788, 0.767 and 0.746.

Conclusion: The mean ofMPV, PDW and PLCR were significantly increased in ACS compared to control group value .MPV has the highest value of sensitivity, specificity, NPV and PPV followed by PLCR then PDW. These parameters could be used as a supportive tool for prediction of ACS disease on the top of the clinical diagnosis and advanced tests set up.

Key Words-, Acute Coronary syndrome, Platelet count and indices, Diagnostic predictivevalue

1. Introduction

1.1 Background

Acute Coronary Syndrome (ACS), also referred to as Acute Coronary Artery Disease, is a cardiovascular pathology characterized by ischemia resulting from inadequate oxygen-rich blood supply to the heart as a consequence of blockage or narrowing of the coronary arteries [1, 2].This phenomenon represents the most prominent instance of mortality. ACS represents the observable clinical presentation of coronary heart disease .The phenomenon of ACS arises as a consequence of the accrual of plaque within the coronary arteries. The aforementioned arteries serve to provide the myocardium with blood that is rich in oxygen. Atherosclerotic plaque formation is predominantly attributed to the deposition of lipids, including cholesterol, in addition to other biomolecules, such as calcium in the blood[3].

As time passes, plaque accumulates and solidifies, causing arteries to narrow and decreasing blood flow to the heart. Over time, it is possible for an area of atherosclerotic plaque to undergo a rupture event. This rupture is typically succeeded by the activation of platelets and the formation of a thrombus [4]. The process of activation results in the formation of platelets large in size [5]. Certain factors are crucial in promoting prothrombotic episodes, leading to the formation of blood clots on thrombus-induced plaques,once the clot grows to a significant size, it can impede the flow of oxygen-rich blood to the specific part of the heart muscle fed by the corresponding artery, potentially causing serious problems [6].

The insufficient supply of oxygenated blood to a specific area within the myocardium can result in angina which refers to chest pain or discomfort. Furthermore, such impaired blood flow may trigger the onset of a heart attack, heart

failure, and may also disrupt the regular pace and sequence of heartbeat, medically indicative of arrhythmias or causing death [2] .According to research, the onset of coronary heart disease occurs as a result of specific factors that inflict damage upon the inner layers of the coronary arteries. The causative elements comprise of smoking, elevated levels of specific lipids and cholesterol in the circulation, hypertension, as well as elevated blood glucose levels manifested by insulin resistance or diabetic disorder [2, 3].

There are three distinct categories of ACS, identifiable as ST-segment elevation myocardial infarction (STEMI), non-ST segment elevation myocardial infarction (NSTEMI), and unstable angina (UA) [7]. The management of acute coronary syndrome involves the administration of nitrate medication in order to mitigate heart attacks. Additionally, the use of anticoagulant pharmaceuticals, such as aspirin, has the potential to diminish the likelihood of blood coagulation. Statins and beta-blockers are pharmacological agents that have been proven effective in reducing cholesterol levels and blood pressure, respectively. These agents have also demonstrated their ability to prevent myocardial infarctions and premature mortality in various clinical trials [8].

Percutaneous coronary intervention is one of the procedures done for the ACS patients to open clogged coronary arteries. It involves the introduction of a slender balloon into the occluded coronary artery followed by its inflation, causing the compression of the atherosclerotic plaque and consequent dilation of the lumen to augment the antegrade blood flow. Furthermore, a stent, a diminutive and pliant cylindrical device, is subsequently positioned within the intraluminal space to maintain its patency. The surgical procedure of coronary artery bypass grafting

involves the utilization of a vein obtained from another anatomical location to create a bypass around a singularly obstructed coronary artery [8].

Coronary heart disease (CHD) has emerged as the foremost cause of mortality on a global scale,approximately 3.8 million males and 3. 4 million females die annually [9].

The platelet activation leads to the onset of ACS [1]. The size of platelets is recognized as a reliable indicator of their level of activity, with larger platelets typically being indicative of younger and metabolically more active, which can be attributed to the presence of intracellular granules. Consequently, the larger platelets exhibit an increased tendency to adhere and clump together, attributed to the heightened levels of thromboxane A2 present within them. Moreover, they display elevated quantities of serotonin and β-thromboglobulin, thereby endowing them with a greater proclivity towards thrombosis [10-12]. Consequently, they facilitate the advancement of atherosclerotic lesions and the formation of thrombus, leading to the onset of ACS. Platelets serve as an indicator of their own activation, thus implicating their potential relevance to the clinical presentation of ACS [13-15]. It follows that modifications to platelet morphology and functionality may confer an elevated likelihood of developing vascular disease. The measurement of MPV, or Mean Platelet Volume, offers valuable insights into the functional and active properties of platelets by providing an assessment of their size. The value of the parameter is estimated to be within the range of 7. 4- 10.4 fL [8].

Platelets with a greater mean platelet volume (MPV) exhibit a greater propensity to encourage the formation of blood clots, thus serving as a diagnostic indicator of hypercoagulability when compared to smaller platelets. The MPV is found to be greater in instances where there has been platelet destruction. The Platelet Distribution Width (PDW) is a measure that provides an indication of the variation in

size of platelets within a given sample of blood. The concentration of large platelets is observed to elevate concomitantly with platelet activation, such as in cases of coronary artery disease. It has been postulated that this parameter serves as a superior indicator of platelet activation in comparison to MPV, as the latter is prone to elevation during the process of platelet swelling [16].

Platelet activation led bone marrow releases immature young platelets to the circulation to compensate the demand of platelet turnover. Platelet Large cell Ratio (P-LCR) test is one of the test parameters expressed as the ratio of platelets with platelet volume greater than 12 femtolitres (fL). The ratio of normal P-LCR, i.e. large-volume platelets to all platelets, should be less than 30% and giant platelets and aggregated platelets make PLCR increase, hence larger platelets which are reflected by high P-LCR levels [17]. Plateletrite is measured the total platelet mass and has adirect relation to the platelet count and mean platelet volume and it detects when there is the quantitative abnormalities.as as result of this PCT is increased [18].

1.2 Statement of the problem

In 2019 World Health Organization (WHO) report indicated that ACS is the foremost cause of mortality, accounting for 16% of the global fatality rate [19]. According to the2015 WHO report,indicates a significant burden of ACS in Africa. Age-standardized mortality rates (ASMR) for this disease were estimated at 27% and 25% for men and women, respectively [20].

According to the 2018 WHO report, it has been determined that in Ethiopia, the percentage of total deaths caused by ACS has reached 7.81%.The mortality rate, adjusted for age, was calculated at 112. 44 Individuals per100, 000 members of the population and Ethiopia holds the 112th global ranking [21].

World Heart Association recommend cardiac troponin I and T tests arethe preferred biochemical cardiac biomarkers for diagnosing ACS. However, the diagnostic efficiency of cardiac troponin within 2-4 hours of the symptom on site is limited [22].Therefore other laboratory biochemical tests that successfully reduced emergency department delays in provision of immediate care for cardiac pain patients are required in conjunction with established markers [23].

The platelet plays a significant role in the pathogenesis and advancement ofACS, presenting heightened levels of aggregation and early activation in affected individuals [24]. As a result of this platelet parameters change are one of the most commonly identified hematological changes in ACS .Platelet have significant outcome on the prediction of ACS[25]. It is less costly, easily available routine test found in almost all clinical laboratories [26]. Unfortunately, their role as a tool for prediction of ACS had not been extensively studied in Ethiopia.

As a result, performing this study was important in order to provide additional data on this subject of study and also because knowing this role of platelet on ACS patients was allowed to had better diagnosis and for preventive mechanism for patients who were at risk. The aim of this study was to determine platelet parameters diagnostic predictive value on ACS.

1.3 Significance of the study

The study aimed to provide information about the role of platelet count andplatelet indicesasa predictive tool in patients withACS. TASH is one of the oldest referral hospitals and serve countlesspatients each year including ACSpatients. Our findings hopefully will benefit health facilities that do nothave enough capacity to perform, ECG, troponin and CK-MB test as a supportive test along with clinical symptoms and patient history.

This study will also contribute in proving evidence based information of using platelet parameters as useful supportive tool for prediction of ACS for clinicians working in hospitals as well as other health care facilities. By doing so, the findings of this study could play an important role in decreasing morbidity and mortality of ACS patients because of ACS .Furthermore,there is no published studies from Ethiopia; this study could be used as a reference of bench mark study for related studies. It is used for a baseline for policy makers.

2. Literature review

Few researchers have beendone in different parts of the world to determine the diagnostic predictive value of platelet count and indices in patients with ACS and the significant of eachparameter for the acute coronary syndrome. Below i had tried to review some researches and their findings that were closely related to this study.

2.1 Comparisonofplatelet parameters between patients withACS and controls

In India, a comparative analysis conducted by Ranjith MP et al in 2009 aimed to assess alterations in platelet volume indices and count in individuals with coronary heart disease. The sample consisted of 120 patients with Coronary Heart Disease (CHD) and 60 presenting with non-cardiac chest pains. The findings demonstrated that patients diagnosed with coronary syndrome exhibited elevated platelet volume indices and reduced platelet count in comparison to the general population. The

observed alterations in platelet volume indices and count may offer potential value in identifying individuals at heightened risk for acute coronary events [27].

In 2017, a comparative study was conducted by Patil SK et al in India aimed at investigating the correlation between platelet indices and the incidence ACS. The present study investigates the distribution of patients diagnosed with STEMI, NSTEMIand non-cardiac chest pain, with each group comprising 25 individuals within the population under each group. The findings of the study revealed that there were significant elevations in the platelet indices, comprising MPV, PDWand PLCR, among patients diagnosed with STEMI and NSTEMIcompared to those with Non-Cardiac Chest Pain. Consequently, it was employed as an indicator for timely interventions aimed at the incidence and/or progression of ACS [28].

In 2016, a cross-sectional study was conducted in India by Ranjani SG et al to examine the significance and function of platelet volume indices in cases of ACS. A group of 100 patients diagnosed with ACS was compared to a control group of 25 healthy individuals. The study's findings suggest that patients with ACS exhibit higher platelet volume indices when compared to healthy non-acute coronary syndrome controls. These indices were identified as a practical and dependable test, indicating their potential use in conducting the preliminary evaluations of individuals admitted with ACS, in conjunction with other cardiac biomarkers [29].

In 2016, Yassir T et al conducted a cross-sectional study at a hospital in Sudan to explore the platelet count and indices in correlation with coronary artery disease among patients from Sudan. In a cohort of 103 individuals with coronary artery disease (CAD), 53 patients presented with acute coronary syndrome (ACS) while the remaining 50 patients presented with chronic stable angina. A cohort study was conducted. 100 individuals presenting non-cardiac chest pain were utilized as the

control group in the study. The obtained data indicated that. The present study revealed statistically significant differences in platelet count, mean platelet volume (MPV), platelet distribution width (PDW), and platelet-large cell ratio (P-LCR) between individuals diagnosed with acute coronary syndrome (ACS) and those in the control group [30].

In 2016 A cross Sectional Study Conducted in Sudan by Abass EA et al evaluated the platelets volume indices in a group of Sudanese patients with CAD and compared with normal populations. There were 53 patients with acute coronary 100 subjects with non-cardiac chest pain were also included as normal controls. Platelets count, Mean platelets volume (MPV), platelets distribution width (PDW), and platelets -large cell ratio (P-LCR) were significantly higher in ACS cases compared to controls (P-values: < 0.05). Evaluation of platelets count and indices might be useful in predicting those patients at higher risk for acute coronary events [31].

2.2 The predictive value of platelet parameters in ACS patients

A study conducted by PalR et al. in 2014 in India, aimed to investigate the potential of MPVas a predictive diagnostic marker for ACS. A prospective comparative analysis of 104 patients in the ACS group and 111 patients in the non-ACS group was undertaken. The findings revealed a statistically significant elevation of MPV levels in the ACS group when compared to the non-ACS group (p-value < 0. 001).These results establish MPV as a potential supportive diagnostic predictor for ACS. The diagnostic efficacy of MPV was assessed, wherein the sensitivity, specificity, positive predictive value, and negative predictive value yielded respective values of 89.42%, 46.84%, 61.18%, and 82.53% [32].

In 2016, Kumar N et al conducted a prospective study in India that aimed to analyze the correlation between platelet volume indices and a spectrum of coronary artery

disease in a cohort of 230 cases. In the study, a cohort of 100 individuals was selected presenting with either myocardial infarction (MI) or unstable angina (UA). A cohort of 100 individuals with stable coronary artery disease was classified as Group II. In this study, a group of 30 healthy individuals who were matched in terms of age with the experimental group (defined as those with acute coronary events) were examined. After conducting the analysis, the researchers determined that the cut off values for predicting acute coronary events usingMPV,PDW and PLCRwere identified as 12.85 fl , 9.65 fland 22.75% ,respectively .The sensitivity of these markers was found to be 80%, 77%, and 97%, while the specificity was determined to be 77.1%,51.4%,62.9%, respectively[33].

In 2017 A comparative prospective conducted in India by Papawil PS etal to assess Mean Platelet Volume and Other Platelet Indices in Patient of Acute Myocardial Infarction with Healthy Control 60 subjects with acute myocardial infarction and 60 healthy individuals of same age and sex were participated . ROC for MPV shows that best cut off value for MPV for predicting MI was 11.65fl. (Sensitivity 66.7% and specificity of 53.33%. 0.76, PDW for predicting AMI was 13.45fl (sensitivity 73.3% and specificity 63.33%.0.687 , P-LCR showed that cut off value for predicting MI was 38.5 units. (Sensitivity 50% and specificity 68.3%).0.686and PCT, predicting AMI was 0.175 units (sensitivity 65% and specificity 13.3%).0.368[34].

In 2020 A Study Conducted byRametek R et al in India to assess the platelet count and platelet indices in patients with coronary artery disease and acute myocardial infarction. On total of 100ACS were enrolled as study group. 130 age and gender matched patients with non-cardiac complaints were acted as control.The Roc Curve the group of ACS the highest sensitivity and specificity was found out MPV (89.6 % and 89.6%)with cutoff value 10.94 followed by PLCR (77.1% and 55) with cut off value 21.89 and PDW (72.9% and 50%) with cut off value 12.05 [35].

In 2019, a study on the clinical utility of MPV and immature platelet fraction in ACS was conducted by Huang HL et al in Taiwan. The study was a single-center investigation involving 104 patients. of those patients, 63 were initially suspected to have ACS and ultimately diagnosed with the condition, accounting for 65. 3% of the total study population. The research findings indicated that patients diagnosed with ACS demonstrated higher MPV and immature platelet fraction levels relative to non-ACS patients. MPV exhibited encouraging diagnostic utility in predicting ACS, evidenced by AUCof 0. 736. MPVhad a sensitivity, specificity and positive predictive value of 54.2%, 82.8%, 86.7%, respectively [36].

In 2017, Hossain M et al. conducted a quasi-experimental study in Bangladesh to investigate the correlation between Platelet Parameters and ACSbefore and after Anti-platelet Therapy. A total of 79 patients diagnosed with ACS and 63 control subjects were included in the study. The findings of the study indicated that the platelet count cut off value is associated with MPV and PDW, which were observed to be greater than 225 x 10^9/L, 10.7 fl, 12.7 fl, correspondingly the metrics for evaluating the diagnostic capabilities of platelet counts, MPV, and PDW were determined to be 83% sensitivity, 28. 1% specificity, 42.3% accuracy, 37.6% positive predictive value, and 64% negative predictive value for platelet counts and 90.6% sensitivity, 49.4% specificity, 64.8% accuracy, 51.6% positive predictive value, and 89. 8% negative predictive value for MPV and PDW had a sensitivity of 94. 3%, specificity of 52.8%, positive predictive value of 54.9%, negative predictive value of 94.1% [37].

In 2014, a prospective study was conducted in Iran by Dehghani MR et al with the aim of ascertaining the diagnostic utility of platelet indices in patients experiencing acute chest discomfort. In this study, data was collected from a sample of 862 patients experiencing acute chest pain, alongside a control group of 184 individuals

who were match-matched in terms of health status. The findings of the study demonstrate the presence of a significant correlation, of a negative nature, between the MPV, PDW, P-LCR values and platelet count. Cut-off values of 9. 15 fL, 11.35 fl, and 20.25% were used for MPV, PDW, and P-LCR, respectively. The AUC for MPV, PDW, and P-LCR were found to be 0. 563, 0.557, and 0.560,the present study determined the sensitivities and specificities of three hematological parameters, namely MPV,PDWand P-LCR, which yielded values of 72% and 40%, 73% and 37%, and 68% and 44%, respectively [38].

In 2019, Luke K et al conducted a retrospective cross-sectional study in London to investigate the predictive value of hematologic indices in the diagnosis of ACS.The study enrolled a total of 191 patients, including 79 individuals with ACS and 112 with stable coronary artery disease. The findings revealed that patients with ACS had significantly higher platelet-to-lymphocyte ratio levels ($p < 0.001$) compared to those with stable coronary artery disease. The difference in MPV between the two groups was found to be 6. 40, with a higher value recorded in the group of individuals with hypertension. The mean corpuscular volume of red blood cells was observed to be significantly lower ($p < 0.001$) in patients diagnosed with ACS. The findings of the study revealed that the analysis of ROC curve demonstrated that MPV exhibited the highest AUC value of 95% for diagnosing ACS.The optimal cut-off point for MPV was determined to be ⩽ 8. 35 Fl. MPV had Sensitivity and Specificity of 93.6%, 97.3%, respectively [39].

A study undertaken by Lippi G et al in Italy in 2009 aimed to investigate the shift in levels of Increased MPV in Patients with ACS. The research was conducted on a cohort of 2304 adult patients admitted consecutively to the emergency department over a duration of 12 months, with 456 patients (19. 8% of the total sample) being diagnosed with ACS. The residual patients were categorized as non-ACS. The

findings indicate that individuals with ACS exhibited lower MPV values compared to non-ACS individuals. The diagnostic precision of MPV was evaluated by computing the area under the ROC curve, and the result yielded a significant value of 0.661(P<0001). It had a cut off value at the 9.0 fl cutoff. MPV had 83% and 43%,negative predictive value and positive predictive value,respectively [40].

In 2016, Shehata IE et al conducted a case-control study in Egypt to investigate the utility of measuring the MPV for assessing patients with ACS. A cohort of 81 consecutively diagnosed patients suffering from ACS. The present study involved the subdivision of patients into two distinct groups, namely group Ia (consisting of 37 Troponin positive patients) and group I b (comprising 24 Troponin negative patients). In addition to these groups, a control group of 20 healthy individuals was included and labeled as group II. The study yielded significant results, revealing that only the MPVexhibited a considerable increase in the likelihood of developing ACS. Moreover, MPV of 11.1 fL or higher was identified as the optimal cut-off value for predicting Troponin positive ACS, exhibiting a sensitivity of 84% and a specificity of 65%. These findings were statistically significant (P = 0. 000) [41].

3. Objectives

3.1 General objective

- To determine the diagnostic predictive value of platelet count and platelet indices in patients with acute coronary syndrome at TikurAnebesa specialized hospital Addis Ababa, Ethiopia from April 2021 – March 2023.

3.2 Specific objectives

- To compare platelet count and platelet indices between patients with acute coronary syndrome and controls.

- To determine the sensitivity, specificity, positive predictive value, negative predictive value of platelet count and platelet indices in ACS patients.

4 Hypothesis

There is no difference in platelet count and platelet indices in ACS patients compared to healthy individuals.

5. Materials and Methods

5.1 Study area

Study was conducted at TikurAnebesa specialized hospital(TASH) found in Addis Ababa a capital city of Ethiopia. It wasestablished in 1972 and is located in

Lideta-sub city. It is the teaching hospital of health Science College, Addis Ababa University. It is the largest specialized hospital in Ethiopia, and serves as a training center for undergraduate and postgraduate medical students, dentists, medical laboratory technologists, nurses, midwives, pharmacists, and radiology technologists. There is about 929 academic staff, 825 nurse professionals, 55 laboratory technologist, 74 pharmacist, 69 midwife professional, 39 anesthesia professional, 14 physiotherapist, 37 radiology technology, 15 biomedical professional, 6 environmental health, 250 medical doctors, 15 others and 891 administrative staff. The hospital has different types of clinic in for out-patients like hematology, oncology, dermatology, neurology, surgical, gynecology, ortho, diabetics, antenatal, GI, chest, staff clinic…etc. The hospital has more than 700 beds . The emergency department also provides services to about 29,000 patients per year and on average 50 patients per day. Three ACS patients are admitted at emergency per day [42].

5.2 Study design and Period

Hospital based comparative cross sectional study was conducted from April 2021 to March 2023.

5.3 Population

5.3.1 Source population

All ACS patientscome to TASH during study period.

5.3.2 Study population

The study populations were classified in two groups.

Patients with ACS who fulfilled inclusion criteria were categorized as a case group.

Apparently healthy individuals who fulfilled the inclusion criteria were categorized as a control group.

5.4 Exclusion criteria and Inclusion criteria

5.4.1Inclusion criteria

Patients with ACS (for cases) – Patients that were volunteers, above the age of 18 and had a confirmed ACS were selected.

For control groups - Apparently healthy individuals who was volunteers and above the age of 18 were selected.

5.4.2 Exclusion criteria

Pregnant women's, Patients took therapy on platelet and anticoagulation, Patients had clotting factor disease, malignancy and a disease of liver and kidney were excluded .

5.5 Study variables

5.5.1 Dependent Variables

- The platelet count (PLT count)and platelet Indices (MPV, PDW, plateletcrite(PCT), PLCR)

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

5.5.2 Independent Variables

- Age, Sex, Type of ACS

5.6 Measurement and Data Collection

5.6.1 Sample size determination: A formula of hypothesis testing for two population means was used to determine the sample size for the case and control groups.

Required information

Standard deviation- SD1and SD 2, σ^2═Varaiance

Level of significance Z α/2 Value═ 1.96

Power of the test 100 (1-β) %, Zβ which is usually set to 80%═0.84, $\sigma1^2$═ $(SD1)^2$, $\sigma2^2$═ $(SD2)^2$,**μ1═10.08 ,μ2═9.4**

-Hypothesis testing for two population means was used to calculate the sample size

- **Sample size in each group**

$$n_1 = \frac{(\sigma_1^{\ 2} + \sigma_2^{\ 2})(Z_{\alpha/2} + Z_{\beta})^2}{(\mu_1 - \mu_2)^2}$$

Anticipated MPV and standard deviations for ACS cases was (10.08±1.3 fl) and that of control was (9.4±0.5 fl), taken from of a study done in Sudan [31] for thiscalculation.Utilizinga0.2 β, a margin of error of 5%, a confidence level of 95%, and accounting for a non-response rate of 15%, the initial sample size for the case group comprising ACS patients was determined to be 38 and the size of the control group, consisting of apparently healthy individuals, was 38. To enhance the statistical power of the test, the number of ACS cases and controls was effectively doubled. A total of 76 cases and controls were provided for analysis under the purview of ACS. In order to enhance the clinical precision of the study, it was determined necessary to augment the number of controls by two-fold. A total of 152 control entities were provided. Ultimately, a total of 228 individuals were recruited to participate in the present investigation, which included 76 instances of ACS patients and 152 controls.

5.6.2 Sampling Method: Convenient sampling method was used.

5.6.3 Data collection procedure: All patients who was above the age of 18 and having a symptom of chest pain attending to emergency section. The emergency doctor was confirmed the ACS patient by using physical examination, Echo Cardiogram (ECG), Troponin,Creatine Kinase Myoglobin binding(CK-MB)test. Patientswhowere categorized as ACSpatients (Cases) showed abnormal ECG result,

increased Troponin and CK-MB. A healthy individual who came for checkup who were free from communicable infectious disease and chronic disease , no history of ACS disease and their Troponin and CK-MB was normal and attended at TASH was used as controls.After a consent form was given to the participants blood samples (5-mL) was collected from the study participants(for ACS patients(cases) and controls) followed on standard operating procedures (SOPs) by qualified laboratory technologists (Principal investigator) used vacutainer tubes containing 2.0 mg/mL ethylenediaminetetraacetic acid (EDTA-K2) with the patient name labeled. For cases blood sample was drawn from patients of ACS before administration of any anticoagulant .The collected blood samples was thronged 10X mixed to avoid clump and clot formation. The CBC(complete blood cell count) was run on SYSMEX KX 21N(Sysmex corporation, Kobe, japan) hematology analyzer machine within 1hour after collection. Platelet counts and indices were taken from CBC result. The clinical information andsocio-demographic of thegroups(ACS and Control) were obtained from review medical record by using data extraction format and in depth interview. The socio demographic information and blood sample for all investigations was collected by principal investigator.CBC was done in hospital laboratory (TASH)lab by qualified laboratory professional.

5.6.4 Principles of Laboratory analysis: Five ml whole blood was collectedby ethylene diaminetetraaceticacid (EDTA) tube and automated hematology analyzerSYSMEX KX 21Nanalyzed the value of the platelet count and indices. SYSMEX-KX21N automated analyzer performed according to the hydro dynamic focusing (Direct Current Detection), flow cytometer (used a semiconductor laser) and traditional impedance technology to improve accuracy of very low and very high PLT counts technology. Three levels of controls are running to assess the performance of the instrument. Samples were analyzed only when the controls are in the accepted range for all parameters.

5.6.4.1Principle of SYSMEX KX 21N

The KX-21N performs speedy and accurate analysis of 18 parameters including a 3-part WBC differential, plus histograms for Red Blood Cell (RBC), PLT and WBC (White blood Cell)in blood. This employs three detector blocks and two kinds of reagents for bloodanalysis. The WBC count is measured by the WBC detector block using the Direct current detection method. The RBC count and platelets are taken by the RBC detector block, also using the Direct current detection method. TheHemoglobin (HGB)detector block measures the hemoglobin concentration using the non-cyanide hemoglobin method.

The principle is that the blood sample is aspirated and measured to predetermined volume, diluted at a specific ratio, and fed into each transducer. The transducer chamber has 2 mini holes called aperture. 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. The number of blood cells is calculated by counting the pulses.

5.7 Data Quality Assurance

Pre-Analytical: Blood collection was performed by well-trained personnel aseptically from the right patient (including the labeling of patient name on the EDTA tube) based on SOP and was arrive in laboratory immediately after 1 hour of collection to avoid dalliance. After collection the personnel gently mix it well then the tube was properly labeled and transported to the testing section room at room temperature.

Analytical: Properly performed daily quality control and properly mix the sample and check for hemolysis, labeled each EDTA tubes with specific codes, clot and any cracks on the tube before running up on the machine. Make sure that the result was reliable by making cross checks and take only platelet parameters result from the CBC. Low platelet count were verified by examining a blood smear stained by wright stain .Quality of the Wright’ s stain was checked by preparing one differential slide daily using a patient sample with a normal Mean corpuscular volume. Mean corpuscular hemoglobin and Mean corpuscular hemoglobin concentrationand total WBC. The stained slide was reviewed for meeting color specifications of thePost-analytical:Platelet parameters results was reported, recorded and interpreted based on the reference range.

Blood sample quality was ensured by collecting and processing according to the SOP. High, normal and low quality control materials were run every morning for (SYSMEX KX 21N, Sysmex corporation, Kobe, japan) analyzer before patient samples are run and the results was kept confidentially. Precision of the KX 21N (Kobe, japan) analyzer was checked by running a sample in replicates of ten following the manufacturer’ s instruction. The data that was obtained from the data collection format and checklist was checked by principal investigator and advisors

for its completeness. Then it was cleaned, coded, entered and analyzed using SPSS version 25.

5.8 Data analysis and interpretation

Data was entered, analyzed using Statistical Package for Social Science (SPSS) program Science version 25 (SPSS INC, Chicago, IL.USA).The normal distribution of the data checked by Kolmogrov-Similronor test and Independent t test was used to compare the means of ACS patients and control group. ROC curve analysis was used to determine the sensitivity, specificity, Negative Predictive Value(NPV) and Positive Predictive Value(PPV) of platelet parameters. P values less than 0.05 was considered as statistically significant.

5.9 Ethical considerations: The thesis was ethically approved by the departmental research and ethics review committee (DRERC) of the department of Medical Laboratory Science, College of Health Science, and Addis Ababa University. In addition, consent form was given for both cases and controls for permission to record the medical history and for collection blood. CBC (MPV, PDW,PLT, P-LCR, and PCT) test could be performed at the TASH. The result was given to the patient without any payment and the participant should know the finding was benefited the participant itself and the society .All information collected in this study was given code numbers and no name was recorded. The key to this code numbers was kept in a locked file and accessible to the authorized staff.

5.10 Dissemination of Result

The result of this study will be presented to the department of Addis Ababa University, College of Health Sciences, School of Allied Health Sciences, Department of Laboratory science at a public defense. A copy of this material will be given and

communicated to TASH, and other concerned bodies. Effort will be made to disseminate the findings to the medical and scientific community by presenting at professional society conferences and the manuscript will be submitted to peer reviewed journals for publication.

5.11 Operational Definitions

Acute Coronary syndrome- the arteries of the heart cannot deliver enough oxygen-rich blood to the heart.

Apparently healthy – A healthy individual who came for checkup who was free from communicable infectious disease and chronic disease, no history of ACS disease and their Troponin and CK-MB was normal.

Mean platelet volume-is the average platelet size in an individual’s blood sample.

Platelet count- is number of platelets in a micro liter of blood.

Platelet distribution width- uniformity of platelets in terms of shape and size

Plateletcrite- it is measure of total platelet mass, detecting platelet qualitative abnormalities.

Platelet large cell volume-is ratio of large platelets from the 12 fl discriminator or larger to total platelet count.

ROC curve analysis- is a graphical plot that illustrates the diagnostic ability of a test or parameter.

6 Results

6.1 The socio demographic data of ACS patients and control group

A total of 228 study participants were included in this study .They were classified in two groups. ACS patients (n=76) as case group and there were 152 apparently healthy individuals as control group**.** The mean age of ACS group(50.91±11.33) years and control group(51.85±11.7) years was matched. Males61%(140) study participants predominated females 38%(88) in all groups .Majority of ACS groupand controlgroup 31% (72) work innon-government sectors.Most of study participants that means ACS group and control group27% (62) finished primary school. The preponderanceof study subjects 63%(143) were get married (Table 2).

Table 1 Age category of study participants

Age	ACS patients (n=76)	Control(n=152)
30-35	9	15
36-41	9	16
42-47	14	28
48-53	10	31
54-59	16	23
60-65	6	17
66-71	12	18
72-77	0	4

Table 2 The socio- demographic data of ACS patients and control group at TASH, Addis Ababa, Ethiopia, from April 2021 – March 2023 (n =228).

Socio demographic variables		Control (n=152)	ACS(n=76)	Total Number (n=228)
Age		51.85±11.7	50.91±11.33	51.38±11.51
Sex	Male	92(60%)	48 (63%)	140(61%)
	Female	60(39%)	28 (37%)	88(38%)
Occupation	Government employee	31(20%)	13 (17%)	44(19%)
	Nongovernment employee	45(29%)	27 (36%)	72(31%)
	Private business	36 (23%)	19 (25%)	55(24%)
	Student	1(1%)	0 (0%)	1(0%)
	House wife	22 (14%)	8(11%)	30(13%)
	Retirement	17(11%)	9 (12%)	26(11%)
Education level	Unable to read and write	16(10%)	2 (3%)	18(8%)
	Read and write	22 (14%)	14 (18%)	36(16%)
	Primary (1-8)	34(22%)	28 (37%)	62(27%)
	Secondary (9-12)	36(23%)	19 (25%)	55(24%)
	College diploma or degree and above	44(29%)	13 (17%)	57(25%)
Marital status	Married	93(61.18%)	50 (66%)	143(63%)

	Single	25(16.44%)	10 (13%)	35(15%)
	Divorced	20(13.15%)	6 (8%)	26(11%)
	Widowed	14(9%)	10(13%)	24(10%)

The result is presented in mean and standard deviation, number and (%).

6.2 The clinical characteristics and clinical outcome of ACS patients

The clinical sign and symptoms of ACS patients were recorded. The chest pain was the leading clinical characteristics in ACS patients. Most of the ACS patients in this study developed stroke (Table 3).Table 3 The clinical characteristics and clinical outcome of ACS patients at TASH, Addis Ababa, Ethiopia, from April 2021 – March 2023 (n =76).

Clinical characteristic and outcome	ACS (N=76)
Chest pain	43(56.57%)
Shortness of breath	37(48.6%)
Dyspepsia	37(48.6%)
Chest tightness	37(48.6%)
Easy Fatigueablity	42(55.2%)
Shock	44(57.89%)
Arrhythmia	45(59.2%)
Stroke	46(60.5%)
Acute limb ischemia	29(38.1%)
Heart failure	20(26.3%)

6.3 Comparisonof platelet count and platelet indices between ACS and control group

The mean value of MPV, PDW, PLCR had statistically significant difference(significantly increased) across the patient and control (p <0.001).The mean of platelet count and Plateletcritewas not significantly increased compared to the control group with its p value (0.351, 0.234),respectively (Table 4).

Table 4Comparison of platelet parameters ofACS patients and control groups at TASH, Addis Ababa, Ethiopia from April2021-may 2023 (n=228).

Platelet parameters	Control group (n=152)	ACS(n=76)	P value
Mean platelet volume (fl)	10.17±1.24	11.66±.1.19	.000
Platelet distribution width (%)	11.52±2.88	15.10±3.53	.000
Platelet count(10^9/L)	294.48±59.33	277.17±121.18	.351
Platelet large cell ratio(%)	26.55±11.20	37.35±9.58	.000
Plateletcrite(%)	.27±058	.21±.014	.234

6.4Comparison between the diagnostic values of platelet Parameters.

In this study a ROC curve analysis was employed as a means of evaluating the diagnostic predictive efficacy of the platelet parameter. The present study determined the sensitivity, specificity, Positive Predictive Value(PPV), and Negative Predictive Value (NPV) of platelet parameters that exhibited statistical significance . The predective values of these platelet parameters were assessed using various cut-

off values. The results indicate that MPV exhibited a greaterAUC relative to PDWand PLCR, withMPV yielding an AUC value of 0. 78, while the AUC values ofPDW and PLCR was0.77 and 0.74, respectively.

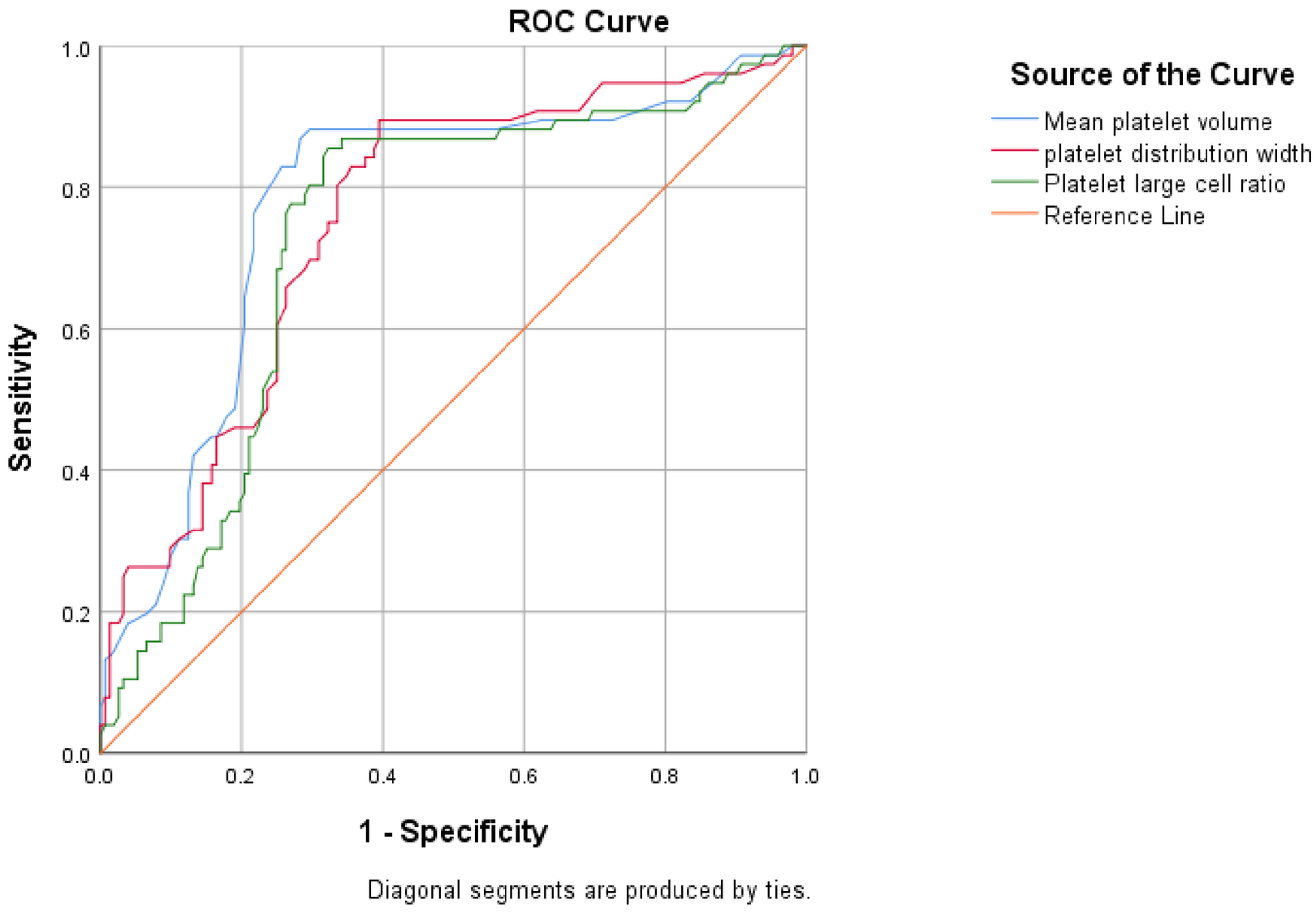

Fig 1 The ROC curve of mean platelet volume(MPV) ,platelet distribution width (PDW)platelet large cell ratio (PLCR)for ACS patients

The cutoff of >10.90fl MPV has the Sensitivity, Specificity, PPV and NPV of 84.2%,75%,66% and 90.4% respectively for differentiating ACS from the apparently healthy groups .The parameters PDW and PLCR with cut off value of >12.75fland >33.20 % respectively can differentiate the ACS groups from healthy ones with sensitivity, specificity ,PPV and NPV of 78.9%,76.3%,67.1%,74.3% ,54.1%,61.1% and 86.3%, respectively(Table 5).

Table 5 The AUC, sensitivity, specificity, positive predictive value (PPV) and negative predictive value(NPV) of mean platelet volume(MPV), platelet distribution width(PDW) and platelet large cell ratio(PLCR) of ACS with different cut off values.

Platelet indices	cut - off	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	AUC(95% CI)
MPV(fl)	>10.90	84.2	75	66	90.4	.788(0.75,0.884)
	>10.65	87.2	72.4	61.5	92.4	
	>9.95	88.0	61.2	89.5	60.5	
PDW(%)	>12.75	78.9	67.1	54.1	86.3	.767(0.701,0.832)
	>12.55	82.9	65.8	54.4	87.7	
	>11.65	83.0	61.8	52.8	89.5	
PLCR (%)	>33.20	76.3	74.3	61.1	86.3	.746(0.677,0.818)
	>32.55	78.9	71.7	65.7	87.8	
	>32.10	81.6	71.1	63.3	88.3	

7. Discussion

The study conducted to figure out the predictive role of platelet count and platelet indices on the diagnosis of ACS. The mean of MPV, PDW and PLCR of the ACS group were significantly increased than control group. The reason for the increment of these parameters is the activation of platelets after the rupture of atherosclerosis led the parametersincrease size and altersits shape [43]. These three parameters had the best differentiating potential in ACS .In the present study the mean of MPV in ACS patients were significantly higher than the mean of control group. In Similar findings Bradier SB et al ,RanjaniSG et al, AbideenZuetal, Sikumbang PR et al ,Pervin S et al[43,29,44,3,4] reported the significantly increment of MPV in ACS patients compared to control .The reason behind the increment of MPV in these studies were the activation and adherence of platelet during the pathophysiology of the disease cause the size and shape of platelet, increased and change, respectively. Moreover the process of aggregation of platelet in the formation of thrombus results increased demand of platelet in the circulation and bone marrow release large immature platelet to the circulation to compensate the shortage. As a result of this the platelet size (MPV) were increased in ACS patients [4]. Functional activity of a platelet directly associated with its size [45].

On the contrary Nataraju G et al [46], Bhayana et al [47],Damodar S et al [48] and Prasad et al [49]found that in ACS Patients the mean of MPV was insignificantly lower compared to control group with significance level of p value (0.23, 0.30, 0.40),respectively .These findings indicated these parameter was not the additional tool to predict the disease. The reason for the cause of this could be the variation of automated machine to measuring Platelet parameters.

In Roc curve analysis the AUC of MPV was 0.778 .The cut off value >10.90 fl can differentiate ACS patients from healthy ones with sensitivity, specificity, NPV,PPV 84. 2%,75%,66% and 90.4%, respectively. This finding concordance with the study of Pipliwal PS et al, had AUC of 0.762 which was a little higher cut off value of 11.65 fland slightly lower specificity and sensitivity of 66.7%, 53.3% respectively [50] .The reason of this slight difference was Pipliwal et al used different cut off values.

A study conducted in china by CHU H et al result was close to the current study, MPV could predict the progression of ACS .It had a the cut off value of 10.35 fl with AUC of 0.800 and could predict and differentiate the occurrence of ACS with low sensitivity of 78.3% and specificity 74.6% compare to the present study [51] .These result agreed with Zhu Y et al reported that MPV had AUC of 0.771 [52]. Ramteke R et al also found out similar finding, MPV had AUC of 0.740 with cut off value of 10.94 fl. It had high Sensitivity and Specificity of 89.6%, respectively [53].The difference in sensitivity and specificity value is due to the variation in the range of selection of AUC.

The PDW of ACS Patients was significantly increased than control group. The current study was Synergistic to Rao S et al, Ranjith MP et al, Abass EA et al , Maheshewari S et al, Patil K et al, Dehghani MR etal , Daimar A et al and Islam MS et al [54,27,55,56,28,38,57,58]which used across sectional comparative study and

Sysmex hematology analyzer which was consistent to the current study. The higher value of PDW in ACS Patients was useful for addition tool for early detection of the disease [53].The significantly increment of this parameter was due to as progressive Platelets activationled increment in size, not only size but also there is a change in shape. That means it changes its shape from discoid (which is normal shape of platelet) to spherical, and pseudopodia formation which cause platelet size vary.Hence PDW is directly measures the size variation of Platelets. The value of PDW increases [59,7, 58,60].

On contrarySusmitha M S et al Adel MH et al,Gunes H etal Studies reported that there was no Significance increment of The Parameter PDW in ACS Patients compared to the apparently healthy Individuals with p value > 0.05 respectively [61, 62, 63] .The reason for the difference is there was a method and instrument variation.

In Roc Curve Analysis >12.75 fl was the cut off value of PDW that separate the ACS patients from the healthy ones with Sensitivity of 78.9%, Specificity 0f 76.3%, NPV of 74.3% and PPV 0f 67.1%. The AUC of PDW was 0.767.Likewise Islam MS et al report that with 12.7flcut off value but it had a high sensitivity of 94.3 % and low specificity of 52% , low PPV of 54% and high NPV of 94% and can differentiate ACS patients from healthy ones [58]. Moreover, Celtinetal AUC (0.800) and Daimer A et al AUC(0.75) reports PDW had a cut off value 15.1fl and 15.50fl respectively which was higher than current study .With this cut off value had 80%,78.4%72.7%,67.7%,sensetivity and specificity , respectively [64,57]. The reason for these cut off value variation, sensitivity and specificity difference was because of it used different cut off values, the difference in automated hematology analyzer and variation in the range of selection of AUC, respectively.

The mean of PLCR in ACS Patients was significant higher than control group .This result Synergic to the finding of Dehghani MR[38],Patil SK et l,[28]AbideenZu et al[44] .This parameter was increased because of afterthe activation of platelet there is a platelet aggregation and bone marrow release platelets which are giant and immature in order to compensate the demand .As a result of this the presence of this platelet aggregation and giant platelet cause the PLCR increases. Hence PLCR reflects the circulatory system in large volume platelet turnover situation [43].

On contrary Susithemal et al[61], khode Vet al [15] reported that there was no a significant increment of ACS Patients than control group. The reason behind this difference is due to the the variation in automated hematology analyzer

PLCR had cut off value of > 33.20 % and could differentiate the ACS groups from healthy ones with sensitivity, specificity, PPV and NPV of, 76.3%, 74.3%, 61.1% and 86.3% respectively. The AUC of PLCR was 0.746.This result was in line with ZhehestovskaDV.et al .PLCR has AUC of 0.713 and cut off value of >32.33%. It could differentiate ACS from healthy individuals with a sensitivity of low 62.7% and specificity of 51% [45] .Meshewari S et al reported different cutoff which was ⩾ 26.15 % for ACS patients had a sensitivity of 72.55%, Specificity of 66.67%, PPV of 68.52%, NPV of 72.34% and it has AUC of 0.701 [56].The reason for this difference wasMeshewari S et al used a different cut off value ,and the difference in sensitivity and specificityof ZhehestovskaDV et al study difference was the variation in the range of selection of AUC .

8. Strength and Limitation of the study

8.1 Strength of Study

The study analyzed the four platelet indices. (MPV, PDW, PLCR, PCT).

The association of the clinical characteristics and outcome of ACS patients to their respective average platelet parameters was assessed in this study.

8.2 Limitation of Study

The study was crosssectional. It was not the better method for assessed the diagnostic predictive role of platelet parameters for ACS patients.

9. Conclusion and Recommendation

9.1 Conclusion

This study showed that MPV, PDW, PLCR had a significant increment in ACS patients compared to controls P value < 0.001. In the group of ACS the MPV had highersensitivity ,specificity, positive predictive value and negative predictive value was found for (84.2%,75%,66%, and 90.4% followed by PLCR (78.9%,76.3%,67.1% and 74.3% and PDW(54.1%,61.1%, and 86.3%) ,respectively.

9.2 Recommendation

MPV,PDW and PLCR could be used as a supportive tool for prediction of ACS disease on the top of the clinical diagnosis and advanced tests set up. As a result of this clinicians must give attention to the platelet parameters when assessing ACS.

Additional researches must be conductedwith more study participants with follow up in order to assess the prognosis of the parameter in ACS patients.

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11 Annexes

Annex I. Participant Information sheet in English Version

Title of the Research Project-The diagnostic predictive value of platelet count and platelet indices in patient with acute coronary syndrome: A comparative cross sectional study at TikurAnebesa specialized hospital, Addis Ababa, Ethiopia

Principal Investigator: Kalkidan Gera (BSc, MSc candidate)

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

Introduction

You are invited to participate as a study subject in a research conducted by MSc candidate, from Addis Ababa University. Your participation is voluntarily. The research teams will include one principal investigator, two advisors; two from Addis Ababa University department of Hematology . Please take as much time as you need to read or listen in the information sheet.

Purpose of the Research Project

We are asking you to take part in this study because we will try to assesThe diagnostic predictive value of platelet count and platelet indices in patients with Acute coronary syndrome.

Purpose of the research:

The health laboratory plays an indispensable role in the health care system. It supports diagnosis (to rule in or rule out a diagnosis), monitoring of response to treatment, epidemiological surveillance, prevention as well as Research (to

understand the pathophysiology of a particular disease process). even though the platelet has great role on the pathogenic of the coronary heart disease .the physician and the lab technologist does not give a different view for it most of the time troponin and CKMB is the prioritizes test orders when in acute corornoryheartdisease.so as many researches done platelet has agreat role of the prognostic and diagnostic effect for the patients

Procedures and the expected participation

If you are willing to participate, you need to understand the purpose of the study and give your consent. Not only this but also specimen collected from you will be used for the research purpose, and the results of your sample will be exposed to some concerned professional staffs as it is needed. The required clinical sample will be collected by residents of hematology department. Then, you are requested to give your consent to the sample collector. After consent, ablood sample will be taken from vein. Moreover, there will be a face-to-face interview for additional questions.

Procedures: blood is collected from vein by using syringe and EDTA tube the mix it then with in 1 hour the CBC is done by using SYSMEX KX21N automated hematology analyzer.and platelet parameters will be reported .

Potential risks and Discomforts

There will be minimal discomfort in giving blood samples. However, there might be some minimal risk and discomfort when we take venous blood. Nevertheless, we will try to minimize the discomfort as much as possible, as the blood samples will be taken by experienced laboratory professionals.

Confidentiality

We respect your privacy and confidentiality. Any information that identifies you will not be shared with anyone else outside the study team. The information we will collect from you as part of the study will be kept in a locked file cabinet, or be protected by a password on the computer only accessible to personnel involved in the study.There is no sensitive issue that you will be asked related with your social desirability but any information that is obtained in connection with this study and that can be identified with you will remain confidential.

Potential benefits to subjects and/or to the society

You will not receive any payment for your participation in this research study as compensation. However, based on the diagnosis result you will be treated in view of that. In addition, the result of the study will be beneficial for the diagnosis and prognosis of coronary heart disease. Hence, you are indirectly benefiting other patients and the society in this respect.

Participation and Withdrawal from the Study

The participation is voluntary and you have the right not to participate in this study. You may withdraw at any time and place without consequences of any kind. You may also reject to give any sample. You can ask any questions regarding to this study and you have a right to get a laboratory diagnosis result free.

Contact information

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

Name Phone: 0923490618 E-mail: kalgera123@gmail.com

Annex II. Participant Information sheet in Amharic Version

የተሳታፊዎችፈቃድናመተማመኛቅፅ

በአዲስአበባዩኒቨርሲቲጤናሳይንስኮሌጅየሕክምናላቦራቶሪሳይንስት/ክፍልበማስተርስድግሪተማሪየመመረቂያጥና ትላይእዲሳተፉተጋብዘዋል::እባክዎበዚህጥናትለመሳተፍከመስማማትዎበፊትከዚህቀጥሎየሚገኘዉንምንባብበ ጥሞናያንብቡግልጽያልሆነልዎትንማንኛዉምሃሳብይጠይቁ::

መግቢያ

የጥናቱርዕስ “The diagnostic predictive value of platelet count and platelet indices in patient with acute syndrome : A comparative cross sectional study at TikurAnebesa specialized hospital, Addis Ababa, Ethiopia ’ ’

የእርስዎበዚህጥናትላይየሚኖርዎትተሳትፎሙሉበሙሉበበጎ ፈቃደኝነትላይየተመሰረተነዉ::በዚህጥናትዉስጥላለ መሳተፍወይምለመሳተፍከወሰኑበኃላለማቋረጥየሚወስኑቢሆንምእንኩዋበዚህሆስፒታልየሚሰጠዉማንኛዉምአ ገልግሎትአይቋረጥም::በጥናቱለመሳተፍየሚስማሙከሆነየስምምነትቅጹላይበጹሁፍወይምበጣትፊርማማስቀመ ጥይጠበቅዎታል::

የጥናቱተሳታፊለመሆንየሚጠበቅበዎትምንድንነው?

በዚህጥናትለመሳተፍየሚስማሙከሆነናሙናዎለጥናቱእንዲሚዉልመስማማትይጠበቅብዎታል::ከተወሰደዉናሙ ናላይየሚገኙመረጃዎችከዚህሆስፒታልዉጭሚገኙናለስራዉአግባብነትላላቸዉሰዎችቢነገርየማይቃወሙመሆኑን መስማማትይጠበቅብዎታል::ይሁንእንጅይህአይነቱመረጃየርስዎንማንነትየሚገልጡመረጃዎችንማለትምስም፤አድ ራሻናየስልክቁጥርየመሳሰሉትንመረጃዎቸንአይጨምርም::ይልቁንምለዚህአገልግሎትብቻየሚዉልእርስዎንለማወቅ የሚያስችልመለያቁጥርጥቅምላይእንዲዉልይደረጋል::በተጨማሪምስለርስዎአጠቃላይየጤናሁኔታለሚቀርቡእንዳን ድተጨማሪጥያቄዎችመልስመስጠትይኖርብዎትአል::

በዚህጥናትመሳተፍየሚያስከትላቸዉቸግሮችምንድንናቸዉ?

ናሙናበሚሰበሰብበትወቅትምንምአይነትየከፋችግርአያጋጥምዎትም::ሆኖምግንናሙናዉንለመሰብሰብልምድያለ ዉባለሙያስለሚመደብናአስፈላጊዉየጥንቃቄእርምጃስለሚወሰድየህመምስሜትአይኖርም::

የህክምናመረጃከሚስጥርተጠብቆመቆየትየሚችለዉእንዴትነዉ?

ስለራስዎየሰጡትማንኛዉምመረጃከተወሰደዉናሙናላይየተገኘዉየላቦራቶሪዉጤትየሚዉለዉለጥናቱአላማብቻ
ነዉ።ይህንማህደርሊያገኙየሚችሉትየተወሰኑየጥናቱተባባሪሰዎችብቻናቸው።ከዚያምበላይስለእርስዎያለውንማን
ኛውንምመረጃየተለየየይለፍቃልባለውየኮምፒውተርየመረጃማህደርውስጥእንዲቀመጥይደረጋል።

በዚህጥናትመሳተፍየሚያስገኛቸውጥቅሞችምንድንናቸው ?

ይህጥናትየማስተርስዲግሪመመረቂያእንደመሆኑመጠንበዚህጥናትበመካፈልዎበገንዘብየሚያገኙትጥቅምባይኖ
ርምከጥናቱበሚገኝውውጤትግንተጠቃሚነዎት።የእርሶዎተሳትፎየእርስዎንናየወገንዎትንናለልብህመምተኛችምር
መራከፍተኛጥቅምይኖረዋል።

በዚህጥናትተሳታፊየመሆንዎመብቶችምንድንናቸው?

በዚህጥናትመሳተፍሙሉበሙሉበእርስዎፈቃደኝነትየተመሰረተበመሆኑበማንኛውምሰዓትናቦታየማቋረጥሙሉመብ
ትየተጠበቀከመሆኑምበላይእራስዎንከጥናቱበማግለልዎምክንያትየሚቀርብዎትምንምአይነትየሆስፒታልአገልግ
ሎትአይኖርም።ከዚህምበተጨማሪጥናቱንበተመለከተማንኛውንምአይነትጥያቄየመጠየቅናገለጻየማግኘትመብትአ
ለብዎት።የላብራቶሪምርመራውጤቱንምበነጻማግኘትይችላሉ።ነገርግንእርስዎበሚሰጡንመረጃችግሩንስፋትለመ
ከላከልእናለመቆጣጠርጠቃሚስለሆነለሚቀርብልዎትጥያቄቀጥተኛመልስይሰጡንዘንድበታላቅአክብሮትእንጠይ
ቃለን።

ጥያቄካለኝወይምችግርቢያጋጥመኝምንማድረግይገባል?

ይህንንጥናትበተመለከተወይምከዚህጥናትጋርበተዛመደመልኩስለሚያጋጥሙድንገተኛአደጋዎችወይምጥያቄካለ
ዎትበሚመለከተውአድራሻይጠቀሙ።

ሞባይል፡0923490618

ኢሜል፡kalgera123@gmail.com

Annex III. Informed consent form in English version

Card no…..……

I had been informed that the objective of this study is todetermine the diagnostic predictive value of platelet count and platelet indices in patients with acute coronary syndrome: A comparative cross sectional study at TikurAnebesa specialized hospital, Addis Ababa, Ethiopia . The results of this study have an importance to treat me and other patients, and to be used as an input for the future development of strategies or guidelines for diagnosing and prognosis of coronary heart disease. I had been also informed about the confidentiality of this study. The principal investigator requested me to participate in the study that would require my willingness to provide the required data that include blood and, and filling questionnaire. Therefore, with full understanding of the importance of the study, I agreed voluntarily to provide the requested samples and my benefit will be only from the free laboratory investigation result/s.

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

Signature: _____

Date_____

Annex IV. Informed consent form in Amharic version

የተሳታፊዎችስምምነትማረጋገጫ

የሚስጥርቁጥር ----------

የተሳታፊዉስም ----------

እኔስሜከላይየተጠቀሰውተሳታፊ. ‘ The diagnostic predictive value of platelet count and platelet indices in patients with acute coronory syndrome: A comparative cross sectional study at TikurAnebesa specialized hospital, Addis Ababa, Ethiopia’ ጥናትላይበቂገለጻተደርጎልኛል።ለጥናቱምደምናሙናእንደሚያስፈልግተገልጾልኛል።ጥናቱንምአላማዎችምተረድቻለሁ።በቃለመጠይቁላይየገለጽኳቸውመረጃዎችበሙሉበሚስጥርየተጠበቁእንደሚሆኑተነግሮኛል።በጥናቱላይያለመሳተፍናማንኛውንምመረጃያለመስጠትእንዲሁምበማንኛውምጊዜከጥናቱራሴንየማግለልመብቴየተጠበቀእንደሆነተገልጾልኛልስለዚህለዚህጥናትመረጃናየስምምነትቃሌንየሰጠሁትበአጠቃላይሁኔታውንበመረዳትናበፍጹምፍቃደኝነትነው፡በተጨማሪምጥያቄለመጠየቅተፈቅዶልኝለማወቅየፈለኩትንያህልማብራሪያአግኝቻለሁ።የዚህጥናትተሳታፊበመሆኔየማገኘውጥቅምየሁሉንምምርመራውጤትበነጻማግኘትእንደሆነተረድቻለሁ።በአጠቃላይእኔከላይበመተማመኛቅፅየተጠቀሱትንሁሉበሚገባናበተረጋጋመንፈስአንብቤዋለሁኝ።ስለዚህበዚህጥናትለመሳተፍፈቃደኛመሆኔንበፊርማዬአረጋግጣለሁ።

ፊርማ----------ቀን ----/---/----------

(የስምምነትቅጹንማንበብለማይችሉተሳታፊዎች)

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Annex V. SOP for SYSMEX KX-21N machine

Analysis Mode

Whole blood mode: This is the mode of analyzing collected blood sample in the whole bloodstatus. The tubecap is opened and the sample is aspirated through the sample probe one after another.

Pre-diluted mode:This mode is used in analyzing a minute amount of child's blood, for instance, collected from the earlobe or fingertip. In this mode, blood sample diluted into 1:26 before analysis is used. The sample aspiration procedure is the same as in the whole blood mode.

PROCEDURES IN EACH ANALYSIS MODE

With this instrument, sample mixing, cap removal, and tube setting are performed manually and Sample analysis can be executed when the instrument is in the Ready status.

Whole Blood (WB) Mode

Samples are processed in the following steps: Collecting and preparing samples , Selecting whole blood mode , Inputting sample No. and Analyzing samples

Collecting and preparing samples

A specified amount of sample, corresponding to the amount of EDTA anticoagulant, is collected from the vein. Tubes up to 80 mm in height should be used. The volume of sample that can be aspirated is 50µl.

Analyzing samples

o Mix the sample sufficiently

o Remove the plug while taking care not to allow blood

o Set the tube to the sample probe, and in that condition, press the start switch

o 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.

o When the LCD screen displays "Ready," prepare the next samples and repeat the above procedures. **Pre-Diluted (PD) Mode**

In this mode, a sample is diluted into 1:26 before analysis. This mode is applied in analyzing a capillary blood collected from the earlobe or fingertip. Samples are processed by the following steps:

o Collecting and preparing samples

o Preparing analysis samples in PD mode (dilution of 1:26)

o Selecting Pre-Diluted mode

o Inputting sample no.

o Analyzing samples

Collecting and preparing samples: Dilute samples to the ratio of 1:26 using CELLPACK dispensed beforehand in containers. [Example] 20 µL of blood is diluted in 500 µL of CELLPACK.

Tubes up to 80 mm in height should be used. The volume of sample is as follows:

Volume of whole blood required Approx. 20 µL or more

Volume of sample aspirated Approx. 200 µL

Preparing analysis samples in PD mode (1:26 Dilution)

o Clean a container such as erlenmeyer flask, beaker, etc. with CELLPACK and removeany dirt.

o Using a syringe, etc., take CELLPACK into a cleaned container.

o Using a 500 µL transfer pipette, dispense 500 µL of CELLPACK into a micro tube.

o Using a capillary tube, etc., collect 20 µL of blood and dispense it into the micro tube.

o Attach the cap and mix well.

When preparing a 1:26 dilution sample, use the tools listed below:

o Diluent (CELLPACK)

o Micro-tube (MT-40, etc.)

o Capillary tube o A 500 µL transfer pipette

o A container, such as erlenmeyer flask or beaker

o A syringe Analyzing Samples:

When preparing a 1:26 dilution sample, use the tools listed below:

o Diluent (CELLPACK)

o Micro-tube (MT-40, etc.)

o Capillary tube

o A 500 µL transfer pipette o A container, such as erlenmeyer flask or beaker

o A syringe

Analyzing Samples:

to analyze the sample in Prediluted Mode, first switch the analyzer to Prediluted mode and follow the procedure as Whole Blood analysis.

Reagents of Sysmex

CELLPACK: is ready to use for impedance and photoelectrical analysis of whole blood, its ingredients are: sodium chloride, boric acid, sodium tetra borate, EDTA-2K

STROMATOLYZER WH: is ready to use lysing reagent to analyze the leucocytes by

lysing the RBC and left the WBC Free and easy to count; whole blood sample by resistance 50 measurement and photometric measurement, and its ingredients are: non ionic surfactant, organic quaternary ammonium salt

CELLCLEAN: is a strong alkaline detergent to remove lysing reagents, cellular residuals and blood proteins remaining in the hydraulics of sysmex analyzer. Is a detergent to clean the instrument, to remove residual and blood proteins from the hydraulic systems, transducer, sample rotor valve, whole blood aspiration tube and Hgb flow cell Ingredients: sodium hypochlorite

Annex VII.Data extraction format

1. Check list

Result reporting form

Serial no	Card no	Age	Sex	MPV	Platelet count	PDW	PCT	PLCR

1. Educational level: 1. Unable to read and write 2.Read and write 3.Primery (1-8 years) 4.Secondery(9-12) 5.CollageDiploma/Degree and above

2. Occupation: 1Governement employee 2. Non-government employee 3.Private business 4.Student 5. House wife

3. Marital status: 1.Married 2.Single 3.Divorced 4.Windowed

Name of health facility----------

Date: _____

Signature of the laboratory technologist_____

AnnexVIII. Declaration

The undersigned declares that this thesis complies with the regulations of the University and meets the accepted standards with respect to originality and quality. PI also agrees to accept responsibility for the scientific ethical and technical conduct of the research project and for provision of required progress reports.

M.Sc. candidate: Kalkidan Gera (B.Sc.)

Signature:_____

Date of submission:_____

This thesis has been submitted with our approval as advisors.

Advisor:

FikaduUrgessa(MSc, PhDcandidate)

Signature: _____

Date: _____

Place: Addis Ababa, Ethiopia.

Advisor: MelatworkTibebu (MSc, PhD candidate)

Signature: _____

Date: _____

Place: Addis Ababa, Ethiopia.