ADDIS ABABA UNIVERSITY

COLLEGE OF HEALTH SCIENCE

DEPARTMENT OF MEDICAL LABORATORY SCIENCE

Assessment of Platelet to Lymphocyte ratio and Neutrophil to Lymphocyte ratio as potential biomarkers in patients with rheumatoid arthritis at St. Paul's Hospital Millennium Medical College, Addis Ababa, Ethiopia.

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SCHOOL OF GRADUATE STUDIES

This is to certify that the thesis prepared by Senait Abate, entitled: *Assessment of Platelet to Lymphocyte ratio and Neutrophil to Lymphocyte ratio as potential biomarkers in patients with rheumatoid arthritis at St. Paul's Hospital Millennium Medical College, Addis Ababa, Ethiopia* submitted in partial fulfillment of the requirements for Master of Science degree in Clinical Laboratory Sciences (Hematology and Immunohematology) complies with the regulations of the University and meets the accepted standards with respect to originality and quality.

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Abbreviation

ACR	American College of Rheumatology
AS	Ankylosing spondylitis
AUC	Area under the curve
BD	Becton Dickinson
CBC	Complete blood count
CD	Cluster of differentiation
CRP	C –Reactive protein
CV	Cardiovascular
DAS 28	Disease activity score in 28 joints
ESR	Erythrocyte sedimentation rate
EULAR	European League Against Rheumatism
FMOH	Federal Ministry of Health
IQR	Interquartile range
MTX	Methotrexate
NET	Intracellular traps
NET	Net intracellular traps
NLR	Neutrophil to lymphocyte ratio
PI	Principal Investigator
PLR	Platelet to lymphocyte ratio
RA	Rheumatoid arthritis
RF	Rheumatoid factor
ROC	Receiver Operating characteristic

ROS	Reactive oxygen species
SJC	Swollen joint counts
SLE	Systemic lupus erythematosus
SOPs	Standard operating procedures
SPHMMC	St. Paul's Hospital Millennium Medical College
SS	Sjögren’s syndrome
VAS	Visual Analog Scale
WHO	World health organization

Abstract

Background: Rheumatoid Arthritis (RA) is a chronic, systemic autoimmune disease involving inflammation of synovial joints. The interest in the study of platelet to lymphocyte ratio (PLR) and neutrophil to lymphocyte ratio(NLR) has grown recently because they have been found to be predictive of the prognosis of patients with diverse inflammatory and ischemic conditions and these ratios are easily calculated from hematology analyzer printout. However, in Ethiopia no published study is available in this regard.

Objective: This study aimed to assess platelet to lymphocyte ratio (PLR) and neutrophil to lymphocyte ratio (NLR) as potential biomarkers in patients with rheumatoid arthritis at St. Paul's Hospital Millennium Medical College, Addis Ababa, Ethiopia from April-July, 2019.

Method: This comparative cross-sectional study enrolled 61 newly diagnosed adult rheumatoid arthritis patients and 61 sex and age matched healthy controls. Blood sample was collected from all study participants using convenient sampling technique. CBC was measured using Mindray BC5300 hematology analyzer and BD sedintainer was used for ESR measurement, while RF and CRP were done based on latex agglutination method. Data analysis was done using *SPSS version 20*. Level of significance between groups was analyzed using independent student t-test and Mann-Whitney U test. Receiver operating characteristic (ROC) curves was used to evaluate the performance of PLR and NLR. P values less than 0.05 was taken as statistically significant.

Result: The median PLR and NLR were significantly increased in RA patients compared with healthy controls 195(IQR, 150-242) vs 125(105-150), $P<0.05$; 2.4(1.85-3.5) vs. 1.6(1.3-1.9), $P<0.05$ respectively). ROC curves analysis showed that PLR had area under the curve(AUC) of 0.859 with 85.2% sensitivity and 66% specificity as a marker of rheumatoid arthritis and NLR had AUC of 0.80 with sensitivity and specificity of 78.3% and 68% respectively.

Conclusion: There was a statistically significant increment in the level of Platelet to lymphocyte ratio and Neutrophil to lymphocyte ratio among new RA patients compared with healthy controls. In addition ROC curves analysis revealed a good diagnostic performance. Therefore, PLR and NLR can be added to routine diagnostic parameters as indicators in the assessment of rheumatoid arthritis patients**.** So, further studies with larger numbers of RA cases is needed to better understand the role of PLR and NLR in patients with RA.

Key Words: Rheumatoid arthritis, Platelet to lymphocyte ratio, Neutrophil to lymphocyte ratio.

1.Introduction

1.1.Background

Rheumatoid Arthritis (RA) is a chronic, systemic autoimmune disease of unknown etiology which involves inflammation of synovial joints and it has an incidence of 1% worldwide[1,2]. It leads to progressive damage and degeneration of the joint, mainly when inflammation is persistent [3]. Rheumatoid arthritis most commonly affects smaller joints, such as the joints in the hands and feet. However, larger joints such as the hips and knees can also be affected [4]. The disease also affects many other parts of the body (extra-articular tissues) including the skin, blood vessels, heart, lungs, kidney, and muscles. It arises more frequently in females than males and being predominantly observed in elderly Peoples [5].

Diagnosis of rheumatoid arthritis is not confirmed by single test. Thus, a combination of clinical, laboratory and radiological investigation is used [6]. Early diagnosis and treatment of RA can avert or substantially slow progression of joint damage in up to 90% of patients, thereby prevent irreversible disability [7].

There is no specific laboratory test for direct diagnosis of RA, Hence, multiple laboratory tests are often conducted to diagnose RA. Inflammation is the clue determinant and primary basic mechanism resulting in disability and increased mortality in RA patients. Hereof, assessment of inflammation in RA with trusted markers is important to detect long-term outcomes [8]. Systemic inflammation is associated with changes in circulating blood cell quantity and composition. Indeed an increase in neutrophil count, thrombocytosis, anemia, and decreased lymphocyte count occur with diverse inflammatory conditions. Hence, components of circulating blood cells could be used for the evaluation of inflammatory activity [9].

The most commonly used laboratory tests in RA patients are, inflammatory markers such as, acute-phase proteins C-reactive protein (CRP), non-specific tests the erythrocyte sedimentation rate (ESR), and serological markers like rheumatoid factor (RF), and anti-cyclic citrullinated peptide(Anti-CCP) antibody have been considered to be diagnostic indicators and markers that estimate disease activity in patients with RA [10,11]. Additionally, CBC(complete blood count) is a very common test that is often used as a screening test that gives a picture of a person's health status and can be used as the first step to screen for a wide range of medical conditions

including inflammatory disease. Parameters of complete blood count(CBC), especially including immune system elements, have a significant place in the assessment of various diseases. Immune system elements involve the neutrophils, lymphocytes, and platelets that have a role in the control of inflammation [12].

Platelets play a major role in inflammation and immune-modulation. Activated platelets release of proinflammatory cytokines including IL-1, and bind other platelets and leukocytes in the bloodstream that contribute to the development of systemic inflammatory response. Platelet counts are elevated in patients with chronic inflammatory and autoimmune diseases. Elevated neutrophils are also essential mediators of host defense. They can produce many inflammatory mediators and cytokines and bridge the innate and adaptive immune responses in autoimmune diseases. Lymphopenia is significantly and independently associated with rheumatoid factor positivity and emerged as a possible marker for the severe disease [13,14]. Platelet-to-lymphocyte ratio (PLR) which can be derived from the complete blood count is a novel index reflecting a systemic inflammatory burden that combines prognostic values of an individual's platelet and lymphocyte count [15,16]. The prognostic importance of PLR has also been noted in coronary artery diseases and hepatobiliary malignancies. In these conditions, elevated values of PLR confer poor prognosis [17].

Neutrophil to lymphocyte ratio (NLR), defined as the absolute neutrophils count divided by the absolute lymphocyte count is a simple parameter to assess easily the inflammatory status of a subject [18]. The interest in the study of NLR and PLR has grown recently because they have been found to be predictive of the prognoses of patients with diverse inflammatory and ischemic conditions. These ratios are easily calculated based on the full blood count and have been recognized as convenient, reliable and inexpensive markers [19,20]. Therefore, PLR and NLR do not require additional tests or costs so that they can readily be introduced in clinical practice and in the diagnosis process of rheumatoid arthritis as these parameters are easily calculated from the haemogram. Thus, this study assessed the Platelet to lymphocyte ratio and Neutrophil to lymphocyte ratio as potential biomarkers in patients with rheumatoid arthritis at St. Paul's Hospital Millennium Medical College. In particular, this study put forward that the mean PLR and NLR value of rheumatoid arthritis patients will be higher than healthy controls.

1.2. Statement of the problem

Rheumatoid Arthritis which is a systemic autoimmune disease leads to progressive damage and degeneration of the joint, mainly when inflammation is persistent, thereby decreases the quality of life [1-3]. Early detection and treatment of RA can avert or substantially slow progression of joint damage and prevent irreversible disability [7]. However, no single marker is outstanding for the diagnosis of RA. As a result, a combination of clinical, laboratory and radiological investigation is utilized in clinical care [6].

The utilization of Platelet to lymphocyte ratio and neutrophil to lymphocyte ratio for rheumatoid arthritis is absolutely low. Evidence has suggested that the platelet to lymphocyte ratio (PLR) and the neutrophil to lymphocyte (NLR), are potential indicators of systemic inflammation and immune response [21,22]. However, it has been assumed that they are not utilized as regular as they should be in medical practice in Ethiopia. If confirmed in our patients they can be easily introduced as an additional inflammatory marker at no cost

Recent studies have shown that PLR is used as an index for differential diagnosis or prognostic prediction of diverse diseases such as cancer, [23] metabolic syndrome [24] and inflammatory diseases, especially for evaluating cardiovascular risk and events [25]. Also NLR is elevated in autoimmune diseases such as psoriasis [26], ulcerative colitis [27], systemic lupus erythematosu(SLE), Sjögren's syndrome (SS) [28] and Behçet's disease [29]. However, Limited studies were done on PLR and NLR with rheumatoid arthritis patients .In addition there is no published study is available in our country in this regard. Therefore, there is a need for investigating additional biomarkers that can assist in the management of patients with rheumatoid arthritis. Hence, this study aimed to assess platelet to lymphocyte ratio and neutrophil to lymphocyte ratio as a potential biomarkers in rheumatoid arthritis patients.

1.2. Significance of the study

This study will provide information on the significance of platelet to lymphocyte ratio and neutrophil to lymphocyte ratio in the diagnosis process of rheumatoid arthritis disease. Hence, the study identifies additional markers from the complete blood count analysis which is one of the most routinely requested tests at no additional cost. This study can help patients as this parameters are easily available with no additional cost and help clinicians as additional inflammatory marker in the diagnosis of RA patients. To the best of our knowledge such kind of study was not conducted in our country and it can be serve as baseline data for further study in our settings. Also, the result obtained from this study could be help policy makers to introduce in patient management practice.

2. Literature Review

Recently, several reports have suggested the potential utility of the platelet to lymphocyte ratio (PLR) and the neutrophil to lymphocyte ratio (NLR), as diagnostic biomarkers in RA and other rheumatic diseases [30].

2.1.PLR and Rheumatoid arthritis

Substantial data are emerging on the pathogenic involvement of platelets in inflammatory arthritis and autoimmune diseases, indicating the existence of crosstalk between the coagulation and inflammation system. Upon activation, platelets release pro-inflammatory platelets microparticles, which interact with leucocytes leading to joint and systemic inflammation in rheumatoid arthritis [31].

According to study conducted by Zamora C, *et al.* in Spain in 2015, the presence of CD36+ CD4+Tlymphocytes in RA patients and the percentage of CD4+T lymphocytes with bound platelets was higher on RA patients than in healthy donors [32]. Boilard E, *et al*.2012, also have shown that platelets are not only critical haemostatic cells but also participate in the inflammatory response and seem to be key participants in the development and maintenance of joint inflammation, via IL-1-bearing microparticles, synthesis of PGI2 precursors and by promoting vascular permeability [33]. In 2019, Gasparyan AY, *et al*. assessed that the Platelet-to-lymphocyte ratio as an inflammatory marker in rheumatic diseases. They summarized as PLR is an emerging inflammatory marker that, in combination with NLR and other hematologic indices, can help in the diagnosis and assessment of the activity and severity of several rheumatic diseases [34].

Moreover, a hospital-based cross-sectional study was conducted in 2015 by Peng YF. *et al.* in china to evaluate whether NLR and PLR are associated with RA and their finding showed that NLR and PLR were significantly higher in RA patients compared with healthy controls (3.20±2.06vs. 1.56±0.47, P<0.01; 192.85±101.78 vs. 103.49±28.68, P<0.01) respectively. Even so, Receiver operating characteristic (ROC) curves analysis showed that PLR values higher than 115.7 evaluated RA with a sensitivity of 82.5%, a specificity of 74.8% and area under the curve (AUC) of 0.847. So the study concluded that PLR is associated with RA [35]. In the same country a Meta-analysis was conducted by Hao X, *et al.* in 2017 on the relationship between

hematological indices and autoimmune rheumatic diseases. When compared to the healthy control group, NLR was increased in patients with ankylosing spondylitisn(AS), Behçet's disease(BD), and RA(SMD = 0.33; 95% CI: 0.19 to 0.47; SMD = 1.90; 95% CI: 0.13 to 3.67; SMD = 0.75; 95% CI: 0.23 to 1.28) and PLR was found increased in RA and SLE (SMD = 33.91; 95% CI: 20.50 to 47.32; SMD = 59.11; 95% CI: 4.46 to 113.76). So their finding indicated that NLR and PLR could be recommended as inexpensive diagnostic biomarkers for Autoimmune Rheumatic Diseases [36].

Furthermore, several studies have investigated the diagnostic value of the NLR and PLR in Rheumatoid arthritis (RA) patients. Their studies suggested that the value of NLR and PLR positively correlated with RA. So NLR and PLR in peripheral blood had a high diagnostic value and can be used as an additional inflammatory marker in patients with RA [37-40].

Abd-Elazeem M I. *et al.* 2018, also studied the neutrophil-lymphocyte and platelet-lymphocyte ratios in rheumatoid arthritis patients in Egypt. They assessed that there was a significant difference regarding NLR and PLR between active patients and control (2.1 ± 0.59 and 1.27 ± 0.46; p = .03 and p = .04 respectively). The study concluded that NLR and PLR are two emerging inflammatory biomarkers that could be used to evaluate disease activity in active RA patients [41]. In the same way Helal *et al*. 2019, assessed that Neutrophil-lymphocyte ratio (NLR) and platelet-lymphocyte ratio (PLR) as markers of disease activity in rheumatoid arthritis. Finally, they concluded that NLR and PLR of RA patients were significantly higher when compared to that of the controls and a significant correlation between NLR and the DAS 28 score was observed as well [42].

2.2. NLR and Rheumatoid arthritis

The cross-sectional study was conducted in India, in 2017 by Chandrashekara, *et al.* on the characterization of neutrophil-to-lymphocyte ratio as a measure of inflammation in rheumatoid arthritis patients. Their study suggested that distribution of NLR values corresponded with DAS28-CRP(3) rather than CRP and ESR and A significant difference in the Visual Analog Scale(VAS), swollen joint counts (SJC-28), inflammatory parameters and general health outcome measures were observed among the NLR groups. In addition the NLR cut-off value of 1.4 classified the patients in deep remission with 90% specificity, 24% sensitivity, likelihood ratio positive (LR+)2.46 and likelihood ratio negative (LR-) 0.84. of NLR. So they concluded that NLR may serve as a less expensive and effective measure of inflammation [43].

On the contrary, another study was conducted in Bagdad by Mikhael EM. *et al.* in 2013, to evaluate the usefulness of NLR to detect inflammation in RA, and its correlation to RA disease activity indices and some hematological parameters. NLR was positively correlated with ESR and inversely correlated with Hb, but it did not show any correlation with other clinical and laboratory parameters. They concluded that NLR is less correlated with inflammation and not suitable to monitor disease activity in RA patients using methotrexate(MTX)[44].

Mercan R, *et al.* in 2016 conducted a study on the association between neutrophil/ lymphocyte ratio and disease activity in rheumatoid arthritis and ankylosing spondylitis (AS). Their study shows that NLR was higher both in RA (2.53 ±1.4 vs. 2.16±1.0,p=0.019) and AS(2.43 ±1.4 vs. 2.16±1.0,p=0.077) when compared to control subject and it is a cheap and readily available marker for the assessment of disease activity in RA [45]. On the other hand, Boulos D, *et al.* conducted study in 2019 on the neutrophil lymphocyte ratio in early rheumatoid arthritis and its ability to predict subsequent failure of triple therapy. Their finding showed that the mean NLR was significantly higher in those who failed triple therapy compared with those who did not (3.7 ±2.8 vs. 2.9±1.5,p=0.02). So the study concluded that NLR may offer an inexpensive, objective and reproducible prognostic marker in RA [46].

In Africa Fawzy R M. *et al.* also conducted a study in Egypt, in 2017. According to this study, NLR was significantly increased in the RA patients (3.28 ± 0.59) when compared to the control (1.7 ± 0.23) ($p < 0.0002$). The parameter was also significantly elevated in highly active RA patients compared to patients with moderate and low disease activity ($p < 0.014$). The study

shows that NLR significantly correlated with disease activity indices in recent-onset RA patients [47].

However, such published studies are lacking in our country. So the search for additional inexpensive and simple parameters is of the greatest importance for RA patients. Therefore, the assessment of PLR and NLR as a potential biomarker in patients with RA were investigated in this study.

3.Objectives

3.1.General objective

To assess platelet to lymphocyte ratio (PLR) and neutrophil to lymphocyte ratio (NLR) as potential biomarkers in patients with rheumatoid arthritis at St. Paul's Hospital Millennium Medical College, Addis Ababa, Ethiopia from April up to July, 2019 .

3.2.Specific objectives

- To assess platelet to lymphocyte ratio (PLR) as a potential biomarker in patients with rheumatoid arthritis.
- To assess neutrophil to lymphocyte ratio (NLR) as a potential biomarker in patients with rheumatoid arthritis.
- To determine the correlation between PLR, NLR, and other laboratory hematological RA parameters.

4.Materials and Methods

4.1.Study area

The study was conducted at St. Paul's Hospital Millennium Medical College which is located western part of Addis Ababa Gulele sub city woreda 9.It is a referral hospital in Addis Ababa under the Ethiopian Federal Ministry of Health (FMOH). Also, it is the second-largest public hospital in the nation and established by the late Emperor Haile Selassie in 1968 with the help of the German Evangelical Church. It was established to serve the economically underprivileged population, providing services free of charge to about 75% of its patients. In 2007 it became a medical college and its core services include the provision of medical care, teaching, and research.

The hospital has 2800 clinical and nonclinical staff members that provide medical specialty services to an average of 1200 patients daily including private wing. The hospital has 25 departments including diagnostic laboratory and most recently kidney hemodialysis and Renal replacement units and more than 700 beds. Many people are referred from all over the country to this hospital.

The hospital's laboratory is equipped with a fully automated hematology analyzer and participates in external quality control; additionally, the laboratory implements a laboratory quality management system and is awarded three stars according to the WHO afro checklist[48].

4.2. Study design and period

A comparative Cross-sectional study was conducted to assess the platelet to lymphocyte ratio and neutrophil to lymphocyte ratio as potential biomarkers in patients with rheumatoid arthritis at the Rheumatology Clinic of St. Paul's Hospital Millennium Medical College. The study was carried out from April up to July,2019.

4.3. Population

4.3.1. Source population

The source populations were patients aged 18years and older as well as newly diagnosed with rheumatoid arthritis disease based on American College of Rheumatology (ACR) and European League Against Rheumatism (EULAR) 2010 classification criteria and healthy controls who visit St. Paul's Hospital Millennium Medical College during the study period.

4.3.2. Study population

The study populations were patients with their cases of rheumatoid arthritis disease and healthy individuals who fulfill the inclusion criteria during the study period.

4.4. Inclusion and exclusion criteria

4.4.1. Inclusion criteria

- Aged 18years and older at the time of the study and volunteer to participate were included.
- Patients suffering from or diagnosed with rheumatoid arthritis
- Sex and age-matched healthy controls who had no previous history of chronic disease and the current history of any disease sign and symptom were included in the study.

4.4.2. Exclusion criteria

- Those patients who are taking anti-inflammatory therapy and with a history of diseases like:
    - Cardia-cerebrovascular disease,
    - Hematologic disease (such as chronic myelogenous leukemia, thrombocytosis),
    - known chronic liver and kidney diseases,
    - Infectious diseases,
    - Malignant tumor diseases,
    - Hypertension, diabetes, Metabolic syndrome,
    - other autoimmune diseases (such as ankylosing spondylitis) and pregnant women were excluded from this study.

4.5. Study Variables

4.5.1. Dependent variables

- ✓ Platelet count,
- ✓ WBC count
- ✓ lymphocyte count
- ✓ Neutrophil count
- ✓ PLR and also
- ✓ NLR

4.5.2. Independent variable

- Age
- Sex

4.6. Sample size calculation and Sampling method

4.6.1. Sample size determination

The maximum number of sample required for this study is determined by using a comparison of two independent means formula, considering the following assumptions:

$$n=2\ SD^2/d^2 *(Z\alpha +Z\beta)2$$

Where:-n, = maximum sample size required for the study

$Z\alpha$=standard normal distribution ($Z\alpha/2$=1.96) with a confidence interval of 95% was used

$Z\beta$ = Standard normal value for power = 0.84 (80%)

SD= Standard deviation value = 2.06 according to the Previous study conducted on Platelet to lymphocyte ratio and Neutrophil to lymphocyte ratio in patients with rheumatoid arthritis[38].

d= Effect size or expected mean difference between RA patients and controls = 1.1

Note: Sample size for NLR and PLR calculated independently and the larger sample size was taken

$$n= \frac{2(2.06)^2*(1.96+0.84)^2}{(1.1)^2} = 54.99 \text{ rounded to } 55 +10\% \text{ contingency= } 61$$

Considering an equal number of rheumatoid arthritis patients and healthy controls a total of 122 participants were included in this study.

4.6.2. Sampling method

Data from all patients who fulfilled the inclusion criteria and newly diagnosed with rheumatoid arthritis and healthy controls selected from the patients attendant who visits St. Paul's Hospital Millennium Medical College during the study period were utilized in this study. A convenient sampling technique was used to recruit 61 cases and 61 age and sex-matched controls. All volunteer individuals consecutively recruited until calculated sample size reached

4.7.Measurement and Data collection

4.7.1.Data Collection Procedure

In this study, 61 newly diagnosed rheumatoid arthritis patients and 61 healthy controls with full socio-demographic data were included. The aim of the study was explained to all study participants and informed consent was obtained from all individuals included in this study. Each study participant provided an adequate amount of blood for CBC, ESR, RF and CRP determinations.

4.7.2 Laboratory analysis

- **Complete blood count and Erythrocyte sedimentation rate test**

Venous blood sample of 2 ml was collected from all study participants with EDTA vacutainer tube for complete blood count determination and 0.8ml blood sample was collected with BD sedintainer tube for ESR determination. CBC were measured by using Mindray BC5300 hematology analyzer.

Mindray BC-5300 Hematology analyzer

It provides 27 parameters, for RBC/PLT enumeration electrical impedance method is used and for WBC 4 parts (lymphocyte, monocyte, neutrophils, and eosinophils) differentiation, chemical dye, flow cytometry, and laser scatter are applied. For Basophil and total WBC count, the cells are first treated with chemical dye, and then enumerated by impedance method and Cyanide-free hemoglobin determination method [47].

N.B. Platelet/Lymphocyte ratio and Neutrophil/Lymphocyte ratio was determined from the CBC data obtained from Mindray BC-5300.

Erythrocyte Sedimentation rate

BD Sedintainer was designed for blood sedimentation without having to use sedimentation pipettes. Blood is drawn directly into the BD Sedintainer then inverted and returned upright 10 times(mixed) and then placed in the BD Sedintainer stand. The rate at which they settle is measured as the number of millimeters of clear plasma present at the top of the column after one hour(mm/hr**)**. The resulting reading is per the Westergren method.

Rheumatoid factor and C- reactive protein

About 2 ml of blood sample was collected in sterile plain vacutainer tube. Then, the sample was allowed to clot for 60 minutes and centrifuged at 3000 rpm for 5 minutes to obtain the serum. The serum was separated and used for RF and CRP determination.

Rheumatoid factor

The RF reagent is a suspension of polystyrene latex particles sensitized with specially prepared human IgG. The reagent is based on an immunological reaction between human IgG bound to biologically inert latex particles and rheumatoid factors in the test specimen. When serum-containing rheumatoid factors is mixed with the latex reagent, visible agglutination occurs [48].

C- reactive protein

The C-Reactive protein test is based on the principle of the latex agglutination. When latex particle complexed human anti-CRP are mixed with a patient's serum containing C reactive proteins, a visible agglutination reaction will take place within 2 minutes [49].

4.8. Data Quality Assurance

In order to get an accurate result, Standard Operating procedures (SOPs) were followed during sample collection, transportation, and examination. The label and the collected samples were checked if there has clots, hemolysis or not before participant leaves, the collected blood sample was transported to hematology and serology laboratory for analysis of CBC, ESR, and RF, CRP respectively. Rheumatoid factor and C- reactive protein reagents were checked daily with known positive and negative control. Known three levels of control(Low, Normal and High) were done daily to check the proper functionality of the BC5300 hematology analyzer. All reagents and samples were used, stored and analyzed based on St. Paul's Hospital Millennium Medical College SOPs. Finally, the result was verified by senior laboratory professionals.

4.9. Data analysis and interpretation

Collected data were cleaned, edited and checked for completeness and analyzed using *SPSS version 20* (Statistical Package for social sciences, SPSS) statistical software for analysis. Qualitative data were expressed in numbers and percent, as well as quantitative data, were expressed as means ± standard deviation and median (IQR). Assessment of normality was performed by Kolmogorov-Smirnov test. The student's t-test for parametric data, Mann-Whitney U test for non- parametric data, and chi-square test were used for the comparison between the groups. Pearson's correlation coefficient was used to determine the relationship between PLR, NLR and other laboratory hematological parameters for RA. Finally, the receiver operating characteristic (ROC) curves were evaluated to measure the performance of the PLR and NLR. P-value of <0.05 was considered as statistically significant.

4.10. Operational Definitions

Rheumatoid Arthritis (RA): Rheumatoid arthritis is an autoimmune disease that causes chronic inflammation of the joints.

Biomarker: Is a measurable indicator of severity or presence of some disease state

Platelet to lymphocyte ratio (PLR): Platelet to lymphocyte ratio was defined as the ratio of absolute platelet count divided by the absolute lymphocyte count .

Neutrophil to lymphocyte ratio (NLR): Is the ratio of absolute neutrophil count to absolute lymphocyte count .

ROC curve :It is a graphical technique and effective method of evaluating the performance of diagnostic tests

Area under the curve (AUC) is considered as an effective measure of a validity of a screening test and can evaluate the discriminability of a test, to identify people with the disease or not

Cutoff Value: Is the dividing points on measuring scales where the test results are divided into different categories, like positive or negative .

High PLR cut off: A value with a PLR greater than 141 cut off value was defined as high PLR
High NLR cut off: A value with a NLR greater than 1.77 cut off value

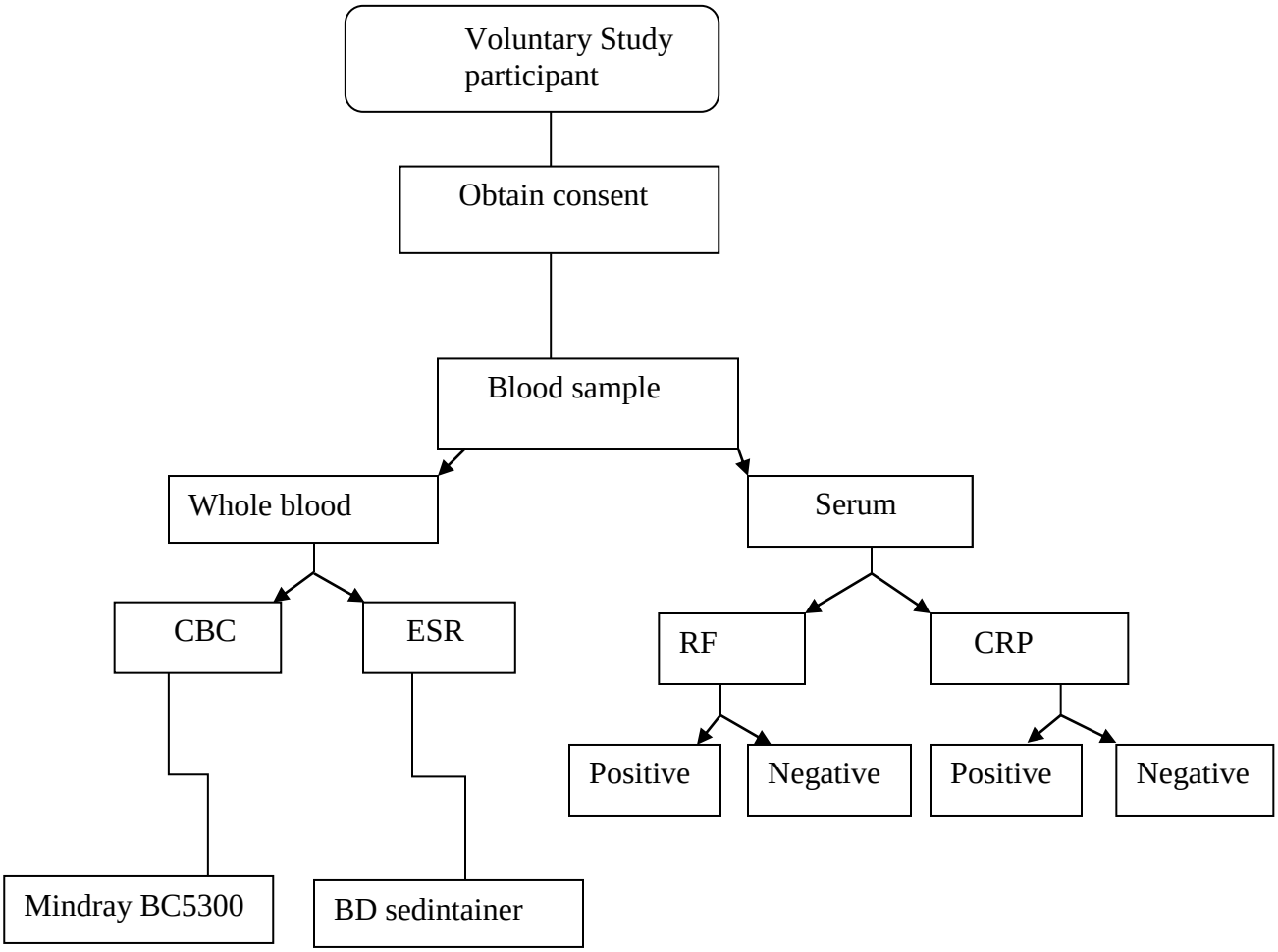
4.11. Ethical considerations

Ethical clearance was obtained from the Department of Research and Ethical Review Committee (DRERC) of Medical Laboratory Science, College of Health Science, Addis Ababa University and St Paul's hospital millennium medical college. The aim of the study, benefits, and rights was explained to study participants and informed consent was obtained. The permission from the hospital was obtained. Blood samples were taken from those individuals who were volunteers and signed on the consent form. Participants with abnormal results were treated. Any information that was obtained during the study were kept confidential by password protection of electronic files and locking of hard copies.

4.12. Dissemination of the result

The finding of this study will be disseminated to Addis Ababa University, College of Health Sciences, Department of Medical Laboratory Sciences. The result will also be communicated to St. Paul's Hospital Millennium Medical College, and presented during annual conferences of professional societies and other concerned bodies. The finding of the study will also be presented to the medical scientific community and the manuscript will be submitted to peer-reviewed journals for publication.

5. Overall Laboratory Workflow



6. Result

6.1. Socio-demographic characteristics of study participants

The current study included a total of 122 participants aged 18-69 years, of whom 61 were newly diagnosed RA patients and 61 sex and age-matched healthy controls. Sex and age distribution data are summarized in Table 1. The mean±SD age of patients was 42.1±13.1 years which was comparable with that of the control 41.8 ± 12.7 (p-value was = 0.92). There was no difference in gender as well; rheumatoid arthritis patients were 47 (77%) females and 14 (23%) males which were similar in healthy controls.

Table1.Socio-demographic characteristics of study participants at St Paul's Hospital Millennium Medical College, April-July, 2019

Parameters	**RA patients (n=61)**		**Healthy control (n=61)**	
Sex	Frequency	%	No.	%
Male	14	23.0	14	23.0
Female	47	77.0	47	77.0
Age(years)				
18-25	8	13.1	7	11.5
26-35	11	18.1	13	21.3
36-45	21	34.4	20	32.8
46-55	8	13.1	10	16.4
55 and above	13	21.3	11	18.0
Age Mean ± SD	42.1± 13.1		41.8 ± 12.7	

RA: rheumatoid arthritis; SD=standard deviation

6.2. Comparison of Mean±SD and Median of selected laboratory investigations between rheumatoid arthritis patients and controls

As shown in Table 2, Figure1 and 2, The median PLR and NLR were significantly increased in RA patients compared with healthy controls 195(IQR, 150-242) vs 125(105-150), P<0.001; 2.4(1.85-3.5) vs. 1.6(1.3-1.9), P<0.0001 respectively)). In addition, there were significant differences in mean±SD regarding the following variables between RA patients and healthy controls: the of total white blood cell count (8.21±3.08 vs 5.70±1.46), absolute neutrophil count(5.41±2.56 vs 3.13±1.09); platelets (404.86±138.87 vs 233.19±44.82); ESR(58.39±35.04 vs 10.42±4.14), P<0.001 for all parameters except platelet (P= 0.00). Moreover,59(96.7%) patients were positive for RF while2(3.3%) were negative. CRP was positive in 48(78.7%) of the patients while 13(21.3%) had negative CRP. However, there was no significant difference in the mean±SD regarding absolute lymphocyte count between RA patients with healthy control, (2.03±0.56 vs 1.90±0.48, P= 0.17.) On the other hand, all controls were negative for RF and CRP

Table2. Comparison of Mean±SD and Median (IQR) of selected laboratory investigations between rheumatoid arthritis patients and controls at St Paul's Hospital Millennium Medical College, April-July, 2019

Parameters	**RA patients (n=61)**	**Healthy control (n=61)**	**P value**
WBC ($x10^9$/L)	8.21±3.08	5.70±1.46	**P<0.001**
Absolute Neutrophil count($x10^9$/L)	5.41±2.56	3.13±1.09	**P<0.001**
Absolute Lymphocyte count($x10^9$/L)	2.03±0.56	1.90±0.48	P=0.17
Absolute platelet count($x10^9$/L)	404.86±138.87	233.19±44.82	**P=0.00**
CRP Positivity	48(78.7)	0(0%)	
ESR(mm/hr)	50(35-70) ®	10(6.5-15)	**P<0.001**
RF Positivity	59(96.7%)	0(0%)	
PLR	195(150-242) ®	125(105-150)	**P<0.001**
NLR	2.4(1.85-3.5) ®	1.6(1.3-1.9)	**P<0.001**

RA: rheumatoid arthritis, SD: standard deviation, WBC:-white blood cell count, CRP:- C-reactive protein, ESR:- erythrocyte sedimentation rate, RF: rheumatoid factor, PLR: platelet to lymphocyte ratio, NLR: neutrophil to lymphocyte ratio ® - Interquartile range

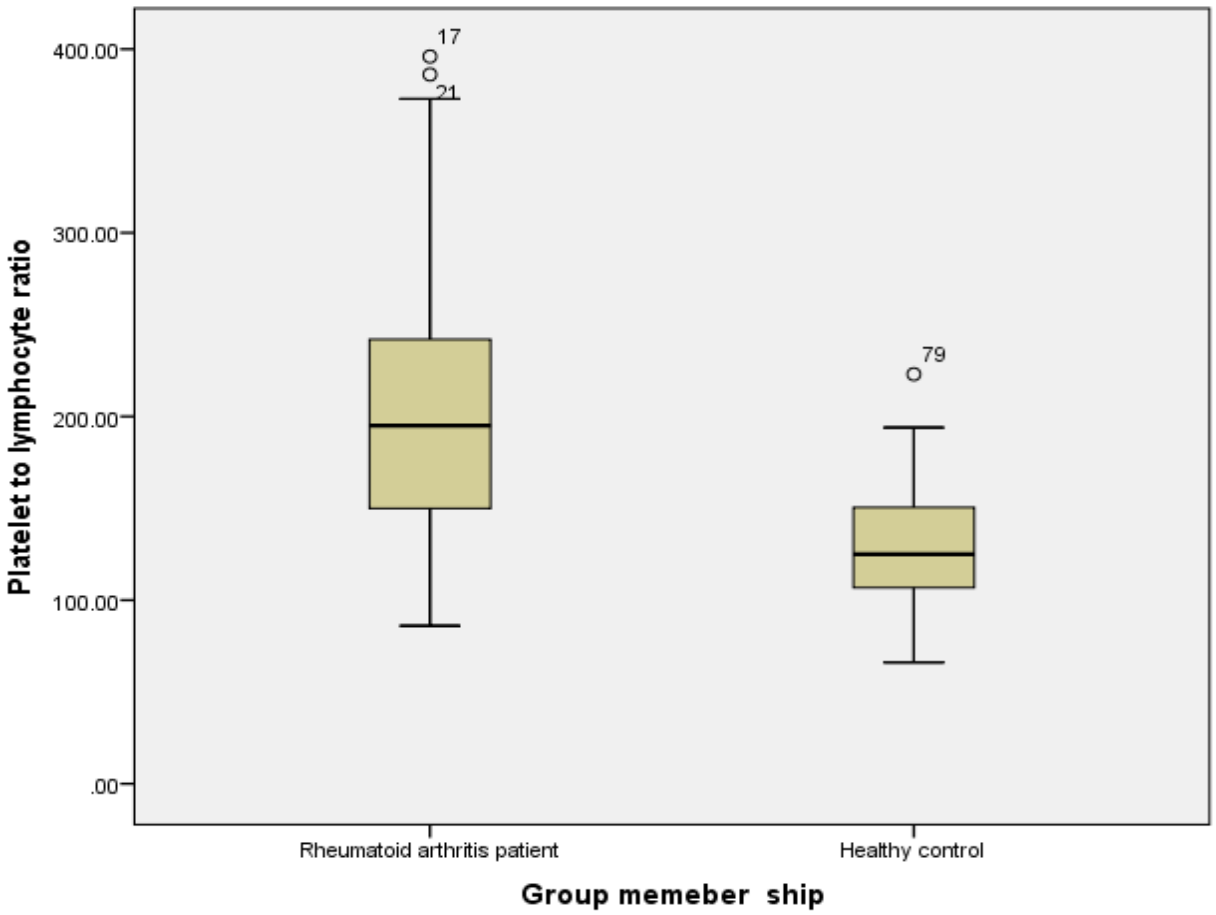


Figure:1.. Box and whisker diagram of platelet to lymphocyte ratio count among rheumatoid arthritis and healthy control. The middle of the box represents the median value. The outer box edges represent the 25th percentiles and 75th percentiles. The ends of the whiskers represent the lower and upper adjacent values. The dots above the upper adjacent values were outliers.

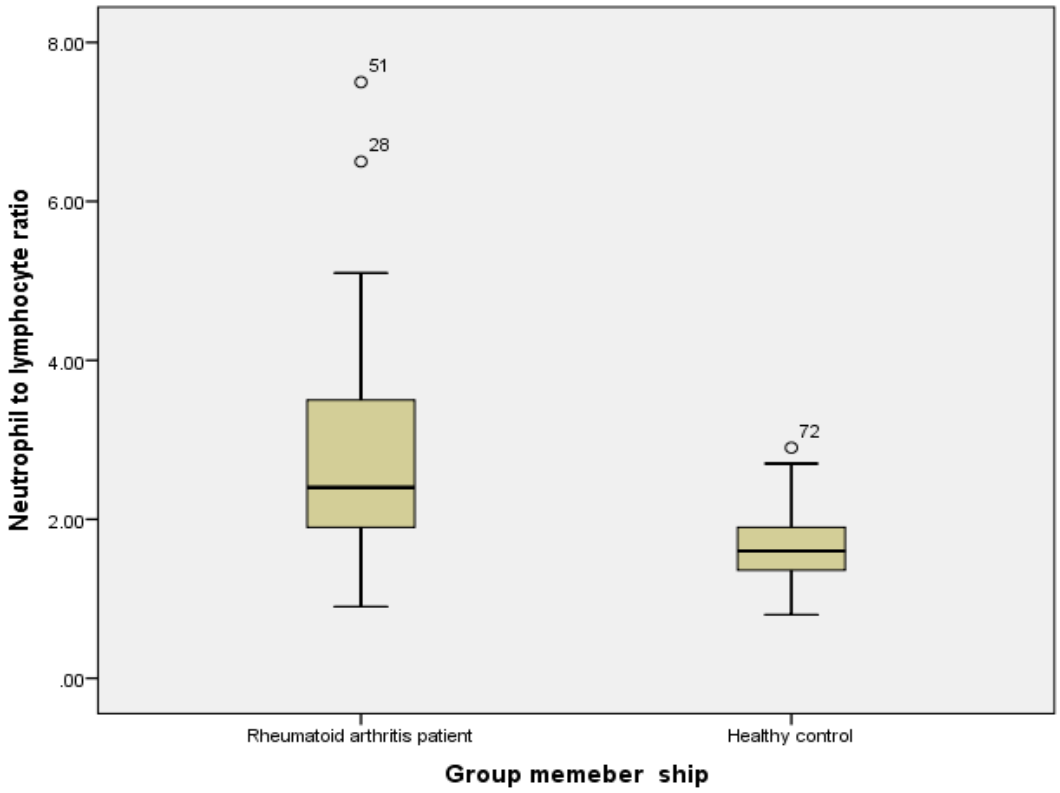


Figure:2. Box and whisker diagram of neutrophil to lymphocyte ratio count among rheumatoid arthritis and healthy control.. The middle of the box represents the median value. The outer box edges represent the 25th percentiles and 75th percentiles. The ends of the whiskers represent the lower and upper adjacent values. The dots above the upper adjacent values were outliers

6.3. Comparison of PLR and NLR between male and female

As shown in Table 3, Comparisons of median between males and females regarding PLR 233(IQR, 180-250) vs 183(147-231) and NLR 2.75(1.9-3.9) vs 2.4(1.7-3.2) respectively) showed no significant difference between the two groups (P = 0.15 and P = 0.47 respectively).

Table 3. Comparison of Median (IQR) of PLR and NLR between male and female at St Paul's Hospital Millennium Medical College, April-July, 2019

Parameters	**Male**	**Female**	**P- value**
PLR	233(180-250)	183(147-231)	**0.15**
NLR	2.75(1.9-3.9)	2.4(1.7-3.2)	**0.47**

6.4. Correlation of PLR, NLR with other hematological parameters

When we evaluate the correlation of PLR and NLR with RA related laboratory variables, both PLR and NLR were positively correlated with ESR (PLR, r= 0.49; P<0.001 and NLR, r=0.34 P<0.001)and platelet count (PLR, r= 0.78; NLR, r=0.34; P< 0.001for both). NLR but not PLR showed positive correlation with WBC (r=0.66) and neutrophil count (r=0.78),P<0.001 for both. In contrast both PLR (r=-0.303, P=0.001) and NLR negatively correlated with lymphocyte, though correlation of the later was weak and did not reach statistical significance (Table 3).

Table 4. Correlation of the PLR and NLR with the Hematological parameters in rheumatoid arthritis patients at St Paul's Hospital Millennium Medical College, April-July, 2019

Parameters	**RA patients n= 61**			
	PLR		NLR	
	R	**P value**	**R**	**P value**
WBC ($x10^9$/L)	0.157	0.084	0.66	**<0.001**
Absolute Neutrophil count($x10^9$/L)	0.25	0.06	0.78	**< 0.001**
Absolute Lymphocyte count($x10^9$/L)	-0.303	**0.001**	-0.107	0.24
Absolute platelet count($x10^9$/L)	0.78	**< 0.001**	0.34	**<0.001**
ESR(mm/hr)	0.49	**< 0.001**	0.34	**< 0.001**

r:- correlation coefficient, WBC-white blood cell count, ESR- erythrocyte sedimentation rate, PLR: platelet to lymphocyte ratio, NLR: neutrophil to lymphocyte ratio

6.5. Performance characteristics of PLR and NLR

Receiver operating characteristic (ROC) curve was used to measure the performance characteristics of PLR and NLR, as shown in Table 5 and Figure 3. Accordingly, PLR had an AUC of 0.859(95% CI, 0.793-0.925; *P*<0.00) and the optimal cut off value was 141.3, with 85.2% sensitivity and 66% specificity in separating RA patients from healthy individuals. The AUC value for NLR was 0.80 (95% CI, 0.720-0.880; *P*<0.0001) and the optimal cut-off value was 1.77, with sensitivity and specificity of 78.3% and 68%, respectively. These results indicated

that the addition of NLR and PLR to other diagnostic parameters revealed higher diagnostic value for discriminating RA patients from healthy individuals.

Table 5: The performance of PLR and NLR as laboratory markers for RA at St Paul's Hospital Millennium Medical College, April-July, 2019

Variables	AUC	Cut off value	Sensitivity	Specificity	P -value	Asymptotic 95% Confidence Interval	
						Lower Bound	Upper Bound
PLR	.859	>139	85.2%	63.9%	0.00	.793	.925
		>141.3	85.2%	66%			
		>143.	82.6%	67.2%			
NLR	.800	>1.73	78.5%	64%	< 0.001	.720	.880
		>1.77	78.3%	68%			
		>1.83	75.4%	73.2%			

PLR: platelet to lymphocyte ratio, NLR: neutrophil to lymphocyte ratio, AUC: area under the curve

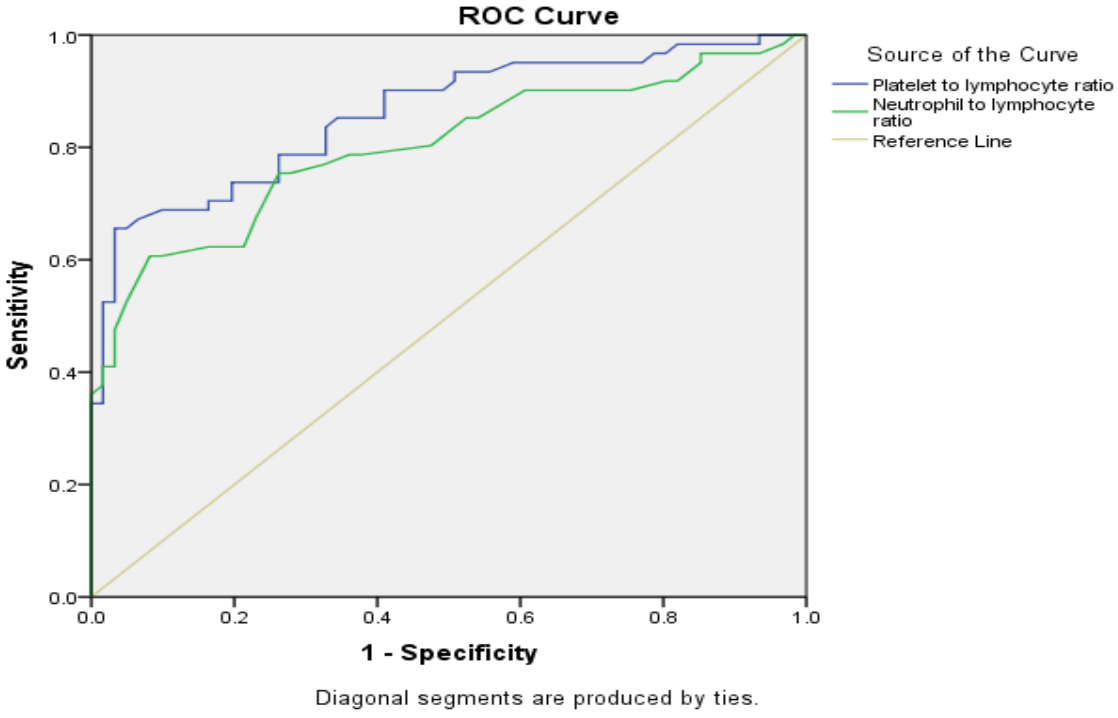


Figure 1: Receiver operating characteristic (ROC) curves showing the performance of PLR and NLR levels for patients with RA

7. Discussion

Rheumatoid Arthritis (RA) is characterized by symmetric joint involvement, erosion, and deformity in the joints as a result of synovial inflammation. Changes in peripheral blood parameters are useful in the disease prognosis of many diseases. NLR and PLR are believed to be a reliable marker of the inflammatory response [36]. This study analyzed the assessment of PLR and NLR as potential biomarkers in rheumatoid arthritis patients.

In the present study both PLR and NLR were significantly increased in RA patients compared with healthy controls. A similar pattern of results was obtained in china by Zhang *et al.*2016 [38]. Their findings that RA patients both in the disease active stage and in the non-active stage have revealed significantly high levels of NLR and PLR compared to healthy individuals. Uslu *et al.*2015[52] also demonstrated a significant difference in NLR and PLR between the patients and controls in their study which included 104 RA patients and 51 matched healthy subjects. They suggested that NLR and PLR are two novel inflammatory markers that must be used to assess disease activity in RA patients. On the other hand, other published reports showed a highly significant increase in NLR in RA patients when compared to healthy controls [47]. In contrast to our results, Sireen *et al.*2018, conducted a study in Sudan [53] that assessed the neutrophil and platelet-to-lymphocyte ratios as diagnostic biomarkers for rheumatoid arthritis. Their study found no significant difference in NLR between rheumatoid arthritis patients and the control group. The contradiction of this study with our result may be because most of their rheumatoid arthritis patients were on treatment.

In the present study, no significant difference was found in PLR and NLR between the gender of RA patients. This is in line with previous studies performed by Kweon *et al.*,2016, [54] and Fawzy *et al.*2017, [47] who reported no significant difference in NLR by gender. Similarly, Abd-Elazeem *et.al*.2018, [41] compared PLR and NLR between gender but no statistically significant difference was noted ($P>0.05$).

On the other hand, this study also concentrated on parameters from routine blood tests done for RA patients and found that WBC, neutrophils, lymphocyte and platelets counts, as well as ESR, were indeed higher for patients with RA when compared to healthy controls. In our study, in agreement with Peng *et al.*2015, [35] and Fu H *et al* .2015[40], we have found that platelet

counts were significantly increased in patients when compared to healthy controls. On the other hand, Zhang *et al.*2016[38] and Zengin O *et al.*2018,[55] did not find a statistically significant difference between patient groups and the control group regarding platelet count. This controversery may be due to study design as their study was a retrospective design. Uslu *et al.*2015 [52] and Mercan *et al.*2016[45] found that there was no significant difference regarding lymphocyte count between patients and control group, which was in agreement with our study. And also there was significantly increase in Neutrophil count in RA patients compared to healthy controls, these results were in agreement with Peng *et al.* 2015 [35] and Fawzy *et al.*2017, [47]. However, the results were in contrast to Zhang *et al,* 2016 [38] and Mercan *et al.*2016, [45], who showed no difference in neutrophil count between the patients and control groups.

The current finding of thrombocytosis and neutrophilian RA patients is scientifically sound and supported by evidences. Platelets play an active role in inflammation. The increased mass of circulating platelets is probably a consequence of chronic inflammation. Activated platelets not only produce growth factors such as platelet-derived growth factor and transforming growth factor-β, but also release chemokines, which have important effects in vascular inflammation leading to thrombosis [56]. Neutrophils infiltrate synovial tissues, release degradative enzymes, reactiveoxygen species, and cytokines, regulate cell-cell immune interactions and produce net intracellular traps(NET) producing autoantigens that act as neo-epitopes (e.g.citrullinated proteins) thus driving the inflammatory process from acute to chronic inflammation [57,58].At the same time lymphopenia is recognized in RA patients with selective apoptosis of T cell subsets. Due to the increased levels of cytokines, lymphocytes play a central role in the pathogenesis of RA [59].

In addition, the current study analyzed the correlations of NLR and PLR with other inflammatory laboratory parameters of RA. Both NLR and PLR were positively correlated with ESR, a finding which is in line with Zhang *et al,*'s 2016, report [38]. Fu *et al.* 2015 [40] and Mikhael *et al.*2013, [44] also showed NLR was positively and significantly correlated with ESR. Furthermore, our study showed that NLR was positively and significantly correlated with neutrophil but inversely correlated with absolute lymphocyte count while the PLR significantly inversely correlated with lymphocytes count. This is consistent with what has been found in Egypt by Abd-Elazeem *et.al.*2018, [41].

Finally, evaluation of the performance of PLR and NLR as an inflammatory marker in RA patients by using ROC curve analysis revealed AUC values of 0.859 and 0.80 for PLR and NLR, respectively. This is consistent with Previous study conduct by Peng *et al.*2015 [35] and Helal *et al.*2019 [42]. All these findings put forward that PLR and NLR could be used to reflect the inflammatory response and can serve as inflammatory marker in patients with RA. However, although automated hematology result printouts contain a minimum of 22 parameters, in most cases, it has been shown that not more than four common parameters are being clinically utilized routinely according to a study conducted in Ethiopia by Birhaneselassie *et al.*2013,[60]. The current study is part of evidence generation and promotion of such underutilized hematological laboratory markers for patient care in our setting with no additional cost.

7.1. Strength of the study

This was the first study in the country focused on Platelet to lymphocyte ratio and neutrophil to lymphocyte ratio regarding rheumatoid arthritis patients, it will be used as a baseline for further studies.

7.2. Limitation of the study

This study has some limitations; as it is a comparative cross-sectional, the variation of platelet to lymphocyte ratio and neutrophil to lymphocyte ratio was not observed after undergoing effective treatment (treatment outcome) for patients with RA. Serological test like Anti-cyclic citrullinated peptide(Anti-CCP) , quantitative method for rheumatoid factor and C- reactive protein was not done as it is expensive.

7.3. Conclusion

There was a statistically significant increment in the level of Platelet to lymphocyte ratio and Neutrophil to lymphocyte ratio among new RA patients compared with healthy controls. In addition ROC curves analysis revealed PLR had 85.2% sensitivity and 66% specificity while NLR had 78.3% sensitivity and 68% specificity in discriminating RA patients from healthy controls. Both PLR and NLR in RA patients were positively correlated with ESR and platelet count. NLR showed positively and significantly correlated with WBC and neutrophil count. In contrast both PLR and NLR negatively correlated with lymphocyte in RA patients. Therefore, PLR and NLR can be added to

routine diagnostic parameters as indicators in the assessment of rheumatoid arthritis patients**.**

7.4. Recommendation

Based on the finding of the current study, the following points were recommended. There is a need of:-

- ✓ Further studies with larger numbers of RA cases to better understand the role of PLR and NLR in patients with rheumatoid arthritis since this study is the first in our country
- ✓ Additional investigations should be done for detecting more markers that can help in the assessment of RA activity, such as Mean platelet volume(MPV), monocyte to lymphocyte ratio(MLR), plateletcrit (PCT) and platelet distribution width(PDW)
- ✓ Prospective cohort study needed to clarify the treatment received and rheumatoid arthritis disease outcome

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Annex

Annex I: Participant Information sheet

My name is_____. You are being asked to take part in a research study on "assessing platelet to lymphocyte ratio and neutrophil to lymphocyte ratio as a potential biomarker in patients with rheumatoid arthritis". The study has been approved by Addis Ababa University Medical Laboratory Science department research ethics committee. The study finding will give information about how many individual platelets to lymphocyte ratio and neutrophil to lymphocyte ratio are associated with RA disease.

In this study, you will be asked to give 5ml of a blood sample, which will be used to assess the platelet to lymphocyte ratio and neutrophil to lymphocyte ratio as potential biomarkers in patients with rheumatoid arthritis. The sample collection procedures take about 10 minutes and you are selected to participate in this study because of your being voluntary to give a blood sample and to answer the questions so, the tests for the blood sample you give will be performed free. I would like to assure you that all of the results and the answers you give will be kept confidential and for the study purpose only. You have the full right to stop being a participant of the research study at any time without explanation. Also, you have the right to have a question about the study to be answered. I will be glad to answer your questions about this study at any time.

You may contact me at e-mail address *senait921@gmail.com* or mobile *+251 913475785*

Department of Medical Laboratory Science research ethics office +251 11 275 5170

i. Participant information sheet (Amharic version)

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::የዚህጥናትዓላማፕላትልትቱሊምፎሳይትረሽዮእናኒዩትሮፊልቱሊምፎሳይትረሽዮ(Platelet to lymphocyte ratio and Neutrophil to lymphocyte ratio) የሚባል የላቦራቶሪ ምርመራ ምን ያህል የሪህበሽታን ለማከም ይረዳል የሚለዉን ለመገምገም ነዉ:: እርስዎ በዚህ የምርምር ሥራ ላይ እንዲሳተፉ በአክብሮት ተጋብዘዋል፡፡ይህ የምርምር ሥራ አዲስ አበባ ዩኒቨርስቲ የላቦራቶሪ ሳይን ስትምህርት ቤት የምርምር ሥራ ሥነ-ምግባር ኮሚቴ ፈቃድ ያገኘ ሲሆን ከጥናቱ የሚገኘዉ ዉጤት በሽታው ንለማጥፋት እና ለመቆጣጠር ይጠቅማል፡፡

በዚህ የጥናትሥራ ለመሳተፍ ፈቃደኛ ከሆኑ 5 ሚሊሊትር የደም ናሙና ይሰጣሉ፡፡ይህ የደም ናሙና ፕላትልት ቱሊምፎሳይት ረሽዮ እና ኒዩትሮፊል ቱሊምፎሳይት ረሽዮ የሚባል የላቦራቶሪምርመራ ምንያህል የሪህበሽታን ለማከምይረዳል የሚለዉን ለመገምገም ይረዳል፡፡ ናሙና ለመውሰድ10 ደቂቃይወስዳል፡፡በዚህ ምርምር እንዲሳተፉ የተመረጡት የደም ናሙና ለመስጠት እና ፍቃደኛ በመሆንዎ ሲሆን በዚህ ጥናት በመሳተፍዎ በሰጡት ናሙና ላይ የሚደረጉ ምርመራዎች በነፃ ይሰራልዎታል እናያገኛሉ፡፡የምርመራውጤቶቹ እና የሰጡትመልስ ሙሉ በሙሉ ሚስጥራዊነቱየተጠበቀ እንደሚሆን ናለጥናቱ ዓላማ ብቻ እንደሚውል ላረጋግጥልዎ እወዳለሁ፡፡በጥናቱ ላይ ያለምንም ማብራርያ ላለመሳተፍም ሆነ በመሀልለማቋረጥሙሉመብትአለዎት፡፡

በዚህጥናትላይያለዎትንጥያቄበማንኛውምሰዓትሊጠይቁናምላሽሊያገኙይችላሉ፡፡

በሚቀጥለውአድራሻሊያገኙንይችላሉ

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ህክምናላቦራቶሪትምህርትክፍልየምርምርሥነምግባርቢሮስልክቁጥር +251 11 275 5170

• • ሰናይትአባተ

የሕክምናላብራቶሪሳይንስትምህርትክፍል፤የጤናሳይንስኮሌጅ፤አዲስአበባዩኒቨርሲቲ

ለተጨማሪመረጃ፡አዲስአበባዩኒቨርሲቲ፤የሕክምናላብራቶሪሳይንስት/ክፍልይጠይቁ፡፡ስልክ+251112755170

Annex II: Informed consent form

I have read the information above, or it has been read to me. I have been given the opportunity to ask questions and my questions have been answered to my satisfaction. **I voluntarily consent that I would participate in this study.**

To collect my blood and be a participant in this study and understand that I have the right to withdraw from the study at any time .

Print name of participant, date and signature or thumb impression of participant

_____ ____ /____ /____ (dd/mm/yy) _____

If illiterate;

Print name of independent literate witness, date and signature of witness (if possible, this person should be selected by the participant and should have no connection to the research team)

_____ ____/____/_____ (dd/mm/yy) _____

Phone number_____

Print name of researcher, date and signature of researcher

_____ _____/____/_____ (dd/mm/yy) _____

i. Informed consent form (Amharic version)

ተሳታፊው በጥናቱ ለመሳተፍ ሙሉ ፈቃደኛ መሆኑን የሚገልጽበ ትቅጽ

የተሳታፊውስም_____

ስለጥናቱአስፈላጊየሆኑትንመረጃዎችአንብቤወይምተነቦልኝእናምየጥናቱዐላማእናአስፈላጊነትተረድቻለሁ፡፡
በማንኛውምሰዓትከጥናቱየመውጣትመብትእንዳለኝተነግሮኛል፡፡
ከጥናቱጋርየተያያዙጥያቄዎችንምጠይቄማብራሪያዎችተሰጥተዉኛል፡፡
እናምፍቃደኛመሆኔከታችበመፈረምአረጋግጣለሁ፡፡

እኔ_____

የተባልኩየተጠየቁትንጥያቄመልስእናናሙናለ_____

_____ ለመስጠትፈቃደኝነቴንገልጫለሁ፡፡

የተሳታፊውፊርማ_____

ቀን_____

የተመራማሪዉፊርማ_____

ቀን_____

Annex III: Laboratory test Procedure

Mindray BC5300 hematology analyzer

Principle

Mindray BC 5300 provie about 27 parameters. RBC/PLT, WBC/Basophil are counted and sized by electrical impedance method and for WBC 4 parts (lymphocyte, monocyte, neutrophils, and eosinophils) differentiation, chemical dye, flow cytometry, and laser scatter are applied. Hemoglobin is determined by Cyanide-free colorometric method

Sample collection

1. Use clean K2EDTA anticoagulant collection tubes to collect blood samples
2. Be sure to collect at least 0.5ml blood every time
3. Mix the sample accordingly

Sample Analysis

Entering work list information

1. Entering the sample ID
2. Selecting analysis mode
3. Entering the draw time and delivery time
4. Entering patient information and content of diagnosis
5. Saving

Running the sample

1. Mix the sample well and run it through the sample probe
2. Remove the sample tube after you hear the beep sound
3. After one minute record the result

ESR test

Principle

BD Sedintainer was designed for blood sedimentation without having to use sedimentation pipettes. Blood is drawn directly into the BD Sedintainer then inverted and returned upright 10 times(mixed) and then placed in the BD Sedintainer stand. The rate at which they settle is measured as the number of millimeters of clear plasma present at the top of the column after one hour(mm/hr). The resulting reading is per the Westergren method

Procedure for ESR

1. Collect the blood directly from the patient into BD vacutainer glass tubes.
2. Thoroughly mix blood specimens.
3. Place the tube vertically into a leveled BD Sedintainer stand and set a timer for 60 minutes.
4. At the end of exactly 60 minutes record in millimeters the distance from the zero lines to the top of the erythrocyte sedinteiner in the tube.
5. After reading the result decontaminate the tube and rack, discard all contaminated devices into an approved biohazard waste container.

CRP test

Principle

CRP usually appears in the sera of patients in the acute stages of a number of inflammatory conditions such as most bacterial and some viral infections; acute rheumatoid fever with or without carditis; rheumatoid arthritis and most other collagen diseases; and in a number of other conditions characterized by inflammation. CRP is considered to be a sensitive indicator of inflammation. Changes in the serum level of CRP with time from the same patient can be used as an index of recovery. The principle of this test is based on the immunological reaction between CRP as an antigen and the corresponding antibody coated on the surface of biologically inert latex particles. When latex particle complexed human anti-CRP are mixed with a patient's serum containing C reactive proteins, a visible agglutination reaction will take place within 2 minutes.

Material and Components

1. **CRP Latex Reagent:** Contains polystyrene latex particles coated with anti-human CRP in a stabilized buffer with less than 0.1% sodium azide as preservative.

2.**CRP Positive Control:** Human Serum that contains more than 6mg/L CRP and less than 0.1% sodium azide as preservative.

3.**CRP Negative Control:** Human serum that has been diluted and stabilized with buffer and contain less than 0.1% sodium azide as preservative.

4.**Glycine-saline Buffer:** To be diluted 1:20 with distilled water.

(20X) Concentrate

5.Disposable pipettes and test slides

Procedures

1. Allow each component to reach room temperature
2. Gently shake the latex reagent to dispense the particles
3. Place a drop of undiluted serum onto the circle of the test slide using the disposable pipettes provided
4. Add one drop of the latex reagent next to the drop of serum
5. Using the other end of the pipette spread the reagent and serum sample over the entire area of the test circle
6. Gently tilt the test slide backward and forwards approximately once every two seconds for two minutes

INTERPRETATION TEST RESULT

- The presence of agglutination indicates a level of CRP in the sample equal to or greater than 6mg/l.
- The lack of agglutination indicates a CRP level of less than 6mg/l in the sample.

RF test

Principle

The RF reagent is a suspension of polystyrene latex particles sensitized with specially prepared human IgG. The reagent is based on an immunological reaction between human IgG bound to biologically inert latex particles and rheumatoid factors in the test specimen. When serum containing rheumatoid factors is mixed with the latex reagent, visible agglutination occurs. The RF latex reagent sensitivity has been adjusted to detect a minimum of 8 IU/mL of rheumatoid factors according with the WHO International Standard without previous sample dilution

MATERIALS AND COMPONENTS

1. **RF Latex Reagent**: A suspension of uniform polystyrene particles coated
with IgG (human) in glycine buffer, pH 8.2; reagent sensitivity is standardized with the WHO RF Standard. Mix well before using
2. **RF Positive Control Serum**: A stabilized, prediluted human serum containing at least 8 IU/mL of RF.
3. **RF Negative Control Serum**: A stabilized, prediluted human serum containing less than 8 IU/mL of RF.
4. **Glycine-Saline Buffer (20x)**: pH 8.2 ± 0.1M glycine and 0.15M NaCl
5. Reaction Slide.
6. Pipette/Stir Sticks.

Sample collection and Preparation

1. Use fresh serum collected by centrifuging clotted blood.
2. If the test cannot be carried out on the same day, the serum may be stored between 2 - 8°C for no longer than 72 hours after collection.
3. For longer periods the sample must be frozen.
4. As in all serological tests, the hemolytic or contaminated serum must not be used.

Testing

1. Bring reagents and specimens to room temperature before use.

2. Using pipettes provided. Place one drop of the undiluted specimens on successive fields. Retain Pipette Stir Sticks for the mixing step.

3. Gently resuspend the RF Latex Reagent and add one drop to each test field. Use pipette Stir Stick to spread the reaction mixture over the entire test field.

4. Rotate the slide manually or with a mechanical rotor at 80-100 rpm for 2 minutes and read immediately under direct light.

5. Presence of agglutination of the latex particle is a positive result

6. Agglutination indicates an RF concentration of equal to or more than 8 IU/ml.

INTERPRETATION

Negative Result: A negative reaction is indicated by a uniform milky suspension with no agglutination .

Positive Result: A positive reaction is indicated by any observable agglutination in the reaction mixture.

Annex IV Data collection format

ID/ ser#	Age	Gender	RF (P/N)	ESR (mm/hr	CRP (P/N	WBC ($x10^{9/}_{L}$	Abs Ne ($x10^{9}$)	Abs Lym ($x10^{3/}_{L}$	Abs PLT ($x10^{3/}_{L}$	PLR	NLR

Annex V. Eligibility criteria of RA Patients

1. ≥18 years of age

2. No anti-inflammatory therapy

A. Yes

B. No

3. History of diseases like:

- Cardia-cerebrovascular disease,

  A. Yes B. No

- Chronic myelogenous leukemia

  A. Yes B. No

- Thrombocytosis

  A. Yes B. No

- known chronic liver and kidney diseases

  A. Yes B. No

- Infectious diseases

  A. Yes B. No

- Malignant tumor diseases

  A. Yes B. No

- Hypertension and diabetes

  A. Yes B. No

- Metabolic syndrome

  A. Yes B. No

- Other autoimmune diseases (ankylosing spondylitis)

  A. Yes B. No

- Pregnant A. Yes B. No

Declaration

I, the undersigned, declare that this M.Sc. thesis is my original work, has not been presented for a degree in this or any other university and that all sources of materials used for the thesis have been duly acknowledged.

M.Sc. candidate: Senait Abate (B.Sc.)

Signature:_____

Date of submission:_____

This thesis has been submitted with our approval as advisors.

Advisor: Aster Tsegaye (MSc, PhD)

Signature: _____

Date: _____

Place: Addis Ababa, Ethiopia.

Advisor: Afework Hagos (MD, Internist, Hematologist)

Signature: _____

Date: _____

Place: Addis Ababa, Ethiopia.