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Predictive value of Platelet to Lymphocyte Ratio, Neutrophil to Lymphocyte Ratio and Mean Platelet Volume in esophageal cancer patients at Tikur Anbessa Specialized Hospital, Addis Ababa, Ethiopia.

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This is to certify that the thesis prepared by Zinashwork Abebe, entitled: ***Predictive value of Platelet to Lymphocyte Ratio, Neutrophil to Lymphocyte Ratio and Mean Platelet Volume in esophageal cancer patients at Tikur Anbessa 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) complies with the regulations of the University and meets the accepted standards with respect to originality and quality.

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Table of content

Contents

List of Tables i
List of Figures ii
Abstract v
1. Introduction 1
1.1. Background 1
1.2 Statement of the Problem 4
1.3 Significance of the Study 6
2. Literature review 7
3. Objectives 10
3.1 General objective 10
3.2 Specific objectives 10
4. Materials and Methods 11
4.1 Study area 11
4.2 Study design and Period 12
4.3 Population 12
4.3.1 Source population 12
4.3.2 Study population 12
4.4 Inclusion and Exclusion criteria 12
4.4.1 Inclusion criteria 12
4.4.2 Exclusion criteria 12
4.5 Study Variables 12
4.5.1 Dependent variables 12
4.5.2 Independent variables 13
4.6 Measurement and Data collection 13
4.6.1 Sample size determination 13
4.6.2 Sampling method 13
4.6.3 Data collection procedure 13
4.6.4 Principles of Laboratory analysis 14
4.7 Data Quality Assurance 16
4.8 Data analysis and interpretation 16

4.9 Ethical considerations 17
4.10 Dissemination of Results 17
4.11 Operational Definitions 17
5. Results 19
5.1 Sociodemographic and clinical characteristics of esophageal cancer patients 19
5.2 Comparison of NLR, PLR and MPV between cases and controls 21
5.3 Predictive values of NLR, PLR and MPV among esophageal cancer patients at TASH ... 22
6. Discussion 25
Strength of the study 27
Limitation of the study 27
7. Conclusion 28
8. Recommendation 28
9. References 29
Annexes 36
Annex I. Information sheet in English Version 36
Annex II. Informed consent form in Amharic version 39
Annex III. Informed consent form in English version 41
Annex IV Questionnaires 42
Annex V. Questionaries (Amharic Version) 44
Annex VI: Questionaries for Apparently healthy individuals 46
VII. Declaration 48

List of Tables

Table1. Sociodemographic characteristics of esophageal cancer patients at Tikur Anbessa Specialized Hospital, Addis Ababa, Ethiopia (n=112)……19

Table 2. Level and type of cancer among esophageal patients at Tikur Anbessa Specialized Hospital, Addis Ababa, Ethiopia ……20

Table 3. Comparison of mean and Std. Deviation of selected hematological parameters between esophageal patients and controls at Tikur Anbessa Specialized Hospital, Addis Ababa, Ethiopia …….22

Table 4. Sensitivity, Specificity, Positive and Negative predictive values of NLR, PLR and MPV as predictors of esophageal cancer at TASH, Addis Ababa, Ethiopia……24

List of Figures

Figure 1. Level and type of cancer among esophageal patients at Tikur Anbessa Specialized Hospital, Addis Ababa, Ethiopia ……..20

Figure 2. ROC curve analysis of NLR, PLR (A) and MPV (B) of esophageal cancer patients at Tikur Anbessa Specialized Hospital, Addis Ababa, Ethiopia……23

Abbreviations

AAU	Addis Ababa University
AC	Adinocarcinoma
AUC	Area under the curve
CBC	Complete blood count
CCRT	Concurrent chemoradiotheraphy
COP-NLR	Combination of platelet count and Neutrophil lymphocyte ratio
CRC	Colorectal cancer
CSS	Cancer specific survival
CTC	Circulating tumor cells
EC	Esophageal cancer
EDTA	Ethylene diamine tetraacetic
EGJ	Esophageal gastric junction
ESCC	Esophageal squamous cell carcinoma
ESD	Endoscopic submucosal dissection
GERD	Gastroesophageal reflux disease
GPS	Glasco prognostic score
mGPS	modified Glasgow prognostic score
MoH	Ministry of Health
MPV	Mean platelet volume
NCD	Non-communicable diseases
NLR	Neutrophil lymphocyte ratio
OS	Overall survival

PB	Peripheral blood
PFS	Progression free survival
PLR	Platelet lymphocyte ratio
PNI	Preneural invasion
RAS	Aphthous stomatitis
RCC	Renal carcinoma
ROC	Receiver operating characteristics
TM	Time to metastasis
TNM	Tumor nodes and metastases
TPD	Time to progressive disease
TRG	Tumor regression grade
VEGF	Vascular endothelial growth factor

Abstract

Background: Esophageal cancer ranked 7th globally in terms of incidences and also 6th in causing death. Inflammation is a major factor in the emergence and spread of malignancies. Though little investigated in our set up, parameters like the Neutrophil count and Neutrophil to Lymphocyte ratio (NLR) and Platelet to Lymphocyte ratio (PLR), and Mean Platelet Volume (MPV) were reported as predictive inflammatory markers of esophageal cancer.

Objective: The aim of this study is to investigate the predictive value of Platelet lymphocyte ratio (PLR), Neutrophil lymphocyte ratio (NLR) and Mean platelet volume (MPV) in esophageal cancer patients at Tikur Anbessa specialized hospital Addis Ababa Ethiopia starting May 2021 to August 2022

Methods: A comparative cross sectional study was collected amongst 112 esophageal cancer patients and 112 controls. Complete blood count (CBC) with differential count of each patient was determined before surgery using Beckman coulter DxH800 automated hematology analyzer. Sample collection and analysis were performed between May 2021 to August 2022 and at the same time the collected data from questionnaires and laboratory analysis were entered to SPSS version 25 for analysis. In order to calculate the Neutrophil-to-lymphocyte ratio (NLR), the total neutrophil count was divided by the total lymphocyte count and for Platelet-to-Lymphocyte ratio(PLR), the platelet count was divided by the absolute lymphocyte count. The ROC curve was used to estimate diagnostic performance of NLR, PLR, and MVP.

Results: Esophageal cancer patients had higher WBC (7.8 vs 7.4, p=0.0001), Absolute Neutrophil (5.62 vs 4.67, p=0.007), lesser MPV (8.53 vs 9.41, p=0.0001), lesser absolute lymphocyte (1.56 vs 2.05, p=0.008), NLR (4.12 vs 2.79, p=0.001), and PLR (19.56 vs 13.62; p=0.004) compared to healthy participants. High diagnosis effectiveness was demonstrated by ROC curve analysis for NLR, PLR and particularly MPV in esophageal cancer patients with threshold values of NLR ≥2.474 (Area under the curve=0.603, 95% CI, 0.529-0.677), with sensitivity of 63.4%, and Specificity of 50% , P=value 0.001), PLR ≥11.6376 (Area under the curve=0.545, 95% CI, 0.469-0.622), Sensitivity 56.3%, and Specificity 55.4%, P= 0.004) MPV≤9.05(Area under the curve=0.783,95% CI, 0.716-0.849), with Sensitivity of 83% and Specificity of 72.3%, P< 0.001.

Conclusion Mean platelet volume gave the highest sensitivity and specificity; together with NRL and PLR may be helpful signs for early finding and recognition of esophageal squamous cell carcinoma and adenocarcinoma.

Key words: Neutrophil-to-lymphocyte-ratio, platelet-to-lymphocyte ratio, mean platelet volume, esophageal cancer, predictive value

1. Introduction

1.1.Background

Cancer is a broad category of disorders that arises when irregular cells proliferate nonstop in virtually any organ or tissue of the body [1]. After atypical changes in the cell's genetic material, which are initiated by connections between environmental and genetic influences, cancer develops from a single cell. Including irregular cell development that has possibilities for invasion and distributed to several bodily regions, carcinogens can be physical, chemical, or biological in nature. Based on how they spread, they are divided into benign and malignant categories. Among the non-communicable diseases (NCDs), one of the most widespread in the globe and a primary reason for death globally is cancer. According to a study, cancer claims more than 7.9 million people globally [2].

Esophageal malignancy is one of the biggest causes of cancer related death worldwide. [2,3]. Esophageal cancer ranked **s**ixth in related to fatalities and seventh considering frequency. In 2018, esophageal cancer is thought to be the cause of 1 in every 20 cancer deaths [1] and the fifth most typical cancer-related cause of deaths in men [4]. Esophageal cancer is easily spread and has the potential to metastasize. Disorders with a propensity for early spread and an absence of symptoms result in the diagnosis of patients at a later stage, which limits available treatments and lowers cure rates [5].

Even with the usual therapy, non-metastatic cancer of the esophagus show an elevated rate of local recurrence [6]. Main reason why esophageal cancer is frequently discovered in its advanced stages is the absence of early clinical symptoms [7].

Esophageal cancer develops through the other layers of the esophagus wall after starting in the inner layer. Based on the cell types that begin it, esophageal cancer can be separated into two categories: adenocarcinoma (AC) and squamous cell carcinoma (ESCC). Adenocarcinoma, which begins in gland cells, is different from squamous cell carcinoma, which starts in the mucosa in the inner layer of the esophagus, which is typically covered in squamous cells [8].

The difficulty and absence of early symptoms of most esophageal cancer makes the diseases difficult to cure. Once it is grown and disseminated and get advanced its diagnosis and severity increased and survival rate will be decreased. Difficulty to swallow, heaviness loss which is unexplained, chest pain, heartburn and indigestion, and cough are some of the symptoms of esophageal cancer [7, 8].

In United States, screening tests have revealed to reduce risk factors for death from esophageal cancer in those who pose a typical risk. But those who are at a high risk of developing esophageal cancer, like those who have esophagus with Barrett's, are frequently monitored to look for early cancers and precancers [9].

Due to dysphagia unintended weight loss is common in patients with esophageal cancer and they may acquire chronic blood loss and that will lead them to iron deficiency anemia from low level of circulating red blood cells. Threat for esophageal cancer includes obesity, cigarettes, alcohol, gastreophageal reflux disease (GERD) [10].

Behavioral, biological, genetic and environmental risks factors are some of factors for the exposure of esophageal cancer. Unhealthy diet and physical inactivity has also a contribution to the disease occurrence in addition to age and sex that have their own role in this regard [11].

Esophageal cancer can be diagnosed through a physical examination, imaging tests, endoscopy, biopsy and blood tests. No professional group currently advises screening the general public for esophageal cancer, or the procedure of screening patients for cancer or pre-cancer while they do not yet have any symptoms of the illness. This is due to the fact that early examination has been demonstrated to reduce threat of cancer of the throat and death in those who have a medium risk. But those who are at a high risk of developing cancer of esophagus like those who have esophagus with Barrett's, are frequently monitored to search for early-stage cancer and precancers [9].

Selected hematological parameters and their derivatives are getting attention as additional simple and easily accessible diagnostic tool. Mean platelet volume (MPV), which is the most popular gauge of size of platelets varies in cancer patients [12, 13].

Although they are a crucial component of hemostasis, additionally, platelets have been connected to the growth of cancer. By releasing metallo proteases, they can aid cancer cell extravasations and encourage development and angiogenesis of the metastatic tumor. Growth factors that promote tumor growth and metastasis, such includes platelet-derived growth factors and angiogenic factors, and vascular endothelial growth factor (VEGF), are released as a result of this activity. Additionally, platelets can defend tumor cells still present after cytolysis by killer T cells. Cancer cells promote an increase in platelet count and activation by secreting platelet agonists and thrombopoietic cytokines [12–14].

By starting and enhancing inflammatory reactions, neutrophils play a crucial part in the first line of defense against infections. Neutrophils can relate with cancer cells in a manner similar to platelets, and they also produce cytokines and effector molecules like VEGF that promote tumor angiogenesis, growth, and metastasis[14].

In order to assess treatment and prognosis for various types of malignancies, the neutrophil to lymphocyte ratio (NLR) is also used as a marker of inflammation associated with cancer [15].

1.2 Statement of the Problem

A non-communicable disease is cancer (NCDs) that currently accounts for large proportion of global mortality, is anticipated to be the primary cause of mortality and present the greatest challenges to raising life expectancy in the twenty-first century [1]. The sixth-highest frequent cause of malignancy-related death and the eighth greatest prevalent cancer in the globe, is cancer of esophagus [6].

Cancer of esophagus (EC) was among the most typical and lethal types of malignancy around the globe in 2015, with 477,900 new cases and 375,000 fatalities. Adenocarcinoma (AC) and Squamous cell carcinoma (SCC) are the two most common pathologic subtypes. SCC the malignant lesion type of esophageal cancer is caused by abnormal proliferating esophageal squamous epithelium or glandular epithelium. Additionally, EC is the primary contributor to cancer-related deaths globally [16]. While AC is more prevalent in Western countries, SCC is the most common histological subtype in Asia [17].

It had a sixth-place death rate and a seventh-place incidence rate in particular in many of Southern and Eastern African nations in 2018. Surgery is considered to be an efficient EC therapy, but since the procedure on the body is more painful, patients must have a higher immune level to tolerate the trauma of surgery, and the prognosis is still very bad overall. The bad outlook and increasing prevalence of EC focus the necessity for better finding and forecasting techniques. In addition, genetic biomarker detection is costly and difficult, particularly for patients in developing nations like Ethiopia. As a result, it is urgent to improve reachable, affordable, and trustworthy markers for predicting esophageal cancer patients' that could enhance the customized care, treatment of EC patients [16].

The difficulty and absence of early symptoms of most esophageal cancer makes the diseases difficult to cure from the beginning. Once it grown and disseminated and get advanced it is diagnosed and severity increased and survival rate will be decreased [18].

The prognosis for esophageal cancer remains dismal despite improvements in diagnosis and therapy because patients frequently receive advanced stage diagnosis. It would be extremely clinically significant if there was a technique that could accurately forecast esophagus cancer prognosis [19].

Africa has high rates of EC incidence and mortality, as well as modifiable and preventable risk factors. Tobacco and alcohol consumption put men as a result of their greater prevalence, at increased risk [20].

Among top five countries of Africa who are scoring high death number due to esophageal cancer is Ethiopia next to South Africa, it scores 4090 deaths in 2015 [21]. Ethiopia a country found in the eastern part of Africa with a population of (123,105,762 million) which is among populations at risk of developing esophageal cancer. Each year an estimated 1622 men and women are diagnosed with esophageal cancer; 1506 die from esophageal cancer related disease [22].

In Ethiopia, cancer accounts for about 5.8% of nationwide mortality overall. Where population-based data are available for Addis Ababa, it is supposed that about 60,960 additional cancer cases are discovered in the nation each year, and over 44,000 people pass away from the disease. Under 75-year-olds have an 11.3 percent chance of developing cancer, and a 94% chance of passing away from it [11]. Late diagnosis is one of the major challenges which necessitate searching for affordable and easily accessible diagnostic tools. With availability of such tools patients at least can be referred to a tertiary care center before it is too late to treat them. Despite the suggestion of markers like NLR, PLR, and MPV as predictors and prognostic markers [6,12-14,17], no published study is available among esophageal cancer patients in Ethiopia investigating the potential of these parameters. This is an opening this finding is trying to discourse.

1.3 Significance of the Study

Health professionals, planners, policy makers, non-governmental organizations, and others involved in determining the predicting value as well as those working in the field of esophageal cancer will all benefit from the study's findings. The study's ability to correlate certain CBC parameters, like platelet to lymphocyte ratio (PLR), mean platelet volume (MPV), and neutrophil lymphocyte ratio (NLR) to EC and their predicting efficacy will alert health personnel before the patient become critically ill. Patients can get better management before it is too late with no additional cost from these calculated parameters of CBC results.

The findings will contribute as an input for esophageal cancer treatment guidelines and may also serve as an indication for upcoming studies.

2. Literature review

Detopoulou *et al.* (2023) showed a systematic assessment of 4258 patients with MPV-related esophageal squamous cell carcinoma. In two studies, MPV was found to be greater in cancer patients related to healthy participants, and a single study, MPV was found to be lower in cancer patients (pre-operatively). The prognostic role of MPV in cancer of esophagus yielded conflicting results because reduced mean platelet volume was associated with both improved survival, advanced cancer stages, and a poor prognosis. Compared to other cancer types, the data for esophageal cancer were less conclusive [23].

In their systematic review on solid tumors, a study done in Toronto, Templeton *et al.* (2014) studied the prognostic role of Neutrophil-to-Lymphocyte Ratio and discovered that the median cutoff for NLR was 4. Overall, NLR above the cutoff was linked to a 1.81 (95% CI = 1.67 to 1.97; P .001) hazard ratio for overall survival, which was present in all disease subgroups, sites, and stages. Cancer free survival (CSS), progression-free survival (PFS), and disease free survival (DFS) hazard ratios for NLR greater than the cutoff were calculated [24].

Esophageal cancer diagnosis is one of the biggest challenges in resource-constrained settings, and as a result, late presentation of patients is a clinical management bottleneck for those patients. The diagnostic value of readily available hematological parameters and their calculated derivatives is therefore being examined by a number of researchers. For instance, Zhou *et al.* (2021) a study done in china investigated the prognostic significance of usual blood examinations early esophageal cancer patients. In their matched case-control study, 329 healthy volunteers served as a comparison group, and 314 developing cancer of esophagus patients were enrolled since 2015 to 2019 to undergo Endoscopic submucosal dissection (ESD). About 219 sets were eventually signed up to relate the blood parameter levels after being matched by age (+/- 2 years old) and gender. The conclusions revealed that patients with early cancer of esophagus had considerably higher MPV (p=0.022), Prothrombin time (p=0.003), PT-INR (p=0.003), Platelet distribution width (p=0.001), and monocyte to lymphocyte ratio (MLR) (p0.001) than the control group [25].

In order to determine if the platelet-to-lymphocyte ratio (PLR) has a clinical effect in esophageal cancer patients receiving curative care, a study by Aoyama *et al.* from Japan was conducted in

2022. The main finding was that in patients of cancer of esophagus who underwent remedial action, the preoperative PLR did seem to have an influence on long-term oncological results. Among 168 patients, 78 (46.4%) fell into the PLR-low category, and 90 (53.6%) fell into the PLR-high category. [26].

An analysis of laboratory tests performed on people with progressive esophageal squamous cell carcinoma and control groups was done retrospectively by Sun *et al*. (2018) in China. Compared to preoperative levels, mean platelet volume (MPV) and mean platelet volume/platelet count ratio (MPR) were significantly lower in comparison to preoperative levels, mean platelet volume (MPV) and mean platelet volume/platelet count ratio (MPR) were meaningfully reduced in patients with locally advanced ESCC results opposed to healthy group (8.14±1.09 fL vs. 10.23±0.78 fL and 0.03875±0.02645 vs. 0.04463±0.00972, respectively. Regarding age and gender influence on long-term oncological results no statistically significant differences were noted across the groups [27].

As indicators of systemic inflammation, pretreatment levels of derivative values of hematological parameters like neutrophil-to-lymphocyte (NLR) and platelet-to-lymphocyte (PLR) ratios are suggested. For instance, Wang *et al.* (2022) in their retrospective study in China studied 283 patients with gastric cancer to examine the relationships among NLR, PLR, and clinicopathological features. According to the designed cutoff points, pretreatment levels of derivative values of hematological parameters like neutrophil-to-lymphocyte (NLR) and platelet-to-lymphocyte (PLR) ratios are suggested as results indicators of widespread inflammation.

The ROC curve's results carefully considered cutoff points defined as increased NLR as 2.38 and increased PLR as 188.1. A total of 196 patients (69.3%) qualified as reduced PLR patients, 183 (64.7%) as increased PLR patients, 100 (35.3%) as low NLR patients, and 87 (30.7%) as high NLR patients. NLR demonstrated a strong correlation with patient tumor regression grade (TRG) among all factors ($p = 0.015$). PLR was connected to TRG and tumor size ($p = 0.05$ and $p = 0.001$, respectively). The greater, Neutrophil lymphocyte ratio and platelet lymphocyte ratio demonstrated that the worse the pathological reaction. Whether in a univariate (OR = 3.457; $p = 0.044$) or multivariate (OR = 6.876; $p = 0.028$) analysis, TRG grade was found to be much poorer in patients who had high NLR and PLR scores. In multivariate investigation, the grade of cancer difference was an independent predictor of TRG (OR = 2.874; $p = 0.037$). In the subgroup

analyses, NLR was related with both results overall survival (OS) and disease-free survival (DFS)) in female patients ($p = 0.04$), while PLR was related by both OS ($p = 0.026$) [28].

NLR with cut-offs of ≥3 and ≥5 predicted progressive disease and worse progression-free survival in a multicenter study of individuals with urothelial carcinoma who received immunotherapy. When the NLR cut-off was ≥5, overall survival was predicted ($p = 0.03$) [29].

Masternak *et al* 2019, from Poland studied MPV levels significantly affect the interval of progression-free survival (PFS) and overall survival (OS) in various different types of solid tumors, and they also have an impact on the prognosis in some lymphoproliferative disorders. This study evaluated the prognostic significance of MPV in people with cancer. Patients with stomach cancer have been found to have elevated MPV levels. The study found that preoperative patients had significantly higher MPV levels than did healthy individuals (patients 8.31 vs. controls 7.85 fL; p: 0.007) [30].

A conclusion given by Shen M *et al* 2014 in their meta- analysis discussed that high level of within a tumor neutrophils are significantly related with in human cancers with negative survival and recurrence [31].

In sum, although there is scarcity of literatures that discuss specifically about predictive value of the markers discussed above in esophageal cancer, the available literatures on esophageal cancer as reviewed above and the body of evidence in other forms of cancer included in this review showed the diagnostic and prognostic significance of simple hematological parameters and their derived ratios as potential markers. This finding is trying to offer the first proof on the predictive potential of NLR, PLR and MPV in esophageal cancer patients.

3. Objectives

3.1 General objective

To investigate the predictive value of Platelet lymphocyte ratio (PLR), Neutrophil lymphocyte ratio (NLR) and Mean platelet volume (MPV) in esophageal cancer patients at Tikur Anbessa specialized hospital Addis Ababa Ethiopia starting May 2021 to August 2022.

3.2 Specific objectives

- To examine the predictive potential of ratios of PLR to NLR in esophageal cancer patients.
- To investigate the predictive potential of MPV in esophageal cancer patients.
- To compare the values of MPV, NLR and PLR between esophageal patients and controls.

4. Materials and Methods

4.1 Study area

The study was carried out at Tikur Anbessa Specialized Hospital (TASH), the biggest general public hospital and teaching hospital in Ethiopia. The Ministry of Health (MoH) gave TASH, also known as Black Lion Hospital and the largest referral hospital in the nation, to Addis Ababa University (AAU) in 1998 so that it could serve as the faculty's primary teaching hospital. The hospital offers tertiary level referral care and is available round-the-clock for emergency services by its most senior specialists with its capacity of about 700 beds The hospital, which is run by Addis Ababa University, The hospital has 929 academic, 825 nurses ,55 medical laboratory, 74 pharmacies, 69 midwife, 39 anesthesia, 14 physiotherapy, 37 radiology, 15 biomedical, 6 environmental health, and 15 administrative staff dedicated to providing health care services. The hospital also has about 950 permanent and contract administrative staff to support the hospital activities.

4.2 Study design and Period

A comparative cross sectional study were done starting May 2021 to August 2022.

4.3 Population

4.3.1 Source population

All esophageal cancer patients who are attending at Tikur Anbessa Specialized Hospital (TASH)

4.3.2 Study population

Esophageal cancer patients who fulfill the eligibility criteria and who are at Tikur Anbessa specialized hospital, Addis Ababa, Ethiopia between May 2021 to August 2022 were prospectively recruited.

4.4 Inclusion and Exclusion criteria

4.4.1 Inclusion criteria

Patients meeting all of the following requirements were eligible for enrollment

- Patients whose pathological stages were determined according to the international tumor nodes and metastases (TNM) classification system for esophageal cancer.
- Patients who did not undergo surgery and histologically confirmed of esophageal cancer.
- Patents untreated earlier diagnosis.

4.4.2 Exclusion criteria

A person with medical treatment, hematological disorders, autoimmune illnesses, systemic inflammatory diseases and other cancers.

4.5 Study Variables

4.5.1 Dependent variables

Neutrophil to lymphocyte ratio, platelet lymphocyte ratio, mean platelet volume as predicting value towards esophageal cancer.

4.5.2 Independent variables

Socio demographic profiles such as; age, sex, region, stage of cancer.

4.6 Measurement and Data collection

4.6.1 Sample size determination

$$n = \frac{(z\alpha/2)^2 * p(1-p)}{d^2}$$

$$n = \frac{(1.96)^2 \times 0.91 \times 0.09}{0.5^2} = 126$$

126 case + 126 controls = 252

By considering 10% contingency the final sample size will be 277

P is taken from a study conducted at Tikure Anbessa specialized hospital in which the prevalence of esophageal cancer in male patients was 9.1% whereas the prevalence in female patients was 5.6% [32].

Where **Z** is the value from the standard normal distribution reflecting the confidence level that will be used (e.g., **Z** = 1.96 for 95%), σ is the standard deviation of the outcome variable

P is the estimated proportion of the population

D is the margin of error

4.6.2 Sampling method

Medical history of 112 patients' consecutive participants who did not underwent partial or complete esophactomy at Tikur Anbesa Specialized Hospital, and 112 apparently healthy volunteers.

4.6.3 Data collection procedure

Important variables like sociodemographic characteristics of the patients' clinical characteristics (such as age, sex, marital status, level of education, religion, place of residence, and occupation) were gathered using a structured data extraction tool (such as date of diagnosis, when it

expanded, stage and histological type of cancer, type of treatment provided, current status and last date of follow up).

For CBC analysis blood is drawn from a vein, usually from the inside of the elbow or the back of the hand, after being sure the participants seated comfortably, the skin where cleaned with 70% alcohol before sample collection and by syringe method 2 to 3ml of venous blood were drawn and dispense to EDTA tubes and mix it well and label it as well.

4.6.4 Principles of Laboratory analysis

CBC Analysis

The Coulter method, which accurately counts and measures the sizes of cells by detecting and measuring changes in electrical resistance when a particle, such as a cell, in a conductive liquid passes through a small aperture, was used to perform the CBC analysis. Each cell serves as an insulator when it is suspended in a conductive liquid (diluents).

Every cell will temporarily increase the resistance of the electrical path between the submerged electrodes on either side of the aperture as it passes through a detectable electronic pulse results from this. For research, a controlled volume of vacuum should be used to push the diluted suspension of cells through the aperture. The number of particles is inversely proportional to the number of pulses. The electrical pulse's peak rises in direct proportion to volume.

These three measurements are made simultaneously on each individual white cell to classify it using differential analysis as the sample that has been prepared for differential analysis flows through the flow cell:

Volume is measured by low-frequency current.

The surface, shape, and reflectivity of the individual WBC cells are identified by the laser light that reflects off them.

High-frequency current detects changes in physical phenomena or conductivity to determine cellular internal content.

White Blood Cell Count is the direct measurement of leukocytes, multiplied by the calibration constant, and expressed as n x 10^9 cells/L with a normal value of 35 fL.

Count of Platelets: this is the number of thrombocytes calculated by multiplying the Platelet Histogram by a calibration factor. The expression for this quantity is: n x 10^9 cells/L, normal range, 2 to 20 fL.

The average volume of a platelet is known as the mean platelet volume (MPV), which is derived from the platelet histogram. It is expressed in femtoliters and represents the mean volume of the platelet population under the fitted platelet curve multiplied by a calibration constant. Mean platelet population volume under the x constant-fitted curve.

The WBC count and the differential share parameters are used to compute the numbers of leukocytes in each class that make up neutrophils count of NE%/100xWBC. Leukocyte numbers in each class are calculated using the WBC count as well as the differential share parameters, and the lymphocyte range is the sum of those numbers. Number Ly%/100xWBC in the absolute sense [33].

A subpopulation of young, metabolically and enzymatically more active platelets taking part in homeostasis is indicated by increased MPV. Two illustrative measures of systemic inflammation are NLR and PLR [34].

While lymphocytosis or neutropenia causes low NLR, neutrophilia or lymphopenia causes high NLR. High NLR suggests that inflammatory factors predominate in the pathogenesis of conditions and may identify patient subgroups that will benefit from anti-inflammatory medication [35].

As a result of systemic inflammation, the neutrophil-lymphocyte ratio (NLR), which is easily calculated by dividing the absolute neutrophil count by the absolute lymphocyte count from a complete blood count with differential, rises.

An average NLR is about 1-3. A NLR of 6 to 9 denotes light stress (e.g. a patient with uncomplicated appendicitis). Patients in critical condition frequently have an NLR of 9 or higher (occasionally reaching values close to 100).

A novel inflammatory marker called the platelet-lymphocyte ratio (PLR) may be used to predict inflammation and mortality in many diseases. The PLR is easily calculable and widely accessible, but it can be impacted by a number of inflammatory diseases. The 95%

reference range of NLR in normal male and female are 0.43~2.75 and 0.37~2.87, **PLR** are 36.63~149.13 and 43.36~172.68, respectively [35].

By dividing the platelet count by the lymphocytes, PLR is calculated. Thrombosis, atherogenesis, and inflammation are all significantly impacted by platelets.

4.7 Data Quality Assurance

Pre analytically the data quality were kept through pre-test for the questionnaires. After the questionnaires were filled it was checked all information was included or completely answered. The right tube (EDTA) was used for collection of sample) and analysis was done within two hours of specimen collection at the TASH main laboratory.

To keep the quality of analytical part three levels known commercial controls from Beckman Coulter with low, normal and high results were run. The results were within acceptable ranges of the Beckman Coulter for low and high controls.

Post analytically results were interpreted against the company provided values and quality of the data were checked through cleaning and double entry.

4.8 Data analysis and interpretation

The information collected were cleaned, implied and registered to computer consuming version 25 SPSS for software analysis. To compare patients and controls independent t test were used, ROC curve analysis was used to measure the predictive assessment of the intended parameters and to calculate sensitivity, specificity, positive and negative predictive values. KS test (kolmogrov-simirnov) were used to determine normality of variables. The result was expressed in percentage and frequency and presented in tables and graphs [36]. Less than 0.05 P values were considered as statistically significant.

4.9 Ethical considerations

To carry out the study ethical clearance was obtained from Addis Ababa University (AAU) College of Health Sciences; Department of Medical Laboratory Sciences research ethics review committee on 02/02/2021 DRERC/606/21/MLS. Letter of approval to collect the study was found from Tikur Anbessa specialized hospital Administration office. Participants were enrolled after obtaining informed consent granting them their right to drop from the study any time. CBC results were immediately delivered to their attending physicians. Personal identifying was not used, only code numbers were utilized.

4.10 Dissemination of Results

The thesis will be submitted to the Department of Medical Laboratory Sciences, defended in public and copies will be kept at the library for future reference. The result will also be disseminated to the appropriate bodies. The findings will be published in local or international peer reviewed journals.

4.11 Operational Definitions

Predicting value: the probability that a positive test result means a disease exists or that a negative test result rules out diseases.

Negative Predictive Value: the probability that a person does not have a disease or condition, given a negative test result, is known as the NPV. It is the quantity of true negatives divided by the total of true negatives and false negatives in a population.

Positive Predictive Value: The percentage of cases with positive test results that are already patients is known as the positive predictive value. It is the proportion of patients with accurate diagnoses to everyone else with a positive test result, including healthy individuals who were misdiagnosed as patients [37].

The neutrophil-to-lymphocyte ratio (NLR): calculated as a simple ratio between the neutrophil and lymphocyte counts measured in peripheral blood, is a biomarker which conjugates two faces of the immune system: the innate immune response, mainly due to neutrophils, and adaptive immunity, supported by lymphocytes [38].

Platelet lymphocyte ratio (PLR): is a ratio between the absolute platelet count and the absolute lymphocyte count [39].

Mean platelet volume (MPV): is a blood test measures the average size of platelets in a blood, the platelets that help our blood clot. When considered alongside other test results on a complete blood count [40].

Stages of cancer: Stage 0– the cancer is where it started and hasn't spread

Stage 1 – the cancer is small and hasn't spread anywhere else.

Stage 2 – the cancer has grown, but hasn't spread.

Stage 3 – the cancer is larger and may have spread to the surrounding tissues and/or the lymph nodes (or "glands", part of the immune system)

Stage 4– the cancer has spread from where it started to at least 1 other body organ, also known as ″ secondary″ or ″metastatic″ cancer [41].

5. Results

5.1 Sociodemographic and clinical characteristics of esophageal cancer patients

A total of 112 adult esophageal cancer patients participated in the study. Female were the predominant patients 72(63.3%) and the most of patients were in the age group 50-60 years, 41(36.6%) (Table1). The mean age of patients was 51.9 year. When characterized by esophageal cancer type, 81(72.3%) were esophageal squamous cell carcinoma (ESCC) patients while the rest 31(27.7%) were adenocarcinoma (AC) patients (Figure 1) 42(37.5%) secondary level of cancer patients (Table2). Most of the participants came from Oromia region 44.6%.

Table1. Sociodemographic characteristics of esophageal cancer patients at Tikur Anbessa Specialized Hospital, Addis Ababa, Ethiopia (n=112) and control (n=112)

Variables	Category	Cases		Controls	
Sex	Male	**Frequency**	**%**	**Frequency**	**%**
		40	35.7	54	48.2
	Female	72	64.3	58	51.78
Age (Years)	17-27	4	3.6	2	1.78
	28-38	13	11.6	2	1.78
	39-49	29	25.9	50	44.64
	50-60	41	36.6	55	49.1
	>60	25	22.3	3	2.67
Educational status	Cannot read and write	80	71.4	1	0.89
	Primary	20	17.9	34	30.35
	Secondary	5	4.5	33	29.46
	Diploma	3	2.7	13	11.6
	Degree	4	3.6	25	22.3
	Above 1st degree	0	0	6	5.35
Residence	Oromia	50	44.6	17	15.17
	SNNPR	30	26.8	11	9.82
	Amhara	13	11.6	4	3.57
	Addis Ababa	12	10.7	76	67.85
	Harar	5	4.5	1	0.89
	Somali	2	1.8	4	3.57

Table 2. Level and type of cancer among esophageal patients at Tikur Anbessa Specialized Hospital, Addis Ababa, Ethiopia

Type of esophageal cancer	Level of esophageal cancer				Total
	Primary	Secondary	Tertiary	Quaternary	
Esophageal squamous cell carcinoma	16	27	22	16	81
Adino carcinoma	5	15	6	5	31
Total	21	42	28	21	112

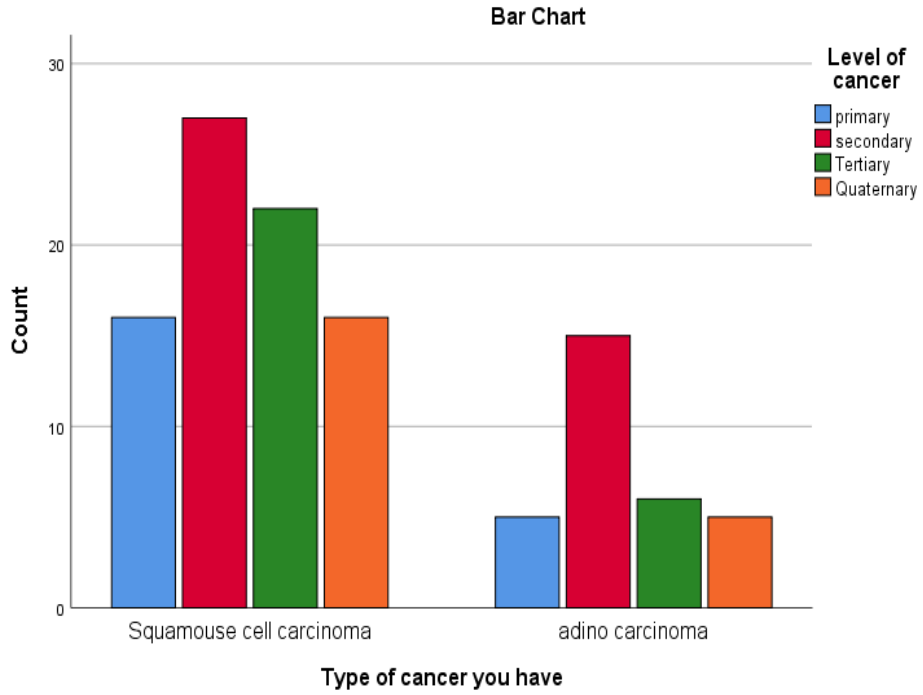


Figure 1. Level and type of cancer among esophageal patients at Tikur Anbessa Specialized Hospital, Addis Ababa, Ethiopia

5.2 Comparison of NLR, PLR and MPV between cases and controls

Comparison of the inflammatory markers NLR, PLR and MPV as well as WBC, absolute neutrophil count, platelet count, absolute lymphocyte count was made between cases and control as displayed in Table 2. Esophageal cancer patients had significantly higher WBC (case 7.8 vs control 7.4, p=0.0001), Absolute Neutrophil (case 5.62 vs control 4.67, p=0.007), lower MPV (case 8.53 vs control 9.41, p=0.0001), lower absolute lymphocyte (case 1.56 vs control 2.05, p=0.008), higher neutrophil to lymphocyte ratio (NLR) (case 4.12 vs control 2.79, p=0.001), and platelet to lymphocyte ratio (PLR) (case 19.56 vs control 13.62; p=0.004) related to healthy controls (Table 2).

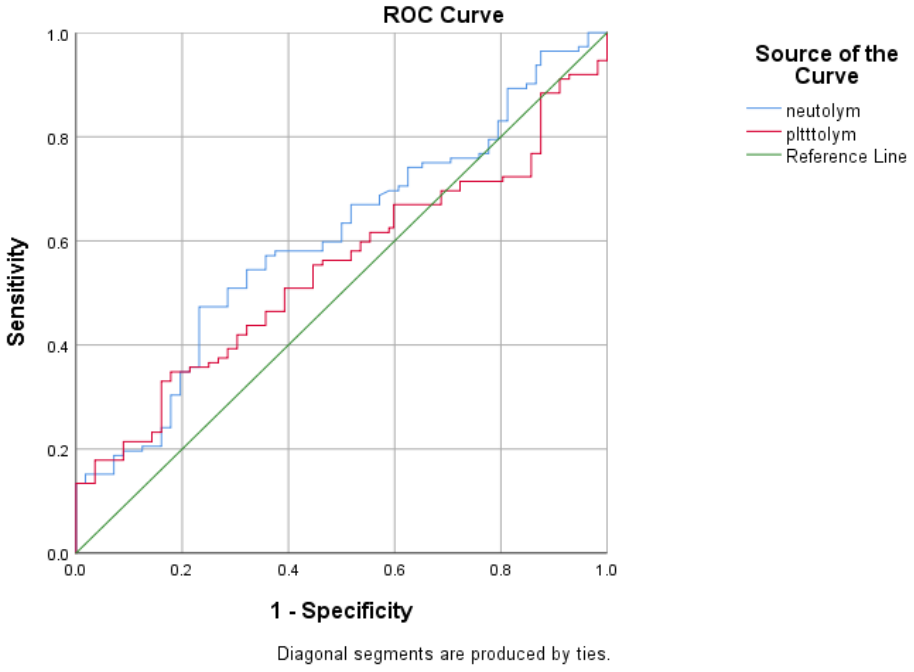
Table 3. Comparison of mean and Std. Deviation of selected hematological parameters between esophageal patients and controls at Tikur Anbessa Specialized Hospital, Addis Ababa, Ethiopia

Variables	Mean		Std. Deviation		P value
	Patients	Control	Patients	Control	
White blood cell (x 10^9/L)	7.8	7.4	4.1	1.9	**0.0001**
Absolute neutrophil (x 10^9/L)	5.62	4.67	4.11	1.69	**0.007**
Platelet (x 10^9/L)	308.5	317.1	115.6	54.7	0.511
Absolute Lymphocyte (x 10^9/L)	1.56	2.05	0.60	0.97	**0.008**
Mean Platelet Volume (fl)	8.53	9.41	1.22	0.44	**0.0001**
Neutrophil to Lymphocyte Ratio	4.12	2.79	3.95	1.60	**0.001**
Platelet to Lymphocyte Ratio	19.56	13.62	20.65	6.93	**0.004**

5.3 Predictive values of NLR, PLR and MPV among esophageal cancer patients at TASH

ROC curve analysis of specificity and sensitivity with the area under the curve (AUC) showing the probability that patient with esophageal cancer has a higher value of the measurements platelet lymphocyte ratio (PLR), neutrophil lymphocyte ratio (NLR) and mean platelet volume (MPV) is shown below in Figure 3 and Table 3. AUC for MPV was 0.783 (0.716-0.849) with cutoff value of MPV≤ 9.05 and 83% sensitivity, 72.3% specificity, 81% PPV and 75% NPV. The AUC for Neutrophil to lymphocyte ratio (NLR) was 0.603 (0.529-0.677) when NLR was ≥2.474 with 63.4% sensitivity, 50% specificity, 57.7% PPV and 55.9% NPV. The Neutrophil to Lymphocyte ratio AUC was 0.603 (0.529-0.677) at a cutoff value of NLR ≥2.474 with 63.4% sensitivity, 50% specificity, 57.7% PPV and 55.9% NPV.

A. ROC NLR and PLR



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B. **ROC MPV**

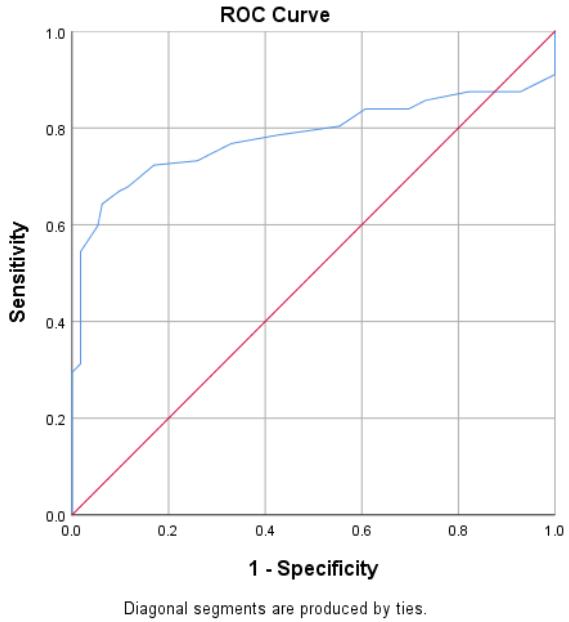

Figure 2. ROC curve analysis of NLR, PLR (A) and MPV (B) of esophageal cancer patients at Tikur Anbessa Specialized Hospital, Addis Ababa, Ethiopia.

Table 4. Sensitivity, Specificity, Positive and Negative predictive values of NLR, PLR and MPV as predictors of esophageal cancer at TASH, Addis Ababa, Ethiopia

	Sensitivity	**Specificity**	**PPV**	**NPV**
Platelet to Lymphocyte Ratio (PLR)	56.3%	55.4%	55.0%	54.8%
Neutrophil to Lymphocyte Ratio (NLR)	63.4%	50%	57.7%	55.9%
Mean Platelet Volume (MPV)	83.0%	72.3%	81.0%	75.0%

6. Discussion

Esophageal cancer is one of the cancer types which ranked the 7th cancer globally in terms of incidences and also ranked 6th in causing death throughout the world [42]. Although no concrete data is available regarding the burden of esophageal cancer in Ethiopia, Oromia region has been reported as a high burden area in the country [43] which is also seen in the current study where the most of cancer patients were from Oromia (44.6%). Cancer diagnosis and management in resource limited settings is challenging and patients usually present at late stages. As a result, there is a high need to look for additional simple parameters which can be used as forecasters of cancer. This study expected to investigate the predictive potential of Neutrophil-to-Lymphocyte ratio, Platelet-to-Lymphocyte ratio and Mean Platelet Volume esophageal cancer patients at Tikur Anbessa Specialized Hospital. The findings showed esophageal cancer patients had significantly higher WBC, Absolute Neutrophil count, Neutrophil to Lymphocyte ratio, Platelet to Lymphocyte ratio related to healthy participants. On the other hand, patients had significantly lower MPV and total lymphocyte counts compared to controls.

The findings of higher WBC, Neutrophil count and NLR can be explained by the potential of cancer cells in generating granulocyte colony-stimulating factor, tumor necrosis factor-alpha, interleukin-1, and interleukin-6, which can increase WBC and neutrophil counts as stated by Nowarski *et al* 2013 [44].

The finding of significantly lower mean platelet volume (MPV) of 8.5±1.2 fL among esophageal cancer patients in patients compared to healthy controls (MPV 9.4 ±0.4 fL) was consistent with reports by Sun *et al* 2018 [27]. In their retrospective study Sun *et al* found MPV of case 8.14 ± 1.09 fL vs. control 10.23 ± 0.78 fL, $P < 0.0001$, respectively, in esophageal cancer patients compared to controls [27].

AUC for MPV in the current study was 0.783 (0.716-0.849) with sensitivity of 83% and specificity of 72.3%when MPV cut off value ≤ 9.05 with positive disease prediction. Evidences so far regarding the predictive potential of MPV in esophageal cancer are contradicting. Our finding of low MPV predicting esophageal cancer is consistent with studies from China where low MPV value is revealed to be related with reduced outcome of esophageal cancer [27, 45].

Unlike our finding others in the contrary have documented increased MPV in esophageal cancer patients [46, 47] as well as low MPV with higher survival [48].

Lower MPV value has also been reported in other forms of cancer like lung cancer to be associated with poor survival indicating the usefulness of this easily available marker in cancer management [49], although no association [50], contradicting association [51] have also been reported suggesting the generation of more evidences under different contexts.

In the current study, Platelet to lymphocyte ratio (PLR) AUC was 0.545 (0.469-0.622). With 56.3% sensitivity, and specificity 55.4% the PLR cutoff ≥11.63 predicts disease occurrences. In this study mean of patients PLR was 19.5± 20.65 vs 13.6±6.93 in the control group. The role of platelet-to-lymphocyte ratio (PLR), and neutrophil-to-lymphocyte ratio (NLR) in predicting poor prognosis in esophageal cancer patients has been reported by Wu *et al* 2019 [52].

The Neutrophil to lymphocyte ratio in the current study showed AUC of 0.603 (0.529-0.677) and 63.4% sensitivity and 50% specificity with NLR cutoff ≥2.47 predicting disease occurrences. In this study mean of patients NLR was 4.1± 3.9 vs 2.7± 1.60. This finding reflects NLR can be a potential parameter in monitoring responses to chemotherapy as also evidenced by a study from China. This was shown by Zhu *et al* 2016 by studying 114 esophageal cancer patients and demonstrated that low NLR value as predictor of high response to chemotherapy [53]. In their search for simple inflammatory biomarker Liu *et al* 2015 reported a consistent areas under curve (AUC) for NLR as our study which was 0.680 (P<0.001) while their PLR AUC was higher than the current study which was 0.701 (P<0.001) [54].

Strength of the study

The study showed Platelet lymphocyte ratio, Neutrophil lymphocyte ratio, and Mean platelet volume have the predictive value of esophageal patients and this research topic predictive value is the first to be done in this area.

Limitation of the study

There is scarcity of literatures that discuss specifically about predictive value of the markers discussed above in esophageal cancer,

7. Conclusion

In conclusion these parameters in particular NLR, PLR and MPV can predict early esophageal cancer patients from delayed diagnosis and spread of the illness. Cutoff values of NLR and PLR need to be established on the given laboratory set up in order to reduce the risk of severity in esophageal cancer. This research backs up the idea that the development of EC cancer is significantly influenced by inflammation, given that routine hematology analysis is currently a component of a standard blood test analysis. Daily independent screening using NLR, PLR, and MPV is possible. Since these parameters can easily be calculated from the CBC routine result print out.

8. Recommendation

The three parameters can be used as markers in the prediction of esophageal cancer patients where capacity for diagnosing it is limiting. At least by looking into these derived values, physicians could consider referring their patients where better diagnostic capacity exists. If more large scale studies regarding inflammatory markers could be done in the area of cancer related diseases, it could help to minimize cost, level of severity, and patients suffering from the disease outcomes.

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Annexes

Annex I. Information sheet in English Version

Title of the Research Project: Predicting value of PLR, NLR and MPV in esophageal cancer patients at Tikur Anbessa specialized hospital Addis Ababa Ethiopia.

Principal Investigator: Zinashwork Abebe (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. 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 see either neutrophil lymphocyte ratio, platelet lymphocyte ratio and mean platelet volume helps as predicting value of esophageal cancer.

Purpose of the research:

These days victims of esophageal cancer individuals are increasing in Ethiopia, so you have been chosen for this study. Therefore, we invite you to take part in this study and have your own contribution.

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,

a sample will be taken from venous puncture. Moreover, there will be a face-to-face interview for additional questions.

Procedures: After agreeing that you can take part, one or more of our research staff will ask you some questions.. We will also collect 5ml of venous blood from you by sterile-disposable syringe in tube containing EDTA. We will conduct laboratory examination to determine different hematological parameters.

Potential risks and Discomforts

There will be minimal 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 confidentially.

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, the result of the study will be beneficial for the detection and managing of esophageal cancer 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 blood 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:**	**E-mail:**
1.Aster Tsegaye (Pr.)	+251 911 696085	tsegayeaster@yahoo.com
2.Zinashwork Abebe	+251 912273429	zinashab2016@gmail.com

Annex II. Informed consent form in Amharic version

የተሳታፊዎች ፈቃድና መተማመኛ ቅፅ

በአዲስ አበባ ዩኒቨርሲቲ ጤና ሳይንስ ኮሌጅ የሕክምና ላቦራቶሪ ሳይንስ ት/ክፍል በማስተርስ ድግሪ ተማሪ የመመረቂያ ጥናት ላይ እዲሳተፉ ተጋብዘዋል።እባክዎ በዚህ ጥናት ለመሳተፍ ከመስማማትዎ በፊት ከዚህ ቀጥሎ የሚገኘዉን ምንባብ በጥሞና ያንብቡና ግልጽ ያልሆነልዎትን ማንኛዉም ሃሳብ ይጠይቁ።

መግቢያ

የጥናቱ ርዕስ Predicting value of PLR, NLR and MPV in esophageal cancer patients at Tikur Anbessa specialized hospital Addis Ababa Ethiopia.

የእርስዎ በዚህ ጥናት ላይ የሚኖርዎት ተሳትፎ ሙሉ በሙሉ በበጎ ፈቃደኝነት ላይ የተመሰረተ ነዉ። በዚህ ጥናት ዉስጥ ላለመሳተፍ ወይም ለመሳተፍ ከወሰኑ በኃላ ለማቋረጥ የሚወስኑ ቢሆንም እንኩዋ በዚህ ሆስፒታል የሚሰጠዉ ማንኛዉም አገልግሎት አይቋረጥም።በጥናቱ ለመሳተፍ የሚስማሙ ከሆነ የስምምነት ቅጹ ላይ በጹሁፍ ወይም በጣት ፊርማ ማስቀመጥ ይጠበቅዎታል።

የጥናቱ ተሳታፊ ለመሆን የሚጠበቅበዎት ምንድን ነው?

በዚህ ጥናት ለመሳተፍ የሚስማሙ ከሆነ ናሙናዎ ለጥናቱ እንዲሚዉል መስማማት ይጠበቅብዎታል።ከተወሰደዉ ናሙና ላይ የሚገኙ መረጃዎች ከዚህ ሆስፒታል ዉጭ ለሚገኙና ለስራዉ አግባብነት ላላቸዉ ሰዎች ቢነገር የማይቃወሙ መሆኑን መስማማት ይጠበቅብዎታል።ይሁን እንጅ ይህ አይነቱ መረጃ የርስዎን ማንነት የሚገልጡ መረጃዎችን ማለትም ስም፤አድራሻና የስልክ ቁጥር የመሳሰሉትን መረጃዎቸን አይጨምርም። ይልቁንም ለዚህ አገልግሎት ብቻ የሚዉል እርስዎን ለማወቅ የሚያስችል መለያ ቁጥር ጥቅም ላይ እንዲዉል ይደረጋል።በተጨማሪም ስለርስዎ አጠቃላይ የጤና ሁኔታ ለሚቀርቡ አንዳንድ ተጨማሪ ጥያቄዎች መልስ መስጠት ይኖርብዎታል።

በዚህ ጥናት መሳተፍ የሚያስከትላቸዉ ችግሮች ምንድን ናቸዉ?

ናሙና በሚሰበሰብበት ወቅት ምንም አይነት የከፋ ችግር አያጋጥምዎትም። ሆኖም ግን ናሙናዉን ለመሰብሰብ ልምድ ያለዉ ባለሙያ ስለሚመደብና አስፈላጊዉ የጥንቃቄ እርምጃ ስለሚወሰድ የህመም ስሜት አይኖርም።

የህክምና መረጃ በሚስጥር ተጠብቆ መቆየት የሚችለዉ እንዴት ነዉ?

ስለራስዎ የሰጡት ማንኛዉም መረጃና ከተወሰደዉ ናሙና ላይ የተገኘዉ የላቦራቶሪ ዉጤት የሚዉለዉ ለጥናቱ አላማ ብቻ ነዉ። ይህን ማህደር ሊያገኙ የሚችሉት የተወሰኑ የጥናቱ ተባባሪ ሰዎች ብቻ ናቸዉ።ከዚያም በላይ ስለ

እርስዎ ያለውን ማንኛውንም መረጃ የተለየ የይለፍ ቃል ባለው የኮምፒውተር የመረጃ ማህደር ውስጥ እንዲቀመጥ ይደረጋል ።

በዚህ ጥናት መሳተፍ የሚያስገኛቸው ጥቅሞች ምንድን ናቸው ?

ይህ ጥናት የማስተርስ ዲግሪ መመረቂያ እንደመሆኑ መጠን በዚህ ጥናት በመካፈልዎ በገንዘብ የሚያገኙት ጥቅም ባይኖርም ከጥናቱ በሚገኝው ውጤት ግን ተጠቃሚ ነዎት። የእርሶዎ ተሳትፎ የእርስዎንና የወገንዎትን የደም ካንሰር ለማወቅና ለማከታተል ከፍተኛ ጥቅም ይኖረዋል።

በዚህ ጥናት ተሳታፊ የመሆንዎ መብቶች ምንድን ናቸው ?

በዚህ ጥናት መሳተፍ ሙሉ በሙሉ በእርስዎ ፈቃደኝነት የተመሰረተ በመሆኑ በማንኛውም ሰዓትና ቦታ የማቋረጥ ሙሉ መብት የተጠበቀ ከመሆኑም በላይ እራስዎን ከጥናቱ በማግለልዎ ምክንያት የሚቀርብዎት ምንም አይነት የሆስፒታል አገልግሎት አይኖርም ።ከዚህም በተጨማሪ ጥናቱን በተመለከተ ማንኛውንም አይነት ጥያቄ የመጠየቅና ገለጻ የማግኘት መብት አለብዎት።የላብራቶሪ ምርመራ ውጤቱንም በነጻ ማግኘት ይችላሉ። ነገር ግን እርስዎ በሚሰጡን መረጃ የቸግሩን ስፋት ለመከላከል እና ለመቆጣጠር ጠቃሚ ስለሆነ ለሚቀርብልዎት ጥያቄ ቀጥተኛ መልስ ይሰጡን ዘንድ በታላቅ አክብሮት እንጠይቃለን።

ጥያቄ ካለኝ ወይም ችግር ቢያጋጥመኝ ምን ማድረግ ይገባል?

ይህንን ጥናት በተመለከተ ወይም ከዚህ ጥናት ጋር በተዛመደ መልኩ ስለሚያጋጥሙ ድንገተኛ አደጋዎች ወይም ጥያቄ ካለዎት በሚመለከተው አድራሻ ይጠቀሙ።

ስም	**ሞባይል፡**	**ኢሜል፡**
1. **ፕ/ር አስቴር ፀጋዬ**	+251 911 696085	tsegayeaster@yahoo.com
2. . **ዝናሽወርቅ አበበ**	+251 912273429	zinashab2016@gmail.com

Annex III. Informed consent form in English version

Card no…..……

I had been informed that the objective of this study is to evaluate Predicting value of PLR, NLR and MPV in esophageal cancer patients. 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 of esophageal cancer in Ethiopia. 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 sample, 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 Questionnaires

1. Card number _____ Age: _____ Sex ; Male ____, Female ____

   Body weight _____ Kg? Height ____ cm

2. Address: _____ Rural _____Urban _____ Addis Ababa _____ Ward _____

3. Level of Educational? Uneducated _____Elementary _____high school _____ Diploma_____ Degree_____ above_____

4. Type of malignancy EAC_____ ESCC_____ stage of malignancy_____

5. Have you taken any antibiotic? YES_____NO_____, If yes, type of antibiotic _____and for how many Day _____Week, _____ Month_____you use?

6. Do you have Previous Exposure or infection of H. *pylori*? yes _____NO____ IF Yes before how many days_____ or week _____and are you treated _____?

7. Do you Drink alcohol? YES_____NO _____ If yes, how much you consume? low _____high ____, what type _____? Frequency? Daily____ weekly_____ Monthly _____ Holiday_____

8. If the answer for question NO 8 is YES when you start drinking alcohol? Current _____ before a year_____ any other time _____

9. Do you use any tobacco? YES ____NO_____, If yes what type? Cigarate _____ any other_____ How much a day in NO____ One pack_____ More ____

10. Do you take Hot Beverages commonly? YES _____NO _____if yes how much per Day___ OR Week _____Do you eat hot food? YES_____NO _____ if yes what type? _____ degree of hotness, Much warmer _____Medium _____ low _____

11. Do you have any esophageal related disease? YES _____NO_____, If yes, what type? Gastric cancer _____, Gastritis_____ colon cancer_____ other _____

12. Do you eat fruits and vegetables? YES_____NO_____ If yes , Low _____, medium ____, high_____

13. Do you have family history of esophageal cancer? YES_____NO_____

14. What type of Job you work? _____your work place_____
15. What is your economy level or income? Lower _____middle _____ higher ____
16. Do you practice a physical exercise? YES ____ NO ____
17. Do you have swallowing problem? YES ___NO____, If yes, what type of food, Solid_____, Semisolid_____, Liquid_____ Saliva _____
18. DO you have a history of radiotherapy? YES ____ NO_____
19. Do you eat red meat? YES ____ NO ___, if yes, is it frequent? YES ___ NO ____
20. Have you done surgery before? YES ___ NO ____
21. Have you ever been hospitalized? When? YES ___ NO ____
22. When the feeling of the pain starts? ____
23. What is your blood group type? ____

Annex V. Questionaries (Amharic Version)

አዲስ አበባ ዩኒቨርስቲ ጥቁር አንበሳ ስፔሻላይዝድ ሆስፒታል

ለማስተርስ መመረቂያ ጽሁፍ የሚሆን ቃለ መጠይቅ

1. ካርድ ቁጥር _____ ዕድሜ _____ ጾታ _____ ክብደት _____
2. የመኖርያ ቦታ _____ የሚታከሙበት ሆስፒታል _____
3. የሚታከሙበት ክፍል _____
4. የትምህርት ደረጃዎትን ይግለጹ? ያልተማረ የመጀመርያ ደረጃ ☐ ሁለተኛ ደረጃ ዲፕሎማ ዲግሪ ☐ ዶክትሪት ☐ ☐ ☐ ☐
5. የታመሙትን የካንሰር አይነት ይግለጹ _____
6. ከዚህ በፊት መድሀኒት ወስደው ነበር? አዎ አይደለም ☐ ☐
   አዎ ከሆነ መልስዎት የምን አይነት መድሀኒት ወሰዱ_____ ለምን ያክል ጊዜ? _____
7. ከዚህ በፊት የጉሮሮ ካንሰር ነበረብዎት ? አዎ አይደለም ☐ ☐
   ከነበረብዎት ታክመው ነበር?_____ ምን አይነት የጉሮሮ ካንሰር ነው?_____
8. ከዚህ በፊት በሄሊኮባክተር ፓይሎሪ ባክቴሪያ ተይዘው ነበር? አዎ አይደለም ☐ ☐
   ከተያዙ ከስንት ቀን በፊት _____መድሀኒትስ ወስደው ነበር_____ ለምን ያክል ጊዜ _____
9. አልኮል ይጠጣሉ? አዎ አይ☐ ☐
   ከጠጡ ምን አይነት _____
10. ሲጋራ እና ሌሎች አደንዛዥ እጽ ይጠቀማሉ? አዎ አይደለም ☐ ☐
11. ትኩስ ነገር ወይም ሞቅ ያለ መጠጥ ያዘወትራሉ ወይም ይጠቀማሉ? አዎ አይደለም ☐
12. ትኩስ ምግብስ አ☐ረው ይመገባሉ ? አዎ አይደለም ☐ ☐
   አዎ ካሉ የምግቡን አይነት ይጥቀሱ_____
13. ሌሎች ከጉሮሮ ካንሰር ጋር ተያያዥነት ያላቸው በሽታዎች አለብዎት? አዎ አይደለም ☐
   ካለብዎት ☐ይነት በሽታ_____
14. አትክልት መመገብ ያዘወትራሉ? አዎ አይደለም ☐ ☐
   አዎ ካሉ በምን ያህል ጊዜ _____
15. ከቤተሰብዎ መካከል የጉሮሮ ካንሰር ያለበት ሰው አለ?አዎ አይደለም ☐ ☐
16. ምን አይነት ስራ ነው የሚሰሩት _____
17. የገቢ መጠንዎ ዝቅተኛ መካ☐ ከፍተኛ ☐ ☐
18. ያካል ብቃት ንቅስቃሲ ደርጋሉ ? አዎ አይደለም ☐ ☐
19. ጨረር ህክምና ታክመው ነበረ; አዎ አይደለም ☐ ☐
20. ቀይ ስጋ ያዘወትራሉ? አዎ አይደ☐ ☐
21. የቀዶ ህክምና አድርገው ያውቃሉ? መቼ? _____

22. በህመም ምክንያት ሀኪም ቤት ተኝተው ያውቃሉ? _____

23. የህመም ስሜት መቼ ጀመረዎት? _____

24. የደም አይነትዎ ምን አይነት ነው? _____

Annex VI: Questionaries for Apparently healthy individuals

1. Card number _____ Age: _____ Sex _____Body weight _____ Kg?
2. Address: _____Hospital _____Serial number: _____Ward _____
3. Level of Educational? Uneducated _____Elementary _____high school _____ Diploma_____ Degree_____ Masters____ Phd ___
4. Have you taken any antibiotic? YES_____NO_____, If yes, type of antibiotic _____and for how many Day _____Week, _____ Month_____you use?
5. Do you have a previous history of esophageal cancer? Yes ___NO ___ If "yes" have you been treated? _____
6. Do you have Previous Exposure or infection of H. pylori? yes _____NO____ IF Yes, before how many days_____ or week _____and are you treated _____?
7. Do you use alcohol? YES_____NO _____ If yes, what type _____?
8. Do you use any tobacco? YES ____NO_____, If yes what type? Cigarate _____ any other_____
9. Do you take Hot drink commonly? YES _____NO _____
10. Do you eat hot food? YES_____NO _____ if yes what type? _____
11. Do you have any esophageal related disease? YES _____NO_____, If yes, what type? Gastric cancer _____, Gastritis_____ colon cancer_____ other _____
12. Do you eat fruits and vegetables? YES_____NO_____ if yes for how many times per month_____ or week _____, if NO, what food you commonly eat? _____
13. Do you have family history of esophageal cancer? YES_____NO_____
14. What type of Job you work? _____
15. What is your economy level or income? Lower _____middle _____ higher ____
16. Do you practice a physical exercise YES ☐ NO? ☐
17. Do you practice a physical exercise? YES ____ NO ____
18. Do you have swallowing problem? YES ___NO____, If yes, what type of food, Solid_____, Semisolid_____, Liquid_____ any other _____
19. DO you have a history of radiotherapy? YES ____ NO_____
20. Do you eat red meat? YES ____ NO ___, if yes is it frequent? YES ___ NO ____

21. Have you ever been hospitalized? YES ___ NO ____

SOPS Procedures

1. cleans your skin with an antiseptic wipe
2. places tourniquet, around your upper arm to help the vein swell with blood
3. inserts a needle in the your and collects a blood sample in one or more vials
4. removes the elastic band
5. covers the area with a bandage to stop any bleeding
6. Label your sample and send it to a lab for analysis

VII. 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: Zinashwork Abebe (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.

Elias Bisrat (MSc CLS, Hematology and Immunohematology specialty)

Signature: _____

Date: _____

Place: Addis Ababa, Ethiopia.