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Assessment of hematological parameters among asthmatic patients at Tikur Anbessa Specialized Hospital, Addis Ababa, Ethiopia

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This is to certify that the thesis prepared by **Abdu Jemal**, entitled:

Assessment of hematological parameters among asthmatic 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 Contents

List of Table

Abbreviations

BMI	Body Mass Index
CBC	Complete Blood Count
CI	Confidence Interval
COPD	Chronic Obstructive Pulmonary Disease
EDTA	Ethylenediamine tetraacetic acid
ESR	Erythrocyte Sedimentation Rate
Hb	Hemoglobin
Hct	Hematocrit
ICS	Inhaled Corticosteroids
IgE	Immunoglobulin E
IL	Interleukin
MCV	Mean Cell Volume
MCH	Mean Cell Hemoglobin
MCHC	Mean Cell Hemoglobin Concentration
MPV	Mean Platelet Volume
PDW	Platelet Distribution width
RBC	Red Blood Cell
RDW	Red cell distribution width
SOP	Standard Operating Procedure
SPSS	Statistical Package for Social Sciences
TASH	Tikur Anbessa Specialized Hospital
WBC	White Blood Cell
WHO	World Health Organization

Abstract

Background: Asthma is a chronic inflammatory disease which belongs to the leading causes of mortality globally. Airway inflammation involves an interaction of many blood cells. Consequently, asthmatic condition can alter blood parameters mainly neutrophil and eosinophil. However, the role of other major leukocyte parameters, red blood cell (RBC) indices and mean platelet volume (MPV) are less investigated.

Objective: To assess hematological parameters among asthmatic patients at Tikur Anbessa Specialized Hospital, Addis Ababa, Ethiopia

Methods: A comparative cross-sectional study was carried out from January to May 2023 at TASH in Addis Ababa, Ethiopia. A total of 320 study participants consisting of 160 asthmatic patients and 160 healthy controls were included using convenient sampling technique. A completed blood count (CBC) was analyzed by using Beckman coulter hematology analyzer. To determine the erythrocyte sedimentation rate (ESR) Westergren method was used. Data was analyzed using the SPSS software (version 26). Independent t-test was used to compare the mean hematological values of the asthmatic patients and healthy controls. Binary logistic regression was used to assess the association of asthmatic patients with selected risk factors. Statistical significance was determined at P value less than 0.05.

Results: The mean age, percentage of females and resident in urban areas of study participants were 49.53 and 40.72 years old, 55.0% and 51.2% and 84.4% and 89.4% for cases and controls, respectively. The mean values of all WBC parameters, MPV and ESR with the exception of platelet count in asthmatic patients had significant mean difference compared with control groups. ESR value, MPV, total WBC count and absolute and relative counts of neutrophil, eosinophil and basophil were significantly high in asthmatic patients compared to controls. On the other hand absolute and relative counts of lymphocytes and monocytes were significantly lower in asthmatic patients.

Conclusion: This study demonstrated a statistical significant difference in most of hematological parameters among asthmatic patients than the controls. Therefore, hematological parameters showed significant mean difference should be considered for proper management of asthma.

Key words: Hematological abnormalities, Asthma, Neutrophilia, Eosinophilia

1. Introduction

1.1 Background

Asthma is a complex heterogeneous illness characterized by chronic inflammation of the airway of the lungs, wheeze, shortness of breath, cough, increased mucus production and multicellular inflammation. Regardless of age and geographic location, it affects 1-18% of the global population. The disease symptoms and effect on the airway vary over time as well as intensity. Triggering factors include allergen or irritant exposure, physical activity/exercise, change in weather or respiratory viral infections (1,2).

Environmental as well as genetic causes are significant for developing asthma. Common inhaled allergens and indoor plus outdoor air pollution that can vary by geographical regions are all triggers of the disease of asthma (2). Moreover, smoking, certain type of foods or beverages, viral infections and “common cold” are among the common risk factors. Infection with helminthes is also associated with asthma. These infections are relatively common in Africa. The rise in the level of immunoglobulin E (IgE) and a prominent T helper2 immune response against helminthic infections could most probably be the cause (3).

Cells like eosinophils, neutrophils, mast cells, T lymphocytes, and dendritic cells play a crucial role in the pathogenesis of asthma. The generation of interleukin-4 (IL-4), IL-5, and IL-13 which are T-helper 2 cytokines can also describe the overproduction of IgE, presence of eosinophils and the development of asthma. The key factor in the pathophysiology of asthma is inflammation. Several cell types and mediators involve in the inflammatory reactions of the airways. This interaction ultimately causes the pathophysiological characteristic features of asthma (4,5).

For instance, eosinophils release their granule proteins which are cytotoxic when they reach the target organ. Once attracted to the inflammatory site, eosinophils partake in the modulation of immune response, induction of airway hyperresponsiveness and remodeling, characteristic features of asthma. Therefore, eosinophilia often correlates with enhanced asthma severity. On the other hand, activation of mucosal mast cells results in the release of large number of cytokines and bronchoconstrictor mediators such as histamine and prostaglandin. The synthesis of these mediators changes the airway environment and stimulates inflammation. Changes in the

airway structure is influenced by cells such as T lymphocytes as well as other airway residing cells (5-7).

Severe form of chronic asthma is characterized by accumulation of Neutrophils in the airway. This increment of neutrophils is responsible for chronic airway narrowing and aggravation of acute severe asthma (8,9). Since granulocytes cells such as mast cells, eosinophils and neutrophils reach the airway through the circulation, they are useful parameters to consider in the diagnostic workup of asthmatic patients in addition to the T-helper 2 cells (10).

Besides platelets (PLTs) are also critical cells in the pathogenesis of asthma. This is because PLTs produce numerous inflammatory substances when they get activated. The mean platelet volume (MPV), a parameter which is generated with the routine CBC count, can also be used as a marker of this activation (11). Furthermore, mast cells and macrophages produce IL-1 and Tumor Necrosis Factor alpha (TNF α) which stimulates the liver to produce acute-phase proteins. A rise in acute phase proteins decreases a negative charge of RBC surface and increases the rouleaux formation of RBCs, then increases Erythrocyte Sedimentation Rate (ESR) (9).

Hematological parameters are quantifiable indices of blood that can be utilized as markers for the diagnosis and monitoring of certain physiological and pathological variations from the normal condition. Analysis of routine blood cell biomarkers and erythrocyte sedimentation rate is essential for the diagnosis of asthma (12).

1.2 Statement of the Problem

Asthma is one of the chronic diseases of the respiratory system that belongs to the leading causes of mortality globally. As per the global estimate, more than 300 million people have asthma. With this trend, by 2025, about 100 million people may be living with the disease. According to the World Health Organization (WHO), an estimated 250,000 deaths attributed to asthma occur annually particularly in low- and middle-income countries (3).

Centers for Disease Control and Prevention (CDC) also estimated a 1.1 death per 100,000 individuals in the year 2015 as a result of complications attributed to asthma. In 2016, It ranked 23rd among the leading cause of premature mortality. According to 2018 estimates, globally 1000 people die from asthma daily (13). In the developing countries, its prevalence is still increasing, greatly affecting public health, society as well as economic status due to poor disease management, monitoring and evaluation of the disease progress. All in all the need to better understand predisposing factors to envisage its occurrence and severity are among the priority areas (14).

Studies are showing that the common contributing factors such as family history of asthma, air pollution, allergen sensitization, and behavioral factors like cigarette smoking, limited habit of physical exercise and changes in the dietary pattern are suggested to be responsible for the increasing trend in developing countries. The allergens affecting the respiratory system differ from place to place. They induce asthma by eliciting allergic inflammation of the nasal as well as the bronchial mucosa (15). Asthma is also still a critical challenge in the developed nations as a result of enhanced industrialization and increasing urban pollution (16).

The changing life style and urbanization which is increasing at an alarming rate in Africa are responsible for the rising of many chronic diseases including asthma and other allergic diseases. This increasing trend may even lead to a higher magnitude than in the developing countries as a result the conducive conditions in the African continent. The immune triggering effects of infection with helminthes and their capability to cause allergen city and pulmonary eosinophilia, are the main reasons as these infections are prevalent in many African settings (3).

Asthmatic condition can alter blood parameters mainly the complete blood count as a result of the immune response and hematopoietic physiology during a disease state. Predominantly eosinophils and neutrophils play major roles in pathogenesis of asthma. However the relation

between peripheral blood cell counts of other major leukocyte groups, Red Blood Cell (RBC) indices, Hemoglobin (Hb), Erythrocyte Sedimentation Rate (ESR) in asthmatic patient is less clear (17). Moreover, although platelets are indicated in the pathogenesis of asthma, the role of their derivative parameters including MPV is less investigated (11).

The hematological profile varies in patients with an allergic disorder including asthma. Therefore, routine blood tests should be mandatory in all allergic conditions including in asymptomatic or mild conditions to facilitate early diagnosis and treatment. Moreover, hematological analysis is useful for better patient follow-up and clinical management (18).

Studies on asthma and its effect on hematological parameters with the predisposing factors are limited in Ethiopia. Specifically in Addis Ababa there is no published data. So, the aim of this study was to assess the hematological parameters among asthmatic patients at Tikur Anbessa Specialized Hospital. In addition, the study aimed to investigate the hematological abnormalities among asthmatic patients and the common predisposing conditions.

1.3. Significance of the study

This study will provide useful up-to date data for the hematological parameters among asthmatic patients comparatively with the control subjects which can be an input for clinicians in the patient management process.

The study can serve as baseline for the scientific community to conduct further studies, to determine relatively precise information and the data will help the health providers to give evidence based intervention, for better monitoring of patients and awareness creation. Furthermore, it will be used for policy makers and other concerned bodies for future planning, allocation of resources and to develop revise guideline on the management of asthmatic patients with hematological abnormalities. The study will also help the study participants to know their hematological parameter abnormalities and to be treated accordingly.

2. Literature Review

Asthma is among the main public health challenge causing remarkable global morbidity, mortality, as well as increased health care costs. Asthma places a tremendous burden on many nations' social, economic, and health care levels. In addition, the inflammatory conditions can cause a change in hematopoietic physiology, and increased severity of asthma results in hematological abnormalities (19).

A cross-sectional study conducted in Mexico in 2020 on adult asthmatic and healthy individuals showed that the magnitude of hematological abnormalities was high among asthmatic patients. The study indicated that the prevalence of eosinophilia was 37.7%, whereas in the control group the prevalence of eosinophilia was 4.6%. Asthmatic patients had higher odds of having eosinophilia (OR= 12.61, $p < 0.0001$). Moreover, the study indicated that asthmatic patients had higher levels of basophils. Overall, the results revealed that patients with asthma had a higher concentration of eosinophils and basophils, fewer monocytes in their blood (20).

Another cross-sectional study conducted in Mexico in 2018, on adult asthmatic patients (≥18 years) indicated that the prevalence of eosinophilia was 37.8%. The study also assessed the factors associated with eosinophilia. The study indicated that patients with an age <50 years were about three times more likely to develop eosinophilia than older individuals (OR= 3.25; 95% CI: 1.33-7.94) (21).

Another cross-sectional study carried out in 2000 at the Royal Brompton Hospital, London found that the activation of neutrophil and higher eosinophil count in the peripheral blood of asthmatic patients (22).

According to a historical cohort study of asthmatic patients aged 12- 80 years in the UK from 1990-2013 indicated that about 20,929 (16%) of the total of 130,248 patients had eosinophilia (eosinophil count > 400 cells per microliter). In patients with increased eosinophil count, asthma exacerbation and acute respiratory events were significantly greater than patients with an eosinophil count of less than 400 cells/μl (23).

A pilot study conducted in 2015, Boston, USA on all hospitalized patients for asthma exacerbation demonstrated that about 40% of patients had eosinophilia and a higher eosinophil

count was linked to frequent asthma attacks. The study indicated that patients with eosinophilia had more asthmatic attacks than patients with low eosinophil counts (24).

A comparative study done in Ukraine on the changes of RBC indexes in children showed that a significant variation in MCV between pediatric patients with asthma compared to health children. Although no gender difference in MCV values was noted in the study, there was a significant age difference. Children with microcytosis were 3 times more likely to have severe asthma than children with normocytic blood picture as compared to those having persistent mild and moderate asthma (25).

A comparative study reported by Kemona-Chętnik *et al* in 2007, from Poland, on adult asthmatic and healthy study individuals showed that thrombocytes were statistically higher in allergic asthma patients than control groups. The study showed that thrombocytes could be involved in allergic inflammation and remodeling of airways (26).

Based on the retrospective study done in Turkey, the hemoglobin concentration was found to be higher in patients than controls though the study reported no difference in packed cell volume. The study group had a decreased MCV; again this difference did not reach statistical significance. Although the patients' WBC and platelet counts were higher than the values in controls, these differences were not statistically sound. On the other hand, the mean platelet volume was significantly decreased in the patients. Inverse correlation was noted between MPV and thrombocyte counts. The MPV values were also significantly higher in females and patients under the age of six (27).

A similar retrospective study from Turkey, in 2018 on adult patients showed that study subjects with an asthma exacerbation had lower mean MPV values (9.2 ± 0.9 fL) than those patients without asthma exacerbation (9.6 ± 1.3 fL). WBC count and absolute and relative neutrophil counts were significantly increased in those with asthma exacerbation than patients without as well as healthy controls (28).

A retrospective observational study conducted in Turkey on adult asthmatic patients hospitalized due to asthmatic attacks between 2015 and 2017 revealed higher count of leukocytes and neutrophils. The counts were significantly elevated in patients hospitalized with asthma exacerbation. The ratio of neutrophils to lymphocytes was statistically significantly associated with repeated Emergency Department admissions (29).

The comparative cross-sectional study conducted in 2017, on adult asthmatic patients in Pakistan showed that many hematologic parameters (hemoglobin, WBC, absolute lymphocyte count, absolute and relative neutrophil counts) were higher, while mean platelet volume and relative lymphocyte count were lower. Most WBC parameters were higher in asthma patients (30).

A study reported from India in 2013, recruited 50 adult asthmatic patients 100 with other allergic disorders and 50 controls at Assam Medical College, using a cross sectional study design. there was a significant increment in the total leukocyte count, absolute eosinophil count, ESR and the percent neutrophil and eosinophil counts where as a significantly decreased lymphocyte count was observed among the patients (17).

A report from Nigeria in 2019 showed increase in WBC, decrease in MCV, MCH, MCHC and no significant variation in Neutrophils, Lymphocytes, Monocytes, Eosinophils, erythrocytes, Hemoglobin, hematocrit and thrombocytes of patients with asthma as compared to healthy participants. The findings revealed lower number of Neutrophils, Monocytes and elevated Lymphocytes, mean cell hemoglobin but no significant difference by gender was noted for WBC, Eosinophils, Platelets and RBC parameters (RBC, Hb, HCT, MCHC) (31).

Another comparative cross-sectional study conducted in Nigeria in 2021 involves 108 adult asthmatic patients taking inhaled corticosteroids. Compared to similar number of healthy participants, the neutrophils, eosinophils and basophils counts were elevated in asthmatic patients. Whereas monocytes, lymphocytes, HCT, RBC and Hb were significantly lower in asthmatic patients than the healthy counterparts (32).

A research finding reported in 2022 from Northwest Ethiopia revealed that the increased magnitude of neutrophil, eosinophil, thrombocyte, and leukocyte in asthmatic patients. Based on the result of the study, factors such as poor culture of physical exercise and using drugs other than asthmatic drugs before three months were related with neutrophilia. Eosinophilia and thrombocytopenia were attributed to being admitted to the emergency unit was found to be related with (33).

A research finding from southwest Ethiopia revealed that the absolute and relative counts of eosinophil, neutrophil, and basophil, total WBC as well as ESR were significantly higher in asthmatic patients. However, in asthmatic patients, the absolute and relative monocyte and lymphocyte values were significantly decreased (18).

In general, the hematological parameters among asthmatic patients are assessed in different studies and demonstrated a change of hematological parameters in patients rather than in control of apparently healthy subjects. Asthmatic diseases are widespread and affecting humans in different regions and this leads to a continuous change of hematological profiles based on how severe asthma is and its duration. This hematological abnormality may lead to hyper-reactive state may have the potential to cause serious life-threatening condition. Thus, patients with underlying risk factors should be suspicious of having hematological abnormalities. Cognizant of this, the current research tried to contribute to the existing gap of data limitation in Ethiopia particularly in Addis Ababa and offer supplementary data to expand the existing scientific information worldwide.

3. Objectives

3.1 General Objective

- To assess hematological parameters among asthmatic patients at Tikur Anbessa Specialized Hospital Addis Ababa, Ethiopia, 2023.

3.2 Specific Objectives

- To assess hematological abnormalities among asthmatic patients and healthy controls
- To compare the magnitude of hematological parameters in subjects having asthmatic diseases with that in normal subjects

4. Materials and Methods

4.1 Study Area

The study area was Tikur Anbessa Specialized Hospital, which was established in 1973 G.C. The hospital is found in Lideta sub city, Addis Ababa, the capital city of Ethiopia. It serves as a tertiary level referral hospital and also as the main teaching hospital of the College of Health Sciences of Addis Ababa University. It has a bed capacity of more than 700 beds and serves approximately 400,000 patients per year. The Hospital is staffed with 201 doctors, 627 nurses, 55 laboratory professionals and more than 115 other health professionals who provide the health care services in the various specialty and subspecialty clinics including the chest clinic. The chest clinic at TASH every month sees a large number of asthmatic patients, with over 150 visits per month. TASH laboratory has different departments including the hematology department. The hematology laboratory is one of the loaded laboratories of TASH which receives on average 350-400 samples during the day shift (34).

4.2 Study Design and Period

Hospital based comparative cross-sectional study was conducted at Tikur Anbessa Specialized Hospital (TASH) from January to May, 2023.

4.3 Population

4.3.1 Source Population

All asthmatic patients and apparently healthy patient's caregiver relatives at Tikur Anbessa Specialized Hospital (TASH) was considered as a source of population for cases and apparently healthy controls respectively.

4.3.2 Study Population

Asthmatic patients and apparently healthy patient's caregiver relatives with all age group, who visit Tikur Anbessa Specialized Hospital (TASH) and meet the eligibility requirements during the data collection time was the study population.

4.4 Inclusion and Exclusion Criteria

4.4.1 Inclusion Criteria

Asthmatic patients and apparently healthy patient's caregiver relatives attending TASH during the study period and willing to participate in the study were included.

4.4.2 Exclusion Criteria

Patients like pregnant women as well as a disease conditions such as chronic obstructive pulmonary disease (COPD) other than asthma, chronic diseases like HIV/AIDS, chronic kidney disease, hematological disorders like bleeding disorders and hematological malignancies, tuberculosis, helminthic infections and others were not included in the study by checking their patient's charts as well as interviewing.

4.5 Study Variables

4.5.1 Dependent Variables

- Hematological abnormalities

4.5.2 Independent Variables

- Age
- Sex
- Educational status
- occupation
- Alcohol drinking habit
- Type of energy source for cooking

4.6 Sample Size Calculation and Sampling Methods

4.6.1 Sample Size Calculation

The difference between two populations means was used to calculate sample size. The level of significance which is usually set to α level of 0.05, respective Z value is 1.96 and power of the test: 100 (1-β) %, 80% which is equal to 0.842 and 1:1 ratio of case to control. Thus, the following formula was sued:

$$N = 2\sigma^2 [Z_{1-\alpha/2} + Z_{1-\beta}]^2 / (\mu_1-\mu_2)^2$$

Where, σ is pooled standard deviation of the two groups ($\sigma^2= S_1^2+S_2^2 / 2$)

The mean and standard deviation of platelet count of the two groups, which gives the highest sample size compared to the other parameters, are taken from the previous similar study that is 252.77x10^9/L and 77.23 for cases respectively and 275.62 x109/L and 60.48 for controls respectively (18).

Based on the given information, $\sigma^2 = 77.23^2 + 60.48^2 / 2 = 4811.15165$

$n = 2(4811.15165) [1.96 + 0.84]^2 / (32.84-36.95)^2 = 75438.857872 / 522.1225$

n = 145 + 10% non-response rate (15) = 160

Therefore, the minimum sample size required for case group was 160.

The total sample size to be taken (N) = 320

4.6.2 Sampling Methods

A convenient sampling technique was used to select the study participants until the sample size was satisfied.

4.7 Measurement and Data Collection

4.7.1 Data Collection Procedure

Through an interview, data related to socio-demographic and behavioral characteristics were collected using a pre-tested structured questionnaire. Clinical data of study participants was collected through reviewing medical records and also face to face interview of study participants. Anthropometric variables such as weight and height were measured by clinical nurses. Then, body mass index (BMI) was computed as weight in kilograms divided by height in meters squared. After the data collection was completed, whole blood samples were collected from each study participant.

4.7.2 Laboratory Analysis

Blood sample collection

From participants who provide consent, 5ml venous blood sample was collected using ethylene diamine tetra acetic acid (EDTA) vacutainer tubes and transported to the hematology laboratory as soon as possible for the investigation of CBC and ESR. Laboratory technologists and

phlebotomists at Tikur Anbessa Specialized Hospital collected the blood according to the blood collection standard operating procedures (SOPs) of the laboratory under the supervision of the principal investigator (PI).

Hematological parameter analysis

Blood samples were run for complete blood count within two hours of sample collection. Total WBC, absolute and relative eosinophil, neutrophil, lymphocyte, monocyte, and basophil counts, RBC, Hb, MCV, MCH, MCHC, RDW, Platelet, MPV and PDW of the study participants was performed by using five differential Unicel DxH 800 (Beckman Coulter, Ireland) automated hematology analyzer. The coulter analyzes the specimen via the principle of impedance, optical method and spectrophotometry as explained in the Annex part (Annex V). The results were printed and hematological parameters were summarized.

ESR measurement

For ESR measurement the EDTA blood was further diluted with 3.1 g/L tri–sodium citrate in a ratio of 1:4 ratios, where 1 part of citrate was used to 3 parts of blood. The diluted blood samples were filled in westergreen tubes and allowed to stand for an hour following the standard Westergreen method of ESR measurement and the result was reported in mm/hr.

4.8 Data Quality Assurance

Pre-analytical

Data quality was ensured by giving short training to data collectors and by strictly adhering to SOPs. The data collection tool was pretested on 5% of asthmatic patients and validated before one week of the study time. The questionnaire was evaluated during pretesting and modified accordingly before actual data collection is started. The questionnaire was translated to Amharic language and at the end translated back to English. Patients were properly identified and specimens were properly labeled. Samples were properly collected and transported immediately to hematology laboratory to ensure the quality. The specimen rejection criteria of the laboratory was routine checked so that clotted samples, wrong anticoagulant samples and all listed in the sample rejection criteria list are rejected. Data extracted from charts and from interview were checked for clarity, consistency and completeness. Moreover reagents quality like expiry date and instruments preventive maintenance and calibrations logs were checked.

Analytical

Before analyzing patient samples, three levels of quality controls, low, normal and high were run daily as per the manufacturers guideline and as stipulated in the SOPs.

Post-analytical

The results of laboratory examination were reviewed and recorded on well-prepared format carefully. Leftover samples were handled as per the laboratory regulation of specimen retention and disposal.

4.9 Data Analysis and Interpretation

Data was cleaned, entered and analyzed using the statistical package for social science (SPSS) software (version 26). Descriptive statistics like frequency, mean and percentage was calculated for the description of the data as appropriate. Independent t-test was used to compare the means of the asthmatic patients and apparently healthy controls. A chi-square test was performed to assess the relationship of selected hematological abnormalities between asthmatic patients and healthy controls. Both bivariate and multivariable logistic regressions were used to assess the association of asthmatic patients with important selected variables. Those variables with a P-value of < 0.25 in the bivariable logistic regression analysis were fitted in to multivariable logistic regression analysis. P value of less than 0.05 was used to declare statistical significance.

4.10 Operational Definitions

- **Asthmatic patients**: Patients with a clinical asthma diagnosis and who experienced an asthma attack
- **Stages of asthma:** are classified as intermittent, mild persistent, moderate persistent and severe persistent based on global initiative for asthma (GINA) 2022 guideline.
- **Mild asthma:** Well-controlled with as-needed reliever medication alone or with low-intensity controller treatment such as low-dose inhaled corticosteroids (ICSs), leukotriene receptor antagonists, or chromones
- **Moderate asthma:** Well-controlled with low-dose ICS/long-acting beta2-agonists
- **Severe asthma:** Requires high-dose ICS to prevent it from becoming uncontrolled, or asthma that remains uncontrolled despite this treatment
- **Leukocytosis:** is defined as an increase in total WBC count (NR = 3.6 – $10.2\times10^3/\mu l$)

- **Neutrophilia:** is defined as an increase in neutrophil count (NR = 2 – 7×103/µl)
- **Eosinophilia:** is defined as an increase in eosinophil count (NR = 0.0 – 0.5×103/µl)

4.11 Ethical Considerations

The study was carried out after the study protocol is reviewed and ethically approved by the departmental research and ethical review board, ethical committee of the Department of Medical Laboratory Sciences of College of Health Sciences of Addis Ababa University. Letter of cooperation was written to the respective administrators and workers of the Tikur Anbessa Specialized Hospital. Respondents were informed about the objectives and purpose of the study and verbal consent was obtained from each respondent. Their privacy and confidentiality of the information was assured throughout the study procedure. Besides, participants were made aware of their full right to participate or decline from the study anytime. Data that was collected for this study was not used for other purpose without approval of each participant. All international and institutional research ethics conventions were strictly obeyed during our study process. All COVID-19 protocols to protect both study participants and data collectors were adhered.

4.12 Dissemination of the result

After completion, final thesis results was presented and discussed with department of MLS staffs and students. The final thesis was submitted to Addis Ababa University College of Health Sciences, Department of Medical Laboratory Sciences. Tikur Anbessa Specialized Hospital will be informed of the findings. In addition, the findings will be published on reputable and highly accessed journals as well as presented at scientific conferences. Findings will be shared with the medical community on annual conferences.

5. Results

5.1 Socio-demographic and behavioural characteristics of the study participants

This study recruited 160 asthmatic patients and 160 apparently healthy controls. The mean age of study participants were 49.53 ± 19.68 ranged from 18 to 90 years old and 40.72 ± 18.36 ranged from 18 to 73 years old for cases and controls, respectively. Over half (55.0%) of cases and 51.2% of the controls were females. Among study participants, majority (84.4%) of the asthmatic patients and 89.4% of the apparently healthy individuals were residing in urban areas. Regarding marital and educational status, 66.9% of the patients and 60.0% of their counterpart were married. However, about 31.9% of the patients and 10% of controls cannot read and write, while 40% of the controls versus 20.6% patients had at least college and above diplomas or degrees. Private workers accounted 42.5% in the patients group, but 30.6% of the controls were housewives.

From the study participants, 3.8% of asthmatic patients and 2.5% of the controls were cigarette smokers, and 13.8% of the cases and 5.6% of the controls had a habit of alcohol drinking. Moreover, 18.8% of the cases and 20% of the controls had a habit of practicing physical exercise. Majority of asthmatic (69.4%) and control (78.8%) study participants were replied as they used electricity to the question about the source of energy for cooking. Among study participants, 19.4% and 25% had high body mass index (BMI) for cases and controls respectively (Table 1).

Table1. Socio-demographic and behavioural characteristics of the study participants at Tikur Anbessa Specialized Hospital, Addis Ababa, Ethiopia, 2023

Variables		Asthmatic patients	Control group
		N (%)	N (%)
Age (years)	1-20	10 (6.3)	25 (15.6)
	21-40	55 (34.4)	69 (43.1)
	41-60	31 (19.4)	30 (18.8)
	>60	64 (40)	36 (22.5)
Sex	Male	72 (45)	78 (48.8)
	Female	88 (55)	82 (51.2)
Religion	Orthodox	92 (57.5)	85 (53.1)
	Muslim	36 (22.5)	41 (25.6)
	Protestant	27 (16.9)	30 (18.8)
	Catholic	5 (3.1)	4 (2.5)
Ethnicity	Amhara	14 (8.8)	15 (9.4)
	Oromo	31 (19.4)	28 (17.5)
	Tigre	6 (3.8)	4 (2.5)
	Addis Ababa	104 (65.0)	111 (69.4)
	SNNPR	5 (3.1)	2 (1.3)
Residence	Urban	135 (84.4)	143 (89.4)
	Rural	25 (15.6)	17 (10.6)
Marital Status	Married	107 (66.9)	96 (60.0)
	Single	46 (28.7)	48 (30.0)
	Divorced	7 (4.4)	16 (10.0)
Educational Status	Cannot read and write	51 (31.9)	16 (10.0)
	Elementary school	44 (27.5)	41 (25.6)
	Secondary school	32 (20.0)	39 (24.4)
	College and above	33 (20.6)	64 (40)
Occupation	Employee	17 (10.6)	28 (17.5)
	Student	25 (15.6)	40 (25.0)
	House wife	50 (31.3)	49 (30.6)
	Private	68 (42.5)	43 (26.9)
Family Size	≤4	92 (57.5)	98 (61.3)
	5 – 8	54 (33.8)	43 (26.9)
	>8	14 (8.8)	19 (11.9)
Cigarette Smoking	Yes	6 (3.8)	4 (2.5)
	No	154 (96.3)	156 (97.5)
Alcohol drinking	Yes	22 (13.8)	9 (5.6)
	No	138 (86.3)	151 (94.4)
Physical Exercise	Yes	30 (18.8)	32 (20.0)
	No	130 (81.2)	128 (80.0)
Cooking Material	Charcoal	28 (17.5)	19 (11.9)
	Wood	21 (13.1)	15 (9.4)

	Electricity	111 (69.4)	126 (78.8)
BMI	<18.5 (Underweight)	21 (13.1)	-
	18.5-24.9 (Normal)	100 (62.5)	120 (75)
	25-29.9 (Overweight)	31 (19.4)	40 (25)
	≥30 (Obesity)	8 (5)	-

5.2 Clinical characteristics of the study participants

The table presented below shows that, about 56 (35%) of asthmatic patients were living with asthma 5 years and below. More than half 89 (55.6%) the patients had a family history of asthma. Asthma with acute exacerbation was seen in 86 (53.8%), while 59 (36.9%), 55 (34.4%) had mild and moderate severity, respectively. Regarding symptoms of asthma, 61 (38.1%), 43 (26.9%) had shortness of breath and more than one symptoms of asthma respectively. About 152 (95%) of the asthmatic patients had taken asthmatic drugs (Table 2).

Table2. Clinical characteristics of the study participants at Tikur Anbessa Specialized Hospital, Addis Ababa, Ethiopia, 2023

Variables		Asthmatic patients	
		Frequency	**Percent**
Duration of asthma (in years)	≤ 5	56	35.0
	6 – 10	53	33.1
	> 10	51	31.9
Family history	Yes	89	55.6
	No	71	44.4
Asthma exacerbation	Acute	86	53.8
	Chronic	74	46.
Severity of asthma	Intermittent	22	13.8
	Mild persistent	59	36.9
	Moderate persistent	55	34.4
	Sever persistent	24	15.0
Symptoms	Wheezing	20	12.5
	Shortness of breath	61	38.1
	Runny nose	15	9.4
	Cough	21	13.1
	More than one symptoms	43	26.9
Asthmatic drug	Yes	152	95.0
	No	8	5.0

5.3 Hematological parameters of asthmatic cases and controls

An independent sample t- test was conducted to determine if there is a significant difference of means of haematological parameters between asthmatic patients and apparently healthy control groups. There was no statistically significant difference in the total red cells and indices values. However, there was a statistically significant difference between the patients and controls regarding WBC differential values. Asthmatic Patients had statistically significantly higher values for total Leucocytes, Neutrophil, Eosinophil, Basophil absolute and relative counts. Whereas lower values in patients were detected for Lymphocytes and Monocytes.

The mean values with standard deviation of the red blood cell count (RBC), mean cell volume (MCV), haematocrit (HCT), hemoglobin concentration (Hb), mean cell haemoglobin (MCH) and mean cell hemoglobin concentration (MCHC) of both the asthmatics and normal healthy controls were 4.78± 1.10 and 4.82±0.39, 86.89±6.08 and 87.19±4.16, 41.28±8.64 and 42.13±3.29, 13.41±2.65 and 13.56±1.19, 28.79±2.58 and 29.06±1.16, 32.65±1.18 and 32.79±0.79 respectively.

On the other hand, the mean±SD of total WBC, absolute neutrophil, eosinophil and basophil counts for the asthmatic participants and normal healthy control groups were 8.39±3.96 *vs* 7.20±1.17, 5.84±3.46 *vs* 4.12±1.05, 0.52±0.42 and 0.32±0.13, 0.10±0.13 and 0.06±0.04 respectively. The respective values for Lymphocyte were 1.61±0.78 *vs* 2.07±0.66, and Monocyte were 0.32±0.27 *vs* 0.62±0.19. The relative counts (%) of neutrophil, eosinophil, basophil which are significantly higher and lymphocyte and monocyte which are lower in asthma patients compared to healthy controls were 67.16±12.73 *vs* 56.82±9.14, 6.22±3.86 *vs* 4.49±1.79, 1.13±0.89 *vs* 0.85±0.55, 20.86±9.89 *vs* 29.07±9.11, 4.24±3.18 *vs* 8.76±2.62, respectively. The mean platelet count was not significantly different between patients and healthy participants (268.54±112.66 *vs* 271.62±61.91). Whereas MPV (9.28±1.36 *vs* 8.63±0.85) and ESR (26.24±13.55 *vs* 7.42±2.94) were significantly higher in patients compared to healthy controls (Table 3).

Table 3. Comparisons of Mean values of hematological parameters of asthmatic cases and controls at Tikur Anbessa Specialized Hospital, Addis Ababa, Ethiopia, 2023

Parameters	Asthmatic patients (Mean ± SD)	Control groups (Mean ± SD)	P-value
RBC parameters			
RBC count ($\times 10^{12}$/L)	4.78± 1.10	4.82±0.39	0.673
MCV (fl)	86.89±6.08	87.19±4.16	0.618
HCT (%)	41.28±8.64	42.13±3.29	0.245
Hemoglobin parameters			
Hb (g/dL)	13.41±2.65	13.56±1.19	0.527
MCH (pg)	28.79±2.58	29.06±1.16	0.228
MCHC (g/dL)	32.65±1.18	32.79±0.79	0.229
WBC absolute counts ($\times 10^9$/L)			
Total WBC count	8.39±3.96	7.20±1.17	0.001
Neutrophil	5.84±3.46	4.12±1.05	<0.001
Lymphocyte	1.61±0.78	2.07±0.66	0.002
Monocyte	0.32±0.27	0.62±0.19	0.002
Eosinophil	0.52±0.42	0.32±0.13	0.001
Basophil	0.10±0.13	0.06±0.04	0.001
WBC differential count (%)			
Neutrophil	67.16±12.73	56.82±9.14	<0.001
Lymphocyte	20.86±9.89	29.07±9.11	<0.001
Monocyte	4.24±3.18	8.76±2.62	<0.001
Eosinophil	6.22±3.86	4.49±1.79	<0.001
Basophil	1.13±0.89	0.85±0.55	0.001
Platelet parameters			
PLT count ($\times 10^9$/L)	268.54±112.66	271.62±61.91	0.762
MPV (fL)	9.28±1.36	8.63±0.85	0.002
ESR (mm/hr)	26.24±13.55	7.42±2.94	0.001

5.4 Assessment of hematological abnormalities

A chi-square test was performed to assess the relationship of hematological abnormalities between asthmatic patients and health controls. The relationship was significant for all parameters. From the current study, 39 (24.4%) of the cases and 17 (10.6) of the controls were anaemic based on their haemoglobin value. That means asthmatic patients were more likely to be anaemic than health controls. From the 39 anaemic patients, 8.8%, 12.5% and 3.1% were mild, moderate and severe anaemic respectively. On the other hand the magnitudes of leucocytosis, neutrophilia, eosinophilia, Basophilia and increased MPV were assessed. The majority of asthmatic patients had eosinophilia 75 (46.9%) and neutrophilia 68 (42.5%). In addition, an elevated ESR value was observed among 131 (81.9%) of the study participants.

Table4. Distribution of Hematological abnormalities among asthmatic patients at Tikur Anbessa Specialized Hospital, Addis Ababa, Ethiopia, 2023

Parameters	Cases N (%)	Controls N (%)	P-value
No anaemia	121 (75.6)	143 (89.4)	0.002
Mild anaemia	14 (8.8)	17 (10.6)	
Moderate anaemia	20 (12.5)	0 (0)	
Severe anaemia	5 (3.1)	0 (0)	
Leucocytosis	36 (22.5)	5 (3.1)	<0.001
Neutrophilia	68 (42.5)	13 (8.1)	<0.001
Eosinophilia	75 (46.9)	5 (3.1)	<0.001
Basophilia	54 (33.8)	5 (3.1)	0.001
Increased MPV	15 (9.4)	3 (1.9)	0.006
Elevated ESR	95 (59.4)	11 (6.9)	<0.001

N.B. The reference range used for anaemia and its classification is based on the WHO classification of anaemia. Which is for anaemia (HB<12gm/dl), Mild anaemia (HB is 10.9-11.0 gm/dl), Moderate anaemia (HB is 8.0-10.9 gm/dl) and Severe anaemia (HB is <8 gm/dl). Regarding to the other parameters, the reference range is based on the established range of the CBC Beckman-coulter hematology analyzer.

5.5 Association of selected variables for asthma

In this study, a bivariate and multivariable logistic regression was performed to assess the association of age, sex, residence, education, occupation, alcohol drinking habit and source of energy for cooking among cases and controls. The results from multivariate logistic regression analysis showed that being the age of 1-20 and 21-40 years (AOR: 0.08, 95% CI, 0.02-0.29, P = <0.001 and AOR: 0.37, 95% CI, 0.18-0.78, P = 0.009 respectively), not able to read and write (AOR: 17.46, 95% CI, 5.2-58.6, P = <0.001), housewife (AOR: 0.16, 95% CI, 0.04-0.61, P = 0.007) and alcohol drinking habit (AOR: 4.59, 95% CI, 1.74-12.1, P = 0.002) were significant associated predictors for asthma. But gender, residence and source of energy for cooking didn't show significant association with asthma.

Table5. Association of selected variables for asthma at Tikur Anbessa Specialized Hospital, Addis Ababa, Ethiopia, 2023

Variables	Categories	Asthmatic diseases		COR (95% CI)	AOR (95% CI)	P-value
		Yes n (%)	No n (%)			
Age (years)	1-20	10 (6.3)	25 (15.6)	0.23 (0.09-0.52)	0.08 (0.02-0.29)	<0.001*
	21-40	55 (34.4)	69 (43.1)	0.45 (0.26-0.77)	0.37 (0.18-0.78)	0.009*
	41-60	31 (19.4)	30 (18.8)	0.58 (0.31-1.11)	-	
	>60	64 (40)	36 (22.5)	1	1	
Sex	Male	72 (45)	78 (48.8)	1	1	
	Female	88 (55)	82 (51.2)	1.16 (0.75-1.80)	1.97 (0.98-3.93)	0.056
Residence	Urban	135 (84.4)	142 (88.8)	0.64 (0.33-1.24)	-	
	Rural	25 (15.6)	18 (11.3)	1		
Education	Cannot read and write	51 (31.9)	16 (10.0)	6.18 (3.07-12.5)	17.46 (5.2-58.6)	<0.001*
	Elementary school	44 (27.5)	41 (25.6)	2.08 (1.15-3.78)	5.98 (1.91-18.8)	0.002*
	Secondary school	32 (20.0)	39 (24.4)	1.59 (0.85-2.98)	-	
	College and above	33 (20.6)	64 (40)	1	1	
Occupation	Employee	17 (10.6)	28 (17.5)	1	1	
	Student	25 (15.6)	40 (25.0)	1.03 (0.47-2.25)	-	
	House wife	50 (31.3)	49 (30.6)	1.68 (0.82-3.45)	0.16 (0.04-0.61)	0.007*
	Private	68 (42.5)	43 (26.9)	2.61 (1.28-5.32)	-	
Alcohol drinking	Yes	22 (13.8)	9 (5.6)	2.68 (1.19-6.01)	4.59 (1.74-12.1)	0.002*
	No	138 (86.3)	151 (94.4)	1	1	
Cooking Material	Charcoal	28 (17.5)	19 (11.9)	1.67 (0.89-3.16)	-	
	Wood	21 (13.1)	15 (9.4)	1.59 (0.78-3.23)	-	
	Electricity	111 (69.4)	126 (78.8)	1		

*N.B: **1** shows reference group, *****Shows significant association **COR**= Crude Odds Ratio, **AOR**= Adjusted Odds Ratio*

6. Discussion

Asthma is a major public health challenge regardless of age groups in developing countries. Its burden in terms of mortality is rapidly increasing. A comparative cross-sectional study was conducted to investigate the hematological parameters among 160 asthmatic patients and 160 apparently healthy controls. Most of the hematological parameters had significant mean difference between the two comparative groups. Thus, ESR, MPV, total WBC as well as absolute and relative counts of neutrophil, eosinophil and basophil were significantly higher mean values in asthmatic patients compared to controls. Conversely, the mean absolute and relative counts of lymphocytes and monocytes were significantly lower in participants with asthma.

In this study, the total WBC counts were significantly higher in patients with asthma as compared to healthy controls. This finding is consistent with the findings reported by Dolap U et al. (28), Tokgoz Akyil F et al. (29), Ejaz S et al. (30), Dihingia A et al. (17), Ifeanyi OE et al. (31), Saidu H et al. (32), and Solomon Y et al. (33). Those previous studies showed that the mean values of WBC count were high in asthmatic patients than the healthy control groups. The elevated WBC is mainly attributed to an increase in the three granulocytes (neutrophil, eosinophil and basophil) and this increment could be due to asthma being a chronic disease (28). In addition, leucocytosis may be results from the secretion of inflammatory cytokines and the effect of medications in asthmatic patients especially the corticosteroid drug can cause elevation in WBC and neutrophil parameters (33).

Based on the finding of this study, both the absolute and relative counts of neutrophils, eosinophils and basophils were significantly higher in patients with asthma as compared to the control groups. The increase in neutrophil and eosinophil count in the current study was consistent with many related studies in previous literatures (17, 18, 20, 24, 32, and 33). The increment of neutrophil could be due to the production of cytokine specifically IL-8. In asthma, allergen exposure triggers inflammatory reactions that result in the release of cytokines, which are responsible for attracting and recruiting by binding to the Receptors of neutrophils (8,9). Moreover, neutrophilia is a characteristic feature of asthma (9). Besides, interleukin-4 and interleukin-13 do play a role in the increment of eosinophils in patients suffering from allergic disorders by producing eotaxin that induces the recruitment of eosinophil (18, 33). Regarding to

the high level of basophil in the cases than controls, the basophils have high affinity of IgE receptor and synthesizes histamine which acts on the histamine H4 receptor on basophils and leads to migration of basophils to the tissues (32).

On the contrary, significantly lower of lymphocyte and monocyte counts were recorded in patients with asthma than in healthy controls. This finding is coherent with the report of other studies (17, 18, 32, and 33). This might be due to the involvement IL–8 after neutrophil activation that causes the activation of innate immune mechanisms and monocytes migrate to the inflamed tissue mature into macrophages (18). In addition, the corticosteroid may suppress the lymphopoiesis and monocytopoiesis (32).

To the best of my literature search goes, no similar report was identified in the literature that observed increased MPV level in blood of asthma patients with statistically significant mean difference when compared with the control groups. Subsequently, the current study is the first to report the increment of MPV in blood of asthma patients. This may be due to the decrease of platelet (thrombocytopenia) by its activation and involvement during the exposure to an allergen. Another similar retrospective study reported by Giray D et al. in Turkey revealed significantly lower MPV in the asthmatic patient group (27). The difference might be due to the difference in method and study population. For example in our study, study participants were adult out patients while in the study done in Turkey, study participants were pediatric patients. The other reason might be emanated from laboratory methods as the skin prick test and/or total IgE and/or allergen-specific IgE test results was used while in this study hematological auto–analyzer was used.

Mean platelet count in the current study was lower in the patients in comparison to the control group; but the difference did not reach to statistically significant level. A previous similar study reported the same finding as they observed no significant difference between the platelet count of patients compared with healthy ones (18).

This study has also proven that, the mean ESR value was significantly higher in asthmatic patients as compared to apparently healthy controls. This observation was corroborated by a finding from India (17) and Ethiopia (18). The elevation of ESR in the present study could be due to the involvement of the known pro-inflammatory cytokines and prostaglandins, mainly by

mast cell, basophil, and macrophage. In turn, these factors trigger the liver to produce acute phase proteins, like fibrinogen not to mention immunoglobulins mainly IgE. Then, high amount of fibrinogen and immunoglobulin will cause increased positive charge as well as the formation of rouleaux. Finally, the rouleaux formation enhances the settling of erythrocytes and hence increases the ESR among the asthmatic patient group (18, 33).

This study also determined the association of selected variables for asthma to show which variable is the most associated predictor. The national center for environmental health report that, women adult ages, low education level, the habit of smoking cigarette and drinking alcohol are more likely to have asthma (1). As of this study, young aged, low education status and house wife patients were more likely to develop asthma than their counterpart. This could be due to the limited knowledge to apply appropriate prevention and control mechanisms of asthma and the habit of drinking alcohol might be also causes frequent asthma attacks after once worsened the outcome of the diseases state (21).

7. Limitation of the study

This study is a health facility-based comparative cross-sectional study. As a result, it did not show the hematological changes at the baseline and changes through time. Again because of the study design used and the limitation of the time period for data collection, we did not study the effect of the drug used in the asthma treatment on these haematological parameters. In this study, we included only asthmatic patients but no other chronic obstructive pulmonary diseases other than asthma such as rhinitis, so the study was limited to briefly show the change of hematological parameters due to allergy or not. Moreover key cytokines that mediate proinflammatory cell recruitment such as IL-4 and IL-5 were not measured due to lack of testing materials.

8. Conclusion and Recommendation

Hematological abnormalities are commonly encountered in asthma patients. Specifically, total leucocytes and its granulocytes subpopulation both absolute and percentages as well as the mean platelet volume were significantly higher in patients having asthma. The rise in erythrocyte sedimentation rate was remarkable in the patients group. Whereas the mononuclear cells lymphocytes and monocytes were lower in them. As a result, assessing hematological parameters in asthma patients comparatively with the healthy controls is useful for proper monitoring and management of asthma patients. The findings warrant that clinicians pay attention to the identified parameters to manage asthmatic patients for an improved health. In addition to this, it is important that MPV parameter is as an effective and inexpensive parameter for the diagnosis of asthma that the physicians should give an emphasis on it and this parameter might be used as a supplementary contribution for the diagnosis of asthma.

Health policymakers should prepare guideline for the management of hematological abnormalities among asthma patients. The physician would request a CBC test to investigate the hematological abnormalities among asthmatic patients and to treat patients accordingly. We recommend researchers for a further longitudinal study to assess the association of hematological abnormalities with different environmental and clinical features among asthma patients. The magnitude of hematological abnormalities and associated factors with allergic and non-allergic asthma will be investigated in the future.

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9. Annexes

Annex I: English Version of Participant Information Sheet

Addis Ababa University, College of Health Sciences, Department of Medical Laboratory Sciences

Title: Hematological abnormalities and its associated factors among asthmatic patients at Tikur Anbessa Specialized Hospital, Addis Ababa, Ethiopia

Introduction: This information sheet is prepared by the principal investigator to clarify the study that you are asked to take part in. If there is any unclarity before you decide to participate or not, you can ask freely.

Purpose: We have planned to conduct a study with the objective of Hematological abnormalities and its associated factors among asthmatic patients at Tikur Anbessa Specialized Hospital, Addis Ababa, Ethiopia.

Procedure: If you volunteer to participate, the data collectors will ask you some questions and collect about 5 ml blood for complete blood count analysis. The interview will take about 10 minutes. And the samples taken from you will be used only for research purposes. We will also review your medical records to get information related to your health status. Participation in this study is voluntary and you can stop your participation at any time. Your decision will not in any way affect the services you will get. We hope you will participate in the study since your information is important, and you can ask questions if you do not understand anything.

Confidentiality: Any information that we are going to collect about you during this research will be kept confidential. Your name and identity on the request paper will be changed to confidentiality code for the purpose of this study. Samples and information given by the participants will serve only for this research not for any other purpose.

Risks: There is no risk that you will face by participating in this study except for little discomfort during blood sample collection. However, experienced medical laboratory professionals will be used to collect blood.

Benefit: Findings from this study will be utilized to improve patient care by managers, administrators, and policy makers. So, you are indirectly benefiting yourself, other patients and

the society at large by involving into this quality related laboratory research. Most importantly, the outcome of the study will be beneficial to plan effective management strategies and design correct doses of antihistamines for the treatment of patients

Person to contact

Please direct any questions or problems you may encounter during this study to the principal investigator: Abdu Jemal, Department of Medical Laboratory Sciences

Tel: +251922119620 **email:** abdulghenijemal@gmail.com

Department of Medical Laboratory Sciences Research and Ethics Review Committee (Tel: 0112755170)

Annex II: Amharic Version of Participant Information Sheet

አዲስ አበባ ዩኒቨርስቲ ጤና ሳይንስ ኮሌጅ የህክምና ላቦራቶሪ ትምህርት ክፍል

ርዕስ፡ Assessment of hematological parameters among asthmatic patients at Tikur Anbessa Specialized Hospital, Addis Ababa, Ethiopia

መግቢያ፡ ይህ የመረጃ ወረቀት እንዲሳተፉበት የተጠየቁትን ጥናት ግልጽ ለማድረግ ሲባል በተመራማሪው በኩል ተዘጋጅቶ የቀረበ ጽሁፍ ነው። በጥናቱ ለመሳተፍ ወይም ላለመሳተፍ ከመወሰንዎ በፊት ማንኛውም ያልገባዎት ነገር ካለ በግልጽ መጠየቅ ይችላሉ።

የተሳታፊዎች ምስጢር ስለመጠበቅ

በዚህ ጥናት ስለእርስዎ የምንሰበስበው ማንኛውም መረጃ ሚስጥራዊነቱ ይጠበቃል።ለጥናቱ ሲባል በመጠየቂያው ወረቀት ላይ ያለውን የእርስዎ ስምም ሆነ ማንነት ወደ ሚስጥራዊ ቁጥር ይቀየራል። እንዲሁም የሰጡት ናሙናም ሆነ መረጃ ከዚህ ጥናት ውጪ ለሌላ አላማ ጥቅም አይውልም።

ጥቅም

ከዚህ ጥናት የሚገኘው ውጤት የህሙማንን ጤና ለማሻሻል፣በአስተዳዳሪዎች እና በመመሪያ አውጪዎች ጥቅም ላይ ይውላል። ስለዚህ እርስዎም ከላቦራቶሪ ውጤት ጥራት ጋር በተገናኘ የላቦራቶሪ ጥናታዊ ምርምር ላይ በመሳተፍዎ በቀጥታ እራስዎትን፣በተዘዋዋሪ ደግሞ ሌሎች ህሙማንን፣ እና በአጠቃላይ ህብረተሰቡን ይጠቅማል ማለት ነው።

ጥያቄ ካለዎት

ይህን ጥናት በተመለከተ ወይም ከዚህ ጋራ በተዛመደ መልኩ ስለሚያጋጥሙ ድንገተኛ ችግሮች ወይም ጥያቄ ካለዎት በሚከተለዉ አድራሻ ይጠቀሙ።

አብዱ ጀማል፣ ላቦራቶሪ ክፍል ላቦራቶሪ ቴክኖልጂስት።

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Annex III: English Version of Informed Consent Form

Consent form

I, the undersigned, confirm that, as I give consent to participate in the study, it is with a clear understanding of the objectives and conditions of the study and with recognition of my right to withdraw from the study if I change my mind. I give consent to include me in the proposed research. I have been given the necessary information about the research. I have also been assured that I can withdraw my consent at any time without penalty or loss of benefits. The proposal has been explained to me in the language I understand**.**

Name ----------

Signature: ----------

Annex IV: Amharic Version of Informed Consent Form

ስለ ጥናቱ ማረጋገጫ

እኔ ስሜ ከታች የተገለጸው የጥናቱ ተሳታፊ ለመሆን ስወስን የጥናቱ አላማ፣ አሰራሮች እና ቅድመ ሁኔታዎች በግልጽ በመረዳት እና ለጥናቱ ተሳታፊነት ፍቃደኝነቴን በማንኛውም ደረጃ የማንሳት መብቴን በማረጋገጥ ነው። በመሆኑም በጥናቱ ተሳታፊ ለመሆን ስወስን በጥናቱ ሳቢያ ሊከሰቱ የሚችሉ አደጋዎችን በሚገባ የተረዳሁና ከጥናቱ በማንኛውም ደረጃ እራሴን ለመሰረዝ ብወስን ተገቢ የሆኑ እገዛዎች ሁሉ እንደማይነፈጉኝ በማመን ነው። እነዚህን መረጃዎች ሁሉ በሚገባ በምረዳው ቋንቋ የተገለጸልኝ መሆኑን በፊርማዬ አረጋግጣለሁ።

ሙሉ ስም ---------- **ፊርማ**----------

Annex V: Laboratory materials and procedures

I. Hematological profile determination

The Materials used for hematological profile determination are hematological analyzer, syringe, EDTA test tube and personal protective equipment's (PEP). Reagents used are Unicel DxH 800(diluent, lyser, cleaner, diff pack), Unicell DxH 800 controls

Overview

The hematological profiles were determined by Unicel DxH 800 (BD coulter, Ireland) coulter system hematology analyzer. The UniCel® DxH 800 Analyzer is a quantitative, automated hematology analyzer for in vitro diagnostic use in screening patient populations found in clinical laboratories. The analyzer provides CBC, WBC 5-part differential, reticulocyte and nucleated RBC from a whole blood.

Principle of UniCel® DxH 800 (BD coulter, Ireland) analyzer

The DxH 800 CBC analysis is based on the *Coulter Principle*. The Coulter Method accurately counts and sizes cells by detecting and measuring changes in electrical resistance when a particle (such as a cell) in a conductive liquid pass through a small aperture. Each cell suspended in a conductive liquid (diluent) act as an insulator. As each cell goes through the aperture, it momentarily increases the resistance of the electrical path between the submerged electrodes on either side of the aperture. This causes a measurable electronic pulse. Then the number of pulse and height of pulse are directly proportional to the number and size of cells respectively. *VCS technology* (volume, conductive and light scatter analysis); WBC differential technology using three measurements: individual cell volume, high-frequency conductivity and laser-light scatter. The combination of low-frequency current, high-frequency current and light-scattering technology provided abundant cell-by-cell information that is translated by the sample processing module (SPM) into data plots. Conductivity analysis is when a current pass through the cell wall and each cell interior detects differences in the insulating properties of cell components. The current characterizes nuclear and granular constituents and the composition of the cell interior. Light scatter cell analysis measures particle size and refractivity to the angle of side scatter from a laser beam.

Hemoglobinometry; the lytic reagent used for the WBC prepares the blood so the system can count leukocytes and measure the amount of hemoglobin. The lytic reagent rapidly and simultaneously destroys the erythrocytes and converts a substantial proportion of the hemoglobin to a stable pigment while it leaves leukocyte nuclei intact. The absorbance of the pigment is directly proportional to the hemoglobin concentration of the sample. The WBCs, RBCs and platelet counts are measured by the coulter principle and relative and absolute counts 5-part differential counts are based on VCS technology. Hemoglobin is measured photometrically. Calculated values are derived from measured parameters.

Unicell DxH 800 complete blood count procedure

1. Collect venous blood specimen with EDTA tube and store properly
2. Ensure the specimen processing module (SPM) is set up for the appropriate test for your workflow
3. Mix the specimen
4. Load the specimens into the cassettes
5. Place the cassettes into the input buffer to the right of the SPM. The SPM automatically begins cycling the cassettes
6. After the SPM cycles the samples, review the sample results at the System Manager
7. Hematological parameters are displayed
8. Inspect for the presence of flags and print out the displayed result and record the result

II. ESR determination

The materials used in the study are Westergren tube, Westergren rack, syringe fitted with rubber, syringe and PPE. Reagents used for ESR determination is tri sodium citrate

Definition

ESR is described as a rate at which RBCs sediment in one hour. It is a common hematological test for nonspecific detection of inflammation that may be caused by infection, some cancers and certain autoimmune diseases.

Principle for ESR determination

When anticoagulated blood is allowed to stand in a narrow vertical glass tube, undisturbed for some time, the RBCs – under the influence of gravity- settle out from the plasma. The rate at

which RBCs settle is measured after one hour (in mm/hr) as the plasma is present at the top of the column.

Procedures of ESR determination

1. Collect venous blood from study participant (asthmatic patient)
2. Mix one part of blood with four parts of 3.1g/L trisodium citrate anticoagulant in a 1:4 ratio (1 part of blood with 4 part of anticoagulant).
3. Fit the syringe fitted with rubber
4. Draw the blood into the Westergren tube up to the '0' mark with the help of a syringe fitted with a rubber
5. Wipe out blood from the bottom of tube with cotton
6. Set the tube upright in the ESR stand. Make sure the pipette fits snugly to eliminate possible leakage and the pipette in a vertical position.
7. Leave the tube undisturbed for one hour
8. At the end of one hour, read the result and report in mm/hr.

Annex VI: Questionnaire

I. English version questionnaire

I am undertaking research to assess hematological abnormalities and its associated factors among asthmatic patients attending Tikur Anbessa Specialized Hospital. I want to ask some questions, all information you give will be strictly kept confidential and it will be only used for research purpose. Here is the questionnaire for the socio-demographic characteristics, clinical related variables, hematological parameters, stool examination and ESR. Please respond to the questions accordingly.

Participant Identification

Participant code _____ Year _____

Participants address (Sub city) _____ Telephone _____ signature_____

Data collector's name _____date _____signature_____

Part I. Questionnaire for sociodemographic characteristics and behavioral variables of the study participants

S/N	**Variables**		**Remarks**
001	Age (in years)	_____	
002	Sex	A. Male B. Female	
003	Religion	A. Orthodox C. Protestant B. Muslim D. Catholic E. Other	
004	Ethnicity	A. Amhara C. Tigrayan B. Oromo D. Other	
005	Residence	A. Urban B. Rural	
006	Name of the residence		
007	Marital Status	A. Married C. Divorced	

		B. Single D. Widowed	
008	Educational status	A. Cannot read and write B. Elementary school C. Secondary school D. College and above	
009	Occupational status	A. Employee B. Student C. Housewife D. Private	
010	Family size	_____	
011	Monthly income	_____	
012	Do you have a habit of smoking cigarette?	A. Yes C. Never B. Yes, previously	
013	Do you have a habit of doing exercise which ending in 30 minutes?	A. Yes B. No	
014	Do you have a habit of drinking alcohol?	A. Yes B. No	
015	What do you use for cooking	A. Charcoal B. Wood C. Electricity	

Part II; Questions for clinical related variables

S/N	Variables		Remark
016	How long did you live with asthma (in years)	_____?	
017	Do you have a family member with asthma?	A. Yes B. No	
018	What type of asthma exacerbation do you have?	A. Acute exacerbation B. Chronic exacerbation	
019	Severity of asthma	A. Mild B. Moderate C. Severe	
020	The severity of the diseases	A. Intermittent B. Mild Persistent C. Moderate persistent D. Sever persistent	
021	What type of symptoms do you have during asthma exacerbation? (more than one answer possible)	A. Wheezing B. Shortness of breath C. Runny/ watery nose D. Cough E. Other (specify)_____	
022	Do you have taken anti-asthmatic drugs within past 3 months?	A. Yes B. No	
023	If your answer is 'yes', list the type of drug you have been taking for last three months?	_____	

Part III; Laboratory examination results

S. NO.	Hematological parameters		Result	Remark
01	RBC parameters	RBC count ($\times 10^{12}$/L)		
		MCV (fl)		
		HCT (%)		
		RDW		
02	Hemoglobin parameters	Hb (g/dL)		
		MCH (pg)		
		MCHC (g/dL)		
03	WBC absolute counts ($\times 10^{9}$/L)	Total WBC count		
		Neutrophil		
		Lymphocyte		
		Monocyte		
		Eosinophil		
		Basophil		
04	WBC differential count (%)	Neutrophil		
		Lymphocyte		
		Monocyte		
		Eosinophil		
		Basophil		

05	Platelet parameters	PLT count ($\times 10^3$/ μL)		
		MPV		
		PDW		
06	ESR (mm/hr.)	ESR		

Name of data collector_____ Signature _____

Name of supervisor _____ Signature _____

Date of data collection_____

II. የአማርኛ ቃለመጠይቅ

ክፍል አንድ፡ የማህበራዊ እና በህሪ ነክ ጉዳዮች ቃለመጠይቅ

ተ.ቁ.	መጠይቅ	መልስ	ምርመራ
001	ዕድሜ	_____	
002	ጸታ	A. ወንድ B. ሴት	
003	ሀይማኖት	A. ኦርቶዶክስ C. ፕሮቴስታንት B. ሙስሊም D. ካቶሊክ E. ሌላ ----------	
004	ብሄር	A. አማራ C. ትግሬ B. ኦሮሞ D. ሌላ----------	
005	የመኖርያ ቦታ	A. ከተማ B. ገጠር	
006	የጋብቻ ሁኔታ	A. ያገባ/ች C. የፈታ/ች B. ያላገባ/ች D. በሏ የሞተባት	
007	የት/ት ደረጃ	A. ያልተማረ/ች C. ሁለተኛ ደረጃ B. አንደኛ ደረጃ D. ኮሌጅ እና ከዚያ በላይ	
008	የስራ ሁኔታ	A. የመንግስት ሠራተኛ B. ተማሪ C. የቤት-እመቤት D. የግል ሠራተኛ	
009	የቤተሰብ መጠን	_____(በቁጥር)	
010	ሲጋራ የማጨስ ልምድ አለዎት?	A. አዎ B. የለም C. አዎ ከዚህ በፊት	
011	በ 30 ደቂቃ የሚያልቅ የአካል	A. አዎ B. የለም	

	ብቃት እንቅስቃሴ የማድረግ ልምድ አለዎት?		
012	አልኮል የመጠጣት ልምድ አለዎት?	A. አዎ B. አልጠጣም	

ክፍል ሁለት፡ የክሊኒካል መረጃዎች ቃለመጠይቅ

ተ.ቁ.	መጠይቅ	መልስ	ምርመራ
013	በአስም በሽታ ከተያዙ ስንት አመት ሆኖዎት?	_____አመት	
014	ከቤተሰብ አባላት በአስም በሽታ የተያዛ አካል አለ?	A. አዎ B. የለም	
015	በአሁኑ ሰዓት የአስም ሕመምዎ በምን ሁኔታ ላይ ይገኛል?	A.አጣዳፊ (acute exacerbation) B. የቆየ (ስር የሰደደ) (chronic exacerbation)	
016	የበሽታዉ አስከፊነት	A. ዝቅተኛ B. መከካለኛ C. ከፍተኛ	
017	በሽታዉ ያለበት ደረጃ	A. አገርሺ/አልፎ-አልፎ (intermittent) B. በተወሰነ መልኩ ቀጣይነት ያለዉ (mild persistent) C. በመከካለኛ ሁኔታ ቀጣይነት ያለዉ (moderate persistent) D. በከፍተኛ ሁኔታ ቀጣይነት ያለዉ (severe persistent)	
018	በአሁኑ ጊዜ ምን አይነት የህመም ስሜት/ምልክት ይሰማዎታል?	A. የመተንፈስ ችግር B. ሳል C. ማቃሰት D. የአፍንጫ እርጥበት E. ሌላ	
019	ባለፉት 3 ወራት ዉስጥ	A. አዎ B. የለም	

	የአስም መድሃኒት ወስደዋል?		
020	በጥያቄ ቁ. 019 መሠረት መልስዎ አዎ ከሆነ የወሰዱትን የመድሃኒት አይነት ይግለጹ?		

Name of data collector_____ Signature _____

Name of supervisor _____ Signature _____

Date of data collection_____

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: **Abdu Jemal (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: **Mikias Negash (MSc, PhD)**

Signature: _____

Date: _____

Place: Addis Ababa, Ethiopia.

Advisor: **Dr Amsalu Bekele (MD, Pulmonologist)**

Signature: _____

Date: _____

Place: Addis Ababa, Ethiopia.