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Assessment of Hematological parameters among Adult Leprosy Patients at ALERT Hospital, Addis Ababa, Ethiopia

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This is to certify that the thesis prepared by Bontu Fikadu, entitled: **Assessment of Hematological parameters among Adult Leprosy patients at ALERT 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

Acknowledgements II

List of table VI

List of figure VII

List of Abbreviations VIII

Abstract IX

1. Introduction 1

1.1 Background 1

1.2 Statement of the Problem 4

1.3. Significance of the study 5

2. Literature review 6

3. Objectives 10

3.1 General Objective 10

3.2 Specific Objectives 10

4. Materials & Method 11

4.1. Study area 11

4.2. Study design & period 11

4.3. Population 11

4.3.1. Source Population 11

4.3.2. Study population 12

4.4. Eligibility Criteria 12

4.4.1. Inclusion Criteria 12

4.4.2. Exclusion Criteria 12

4.5. Study Variables 13

4.5.1. Dependent Variables 13

4.5.2. Independent variables 6
4.6. Sample size calculation and Sampling method 6
4.6.1. Sample size calculation 6
4.6.2. Sampling Method 15
4.7. Data collection Procedure 15
4.7.1. Questionnaire 15
4.7.2. Clinical Data Collection Procedures 16
4.7.3. Laboratory Data 16
4.7.4. Sample collection and Processing 16
4.7.5. Principles of hematology analyzer 17
4.8. Data Quality Assurance 17
4.8.1. Pre-analytical 17
4.8.2. Analytical 17
4.8.3. Post analytical 18
4.9. Data Analysis and Interpretation 18
4.10. Operational Definitions 18
4.11. Ethical Considerations 20
4.12. Dissemination Plan 20
5. Results 20
5.1 Socio-demographic and Clinical Characteristics of the Study Participants 21
5.2 Comparison of Hematological Parameters Among the Study Participants 23
5.3 Evaluation of certain red cell and white cell metrics 25
5.4 Determination of Hematological Parameters as Potential Markers 27
6. Discussion 30
7 Strength and Limitation of the Study 34

7.1 Strength 34
7.2 Limitation 34
8 Conclusion and Recommendation 34
8.1 Conclusion 34
8.2 Recommendation 34
9. References 35
10.ANNEXES 39
Annex I: Information Sheet (English and Amharic version) 39
A, Information Sheet (English version) 39
B. Information Sheet Amharic version 41
Annex II. Consent Form 43
Annex III: Questionnaire (English and Amharic version) 45
A. Questionnaire (English version) 45
B, Questioner Amharic Version 49
Annex IV: Venous Blood Sample Collection 53
Annex V: UniCel® DxH 800 Beckman Coulter automated hematology analyzer 58
Annex VI: Declaration 61

List of table

Table 1 Comparison of hematological parameters among cases and healthy control groups with previous studies..... 6

Table 2 Socio-demographic and clinical characteristics of leprosy cases and controls at ALERT Hospital, Addis Ababa, Ethiopia, January–May 2025..... 21

Table 3 Comparison of hematological parameters among patients with leprosy and controls at ALERT Hospital, Addis Ababa, Ethiopia, January–May 2025..... 23

Table 4 Comparison of hematological parameters among patients with leprosy subgroups and controls at ALERT Hospital, Addis Ababa, Ethiopia, January–May 2025..... 24

Table 5 Evaluation of certain red cell and white cell metrics among patients with leprosy and controls at ALERT Hospital, Addis Ababa, Ethiopia, January–May 2025..... 26

Table 6 Comparison of Hematological Parameters Between Non-Reaction and Reaction Groups 27

Table 7 Evaluation of potential markers of hematological parameters for leprosy reaction..... 29

List of figure

Figure 1.Potential of hematological parameters as markers of leprosy reaction 29

Figure 2 ROC curve of WBC..... 29

Figure 3 ROC curve of Neutrophil 29

List of Abbreviations

AFB Acid-fast bacilli

AUC Area Under the Curve

BB Borderline Borderline leprosy

BL Borderline Lepromatous leprosy

BT Borderline Tuberculoid

CBC Complete Blood Count

EDTA Ethylenediaminetetraacetic acid

ENL Erythema Nodosum Leprosum

Hct Hematocrit

Hgb Hemoglobin

LL Lepromatous Leprosy

MB Multibacillary

MCH Mean Corpuscular Hemoglobin

MCHC Mean Corpuscular Hemoglobin Concentration

MCV Mean Corpuscular Volume

MDT Multi Drug Therapy

NLR Neutrophil-to-Lymphocyte Ratio

PB Paucibacillary

PLT Platelet

RBC Red Blood Cell

RDW Red cell Distribution Width

T1R Type 1 Lepra Reaction

T2R Type 2 Lepra Reaction

WBC White Blood Cell

WHO World Health Organization

Abstract

Background: Leprosy is a chronic infectious disease caused by *Mycobacterium leprae*, primarily affecting the skin and peripheral nerves. Hematological assessments can provide insights into disease status and associated immune responses. However, data on the hematological profiles of leprosy patients in resource-limited settings such as Ethiopia remain scarce.

Objective: To assess hematological parameters among adult leprosy patients at ALERT Hospital, Addis Ababa, Ethiopia, from January to May 2025.

Methods: A hospital-based comparative cross-sectional study was conducted on 280 participants, including 140 leprosy patients (38 newly diagnosed, 42 with reactions, 60 on multidrug therapy[MDT]), and 140 controls, enrolled using a convenience sampling technique. Socio-demographic characteristics and clinical information were collected using a structured interviewer-administered questionnaire and patient records. Hematological parameters were measured using the UniCel DxH 800 Beckman Coulter automated hematology analyzer. Data were analyzed using the Statistical Package for the Social Sciences (SPSS) version 27.

Results: The majority (66.8%) of leprosy patients were male and aged between 31–50 years. Red blood cell count, hemoglobin, and hematocrit were significantly reduced in patients on MDT compared to newly diagnosed patients, patient with reactions and controls ($p<0.001$), while mean corpuscular volume was significantly higher. Hemoglobin and mean corpuscular hemoglobin concentration were also significantly lower in newly diagnosed patients and patient with reactions compared to controls. Red cell distribution width was elevated in patients on MDT and those with reactions ($p<0.001$). White blood cell count, absolute neutrophil and neutrophil-to-lymphocyte ratio were significantly higher in patient with reactions ($p<0.05$). White blood cell and neutrophil counts showed good potential as biomarkers for leprosy reaction, with areas under the curve (AUCs) of 0.790 and 0.778, respectively. WBC showed 71.4% sensitivity and 61.2% specificity, while neutrophil count showed 73.8% sensitivity and 73.5% specificity.

Conclusion: Leprosy patients showed significant hematological alterations, especially those on MDT and those with reactions. These findings suggest that routine hematological monitoring may help in the early detection of abnormalities and support better clinical management of patients.
Keywords: Leprosy, hematological profile, *Mycobacterium leprae,* Leprosy reaction

1. Introduction

1.1 Background

Leprosy is a long-term infectious condition caused by the bacterium *Mycobacterium leprae*. It was discovered in 1874 by Dr. Gerhard Armauer Hansen, a Norwegian physician, while investigating skin nodules in leprosy patients, leading to the disease being called Hansen's disease (1). *M. leprae* is an obligate intracellular bacterium that is acid-alcohol-fast, non-motile, and microaerophilic, with a slow multiplication rate of 12–14 days (2). Unlike most pathogens that thrive at 37°C, *M. leprae* prefers cooler areas of the body such as the skin, nasal mucosa, and ears. It primarily affects the skin, peripheral nerves, mucosal surfaces of the upper respiratory tract, and the eyes (3).

The transmission pathways of *M. leprae* are not fully understood. However, evidence suggests that individuals in close proximity to leprosy patients are at higher risk of infection, likely through infectious aerosols from coughing and sneezing, as well as possibly through direct contact (4). Based on the varying immune responses seen in patient lesions, leprosy is classified into five categories (5): tuberculoid (TT), characterized by a strong immune response; borderline tuberculoid (BT), where immune responses are unstable; borderline borderline (BB), with intermediate responses; borderline lepromatous (BL), showing signs of both immune and bacterial activity; and lepromatous (LL), associated with a weak immune response and high bacterial load (5). The WHO further categorizes leprosy into multibacillary (multiple skin lesions with demonstrated presence of bacilli in slit-skin smear) and paucibacillary (1–5 skin lesions with no demonstrated presence of bacilli in slit-skin smear) types based on the number of skin lesions and bacterial status (6).

The diagnosis of leprosy begins with a WHO-recommended physical examination, which identifies three key signs: (1) hypopigmented or erythematous skin lesions with loss of sensation, (2) thickened or enlarged peripheral nerves with sensory loss or muscle weakness, and (3) a positive acid-fast skin smear or biopsy (7). Laboratory diagnostic methods include the slit-skin smear test, where samples from areas like ear lobes or lesions are stained (Fite or Ziehl–Neelsen) to detect AFB (8). Leprosy is a treatable disease, with the current standard treatment involving a combination of three drugs: dapsone, rifampicin, and clofazimine, known as multi-drug therapy.

Treatment duration is six months for paucibacillary cases and twelve months for multibacillary cases. Early detection and timely treatment are crucial for preventing disabilities (9). One of the drugs used in MDT, dapsone, is known to induce hemolysis, which can impact hematological parameters (10).

Leprosy infection falls under chronic diseases. Similar to other chronic infectious diseases, leprosy infection can lead to anemia due to chronic disease, characterized by reduced RBC counts, hemoglobin levels, and hematocrit values below the normal range. In leprosy infection, particularly in BL and LL types, cytokines play a role in the pathogenesis of chronic disease anemia, namely interleukin-1 (IL-1) and tumor necrosis factor-alpha (TNF- α), which work by directly inhibiting erythropoiesis and disrupt iron metabolism (11). Reactional episodes exacerbate these changes, often accompanied by increased red cell distribution width (RDW) (12).

Although *M. leprae* prefers cooler temperatures, it can infect and survive for at least some time in deep tissues. *M. leprae* is, therefore, occasionally encountered in biopsies of the lymph node, liver, or bone marrow. In such cases, *M. leprae* infiltrates the marrow, causing granulomas infiltration that can lead to pancytopenia (low blood cell counts) and, in some cases, persistent anemia(13).

Throughout the course of chronic infection, patients often experience immune-mediated acute inflammatory episodes known as leprosy reactions, which are broadly classified into two main types. Type 1 reaction (T1R), also known as a reversal reaction, is characterized by a T-cell–mediated hypersensitivity response against *M. leprae*, particularly in the borderline spectrum of the disease. Following inoculation in the nasal mucosa, the immune response is initiated through phagocytosis by antigen-presenting cells (APCs). The APCs promote the differentiation of naïve T-cells into T helper 17 (Th17) cells under the influence of IL-6 and transforming growth factor-beta (TGF-β). Activated Th17 cells produce IL-17, facilitating neutrophil recruitment, while interferon-gamma (IFN-γ) activates macrophages, leading to the release of pro-inflammatory cytokines such as TNF-α, IL-1β, and IL-6 plays a key role in sustaining inflammation(14).

In contrast, Type 2 reaction (T2R), also known as erythema nodosum leprosum (ENL), is mediated by a type III hypersensitivity reaction resulting from inadequate clearance of antigen–antibody complexes. This leads to immune complex deposition, inflammation, and leukocyte chemotaxis. Neutrophils play a central role in ENL pathogenesis, with evidence showing increased levels of

low-density neutrophils (LDNs) and abundant formation of neutrophil extracellular traps (NETs), which are also implicated in severe inflammatory conditions. Additionally, the balance between anti-inflammatory IL-10 and pro-inflammatory TNF-α in neutrophils, along with Toll-like receptor 9 (TLR9) mediated recognition of DNA, represents promising biomarkers for ENL (15).

Hematological parameters act as potential markers for leprosy reactions by reflecting the acute systemic inflammation and immunological shifts occurring in the patient. The Neutrophil-to-lymphocyte ratio (NLR) is a valuable marker of systemic inflammation, reflecting the balance between innate and adaptive immunity. An elevated NLR may serve as an early indicator of immune activation and disease progression (16). Eosinophilia, often associated with hypersensitivity reactions, has emerged as a potential biomarker for predicting leprosy reactions. Histological lesions have shown prominent infiltration of eosinophils together with neutrophils, lymphocytes and plasmocytes lodged inside the deep layers of the dermis and subcutaneous tissue superimposed on chronic multibacillary leprosy (17).

Thrombocytosis, or elevated platelet counts, can occur as a reactive response to inflammation in leprosy, particularly during episodes of symptomatic ENL. Platelet counts often rise upon the initiation of treatment, with further increases observed in the first few weeks, typically returning to normal levels within a few weeks. IL-6, a proinflammatory cytokine, is believed to play a key role in this elevation, as it is associated with reactive thrombocytosis in ENL (18). However, gaps remain in understanding how specific hematological changes correlate with clinical manifestations across various forms of leprosy.

1.2 Statement of the Problem

Leprosy is an ancient chronic infectious disease that remains a significant public health issue, especially in many developing countries. Since the introduction of MDT in the 1980s, there has been a dramatic worldwide reduction in registered cases, from more than 5 million to 133,802 cases by the end of 2021 (19). Although considerable progress has been made toward global leprosy elimination, a substantial number of cases continue to occur annually in the African region (20). In 2024, 172,717 new leprosy cases were reported globally, with 19,171 (11%) originating from the African region. Ethiopia reported an increase of 128 more cases in 2024 compared to 2023. Ethiopia continues to be a key contributor to the regional leprosy burden, reflecting its challenges in eliminating the disease (4).

Beyond the infection itself, leprosy causes severe impairments, affecting not only the skin and peripheral nerves but also the hematopoietic, reticulo-endothelial, and endocrine systems (21). Leprosy patients commonly develop hematological complications due to its systemic nature and the chronic inflammatory response induced by *Mycobacterium leprae* (11). Common hematological abnormalities including decreased RBC count, hemoglobin, hematocrit, MCV, MCH, MCHC, platelet count, and lymphocyte count, whereas WBC count, neutrophil count, eosinophil count, NLR, and RDW are often increased, particularly during reactions (12,13,17). These hematological changes may provide useful indicators of disease activity and leprosy reactions. Hematological parameters obtained from routine complete blood count (CBC) tests may serve as simple and cost-effective indicators for identifying disease progression and reactions (22).

However, in Ethiopia, particularly at ALERT Hospital where a large number of leprosy patients receive treatment and follow-up care, evidence regarding hematological abnormalities among adult leprosy patients and their comparison with apparently healthy individuals remains limited. Moreover, data on the potential role of hematological parameters as markers of leprosy reactions are scarce. This lack of evidence limits the effective use of routine laboratory investigations in improving patient care and early detection of complications. Therefore, this study aimed to assess hematological parameters among adult leprosy patients compared with healthy controls and to identify potential hematological markers of leprosy reactions at ALERT Hospital, Addis Ababa, Ethiopia.

1.3. Significance of the study

This study aimed to assess hematological parameters among adult leprosy patients and evaluate their potential to identify leprosy reactions. These hematological parameters among leprosy patients are often under recognized in routine clinical management. Assessing hematological parameters is important for the early detection of complications, monitoring disease progression, identifying leprosy reactions, and improving the overall management and prognosis of leprosy patients. The findings of this study hold significant implications for policymakers and public health initiatives in Ethiopia by providing critical data on the hematological complications associated with leprosy. Furthermore, this study can serve as a reference for future studies on hematological alterations among leprosy patients.

2. Literature review

Leprosy affects hematological parameters as a result of both the chronic inflammatory response to *Mycobacterium leprae* and the side effects of treatment. The immune response to the infection stimulates the production of pro-inflammatory cytokines, which can influence bone marrow function and lead to alterations in blood cell production (23). The progression of hematological parameters in leprosy patients varies greatly, especially in those experiencing reactions; patients in a reactive state often undergo more severe and complex changes that require careful clinical management (17).

Several studies have investigated the hematological alterations associated with leprosy and its reactions in different populations. A cross-sectional study conducted in Brazil by Gomes et al. involving 123 leprosy patients (98 with reactions) found that the NLR was significantly higher in patients experiencing leprosy reactions than in those without reactions. This suggests that NLR could serve as a valuable indicator for leprosy reactions (24). Additionally, a retrospective study conducted by Barboza et al. in Brazil found that, out of 146 patients with BL and LL, 85% experienced reactional episodes, predominantly ENL. Treatment-naive patients who developed ENL had higher median neutrophil counts (4,567 cells/mm³) than those without reactions (3,731 cells/mm³). Neutrophil counts increased with ENL severity, peaking at 10,243 cells/mm³ in moderate/severe cases. Longitudinal assessments confirmed neutrophilic leukocytosis in ENL patients (8,408 cells/mm³ vs. 4,896 cells/mm³ for BL/LL), underscoring the role of neutrophils in the inflammatory processes associated with ENL in leprosy patients. These findings highlight the significant involvement of neutrophils and NLR in the immunological responses during leprosy reactions (25).

A case-control study conducted in Pakistan at the University of Karachi by Shaikh et al. assessed the hematological profile of 110 newly diagnosed, untreated leprosy patients. Clinical examination and slit-skin smear tests were used for diagnosis, and CBC analysis was performed. The results revealed no significant differences in most CBC parameters ($p>0.05$), except for a significant decrease in hemoglobin ($p<0.001$) and red blood cell (RBC) counts ($p<0.004$) in untreated leprosy patients compared to controls (26).

An observational study was conducted in India by Ambalia D, Vaishnani J, and Desai V assessed RDW values in leprosy patients and those experiencing leprosy reactions at a tertiary care center in India between July 2018 and December 2019. A total of 154 patients were enrolled, including 77 newly diagnosed leprosy patients and 77 individuals with other dermatological issues for comparison. The findings revealed that mean RDW was lowest in TT at 13.44 ± 1.14 and highest in T2R at 17.68 ± 2.23. RDW was significantly elevated in lepra reaction cases (17.09 ± 2.09) compared to leprosy cases without reactions ($p < 0.001$) and the comparison group ($p < 0.001$). The study suggests that monitoring RDW could be beneficial in assessing the inflammatory response in leprosy patients and highlights its prognostic role (12).

A retrospective study conducted at a tertiary care center in India by Vaishnani et al. analyzed hematological changes in patients with T2R. The study revealed significant findings, including lower hemoglobin (HGB) levels and elevated total white blood cell count (TWBC), red cell distribution width (RDW), and NLR, with these changes being more pronounced in patients experiencing severe T2R reactions. T2R is an immune-mediated inflammatory response in leprosy patients with a high bacterial load and involves cytokines such as TNF-α, interferon-gamma (IFN-γ), IL-1β, IL-17A, and IL-6, which influence bone marrow-derived cells and lead to alterations in peripheral blood cells. However, the study's small sample size was a key limitation, warranting further research with larger cohorts to validate these findings (27).

A cross-sectional study conducted by Gupta et al. in India, focusing on 32 lepromatous leprosy patients, reported statistically significant reductions ($p < 0.05$) in hemoglobin, platelet count, mean platelet volume, hematocrit, MCH, and MCHC. The study also confirmed the risk of dapsone-induced hemolysis and identified a broader range of hematological abnormalities, particularly in platelet counts and red cell indices. These findings highlight the importance of routine hematological assessments to detect and manage potential hemolysis in leprosy patients undergoing therapy (28).

A study conducted by Bhatt et al. in India evaluated hematological changes in leprosy patients undergoing MDT with dapsone, particularly focusing on hemoglobin fluctuations over three months and the factors influencing these changes. Among 48 patients, the mean Hb decreased from 13.37 g/dL at baseline to 12.08 g/dL at two months, subsequently increasing to 12.34 g/dL by three months. Of the 42 patients with decreased hemoglobin, 27.1% experienced severe (fall >20%),

35.4% moderate (fall 10–20%), and 25% mild (fall <10%) reductions. The study concluded that dapsone induces a maximal hemoglobin decrease of 1.29 g/dL at two months, after which levels start to recover, indicating that discontinuation of the drug is typically unnecessary due to the reversible nature of hemolysis (29). Similarly, a case-control study conducted in Nigeria by Halim NK and Ogbeide E, involving 76 patients, evaluated hematological alterations in leprosy patients treated with dapsone. The study found a significant decrease in hemoglobin levels and noted lymphocytosis both before and after therapy, further emphasizing the need for regular monitoring of blood parameters in these patients (30).

A cross-sectional study conducted in Southern Nigeria by Inyang, Bassey, and Okoroiwu, examined the hematological parameters of leprosy patients, comparing those undergoing MDT, those who had completed treatment, and healthy controls. The study revealed a higher prevalence of leprosy in males, with 40% of patients experiencing leprosy reactions. Key hematological findings included significantly lower red blood cell counts, hemoglobin, and hematocrit levels in leprosy patients compared to controls, while total white blood cell counts and leukocyte subsets (lymphocytes, neutrophils, monocytes, eosinophils) were elevated. Eosinophil counts were notably higher and identified as potential biomarkers for predicting leprosy reactions. Additionally, 65% of leprosy patients were anemic, with sex and absolute eosinophil counts showing significant associations with leprosy reactions. These findings offer valuable insights into the hematological profiles of leprosy patients and suggest that eosinophil counts may serve as predictive markers of leprosy reactions (17).

In addition to hematological investigations, immunological studies have also been conducted to better understand host responses to leprosy. A study at the ALERT, Addis Ababa examined immune responses in leprosy patients by comparing them with healthy controls. Using flow cytometry, researchers examined memory and effector T cells to see how immune responses differ between leprosy patients and healthy controls. Results showed that memory T cells (CD45RO+) on CD3+ cells and activated T cells (CD62L-) on CD4+ cells were significantly higher in PB patients than in MB patients. Additionally, MB patients had a greater number of CD3+ effector T cells compared to healthy controls, while PB patients exhibited significantly higher levels of CD3+, CD4+, and CD8+ effector memory cells than MB patients(31). However, such immunological studies are expensive and require advanced equipment, which can be a challenge

for developing countries like Ethiopia. Given these limitations, simple and affordable hematological tests offer a practical alternative for assessing health in leprosy patients.

Research specifically focusing on hematological parameters in leprosy patients in Ethiopia is limited, even though the country ranks as the second highest in leprosy incidence in Africa, following the Democratic Republic of Congo (32). While detailed studies from Ethiopia are scarce, it is essential to consider the broader context of hematological alterations associated with leprosy, which can be influenced by both the disease itself and its treatment. Therefore, this study aims to assess the hematological parameters of leprosy patients at ALERT Hospital in Addis Ababa, Ethiopia, providing valuable insights into the hematological profile of these patients and contributing to a better understanding of the disease's impact on blood health.

3. Objectives

3.1 General Objective

To assess hematological parameters among leprosy adult patients at ALERT Hospital, Addis Ababa, Ethiopia, from January to May 2025.

3.2 Specific Objectives

- To compare hematological parameters between adult leprosy patients and apparently healthy individuals at ALERT Hospital, Addis Ababa, Ethiopia.
- To determine hematological parameters as potential markers of leprosy reactions at ALERT Hospital, Addis Ababa, Ethiopia.

4. Materials & Method

4.1. Study area

The study was conducted at ALERT Comprehensive Specialized Hospital, located in Kolfe Keranyo sub-city, Addis Ababa, Ethiopia. It has **8°59'11.4"** North latitude and **38°42'43.92"** East Longitude, with an elevation of 2303 meters. ALERT was established in 1934 by the Sudan Interior Mission (SIM) as a leprosarium and later became the All-Africa Leprosy, Tuberculosis, and Rehabilitation Training Centre (ALERT). It is now a leading institution for leprosy healthcare which is recognized by WHO as an international leprosy training since 1988. Administration of ALERT was transferred to the Federal Democratic Republic of Ethiopia 's Ministry of Health in 2002. Renowned as the pioneer and the third-largest hospital in the nation by patient flow, ALERT serves over 1,500 patients daily and provides care to more than 450,000 patients annually. With nearly over 2000 permanent staff members and around 530 inpatient beds, the hospital offers a comprehensive range of services. The dermatology clinic is dedicated to providing holistic care for lifelong healthy skin and an enhanced quality of life. Its services encompass a comprehensive range of specialized care, including evaluation, diagnosis, management, and treatment of both common and complex skin conditions for adults and children. ALERT Hospital provides dedicated services for leprosy patients through the Red Medical Clinic (RMC), providing follow-up care to over 700 patients annually. ALERT Hospital also implements the WHO-recommended MDT regimen. ALERT hospital laboratory comprises key areas, including microbiology, hematology and immunohematology, clinical chemistry and molecular laboratory, side laboratory, laboratory management, and specimen and data management (33).

4.2. Study design & period

A hospital-based comparative cross-sectional study was conducted at ALERT Hospital from January to May 2025.

4.3. Population

4.3.1. Source Population

Leprosy group: All leprosy patients attending ALERT Hospital.

Control group: All apparently healthy adults working or studying at AHRI, ALERT Hospital, Addis Ababa University College of Health Sciences and patient attendants.

4.3.2. Study population

Case group

All leprosy patients who visited ALERT Hospital during the study period, met the eligibility criteria, and volunteered to participate in the study were included.

Comparison groups

All apparently healthy individuals from senior staff and students of AHRI and ALERT Hospital, staff members of Addis Ababa University College of Health Sciences, and patient attendants, who met the eligibility criteria and volunteered to participate in the study were included.

4.4. Eligibility Criteria

4.4.1. Inclusion Criteria

For Leprosy group

- Age between 18 – 60.
- Newly diagnosed patients, Patients on treatment, and those with reaction.
- Individuals willing to provide informed consent and participate in the study.

For control group

- Age between 18 – 60.
- Apparently healthy individuals without any clinical signs of illness.
- Free from known acute and chronic hematological disorder.
- Willing to participate.

4.4.2. Exclusion Criteria

For Leprosy group

- Patients who had completed MDT and were not experiencing reactions
- Pregnancy

- Patients currently taking medications for other chronic diseases
- Patients with a history of alcohol consumption, smoking, or khat chewing as well as those who recently started

For control group

- Pregnancy
- Current use and history of drug that affect blood cell or clotting
- Individuals with a history of chronic skin or nerve-related diseases (not due to injury)
- Individuals with any acute and chronic disease.
- feeling sick on the day of sample collection, taking any antibiotics, or pain reliever medication
- Individual who have a history of alcoholism and smoking as well as those who recently started

4.5. Study Variables

4.5.1. Dependent Variables

Hematological parameters:

- Red blood cell (RBC) count ($\times 10^{12}$/L)
- Hemoglobin (Hgb) concentration (g/dL)
- Hematocrit (Hct) (L/L)
- Mean corpuscular volume (MCV) (fL)
- Mean corpuscular hemoglobin (MCH) (pg)
- Mean corpuscular hemoglobin concentration (MCHC) (g/dL)
- Red cell distribution width (RDW) (%)
- White blood cell (WBC) count ($\times 10^9$/L)
- Absolute neutrophil count ($\times 10^9$/L)
- Absolute lymphocyte count ($\times 10^9$/L)
- Absolute monocyte count ($\times 10^9$/L)
- Absolute eosinophil count ($\times 10^9$/L)
- Absolute basophil count ($\times 10^9$/L)
- Neutrophil-to-lymphocyte ratio (NLR)
- Platelet count ($\times 10^9$/L)
- Mean platelet volume (MPV) (fL)

4.5.2. Independent variables

- Type of leprosy
- Age of the patient
- Sex of the patient
- Treatment status

4.6. Sample size calculation and Sampling method

4.6.1. Sample size calculation

The sample size was calculated using the double population mean difference formula, based on the hemoglobin values for leprosy cases and controls. The two population means of hemoglobin are assumed to differ by 0.52 g/dL, with standard deviations of 1.44 for cases and 1.65 for controls (31). The sample size is calculated for various hematological parameters, and The feasible sample size was chosen according to the study period (12).

Table 1 Comparison of hematological parameters among cases and healthy control groups with previous studies at ALERT.

Parameter	Cases (Mean ± SD)	controls (Mean ± SD)
RBC ($\times 10^{12}$/L)	5.13 ± 0.36	5.08 ± 0.59
WBC ($\times 10^{9}$/L)	5.92 ± 1.49	5.61 ± 1.74
Hemoglobin (g/dL)	15.38 ± 1.44	15.90 ± 1.65
Platelets ($\times 10^{9}$/L)	277.2 ± 49.2	268.7 ± 57.7

The formula for calculating the sample size is:

$$n \geq \frac{(Z_{1-\frac{\alpha}{2}} + Z_{1-\beta})^2 . (\sigma_1^2 + \sigma_1^2)}{(M_1 - M_2)^2}$$

$$n \geq \frac{(1.96 + 0.84)^2 . (1.44^2 + 1.65^2)}{(15.90 - 15.38)^2}$$

$$n = \frac{7.84 \cdot (2.0736 + 2.7225)}{(0.2704)}$$

$$n = \frac{37.6014}{0.2704} = 139.06$$

$$n \approx 140.0$$

Thus, the required sample size was approximately 140 participants for each group (leprosy cases and controls).

Where:

- n= number of samples for each group
- $Z_{1-\alpha/2}$ = 1.96 (for a 5% significance level)
- $Z_{1-\beta}$ = 0.84 (for 80% power)
- M_1 = 15.38 g/dL (mean hemoglobin in leprosy cases)
- M_2 = 15.90 g/dL (mean hemoglobin in controls)
- σ_1 = 1.44 (standard deviation for leprosy cases)
- σ_2 = 1.65 (standard deviation for controls)

4.6.2. Sampling Method

Non probability, convenience sampling method was employed to select leprosy patients attending ALERT Hospital during the study period based on their availability and willingness to participate. Controls were selected using age- and sex-matching from apparently healthy individuals.

4.7. Data collection Procedure

4.7.1. Questionnaire

A structured pre-tested interviewer-administered questionnaire was used to collect socio-demographic and clinical data of the participants. It included information on socio-demographic characteristics such as age, sex, and occupation. Clinical characteristics such as treatment status, duration of MDT and presence of deformities. The questionnaire was prepared in two languages, English and Amharic, and the Amharic version was used to collect all data from the participants **(Annex III).**

4.7.2. Clinical Data Collection Procedures

Clinical data were collected through patient examination and medical record review by senior dermatologists at ALERT hospital. A thorough physical examination was performed to assess skin lesions in terms of size, type, number, and distribution. Leprosy diagnosis was based on cardinal signs, including hypopigmented or erythematous skin lesions with sensory loss, thickened peripheral nerves with sensory or motor impairment, and/or a positive acid-fast skin smear or biopsy to identify new cases. Clinical assessment was used to classify reaction status, where patients with reaction presented with acute inflammation such as redness, swelling, pain, neuritis, or systemic symptoms, while patients without reaction showed stable clinical conditions without acute changes. Deformities were documented, and severity grading based on sensory and functional assessment was performed by physiotherapists at ALERT Hospital. Neurological examination was conducted to evaluate peripheral nerve involvement using palpation and sensory testing to detect nerve thickening, tenderness, and loss of sensation.

4.7.3. Laboratory Data

Laboratory data were collected by trained laboratory technologists and the principal investigator, following standard operating procedures. Patients were welcomed and informed about all procedures involved in collecting laboratory data. Participants who fulfilled the inclusion criteria had blood samples withdrawn for hematological examinations. Blood samples were collected from both leprosy patients and healthy control individuals to assess hematological parameters. The results were reviewed and recorded by the principal investigator to ensure accuracy and reliability.

4.7.4. Sample collection and Processing

Venous blood was collected for a CBC at RMC by first disinfecting the patient's median cubital vein using a swab soaked in 70% alcohol, moving from the center outward to prevent contamination. A sterile needle was then inserted into the vein, and about 2–3 ml of blood was drawn into an ethylenediaminetetraacetic acid (EDTA) tube containing anticoagulant to prevent clotting. Once collected, the tube was gently inverted 8–10 times to ensure the blood mixed properly with the EDTA. The sample was processed at the hematology laboratory of ALERT Hospital using a calibrated Unicel DxH800 Beckman-Coulter automated hematology analyzer, which automatically measured various hematological parameters including WBC, RBC, HB,

HCT, MCV, MCH, MCHC, WBC differential count, RDW, PLT, and PLT indices. The results were then reviewed and recorded for further analysis

4.7.5. Principles of hematology analyzer

The UniCel DxH 800 Beckman Coulter automated hematology analyzer counts and sizes cells in whole blood using the Coulter Principle, which detects cells as they pass through an electrical aperture. In this method, each cell suspended in a conductive diluent acts as an insulator; as it passes through an aperture between electrodes, it momentarily increases electrical resistance, producing a measurable pulse. The number of pulses corresponds to cell count, while pulse amplitude reflects cell volume. For hemoglobin measurement, a lytic reagent lyses red blood cells and converts hemoglobin to a stable pigment, which is measured photometrically at 525 nm. The Coulter Volume, Conductivity, and Scatter (VCS) technology further enables white blood cell differential. Prepared samples are delivered to a flow cell where a diode laser measures light scatter and absorption, alongside cell volume and conductivity. These raw data are processed by the system algorithms to identify distinct cell populations, calculate parameter values, generate histograms, flags, and data plots, ensuring accurate and comprehensive hematological analysis (34).

4.8. Data Quality Assurance

4.8.1. Pre-analytical

To ensure the quality of the data, training was provided to data collectors, and socio-demographic information was collected using a structured interviewer-administered questionnaire. Standard operating procedures were strictly followed during specimen collection. The quality of the samples was maintained by adhering to the sample rejection criteria of the ALERT Hematology Laboratory.

4.8.2. Analytical

Commercially available low, normal, and high quality control reagents (COULTER® 6C Cell Control) were used to assess the reliability (accuracy and precision) of the data generated by the hematology analyzer.

4.8.3. Post analytical

The accuracy and completeness of the data were checked daily by the principal investigator. The data were cleaned, coded, and correctly entered into the Excel.

4.9. Data Analysis and Interpretation

Data were entered into Microsoft Excel and analyzed using SPSS version 27 (IBM Corp., USA). Continuous variables were presented as mean ± standard deviation for normally distributed data and median with interquartile range for non-normally distributed data, while categorical variables were expressed as frequencies and percentages. Normality was assessed using the Shapiro-Wilk test. Differences among controls and leprosy subgroups (newly diagnosed, on MDT, and with reaction) were evaluated using ANOVA for normally distributed variables (RBC, HGB, HCT) and the Kruskal-Wallis test for other non-normally distributed variables, with post hoc tests performed to identify specific group differences. Post hoc comparisons were performed using Dunn–Bonferroni test for Kruskal–Wallis analysis and Tukey HSD for ANOVA. Effect sizes were calculated using eta squared (η^2). Comparisons of hematological parameters between patients with and without leprosy reactions were performed using independent t-tests for normally distributed variables and Mann-Whitney U tests for non-normally distributed variables. The potential of selected hematological markers as indicators of leprosy reactions was evaluated using receiver operating characteristic (ROC) curves. The area under the curve (AUC) and Youden's J index were used to determine optimal cut-offs. A p-value <0.05 was considered statistically significant.

4.10. Operational Definitions

Anemia: Hemoglobin <13 g/dL in males and <12 g/dL in females(35).

Elevated RDW: RDW-CV >15.0%.

Hypochromia: MCH <27.45 pg and MCHC <32.41 g/dL.

Microcytosis: MCV <78.74 fL.

Macrocytosis: MCV >97.03 fL.

Leukocytosis: WBC count >10.1 × 10^9/L.

Leukopenia: WBC count <3.0 × 10^9/L.

Thrombocytosis: Platelet count >414 × 10^9/L.

Thrombocytopenia: Platelet count <149 × 10^9/L.

The hematological cut-off values were based on determined reference intervals from a study of apparently healthy adults in Addis Ababa (36).

The following are based on the Ethiopian National Guidelines Leprosy (37).

Case: An individual diagnosed with leprosy, confirmed through clinical assessment and laboratory tests, receiving treatment at Alert Hospital. This group may include patients exhibiting a range of clinical manifestations associated with the disease.

Patient with Reaction: patient experiencing sudden onset of symptoms such as redness, swelling, or pain in skin lesions or nerves. This includes episodes of inflammation, tenderness, fever, or other systemic features like neuritis.

Patient without Reaction: patient with stable symptoms, showing no new swelling, redness, or pain in skin lesions or nerves. Existing skin patches and nerve issues remain unchanged, with no signs of sudden worsening or flare-ups.

Control: Apparently healthy individuals, matched by age and sex to the leprosy patients for comparison of hematological parameters.

Grade 0 deformities: No disability found. This means there is no loss of sensation or visible deformity or damage.

Grade 1 deformities: There is loss of sensation in the palm of the hand or sole of the foot, but no visible deformity or damage.

Grade 2 deformities: There is visible damage or disability due to leprosy. This includes weakness or paralysis of muscles on the hands and feet, wounds and ulcers as well as visible deformities such as a foot drop or a claw hand or absorption of fingers.

Multibacillary leprosy: characterized by more than five skin lesions (6).

Paucibacillary leprosy: characterized by 1–5 skin lesions (6).

4.11. Ethical Considerations

The study was reviewed and approved by the Departmental Research and Ethics Review Committee (DRERC) of the Department of Medical Laboratory Sciences, College of Health Sciences, Addis Ababa University (Ref. No. DRERC/789/24/MLS). An official letter of request was sent to ALERT Hospital, followed by submission to the ALERT Ethics Committee, to obtain permission and ethical approval to collect samples and conduct hematological analysis in the hematology laboratory. Each study subject was introduced to the purpose, procedures, potential risks, and benefits of the study, and written informed consent was obtained prior to participation. Clinical data obtained were kept only for the purpose of this study. Data confidentiality was maintained by avoiding personal identifiers during data collection.

4.12. Dissemination Plan

The findings of this study will be presented to Addis Ababa University, Department of Medical Laboratory Sciences. The findings will be communicated to the hospital governing body through reports, direct mailing, conferences, seminar presentations, and workshops. Peer-reviewed national or international journals will be considered for the publication of the findings of this study.

5. Results

5.1 Socio-demographic and Clinical Characteristics of the Study Participants

Out of 280 participants, 140 were leprosy cases and 140 were controls. The cases consisted of 38 newly diagnosed patients, 60 on MDT, and 42 with reaction. The majority of participants across all groups were aged 31–50 years. Males constituted the majority (66.8%) of the cases. The majority of cases were farmers (28.7%) and unemployed (21.6%). Clinically, Multibacillary leprosy was the predominant type (93.0%) across all groups. Grade 1 deformity was the most common among leprosy patients (56.4%). The mean duration of MDT was 6.95 ± 3.3 months. Among reaction cases, Type 2 reaction was more common than Type 1 reaction **(Table 1)**.

Table 2 Socio-demographic and clinical characteristics of leprosy cases and controls at ALERT Hospital, Addis Ababa, Ethiopia, January–May 2025.

Variable	Newly diagnosed patients (n=38)	Patients on MDT (n=60)	Patients with Reaction (n=42)	Cases total (n = 140)	Controls (n=140)
Age (years)					
Mean(SD)	41.8±12.32	37.12±13.11	34.43±11.519	37.63 ± 12.57	35.50 ± 10.67
Age group (years)					
18-30	7(18.4)	23(38.3)	18(42.9)	48 (34.3)	58(41.4)
31-50	23(60.5)	27(45.0)	21(50.0)	71 (50.7)	67(47.9)
≥51	8(21.1)	10(16.7)	3(7.1)	21 (15.0)	15(10.7)
Sex					
Female	15(39.5)	16(26.7)	14(33.3)	45 (32.1)	46(32.9)
Male	23(60.5)	44(73.3)	28(66.7)	95 (67.9)	94(67.1)
Marital status					
Married	22(57.9)	36(60.0)	24(57.1)	82 (58.6)	73(52.1)
Unmarried	10(26.3)	20(33.3)	15(35.7)	45 (32.1)	65(46.4)
Divorced	2(5.3)	4(6.7)	1(2.4)	7 (5.0)	2(1.4)
Widowed	4(10.5)	0(0.0)	2(4.8)	6 (4.3)	0(0.0)
Residence					
Rural	21(55.3)	14(23.3)	10(23.8)	45 (32.1)	14(10.0)
Urban	17(44.7)	46(76.7)	32(76.2)	95 (67.9)	126(90.0)
Occupation					
Farmer	15(39.5)	10(16.7)	3(7.1)	28 (20.0)	12 (8.6)
Merchant	4(10.5)	11(18.3)	9(21.4)	24 (17.1)	3(2.1)
Student	1(2.6)	4(6.7)	6(14.3)	11 (7.9)	6(4.3)
Governmental employee	4(10.5)	8(13.3)	2(4.8)	14 (10.0)	110(78.6)

Non- governmental employee	5(13.2)	9(15.0)	11(26.1)	25 (17.9)	3(2.1)
Unemployed	9(23.7)	18(30.0)	11(26.2)	38 (27.1)	6(4.2)
Educational level					
Cannot read or write	19(50.0)	19(31.7)	12(28.6)	50 (35.7)	6(4.2)
College or University	0(0.0)	7(11.7)	3(7.1)	10 (7.1)	105(75.0)
Elementary school	17(44.8)	24(40.0)	25(59.5)	66 (47.1)	12(8.6)
Preparatory school	2(5.3)	10(16.7)	2(4.8)	14 (10.0)	17(12.1)
Type of leprosy (WHO classification)					
Paucibacillay	3(7.9)	5(8.3)	2(4.8)	10 (7.1)	NA
Multibacillary	35(92.1)	55(91.7)	40(95.2)	130 (92.9)	
Grade of deformity					
Grade 0	2(5.3)	20(33.3)	11(26.2)	33 (23.6)	NA
Grade 1	28(73.7)	29(48.3)	22(52.4)	79 (56.4)	
Grade 2	8(21.1)	11(18.3)	9(21.4)	28 (20.0)	
Duration on MDT (months)					
Mean (SD)	NA	6.95 ± 3.3	NA	NA	NA
Range		1 - 12			
Type of Reaction					
Type 1	NA	NA	16 (38.1)	NA	NA
Type 2			26 (61.9)		

Notes: Values are presented as frequency (%). mean ± standard deviation, Range in months.

Abbreviations: MDT, Multidrug Therapy; NA, Not Applicable; SD, standard deviation.

5.2 Comparison of Hematological Parameters Among the Study Participant

Compared with controls, leprosy patients had significantly lower RBC count, hemoglobin concentration, hematocrit, MCHC, and lymphocyte count ($p < 0.05$). In contrast, WBC count, neutrophil count, MCV, RDW and NLR were significantly higher among leprosy patients than controls ($p < 0.05$) (Table 2).

Table 3 Comparison of hematological parameters among patients with leprosy and controls at ALERT Hospital, Addis Ababa, Ethiopia, January–May 2025.

Parameter	Case (n = 140)	Control (n = 140)	P-Value
RBC (×10¹²/L)	4.69 ± 0.70*	5.24 ± 0.49	**<0.001**
Hemoglobin (g/dL)	13.61 ± 1.86*	16.03 ± 1.38	**<0.001**
Hematocrit (%)	42.57 ± 5.74*	46.49 ± 3.87	**<0.001**
MCV (fL)	90.35 (86.75–95.90)	88.80 (86.65–91.60)	**0.002**
MCH (pg)	29.60 (27.50–30.90)	30.75 (30.05–31.50)	0.121
MCHC (g/dL)	32.40 (30.50–33.30)	34.40 (34.10–35.00)	**<0.001**
RDW (%)	13.90 (13.15–14.70)	13.30 (12.90–13.90)	**<0.001**
WBC	6.70 (5.16–8.29)	5.70 (4.20–7.40)	**<0.001**
NEU#	4.44 (3.35–6.20)	3.80 (2.45–5.30)	**<0.001**
LYM#	1.07 (0.60–1.70)	1.30 (1.10–1.60)	**0.007**
MONO#	0.41 (0.30–0.62)	0.50 (0.40–0.60)	0.195
EOS#	0.10 (0.05–0.37)	0.10 (0.10–0.20)	0.658
BAS#	0.00 (0.00–0.02)	0.00 (0.00–0.00)	0.77
PLT	256.50 (216.00–316.00)	246.00(205.50–290.50)	0.095
MPV	9.35 (8.55–10.40)	8.80 (8.30–9.60)	0.086
NLR	3.67 (2.36–10.31)	2.71 (1.79–4.04)	**<0.001**

Notes: *mean ± SD

Abbreviations: RBC, red blood cell; HGB, hemoglobin; HCT, hematocrit; WBC, white blood cell count; RDW, red cell distribution width; MCV, mean cell volume; MCH, mean cell hemoglobin; MCHC, mean cell hemoglobin concentration; MPV, mean platelet volume; N/L ratio, neutrophil-to-lymphocyte ratio.

5.3 Comparison of Hematological Parameters Among the Sub groups of Leprosy

Significant differences were observed among the study groups in RBC, HGB, HCT, MCV, MCHC, RDW, WBC, neutrophil count, neutrophil-to-lymphocyte ratio and monocyte count. HGB and MCHC were significantly lower in all leprosy categories (New case, MDT, and reaction) than in the controls ($p < 0.05$). RBC and HCT values were significantly lower in the MDT group

compared with the other study groups and controls ($p < 0.05$), while new case, reaction, and control groups showed comparable values. MCV in the MDT group was significantly higher than in all other groups ($p < 0.05$). RDW values were significantly higher in the MDT and reaction groups compared with controls ($p < 0.05$), whereas new cases were comparable with controls.

WBC counts, neutrophil counts, and the N/L ratio were significantly higher in the reaction group compared with the control and other study groups ($p < 0.05$), while the new case and MDT groups showed comparable values with the control group. Lymphocyte and monocyte counts were significantly lower in the MDT group compared with the control group ($p < 0.05$), whereas the new case and reaction groups showed comparable values to each other and to the controls (Table 3).

Table 4 Comparison of hematological parameters among patients with leprosy subgroups and controls at ALERT Hospital, Addis Ababa, Ethiopia, January–May 2025.

Variable	Newly diagnosed patients (n=38)	Patients on MDT (n=60)	Patients with Reaction (n = 42)	Control (n=140)	P-value
RBC(×10¹²/L)	5.07± 0.63[a,b]	4.35±0.51[c]	4.85± 0.77[a]	5.24± 0.49[b]	<0.001
HGB (g/dl)	14.28± 1.55[a]	13.12± 1.52[a]	13.70±2.33[a]	16.03±1.38[b]	<0.001
HTC(%)	44.71±5.66[a,b]	41.00± 4.71[c]	42.86±6.56[a,c]	46.49±3.87[b]	<0.001
MCV (fl)	88.25(85.80-90.30)[a]	94.40(91.60-98.28)[b]	87.80(85.25-92.38)[a]	88.80(86.63-91.60)[a]	<0.001
MCH (pg)	27.95(26.60-29.90)	30.40(28.55-32.10)	28.55(27.53-30.00)	30.75(30.03-31.50)	0.094
MCHC (g/dL)	32.55(30.50-33.50)[a]	32.40(30.45-33.08)[a]	31.85(30.50-33.40)[a]	34.40(34.10-35.00)[b]	<0.001
RDW (%)	13.70(13.00-14.70)[a,b]	13.90(13.20-14.70)[b]	14.00(13.18-14.70)[b]	13.30(12.90-13.90)[a]	<0.001
WBC	6.00(5.57-7.20)[a]	5.45(4.30- 6.97)[a]	9.00(6.52- 12.08)[b]	5.70(4.20- 7.40)[a,c]	<0.001
NEU#	4.42(3.27-5.70)[a]	3.73(2.73-4.80)[a]	6.88(4.05-9.51)[b]	3.80(2.63-5.30) [a]	<0.001
LYM#	1.55(0.98-1.80)[a,b]	0.97(0.23-1.62)[a]	1.06(0.60-1.56)[a,b]	1.30(1.10-1.60)[b]	0.002
MONO#	0.45(0.32-0.63)[a,b]	0.33(0.30-0.47)[a]	0.53(0.37-0.70)[b]	0.40(0.30-0.50)[b]	0.012

EOS#	0.17(0.07-0.32)	0.17(0.07-0.32)	0.09(0.01-0.20)	0.10(0.10-0.20)	0. 191
BASO#	0.00(0.00-0.01)	0.00(0.00-0.02)	0.01(0.00-0.02)	0.00(0.00-0.00)	0.074
NLR	3.64(2.24-5.20)[a,b]	3.13(1.92-9.50)[b,c]	6.00(2.92-13.80)[c]	2.71(1.78-4.05)[a]	<0.001
PLT	282.00(242.00-381.00)	246.00(195.25-284.75)	272.50(229.25-311.00)	246.00(204.75-291.25)[b]	0.302
MPV(fl)	8.70(8.18-10.20)	9.50(8.73-10.40)	9.10(8.10-11.00)	8.80(8.30-9.60)	0.417

Notes: mean ± SD for normally distributed variables and median (IQR) for non-normally distributed variables. [a], [b], and [c] represent group comparisons based on post hoc analysis. Values sharing at least one superscript letter are not significantly different, while values with different superscript letters differ significantly based on post hoc analysis. P-values were obtained using one-way ANOVA for normally distributed variables and Kruskal-Wallis test for non-normally distributed variables

Abbreviations: RBC, red blood cell; Hgb, hemoglobin; Hct, hematocrit; WBC, white blood cell count; RDW, red cell distribution width; MCV, mean cell volume; MCH, mean cell hemoglobin; MCHC, mean cell hemoglobin concentration; MPV, mean platelet volume; N/L ratio, neutrophil-to-lymphocyte ratio; MDT, multidrug therapy; g/dL, gram per deciliter; fL, femtoliter; pg, picogram.

5.4 Evaluation of certain red cell and white cell metrics

Using the WHO criteria for anemia (Hgb <12 g/dL for females and <13 g/dL for males), anemia was observed in, 5.71% of controls, 7.9% of newly diagnosed patients, 35.0% of patients on MDT, and 28.6% of patients with reaction. Based on local reference range, hypochromia defined by low MCH (<27.5 pg) was present in 4.3% of controls, 28.9% of newly diagnosed patients, 10.0% of MDT patients, and 21.4% of reaction cases. Similarly, low MCHC (<32.4 g/dL) was detected in 0.7% of controls, 5.3% of newly diagnosed patients, 11.7% of MDT patients, and 11.9% of reaction cases. Macrocytosis (MCV >97.0 fL) was more frequent in MDT patients (15.0%) and reaction cases (7.1%) compared to newly diagnosed patients (0.0%) and controls (0.7%). Anisocytosis was highest in the reaction group (11.9%), followed by MDT (10.0%), controls

(3.6%), and was absent in new cases (5.2%) ($p < 0.05$). Leukocytosis (WBC >10.1×10⁹/L) was markedly higher in patients with reaction (45.2%) compared to newly diagnosed (7.9%), MDT (1.7%), and controls (1.4%).

Table 5 Evaluation of certain red cell and white cell metrics among patients with leprosy and controls at ALERT Hospital, Addis Ababa, Ethiopia, January–May 2025.

Variable	Newly diagnosed leprosy patients n(%)	Patients on MDT n(%)	Patients with Reaction n(%)	Control subjects n(%)	χ^2	p-value
Anemia (Hb<12*/13º g/dL)	3 (7.9)	21(35.0)	12 (28.6)	8 (5.71)	53.481	< 0.001
No anemia	35 (92.1)	39 (65.0)	30 (71.4)	132 (94.3)		
Low MCH (MCH <27.5 pg)	11 (28.9)	6 (10.0)	9 (21.4)	6 (4.3)	22.848	< 0.001
Normal MCH	27 (71.1)	54 (90.0)	33 (78.6)	134 (95.7)		
Hypochromia (MCHC<32.4g/dL)	2 (5.3)	7 (11.7)	5 (11.9)	1 (0.7)	17.204	< 0.001
Normal MCHC	36 (94.7)	53(88.3%)	37 (88.1)	139 (99.3)		
Microcytosis (MCV < 78.7 fL)	2 (5.3)	2 (3.3)	4 (9.5)	4 (2.9)	3.727	0.292
No microcytosis	36 (94.7)	58 (96.7)	38 (90.5)	136 (97.1)		
Macrocytosis (MCV >97.0 fL)	0 (0.0)	9 (15.0)	3 (7.1)	1 (0.7)	21.861	< 0.001
No macrocytosis	38 (100.0)	51 (85.0)	39 (92.9)	139 (99.3)		
Anisocytosis (RDW > 15%)	2 (5.2)	6 (10.0)	5 (11.9)	5 (3.6)	8.529	0.036
Normal RDW	36 (94.8)	54 (90.0)	37 (88.1)	135 (96.4)		
Leukocytosis (WBC>10.1×10⁹/L)	3 (7.9)	1 (1.7)	19 (45.2)	2 (1.4)	81.723	< 0.001
No leukocytosis	35 (92.1)	59 (98.3)	23 (54.8)	138 (98.6)		
Leukopenia (WBC<3.0×10⁹/L)	3 (7.9)	10(16.7)	0 (0.0)	20(14.3)	8.381	0.039
No leukopenia	35 (92.1)	50 (83.3)	42 (100.0)	120 (85.7)		
Thrombocytosis (platelet>414×10⁹/L)	2 (5.3)	2 (3.3)	2 (4.8)	1 (0.7)	4.074	0.254
No thrombocytosis	36 (94.7)	58 (96.7)	40 (95.2)	139 (99.3)		
Thrombocytopenia	0 (0.0)	3 (5.0)	0 (0.0)	2 (1.4)	5.091	0.165

(platelet<149×10⁹/L)						
No thrombocytopenia	38 (100.0)	57 (95.0)	42 (100.0)	138 (98.6)		

***cutoff for females; º cutoff for males**

5.6 Determination of Hematological Parameters as Potential Markers of Leprosy Reactions

Comparison between non-reaction and reaction groups demonstrated that patients with reaction had significantly higher WBC count, neutrophil count, monocyte count, basophil count, and NLR compared with the non-reaction group ($p < 0.05$). In contrast MCV, and MCH were significantly lower in the reaction group ($p < 0.05$) (Table 4). Parameters that showed significant differences between reaction and non-reaction groups were further evaluated using ROC curve analysis.

Table 6 Comparison of Hematological Parameters Between Non-Reaction and Reaction Groups

Parameter	Non-Reaction (n = 98)	Reaction (n = 42)	p-value
RBC (×10¹²/L)	4.63 ± 0.66*	4.85 ± 0.77	0.085
HGB (g/dL)	13.57 ± 1.63*	13.70 ± 2.33	0.749
HCT (%)	42.44 ± 5.39*	42.86 ± 6.56	0.695
MPV (fL)	9.41 ± 1.41*	9.53 ± 1.75	0.683
WBC (×10⁹/L)	5.95 (4.70–7.50)	9.00 (6.70–11.94)	**<0.001**
NEU# (×10⁹/L)	3.90 (2.96–5.03)	6.88 (4.10–9.50)	**<0.001**
LYM# (×10⁹/L)	1.25 (0.50–1.75)	1.16 (0.60–1.55)	0.068
MONO# (×10⁹/L)	0.40 (0.30–0.50)	0.53 (0.39–0.70)	**0.010**
EOS# (×10⁹/L)	0.12 (0.07–0.40)	0.09 (0.02–0.20)	0.110
BASO# (×10⁹/L)	0.00 (0.00–0.01)	0.01 (0.00–0.03)	**0.027**
MCV (fL)	92.10 (88.50–96.80)	87.80 (85.30–91.50)	**0.002**
MCH (pg)	29.90 (27.50–31.40)	28.55 (27.60–30.00)	**0.017**
MCHC (g/dL)	32.40 (30.50–33.20)	31.85 (30.50–33.40)	0.955
RDW (%)	13.85 (13.10–14.70)	14.00 (13.20–14.70)	0.617
PLT (×10³/µL)	251.00(215.00–319.00)	272.50 (234.00–309.00)	0.433
NLR	3.57 (2.05–7.25)	6.00 (3.02–13.28)	**0.008**

Notes: *mean ± SD

Abbreviations: RBC, red blood cell; HGB, hemoglobin; HCT, hematocrit; WBC, white blood cell count; RDW, red cell distribution width; MCV, mean cell volume; MCH, mean cell hemoglobin; MCHC, mean cell hemoglobin concentration; MPV, mean platelet volume; N/L ratio, neutrophil-to-lymphocyte ratio.

When assessing the potential of hematological parameters as markers of leprosy reaction, **WBC and NEU#** showed good discriminatory ability (Figure1), with AUCs of 0.790 (95% CI: 0.709–0.871) and 0.778 (95% CI: 0.690–0.867), respectively (Table 5). A WBC count cutoff ⩾ 6.72 × 10^3 /μL yielded 71.4% sensitivity and 61.2% specificity, while a neutrophil count cutoff ⩾ 4.90 ×10^3 /μL showed 73.8% sensitivity and 73.5% specificity. At a prevalence of 30%, the positive predictive value (PPV) was 44.1% and the negative predictive value (NPV) was 83.3% for WBC, whereas the PPV and NPV for neutrophil count were 54.4% and 86.8%, respectively.

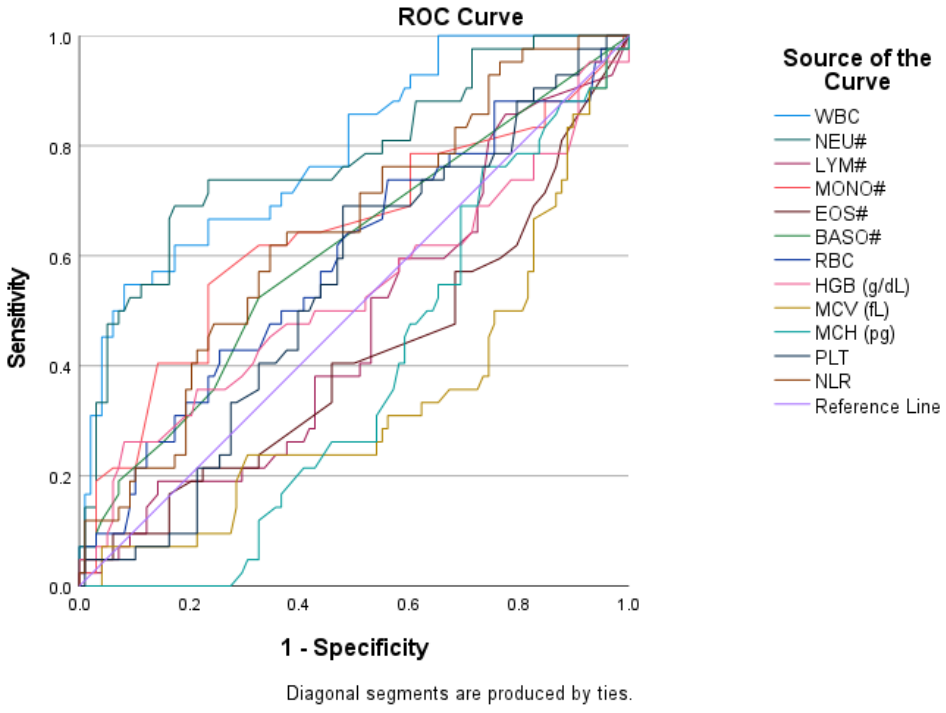


Figure 1 Potential of hematological parameters as markers of leprosy reaction

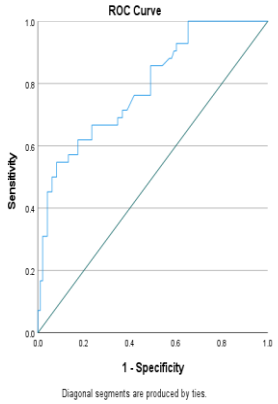


Figure 2 ROC curve of WBC

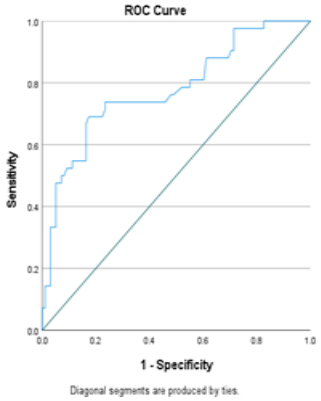


Figure 3 ROC curve of Neutrophil

Table 7 Evaluation of potential markers of hematological parameters for leprosy reaction

Hematological variable	Area under curve	P value	95% CI
Total WBC	0.790	<0.001*	0.709 – 0.871
Absolute neutrophils	0.778	<0.001*	0.690 – 0.867
Absolute lymphocytes	0.467	0.542	0.364 – 0.571
Absolute monocytes	0.638	0.110	0.530 – 0.745
Absolute eosinophils	0.415	0.112	0.308 – 0.522
Absolute basophils	0.603	0.053	0.499 – 0.708
RBC	0.588	0.100	0.483 – 0.693
Hemoglobin	0.529	0.593	0.415 – 0.642
MCV	0.333	0.060	0.233 – 0.434
MCH	0.373	0.105	0.281 – 0.465
Platelet count	0.542	0.433	0.441 – 0.643
N/L ratio	0.642	0.070	0.544 – 0.739

**Notes: ***Statistically significant at 0.05.

Abbreviations: WBC – white blood cells; N/L ratio – neutrophil to lymphocyte ratio; RBC – red cell count.

6. Discussion

Hematological alterations are common among leprosy patients due to chronic inflammation, immune system disturbance, and treatment-related effects. These changes can affect different complete blood count parameters and may reflect the severity and progression of the disease. Some hematological parameters may also serve as potential markers of leprosy reaction, helping in early detection and monitoring of inflammatory episodes (17, 38).

In this study, a male predominance was observed among leprosy patients compared to females. This finding aligns with previous studies conducted in Ethiopia, Nigeria, and Philippines (17, 31, 39). This trend is likely influenced by environmental and socio-cultural factors, as well as inadequate access to healthcare services (40). The majority of the study participants were within the age range of 31–50 years,consistent with finding reported in Brazil (41). The prolonged incubation period of leprosy and extensive social interactions in this age group may contribute to the increased risk of infection (42). Grade I disability was the most common form of disability among the leprosy group. This finding is consistent with report from ALERT (43). The high prevalence of disability indicates challenges in early case detection and timely treatment. The predominance of Type 2 reaction in our study is consistent with report from India (12) but inconsistent with finding reported in Brazil (24). The higher occurrence of ENL among multibacillary cases likely reflects the intense immune complex–mediated inflammatory response characteristic of this reaction type (44).

In the present study, red cell parameters including RBC count, hemoglobin, hematocrit, and MCHC were significantly lower in leprosy patients compared with controls. Similar findings have been reported in Pakistan, India, and Nigeria (17, 28, 30). These hematological changes may be explained by the chronic inflammatory nature of leprosy, which leads to cytokine-mediated suppression of erythropoiesis, impaired iron utilization, and possible drug-related hemolysis during treatment (11).

With respect to subgroup analysis, significantly lower RBC count, hemoglobin, and hematocrit were observed among patients on MDT compared to newly diagnosed patients, reaction and controls. This pattern aligns with studies conducted in Nigeria, Saudi Arabia, India, Bangladesh, and Brazil (17, 45-48) and this may be attributed to dapsone-induced hemolysis during MDT

treatment. Dapsone is metabolized mainly in the liver where it undergoes hydroxylation by cytochrome P-450 enzymes, resulting in the generation of dapsone hydroxylamine (DDS-NOH). This hydroxylamine metabolite is held responsible for some of the major adverse effects of dapsone including methaemoglobin formation and haemolysis (48). The relatively high prevalence of anemia among MDT patients in our study further supports previous evidence that treatment-associated hemolysis and chronic inflammation. The macrocytosis observed among MDT patients in our study may suggests a possible regenerative marrow response following hemolysis (17, 48).

HGB and MCHC were significantly lower in New case than in the controls. Our finding aligns with studies conducted in Pakistan and India(12, 26). This may have occurred due to anemia of chronic disease which caused by persistent *Mycobacterium leprae* infection and inflammatory reactions. Chronic inflammation increases cytokines namely IL-1 and TNF-ά as well as hepcidin production, which restrict iron availability and suppress erythropoiesis, resulting in reduced hemoglobin synthesis and hypochromic red blood cells. Reactional states further intensify inflammation, leading to worsening anemia(11).

RDW in MDT and reaction groups had significantly higher values compared to controls. Similar findings have been reported in Nigeria and India (12, 17). Inflammation contributes to increasing RDW levels by affecting iron metabolism, inhibiting the erythropoietin and premature release of erythrocytes, or shortening red blood cell survival resulting from oxidative damage and indirectly phagocytosis or lysis of RBCs (49). RBCs homeostasis is affected by systemic inflammatory changes, and affects the size, shape and maturity of red cells and reflects as a change in RDW value. Thus, RDW may provide supportive information regarding inflammatory activity in leprosy(12).

In the present study, 30% of leprosy patients presented with leprosy reactions. This proportion exceeds the 10.37% reported from Indonesia (50), while remaining lower than the prevalence observed in Thailand (51) and Brazil (52), where reaction rates of 56.5% and 55.5% were reported, respectively. These differences may be related to variations in multibacillary disease burden, delayed diagnosis and treatment initiation.

Regarding white blood cell parameters, the present study showed significantly increased WBC count, neutrophil count, and neutrophil-to-lymphocyte ratio (NLR) in leprosy patients compared

with controls, while lymphocyte count was significantly reduced. Similar findings have been reported in Brazil, Nigeria, and India (17, 26, 27). These changes reflect an active systemic inflammatory response driven by immune activation against *Mycobacterium leprae*. The increase in neutrophils and WBC count is mainly due to cytokine-mediated stimulation of the innate immune system, while the elevated NLR reflects the imbalance between innate and adaptive immunity, which is commonly associated with disease severity and inflammatory progression in leprosy (15).

Leukocyte alterations were more pronounced among patients with reactions. Reaction cases demonstrated significantly higher total WBC count, absolute neutrophil count, and NLR. This finding is consistent with studies conducted in Ethiopia, Brazil, and Indonesia (24, 25, 53). Rise of WBC count with neutrophilia may suggests systemic inflammatory response and proliferative effect of inflammatory mediators on bone marrow cells, this inflammatory process in the skin and peripheral nerves is intensified by secreting IL-17, TNF-α, and IFN-γ. Release of neutrophilic granular enzymes, other cytokines & NET formation drive the tissue inflammation and tissue injury. Ongoing tissue damage during Type 2 Reaction may further augment inflammation and inflammatory loop may be responsible for progress and severity of T2R (54). The markedly higher prevalence of leukocytosis among reaction patients in our study underscores the acute inflammatory nature of leprosy reactions.

Lymphocyte and monocyte counts were significantly lower among patients receiving MDT compared with controls and other leprosy groups. This finding is consistent with a study conducted in Nigeria (17), while it is inconsistent with findings reported from Bangladeshi (55). Monocytes, which serve as the primary host cells for *Mycobacterium leprae*, are often elevated during active disease due to increased inflammatory and immune activity. Following initiation of multidrug therapy (MDT), monocyte counts may decrease as the bacterial load declines and systemic inflammation subsides(56). Similarly, the reduction in lymphocyte counts after treatment may reflect decreased antigenic stimulation and modulation of the immune response following bacterial clearance.

ROC curve analysis in this study demonstrated that WBC and absolute neutrophil count showed good discriminatory ability for identifying leprosy reactions. While the previous studies in have identified that eosinophil count and NLR as potential biomarkers in Nigeria (17) and in Brazil (24)

respectively, our findings emphasize neutrophil-related parameters as particularly informative indicators of reactional activity. The biological plausibility of neutrophil elevation is supported by evidence showing intense neutrophilic infiltration in ENL lesions and correlation with disease severity (25). *M. leprae* Components, particularly when released after treatment trigger neutrophils to undergo a process called NETosis, where they release their DNA to trap bacteria. This process leads to elevated neutrophil activation markers in the blood (like CD177 and CD64), contributing to a high number of circulating neutrophils. Neutrophils in ENL patients have been shown to have a higher rate of survival (reduced apoptosis) compared to non-reactional leprosy patients, causing them to accumulate in the bloodstream and tissues (54). Differences from previous studies may also be related to variations in sample size, inclusion of different disease stages, and the types of leprosy reactions included in the studies.

7 Strength and Limitation of the Study

7.1 Strength

- Inclusion of newly diagnosed, MDT, and reaction groups, allowing evaluation across different clinical stages of leprosy.

7.2 Limitation

- Cross-sectional design limits assessment of temporal changes.
- Inflammatory biomarkers (e.g., CRP andcytokines) were not included

8 Conclusion and Recommendation

8.1 Conclusion

The present study demonstrated significant hematological alterations among patients with leprosy, particularly those on MDT and those experiencing reactions. Patients on MDT showed a higher prevalence of anemia, macrocytosis, and increased RDW, suggesting treatment-related and inflammatory effects. Reaction cases were characterized by marked leukocyte changes, especially neutrophilia, elevated WBC count, and increased neutrophil-to-lymphocyte ratio. The ROC analysis indicated that WBC and absolute neutrophil count have good discriminatory ability and may serve as potential markers of leprosy reaction.

8.2 Recommendation

Routine hematological monitoring should be strengthened in the management of leprosy patients, particularly for those on MDT and those at risk of developing reactions. Early evaluation of WBC count and neutrophil count may help in identifying reactional episodes promptly. Periodic screening for anemia is also recommended to ensure timely intervention. Further longitudinal studies incorporating inflammatory and immunological biomarkers are recommended to better clarify the role of hematological indices as potential markers of leprosy reaction.

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10.ANNEXES

Annex I: Information Sheet (English and Amharic version)

A, Information Sheet (English version)

Title of the research project: Assessment of hematological parameters among Leprosy patients at ALERT Hospital, Addis Ababa, Ethiopia, 2024

Institution: Addis Ababa University, College of Health Sciences, Department of Medical Laboratory Sciences.

Purpose of the study: The Purpose of this study is to determine hematological parameters among Leprosy patients at Alert Hospital, Addis Ababa, Ethiopia,2024.

Procedures: If you are willing to participate in the study and you sign a consent form, after that some information regarding on socio demographic data will be obtained from you by using pre-tested questioner. you will be asked to receive punctures to draw a 2-3ml blood sample from your vein.

Risk and discomfort: You may experience mild, temporary discomfort during the procedure. All necessary precautions will be taken during sample collection, which will be performed by trained healthcare professionals.

Benefits of the study: It will also make health care providers to be aware of the possible hematological disturbances following Leprosy. If hematological abnormalities will be found in participants, it will be notified for their health care providers to manage those complications. But there will be no any cash payment.

Confidentiality: Any information that will be obtained about you will be kept confidential. This is assured by avoiding use of any identifier about you, and information will be recorded with code numbers only. Data and specimen will be used only for this study.

Voluntary participation: Participation on this study is voluntary basis of you while you have the right to refuse participation at any time. At end, you may ask questions now and in the future if

you do not understand something that is being done. Here are addresses of principal investigator and advisors who you can contact:

Principal investigator:

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   Email: mikiasn2@gmail.com

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Thank you for your participation.

B. Information Sheet Amharic version

ለጥናቱ ተሳታፊዎች የመረጃና ስምምነት ቅፅ (Amharic Version)

የምርምር ጥናቱ ርዕስ: በአለርት ሆስፒታል በስጋ ደዌ ታማሚዎች የየደም ምርመራ ፣ አዲስ አበባ ፣ ኢትዮጵያ፣2024

የድርጅቱ ስም: አዲስ አበባ ዩኒቨርሲቲ፣ ጤና ሳይንስ ኮሌጅ፣ ሕክምና ላብራቶሪ ሳይንስ ትምህርት ክፍል

የጥናቱ ዓላማ: የዚህ ጥናት ዓላማ በአለርት ሆስፒታል በስጋ ደዌ ታማሚዎችን የደም ምርመራ ሁነታዎች ለማወቅ ነው።

ሂደቶች፡- እርስዎ በጥናቱ ለመሳተፍ ፈቃደኛ ከሆኑ እና የስምምነት ቅጽ ከፈረሙ፣ ከዚህ በታች ስለትዘረዘረው ስነ-ሕዝብ መረጃ አንዳንድ መረጃዎች አስቀድሞ ትክክለኛነቱ በተረጋገጠ መጠይቅ በመጠቀም የእርስዎ መረጃ ይሰበሰባል። ለጥናቱ ፈቃደኛ መሆንዎ ከተረጋገጠ በኋላ ከእርስዎ 2-3ሚሊ ሊትር የደም ናሙና በተመደቡ ባለሙያዎች የደም ናሙናዎች የሚሰበሰቡ ይሆናል ።

ተያያዥነት አላቸዉ ተብለዉ የሚታሰቡ ጉዳቶች እርስዎ እዚህ ጥናት ላይ በመሳተፍዎ እና ከላይ የተጠቀሰዉን የደም ናሙና በመስጠትዎ በጤናዎ ላይ የከፋ ችግር አያመጣም፡፡ ነገር ግን መርፌ በሚወጋበት አካባቢ ትንሽ የህመም ስሜት ሊኖር ይችላል፡ :ይሁን እንጅ ይህም በጤና ባለሙያዎቹ እይታ ስር ስለሆነ ለከፋ ጉዳት አይዳርግም፡፡

የጥናቱ ጥቅሞች

ጥናቱ በአለርት ሆስፒታል በስጋ ደዌ ታማሚዎች ሊያግጥሟቸዉ የሚችሏቸውን ይስነደም ሁኔታዎች ለማዎቅ ይረዳል። በተጨማሪም ጥናቱ የጤና አገልግሎት ሰጪዎችን ሊከሰቱ ስለሚችሉ የደም መዛባቶች ማሳሰቢያ ለመስጠት ያግዛል። በጥናቱ ዎቅትም ተሳታፊው(ዋ) የስነደም ችግር አጋጥሞት ቢገኝ፤ ለዚህ ተጓዳኝ ችግሮች የህክምና አግልግሎት እንዲሰጡ ይደረጋል።

የጥናቱ ሚስጥራዊነት

ስለእርስዎ የሚገኝ ማንኛውም መረጃ ሚስጥራዊነቱ የተጠበቀ ይሆናል። ይህም የሚሆነው እርስዎን በሚመለከት ማንኛውንም መለያ ከመጠቀም በመቆጠብ እና ስለርስዎ የሚገኝን መረጃ በኮድ ቁጥሮች ብቻ በመመዝገብ ይሆናል። ሆኖም ስልእርስዎ የሚገኙ መረጃዎችና ናሙናዎች ለዚህ ጥናት ብቻ ጥቅም ላይ የሚዉሉ ይሆናል።

የፈቃደኝነት ተሳትፎ

ይህ ጥናት ብፈቃደኝነት የሚሳተፉበት ሲሆን ፤ በማንኛዉም ጊዜ መሳተፍዎን የመቃወም መብትዎ የተጠበቀ ነው። ስልጥናቱ ያልተረዱት ወይም እንዲረዱት የሚፈልጉት ነገር ካለዎት ከዚህ በታች በተዘረዘሩት ተመራማሪና አማካሪዎች በኩል መጠየቅ ይችላሉ።

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ስልክ: +251 2755170

በጥናቱ ለመሳተፍ ፍቃደኛ በመሆንዎ እናመሰግናለን!!! ቃለ መጠይቁን ያካሄደዉ ሰዉ

Annex II. Consent Form

A. Informed Consent Form (English version)

Participant identification number _____

I have read and/or the information sheet has been read to me fully in my language the information on it. I understood that the research project has got ethical approval from the School of Medical Laboratory Sciences Departmental Research and Ethics Review Committee (DRERC). I also understood the information well about the purpose and benefits of the study, procedures to be carried out, discomforts and risks during sampling as well the confidentiality of the information and other pieces of information regarding the study. I_____ have given my consent freely to participate in the study on Assessment hematological parameters among Leprosy patients at Alert Hospital, Addis Ababa, Ethiopia,2024

Participant(s) signature _____

Data collector name: _____

Signature: _____

Date: __/___/___

B. Informed Consent Form (Amharic version)

Amharic Version Consent form

የተሳታፊ መለያ ቁጥር _____

ስለዚህ ጥናት መረጃ በራሴ በሚገባኝ ቋንቋ አንብቢያለሁ ውይም ተነቦልኛል ። የምርምር ፕሮጀክቱ ከሜዲካል ላብራቶሪ ሳይንስ ትምህርት ቤት ዲፓርትመንታል ጥናትና ስነምግባር ግምገማ ኮሚቴ (DRERC) የሥነ ምግባር ይሁንታ ማግኘቱን ተረድቻለሁ። በተጨማሪም፣ ስለ ጥናቱ አላማ እና ጥቅሞች፣ ስለሚደረጉ ሂደቶች፣ በናሙና ወቅት ያሉ ምቾት ማጣት እና ስጋቶች እንዲሁም የመረጃውን ምስጢራዊነት እና ጥናቱን በተመለከተ ሌሎች መረጃዎችን በደንብ ተረድቻለሁ። በዚህም መሰረት በጥናቱ ለመሳተፍ፣ ስለኔ አስፈላጊዉ መረጃ እና ናሙና እንዲወሰድ ፈቃደኛ ነኝ።

እኔ_____ በአለርት ሆስፒታል በሚሰራው የደም ምርመራ ሁኔታዎች ለማዎቅ በሚሰራው የምርምር ጥናት ለመሳተፍ ነፃ ፈቃዴን በፊርማዬ ያረጋገጥኩ መሆኔን እገልጻለሁ።

የተሳታፊ ፊርማ፡- _____

የመረጃ ሰብሳቢ ስም፡- _____

ፊርማ፡ _____

ቀን፡ _____

Annex III: Questionnaire (English and Amharic version)

A. Questionnaire (English version)

Addis Ababa University

College of Health Sciences

Department of Medical Laboratory Sciences

Questionnaire for data collection on Leprosy patients at Alert hospital, Addis Ababa, Ethiopia, 2024. Fill if dashed lines are provided; otherwise, tick (✓) the appropriate box.

Questionnaire identification number_____, Date: ____/ ___/____

Socio-Demographic Characteristics

1. Age (in years): _____
2. Sex:
   - ☐ Female
   - ☐ Male
3. Residence:
   - ☐ Urban
   - ☐ Rural
4. Marital Status:
   - ☐ Married
   - ☐ Unmarried
   - ☐ Divorced
   - ☐ Widowed
5. Educational Level:
   - ☐ Cannot read or write
   - ☐ Elementary school
   - ☐ Preparatory school
   - ☐ College or University
6. Occupation:
   - ☐ Farmer
   - ☐ Merchant
   - ☐ Student
   - ☐ Non-governmental employee
   - ☐ Governmental employee
   - ☐ Unemployed

Medical and Lifestyle History

1. Do you have any other chronic illnesses?
   - ☐ Diabetes
   - ☐ Tuberculosis
   - ☐ Hypertension
   - ☐ HIV
   - ☐ None
2. Are you currently pregnant or breastfeeding?
   - ☐ Yes
   - ☐ No
3. Do you smoke?
   - ☐ Yes
   - ☐ No
4. Do you consume alcohol?
   - ☐ Yes
   - ☐ No
5. Do you chew khat?
   - ☐ Yes
   - ☐ No
6. Are you taking any other medication besides leprosy treatment?
   - ☐ Yes
   - ☐ No

Clinical Information

1. Are you currently receiving treatment for leprosy?
   ☐ Yes
   ☐ No
2. If yes, how long have you been on MDT? _____
3. Have you ever experienced a leprosy reaction?
   ☐ Yes
   ☐ No
4. If yes, what type of reaction?
   ☐ Type 1 reaction
   ☐ Type 2 reaction
5. Do you have any physical deformities due to leprosy?
   ☐ Grade 0
   ☐ Grade 1
   ☐ Grade 2

Thank you very much for your cooperation

Addis Ababa University

College of Health Sciences

Department of Medical Laboratory Sciences

Questionnaire for Control group data collection at Alert hospital, Addis Ababa, Ethiopia, 2024.

Fill if dashed lines are provided; otherwise, tick (✓) the appropriate box.

Questionnaire identification number_____, Date: ____/ ___/____

Socio-Demographic Characteristics

1. Age (in years): _____
2. Sex:
   - ☐ Female
   - ☐ Male
3. Residence:
   - ☐ Urban
   - ☐ Rural
4. Marital Status:
   - ☐ Married
   - ☐ Unmarried
   - ☐ Divorced
   - ☐ Widowed
5. Educational Level:
   - ☐ Cannot read or write
   - ☐ Elementary school
   - ☐ Preparatory school
   - ☐ College or University
6. Occupation:
   - ☐ Farmer
   - ☐ Merchant
   - ☐ Student
   - ☐ Non-governmental employee
   - ☐ Governmental employee
   - ☐ Unemployed

Medical and Lifestyle History

7. Do you have any chronic illnesses?
   - ☐ Diabetes
   - ☐ Tuberculosis
   - ☐ Hypertension
   - ☐ HIV
   - ☐ None
8. Are you currently pregnant or breastfeeding?
   - ☐ Yes
   - ☐ No
9. Do you smoke?
   - ☐ Yes
   - ☐ No
10. Do you consume alcohol?
    - ☐ Yes
    - ☐ No
11. Do you chew khat?

☐ Yes

☐ No

12. Do you have a history of chronic skin conditions or nerve-related diseases?

☐ Yes

☐ No

13. Have you ever experienced persistent weakness, numbness, or loss of sensation in your hands, feet, or face (not due to injury)?

☐ Yes

☐ No

14. Are you taking any regular medications?

☐ Yes

☐ No

Thank you very much for your cooperation

B, Questioner Amharic Version

አዲስ አበባ ዩኒቨርሲቲ

የጤና ሳይንስ ኮሌጅ

የህክምና ላብራቶሪ ሳይንስ ክፍል

በአለርት ሆስፒታል፣ አዲስ አበባ፣ ኢትዮጵያ፣ 2024 ለሚከናወን በስጋ ደዌ ታካሚዎች የመረጃ ስብስብ መጠይቅ። ክፍት መስመሮች ካሉ ይሙሉ፤ ካልሆነ በተገቢው ሳጥን ውስጥ ምልክት (✓) ያድርጉ።

የመጠይቅ መለያ ቁጥር _____, ቀን: ___/ /_____

የተሳታፊዎች ሶሽዎ-ዲሞግራፊክ መረጃ

1. እድሜ (በዓመት): _____
2. ፆታ:
   - ☐ ሴት
   - ☐ ወንድ
3. መኖሪያ:
   - ☐ ከተማ
   - ☐ ገጠር
4. የጋብቻ ሁኔታ:
   - ☐ ያገባ
   - ☐ ያላገባ
   - ☐ የተፋታ
   - ☐ በሞት የተለየ(ች)
5. የትምህርት ደረጃ:
   - ☐ ማንበብ እና መጻፍ የማይችል
   - ☐ አንደኛ ደረጃ
   - ☐ ሁለተኛ ደረጃ
   - ☐ ኮሌጅ ወይም ዩኒቨርሲቲ
6. ስራ:
   - ☐ ገበሬ
   - ☐ ነጋዴ
   - ☐ ተማሪ
   - ☐ የግል ድርጅት ሰራተኛ
   - ☐ የመንግስት ሰራተኛ
   - ☐ ያልተቀጠረ

የጤና ሁኔታ እና የአኗኗር ዘይቤ

7. ሌሎች የረጅም ጊዜ በሽታዎች አሉዎት?
   - ☐ የስኳር በሽታ
   - ☐ ቲቢ
   - ☐ የደም ግፊት
   - ☐ ኤችአይቪ
   - ☐ የለም
8. በአሁኑ ወቅት የእርግዝና ወይም ጡት የማጥባት ሁኔታ አለ?
   - ☐ አዎ
   - ☐ አይ
9. ታጨሳለህ/ሽ?
   - ☐ አዎ
   - ☐ አይ
10. አልኮል ትጠጣለህ/ሽ?
    - ☐ አዎ

☐ አይ

11. ጫት ትቅማለህ/ሽ?

☐ አዎ

☐ አይ

12. ከስጋ ደዌ ህክምና በተጨማሪ ሌላ መድሃኒት ትወስዳለህ/ሽ?

☐ አዎ

☐ አይ

13. አዎከሆነ፣እባክዎን መድሃኒቱን?

_____

ክሊኒካዊ መረጃ

14. የስጋ ደዌ መዳሃኒት እየወሰደክ/ሽ ነው?

☐ አዎ

☐ አይ

15. አዎ ከሆነ፣ ለምን ያህል ጊዜ መዳሃኒቶን እየወሰዱ ነው? _____

16. የሥጋ ደዌ ምላሽ (reaction) ተፈጥሮ ያውቃል?

☐ አዎ

☐ አይ

17. አዎ ከሆነ፣ የትኛው አይነት?

☐ Type 1 ምላሽ

☐ Type 2 ምላሽ

18. በሥጋ ደዌ ምክንያት አካል ጉዳት አለ?

☐ ደረጃ 0

☐ ደረጃ 1

☐ ደረጃ 2

ስለ ትብብርዎ እናመሰግናለን!

አዲስ አበባ ዩኒቨርሲቲ

የጤና ሳይንስ ኮሌጅ

የህክምና ላብራቶሪ ሳይንስ ክፍል

በአለርት ሆስፒታል፣ አዲስ አበባ፣ ኢትዮጵያ፣ 2024 ለሚከናወን (Control) የመረጃ ስብስብ መጠይቅ። ክፍት መስመሮች ካሉ ይሙሉ፤ ካልሆነ በተገቢው ሳጥን ውስጥ ምልክት (✓) ያድርጉ።

የመጠይቅ መለያ ቁጥር _____, ቀን: ___/___ /___

የተሳታፊዎች ሶሽዎ-ዲሞግራፊክ መረጃ

1. እድሜ (በዓመት): _____
2. *ፆታ*:
   - ☐ ሴት
   - ☐ ወንድ
3. *መኖሪያ*:
   - ☐ ከተማ
   - ☐ ገጠር
4. የጋብቻ ሁኔታ:
   - ☐ ያገባ
   - ☐ ያላገባ
   - ☐ የተፋታ
   - ☐ በሞት የተለየ(ች)
5. የትምህርት ደረጃ:
   - ☐ ማንበብ እና መጻፍ የማይችል.
   - ☐ አንደኛ ደረጃ
   - ☐ ሁለተኛ ደረጃ
   - ☐ ኮሌጅ ወይም ዩኒቨርሲቲ
6. ስራ:
   - ☐ ገበሬ
   - ☐ ነጋዴ
   - ☐ ተማሪ
   - ☐ የግል ድርጅት ሰራተኛ
   - ☐ የመንግስት ሰራተኛ
   - ☐ ያልተቀጠረ

የጤና ሁኔታ እና የአኗኗር ዘይቤ

7. ከሚከተሉት በስሸታዎች በየትኛዎቹ ትይዘው ያውቃሉ?
   - ☐ የስኳር በሽታ
   - ☐ ቲቢ
   - ☐ የደም ግፊት
   - ☐ ኤችአይቪ
   - ☐ የለም
8. በአሁኑ ወቅት የእርግዝና ወይም ጡት የማጥባት ሁኔታ አለ?
   - ☐ አዎ
   - ☐ አይ
9. ታጨሳለህ /ሺ??
   - ☐ አዎ
   - ☐ አይ

10. አልኮል ትጠጣለህ/ሽ?

☐ አዎ

☐ አይ

11. ጫት ትቅማለህ/ሽ?

☐ አዎ

☐ አይ

12. የቆዳ ወይም የነርቭ ችግሮች ታሪክ አለ? (ከጉዳት ውጪ)

☐ አዎ

☐ አይ

13. በእጅ፣ በእግር ወይም በፊት ላይ የሚቆይ ድካም፣ መደንዘዝ (numbness) ወይም የስሜት መጥፋት ተፈጥሯል ያውቃል?

☐ አዎ

☐ አይ

14. በቆሚነት መድሃኒት ትወስዳለህ/ሽ?

☐ አዎ

☐ አይ

15. አዎ ከሆነ፣ እባክዎን መድሃኒቱን? _____

ስለ ትብብርዎ እናመሰግናለን!

Annex IV: Venous Blood Sample Collection

Materials;

- Tourniquet and
- Disposable gloves
- Alcohol (70%) and
- gauze or alcohol wipes
- Sterile disposable needles

Collection procedure

1. Patients will be welcomed and informed about the purpose of taking sample.
2. Materials needed for sample collection will be assembled
3. Visual inspection of both arms will be performed by the phlebotomist assigned to do.
4. Punctures site that is free of bruises, abrasions, and sites of infection will be chosen as site of sample collection.
5. Once the appropriate site has been chosen a tourniquet will be applied just above the area to be punctured.
6. The area chosen for puncturing will be cleaned inside out in a circular fashion with cotton soaked with 70% alcohol.
7. A needle with it bevel faced upward will be inserted gently into the selected site and the tourniquet will be removed as soon as blood begins flowing into the syringes. About 4ml blood will be pooled into the syringe.
8. Once the desired volume taken, and the needle and syringe will be removed from patients' arm and patients will be informed to hold the punctured area with cotton ball for a minute to stop bleeding.
9. Blood withdrawn will be dispensed into a tube containing EDTA.
10. The tube will then be inverted many times in figure of eight to ensure proper mixing of sample to anticoagulant present in the tube.
11. The sample will be labeled with unique identification numbers assigned to match to the corresponding patients.
12. Materials used will be removed from the work area, and patients will be departed with thanks presented by the phlebotomist.

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Annex V: UniCel® DxH 800 Beckman Coulter automated hematology analyzer

Introduction

The UniCel® DxH 800 Analyzer is a quantitative multi-parameter, automated hematology analyzer for in vitro diagnostic use in screening patient populations found in clinical laboratories.

Types of reagent

- COULTER® DxH Diluent
- COULTER® DxH Diff Pack
- COULTER® DxH Cell Lyse
- COULTER® DxH Cleaner

Parameters

Quantitative test for WBC, RBC, HGB, HCT, MCV, MCH, MCHC, RDW, RDW-SD, PLT, MPV, NE%, NE#, LY%, LY#, MO%, MO#, EO%, EO#, BA%, BA# parameters.

Principle

The Coulter Principle of automated cell counting and sizing is used in the analysis of the whole blood. Each cell suspended in a conductive liquid (diluent) acts as an insulator. As each cell goes through the aperture, it momentarily increases the resistance of the electrical path between two submerged electrodes on either side of the aperture. This causes a measurable electronic pulse. While the number of pulses indicates particle count, the amplitude of the electrical pulse is proportional to the cell volume. These pulses are sent to the Signal Conditioner for analog to digital conversion. Pulse counts and digitized pulse measurements are sent to the System 1 Manager for processing by the algorithms where the reported parameter values, flags and histograms are generated.

The lytic reagent used for the white cell count 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. Hemoglobin is measured

photometrically at 525 nm using the sample from the white cell analysis. Clean diluent is introduced into the cuvette during each operating cycle and is used as a blank in the calculation of the HGB.

The COULTER® VCSn technology is used to determine the white cell differential, nucleated red blood cell count and reticulocyte parameters along with associated flags, messages, histograms and data plots. The sample preparation and analysis uses specific reagents and analytical processes for the WBC differential. The prepared sample is delivered to the flow cell for sample detection. As the cells pass through the sensing zone, a diode laser illuminates the particles causing light scatter and light absorption. Simultaneously to the light scatter measurements, cell volume and cell conductivity are also measured. The data collected during each of the analytical processes is transferred to the System Manager where the digital raw values are processed by the algorithm using mathematical approaches designed for finding optimal separation between clusters of data. The identified clusters are used to calculate the frequency of cells within each population, generate parameter values, flags, histograms and data plots.

Carryover

Parameters	Carryover Limit
WBC	CV ≤ 0.50%
RBC	CV ≤ 0.50%
HGB	CV ≤ 1.00%
PLT	CV ≤ 1.00%
DIFF	CV ≤ 200 events

Specimen Identification: Specimen identification is automated or by manual sample identification with the use of a hand held barcode scanner.

Sample Aspiration Volume Automatic, cap-piercing: 165 µL Single tube - open-vial and cap pierce: 165 µL

Throughput: Automated cassette processing CBC/Diff ⩾ 100 specimens per hour.

Calibration:

COULTER® S-CAL® Calibrator (k862122) is used for determining calibration factors to ensure accurate measurements of directly measured CBC parameters. Assigned assay values are traceable to reference methods.

Quality Control:

COULTER® 6C Cell Control (k081822) enables monitoring of system performance for all directly measured and calculated CBC and Diff parameters.

COULTER® LIN-X Linearity Control (k081641) verifies the reportable range, and assesses the calibration of the WBC, RBC, HGB, and PLT parameters.

COULTER® LATRON™ CP-X Control (k885028) determines calibration factors to ensure accurate measurements of directly measured CBC parameters. Assigned assay values are traceable to reference methods.

Annex VI: Declaration

The undersigned declares that this thesis is an original work, has not been submitted for any degree in any other University, and that all sources have been appropriately acknowledged. It complies with the University's regulations and accepted standards of originality and academic quality, and full responsibility is assumed for the scientific, ethical, and technical content of the work.

M.Sc. candidate: **Bontu Fikadu (B.Sc.)**

Signature: _____

Date of submission: _____

This thesis has been submitted with our approval as advisors.

Advisor: **Dr. Mikiyas Negash(PhD)**

Signature: _____

Date: _____

Place: Addis Ababa, Ethiopia.

Advisor: **Dr. Jemal Alemu (PhD)**

Signature: _____

Date: _____

Place: Addis Ababa, Ethiopia.