PREVALENCE AND ASSOCIATED FACTORS OF ANEMIA AMONG ELDERLY PATIENTS AT JIGJIGA UNIVERSITY SHEIKH HASSAN YABARE REFERRAL HOSPITAL, SOMALI REGION, EASTERN ETHIOPIA

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A RESEARCH THESIS SUBMITTED TO THE SCHOOL OF MEDICAL LABORATORY SCIENCES, FACULTY OF HEALTH SCIENCES, INSTITUTE OF HEALTH, JIMMA UNIVERSITY IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN CLINICAL LABORATORY SCIENCE SPECIALTY IN HEMATOLOGY AND IMMUNOHEMATOLGY

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JIMMA, ETHIOPIA

JIMMA UNIVERSITY
INSTITUTE OF HEALTH
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Abstract

***Background**: Anemia is a prevalent condition in geriatrics and its frequency proportionately increases with age and has a much more severe consequences. In these patients, anemia is now recognized as a risk factor for a variety of negative outcomes: hospitalization, morbidity, and mortality. There is limited information regarding the prevalence of anemia and its associated risk factors among elderly patients in the study area (Jigjiga, eastern Ethiopia). Therefore, this study is aimed to assess the prevalence of anemia and its associated factor among elderly patients at Jigjiga University Sheikh Hassan Yabere Referral Hospital.*

***Objective**: To determine the prevalence and associated factors of anemia among elderly patients at Jigjiga University Sheikh Hassan Yabare Referral Hospital from June 20 to July 30, 2022.*

***Methods:** An institution based cross-sectional study was conducted from June 20 to July 30, 2022, recruiting 381 elderly patients by employing convenient sampling technique. Socio-demographic and clinical data were collected through direct interview and review of medical records using a structured questionnaire. Four milliliters (4ml) of venous blood sample was collected from each study participant and analyzed for complete blood count. A stained blood smear and red blood cell indices was used to determine the morphological type of anemia. Descriptive statistical analysis, bivariate and multivariate logistic regression were done using SPSS version 25; $p<0.05$ was considered as statistically significance.*

***Result:** The overall prevalence of anemia among elderly patients in this area was 40.4%. Mild, moderate and severe anemia accounted for 71%, 23.1% and 5.8%, respectively. Morphologic classification of the anemia demonstrated that, 55.13% were normocytic normochromic, 37.18% were microcytic hypochromic and 7.69% was macrocytic normochromic anemia. Age [p=0.009], sex [p=0.003], lower socioeconomic status [p=0.001], and less than once a week of meat consumption [0.015], were significantly associated with anemia in elderly patients.*

***Conclusion and recommendation:** The prevalence of anemia among elderly patients is found 40.4%. Mild anemia was the predominant type. Identified risk factors should be considered for prevention and control of anemia among elderly patients and also screening for anemia among elderly should be a part of their routine management.*

***Keywords: -** anemia, prevalence, elderly, associated factor, Jigjiga, Ethiopia.*

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List of abbreviations

ACD	Anemia Chronic Disease
BMI	Body mass indexes
CKD	Chronic Kidney Disease
CI	Confidence Interval
DM	Diabetic Mellitus
EDTA	Ethylenediaminetetraacetic acid
FL	Femtoliters
GI	Gastrointestinal bleeding
g/dL	Grams per deciliter
HCT	Hematocrit
Hgb	Hemoglobin
IDA	Iron Deficiency Anemia
ID	Iron Deficiency
MCH	Mean corpuscular hemoglobin
MCHC	Mean Corpuscular Hemoglobin Concentration
MCV	Mean corpuscular volume
MNA	Macrocytic Normochromic anemia
NNA	Normocytic normochromic anemia

Chapter one: Introduction

1.1 Background information

Anemia is characterized by a decrease in hemoglobin concentration (Hgb), red blood cell count (RBC), and/or packed cell volume or hematocrit (PCV or HCT), resulting inability of the body to meet the oxygen demands of its tissue (tissue hypoxia) (1). According to the World Health Organization (WHO), anemia is defined as a Hgb concentration less than 12 g/dL (120 g/L) in women and less than 13 g/dL (130 g/L) in men (2). Age, sex, residence, elevation above sea level (altitude), smoking behavior, and different phases of pregnancy influence body physiologic demands (3).

Based on the morphological characteristics of RBCs in a blood smear and RBC indices (mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC)), anemia can be categorized into three different types: microcytic hypochromic anemia, macrocytic normochromic anemia, and normocytic normochromic anemia. Besides that, it can be classified as mild, moderate, or severe anemia based on the severity of the disorder according to hemoglobin value (3,4).

Aging has both medical and social consequences. It is linked to an increase in the prevalence of chronic diseases, impairments, and functional dependency, which leads to increased demand for medical, social, and caregiving services (5,6). The fundamental cause of geriatric anemia is likely multifaceted, and one of the explanations could be found at the hematopoietic stem cell level (7). According to data from two studies performed at the University of Arkansas for medical sciences to examine the hematological situation in an aging population and validate the existence of an age-related change(7).

Anemia afflicted 1.62 billion people worldwide, accounting for 24.8% of the population, with 164 million anemia cases reported among the elderly (8). And its independent risk factor for increased morbidity, mortality, and decreased quality of life in elderly individuals (9). Anemia in the elderly is typically caused by a variety of clinical issues, such as iron deficiency (ID), vitamin B12 and/or folic acid (folate) deficiency, gastrointestinal (GI) bleeding, acute and chronic infections (10,11).

The impact of anemia among the elderly is becoming more visible, particularly in western countries and Asia (12). The mortality risk in the elder anemic adult is significantly higher than in non-anemic elder adults (13). Most commonly, anemia among the elderly is considered to be mild (10–12 g/dL) and gets worse as someone gets older (14).

The etiology of anemia in Ethiopia is not well established and the information available is limited in representativeness of the whole country. Various researchers came up with different conclusions despite the problem being among the ten top morbidities reported by most health institutions in the country. Some studies in the past have documented the problem as being rare in Ethiopia attributed to the consumption of *Eragrostis teff* ("teff") a cereal that has high iron content, while others have concluded the issue as is a mild to moderate public health problem due to factors such as parasitic infections in addition to nutritional deficiencies (15).

Anemia is detected using a variety of techniques, such as complete blood count (CBC) which includes various parameters, and also peripheral blood film morphology, which can reveal abnormal RBC size, shape, and color. The WHO recommends that hemoglobin value is used to diagnose anemia because it is well-validated and widely recognized (3,16).

The treatment comprises removing the source of the problem (for example, iron deficiency, GI hemorrhage) and replacing the nutrient that has been lost(for instance iron and Vit B^{12}(17). On the other hand, chronic illness anemia, chronic kidney disease anemia, and unexplained anemia are all more challenging to treat. Correcting the underlying issue is the first and best therapy option. The majority of anemia in the elderly are mild and do not require treatment. When anemia is severe (hemoglobin level less than 10g/dl), symptoms that necessitate extra therapy frequently emerge. Blood transfusions and erythropoiesis-stimulating medications are two alternatives for treating severe anemia (18).

1.2 Statement of problem

Anemia is a common public health issue that is linked to a higher risk of morbidity and mortality (8). It is thought to play a significant role in the worldwide illness burden. It has an impact on both developed and resource-limited countries, affecting not only human health and productivity, but also a nation's socio-economic growth (19,20). Anemia is a public health problem that is classified as severe (if it affects 40% of the population), moderate (if it affects 20% to 39.9% of the population), and mild (if it affects 5% to 19.9% of the population), as reported by WHO (19). According to the information from worldwide report from WHO, the prevalence of anemia among elderly individuals was 164 million (23.9%) and, the frequency was highest among the lowest category of a national level of socioeconomic development, and vice versa (8).

In a systematic review completed by Gaskell *et al.*, (2008) data from forty-five studies out of 85,409 study participants, indicated that the prevalence of anemia among the elderly person was 17% (3–50%). Overall, 12% (3–25%) in studies based on the community, 47% (31–50%) in nursing homes, and 40% (40–72%) in hospital admissions, with the majority of individuals suffering from mild anemia. According to this study, the prevalence of anemia increased with age and was slightly higher in men than women (21).

Even though anemia is not considered a disease, it can have negative health consequences in these individuals and be linked to a variety of clinical issues such as decreased muscle strength, exercise performance, mental retardation, and dementia (22,23). Anemic older adults have increased hospitalization and mortality rates (24).

According to the demographics studied, the prevalence of anemia among people over 65 years old ranges from 11% to 60% (25–29). Anemia is one of the major risk factors in the community-dwelling elderly population, and it is usually mild. Several observational data from community-based studies have consistently demonstrated that mild anemia is linked to severe negative outcomes in older persons, such as a loss in physical ability (30), decreased mobility (31), cognitive decline (28), increased number of falls (32), increased hospitalization (33,34) and mortality(28,33).

Anemia in the elderly is multifactorial in etiology and complex interaction of many factors, including cancer, chronic renal disease, congestive heart failure (CHF), as well as malnutrition, and iron deficiency (35,36). High-income nations factors including nutritional deficiencies, chronic inflammation, chronic renal disease, and anemia of unknown origin are the most common causes of anemia among elderly individuals (37).

Moreover, anemia among elderly in Africa is likely to be caused by a variety of factors, including soil-transmitted helminths (STH), particularly hookworm infection, chronic illnesses such as HIV, malaria, and nutritional inadequacies (38). In Africa, older people may be more susceptible to iron and vitamin B^{12} insufficiency, which are primarily sourced from animal products, that older people may not be able to buy (39).

Anemia is a significant predictor for elderly people who have had a recent myocardial infarction (MI). The inverse connection between admission hematocrit (HCT) and mortality was observed. Even mild anemia (HCT 33%) was connected to an almost twofold increase in the risk of dying. In nursing home residents without heart disease, women with and without anemia had similar comparable 1-year mortality rates of 8.1% and 8.5%, respectively. On the other hand, anemia was connected to a 27.9% death rate among heart disease patients, compared to 13.1% for non-anemic people with heart disease (40–42).

The prevalence of anemia among elderly people was still high and more vulnerable to anemia due to GI bleeding, hematopoietic stem cell aging, low level of EPO response, intestinal parasite infections, ID, low dietary intake, acute and chronic infections. Therefore, it is a critical health concern among this age group, because it affects physical, mental, and mobility activity. Despite the multiple consequences of this disease, a limited study is conducted on elderly individuals in Ethiopia and no study was conducted in the study area (Jigjiga town) previously. So this study was undertaken to determine anemia prevalence and its possible risk factors which are important to have baseline data to provide evidence for policy formulation and resource allocation to set prevention strategies as well as for early detection and possible intervention, follow-up, and care of the affected elderly people.

1.3 Significance of study

Anemia is one of the most widespread public health problems, especially in developing countries with great risk for elderly individuals. There is also evidence that anemia results in decreased physical activity, mental retardation, increased number of falls, frailty, dementia, hospitalization, and mortality.

The findings of this study will provide information or evidence for policy formulation, resource allocation, to set prevention programs, follow-up and care of elderly people with anemia. Thus, clinicians managing the elderly patients, health professionals, health bureau, officers, and any other concerned bodies hospital administrators, as well as policy-makers will benefit from this information. It can also be used as baseline data for further studies or for researchers interested in doing a similar study.

Chapter two: literature review

2.1 Magnitude and risk factors of anemia among elderly

Anemia among the elderly has a common manifestation of various etiologies such as ID, vitamin $B^{12,}$ and folic acid (folate) deficiency, gastrointestinal bleeding, acute and chronic infections (43). More than 10% of community-population elderly (65 years and older) have WHO-defined anemia. After age 50years, the prevalence of anemia will rise along with age and exceed 20% in those aged 85 years and older. The prevalence of anemia among the elderly was ranged from 9.2% to 23.9% in men and 8.1 to 24.7% in females and most occurrences of anemia were mild (26,28,44,45).

Anemia prevalence, correlation, and impact on elderly individuals was conducted by Bryce *et al.*, (2013) in five Latin American countries (Cuba, Republic, Mexico (rural and urban), Puerto Rico, and Venezuela) out of 8423 studies participants, the prevalence of anemia was ranging from 6.4%-37.3% (19.2%, 37.3%, 6.4%, and 9.2%, 32.1%, and 9.8% respectively) and the commonest type of anemia was normocytic normochromic anemia (NNA). Age, sex, education level, assets, under-nutrition, meat intake, and a high blood creatinine level were found to be significant factors in anemia among the elderly (46).

Also, a cross-sectional study was done by Le et al., (2016) in United States data from five National Health and Nutrition Examination Surveys (NHANES); the prevalence of anemia among the elderly was 13.6%. Age and sex were revealed to be strongly associated factors with anemia (24).

The prevalence of anemia and associated factors among the elderly was 20% in females and 25.2% in males in a community-based cross-sectional study conducted by Orceset al., (2017) in Ecuador. Furthermore, NNA was discovered to be the most common morphological anemia pattern, accounting for 87.4%. Hypoalbuminemia, cancer, chronic renal illness, ID, low-grade inflammation, and being underweight are factors associated with anemia (47).

Institutional based, cross-sectional study was conducted by Bach *et al.*, (2014) in Austria among 19,758 study participants; the prevalence of anemia among the elderly was 21.1%, with advanced age (p=0.001), and male sex (p=0.001), were substantially linked with the prevalence of anemia (48). While another cross-sectional study conducted by Sahin *et al.*, (2016) in Turkey, with a total of 257 study participants, the prevalence of anemia among the elderly was 54.9%. increasing age and nutritional status (malnutrition) were significantly associated with anemia in the study (49). A similar study conducted in Germany, reported by prevalence of anemia among elderly was 54.2% (50).

A cross-sectional study was conducted by Bikbov *et al.*, (2019) in Russia among 5899 study participants; the prevalence of anemia was 23.6%. Sex(male), lower educational level, lower socio-economic status, higher rate ESR, DM and underweight were associated factors of anemia among the elderly (51). On the other hand, a prospective study conducted by Petrosyan *et al.*, (2012) in France, the prevalence of anemia was 53%, while a morphological type of anemia counting 82.1% for NNA, 9.5% for MHA and 8.4% for MNA. Furthermore, mild, moderate, and severe anemia was 61.1%, 33.7%, and 5.2%, respectively. Inflammation (62.1%), iron insufficiency (30.5%), folic acid deficit (21%), chronic renal failure (17.9%), and cobalamin deficiency(11.6%) were the most common causes of anemia (52).

Hospital based, cross-sectional study was conducted by Pautas *et al.*, 2012 in france among 190 study participants; the prevalence anemia was 43.7%. Inflammation, renal impairment, severe malnutrition, and iron deficiency were the most frequent possible causes of anemia (53).

A study conducted by Pathania *et al.*, (2019) in Delhi, India among 335 study participants, the prevalence of anemia among the elderly was 68.7%, with mild, moderate and severe anemia accounting for 47.4%, 47.0%, and 5.6% respectively. Age (advanced age) was found to be a major predictor of anemia (54). Also another study was conducted by Hosseini *et al.*, (2018) in Iran; among 1616 study participants; reported that the prevalence of anemia among the elderly was 19%. Age was a predicted significant factor in anemia (55).

A hospital-based cross-sectional study conducted by Obaidely et al. (2017) in Qatar with a total of 522 study participants, the prevalence of anemia among the elderly patients was determined by age and sex. A man (46%) was higher than women (32.4%) at advanced ages above 80 years, and it was lower than males (28.7%) in females (40.6%) until they reached the age of 80 years. Morphological pattern of anemia were NNA (72.6%), MHA (26.8%), and MNA (0.6%). Besides that, anemia is classified as mild, moderate, and severe with 17.4%, 60.3%, and 21.8%, respectively. Advanced age, chronic kidney disease, and GI bleeding were the significant factors associated with anemia (56).

A hospital-based cross-sectional study conducted in Tanzania by Chamba *et al.*, (2021) among 156 study participants, the prevalence of anemia among the elderly was 79.5% and weighted prevalence levels was mild, moderate, and severe, 23.4%, 42.75, and 33.9% respectively (57). Also another cross-sectional study was done in Uganda by Mugisha *et al.*, 2013 among 1449 study participants; the prevalence of anemia in elderly was 20.3%. Malaria, HIV/AIDS, heavy hookworm infection, low fruit consumption, being unmarried, and hypertension are significantly factors associated with anemia (39).

A cross-sectional study was conducted by Badawy et al., 2017 in Egypt involving 200 study participants, reported that the prevalence of anemia among the elderly was 17.5%, with severity levels of mild and moderate anemia were 16% and 1.5 %, respectively. Age, sex(men), blood loss history, and NSAID were significantly factors associated with anemia (58).

Institution-based cross-sectional study was conducted by Melku *et al.*, 2018 in northern-west, Ethiopia, among 200 study participants; the prevalence of anemia in the geriatric population was 54.5%, with mild, moderate, and severe anemia accounting for 56.9%, 36.7%, and 6.4% respectively. Were morphological type of anemia was NNA (85.3%), MHA (9.20 %), and MNA (5.50%). Vegetarians and geriatrics with a high ESR were more prone to develop anemia (59).

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2.2 Conceptual framework

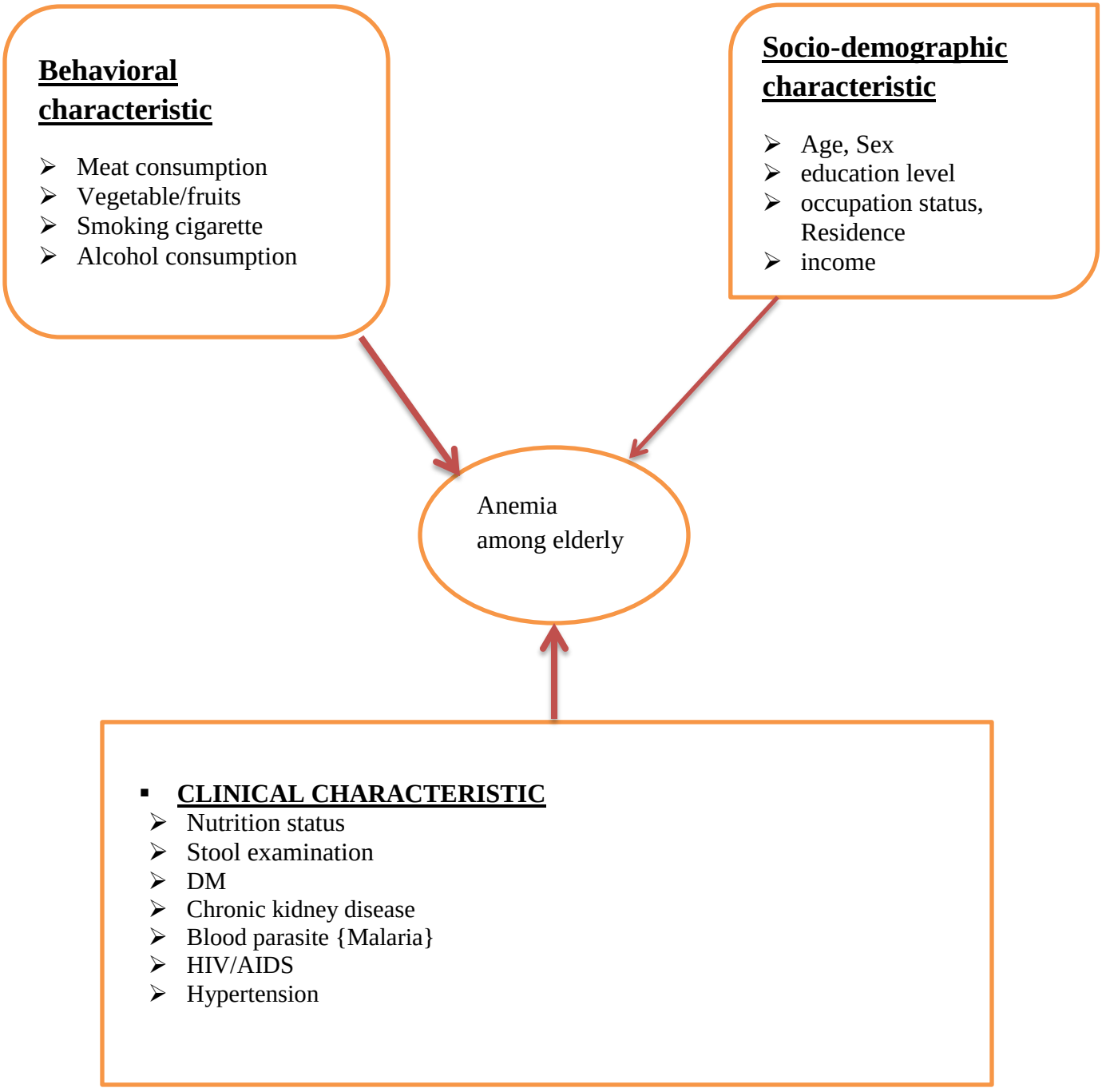

Figure 1:- Conceptual framework of anemia and its associated factors among elderly patients at Jigjiga University Sheik Hassan Yabare Referral Hospital between June 20 and July 30, 2022.

Chapter three: objectives

3.1 General objective

- To determine the prevalence and associated factors of anemia among elderly patients at Jigjiga University Sheikh Hassan Yabere Referral Hospital between June 20 and July 30, 2022.

3.2 Specific objectives

- To determine the prevalence of anemia among elderly patients
- To determine the morphological type
- To determine the severity of anemia among elderly patients
- To identify factors associated with anemia among elderly patients

Chapter four: Material and Methods

4.1 Study area

This study was conducted in Jigjiga town at Jigjiga University Sheik Hassan Yabere Referral Hospital. Jigjiga town is the capital city of the Somali Regional State, 630 km away from Addis-Ababa to the east. The city is located in the Fafan Zone, 60 kilometers (37 miles) away from west of the Somaliland border, with an elevation of 1,934 meters above sea level. Based on the 2007 census conducted by the Central Statistical Agency of Ethiopia, this town has a total population of 277,560, of whom 149,292 are men and 128,268 are women (60,61).

Jigjiga University Sheik Hassan Yabere Referral hospital was inaugurated in January 2017 and it has a total of 342 beds, a 13-bed intensive care unit (five functional now), 3 operation theatres, 24hrs emergency care units, clinical laboratories, and pharmacy facilities are available. The service available in the referral hospital are OPD(outpatient department) medical service (adult & pediatrics), surgical service (adult and pediatric), OPD Gynecology and Obstetrics, OPD Psychiatry, General Surgery, OPD Dermatology, 24hr emergency service (adult and pediatric), inpatient service, labor, and delivery service, Ophthalmology, Dental care unit, Adult and pediatric intensive care, Neonate intensive care, TB and HIV Testing and treatment and Ambulance services are available in the Hospital (62).

4.2 Study design and period

Institution-based cross-sectional study design was conducted between June 20 and July 30, 2022.

4.3 Population

4.3.1 Source population

All elderly individuals attending OPD at Jigjiga University Sheik Hassan Yabare Referral Hospital were considered as the source population for this particular study.

4.3.2 Study population

All elderly individuals attending OPD at Jigjiga University Sheik Hassan Yabere Referral Hospital during the study period and fulfilling the selection criteria was the study population.

4.4 Inclusion and exclusion criteria

4.4.1 Inclusion criteria

- All elderly individuals attending OPD at Jigjiga University Sheik Hassan Yabere Referral Hospital during the study period were included in the study.

4.4.2 Exclusion criteria

- Elderly individuals who had been transfused with red cell/ whole blood within the past three months.
- Elderly individuals who had major surgical intervention within the past three months.
- Elderly individuals who received therapy for anemia in the last three months

4.5 Sample size determination and sampling technique

4.5.1 Sample size determination

The sample size was calculated using a single population proportion formula based on the assumption of 5% expected margins of error, by considering a 95% confidence interval, and by taking the prevalence of 54.5% from a previous study conducted at the University of Gonder, Northwest Ethiopia (59).

By using the following formula:

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

Where:

- n = sample size
- z = statistic for level of confidence
- p = previous prevalence
- d= margin of error

$$n = \frac{(1.96)\ ^\wedge 2 \times (0.545\,(1-0.545))}{(0.05)\ ^\wedge 2} = 381$$

Therefore, the final minimum study subjects for this study were **381**.

4.5.2 Sampling technique

Convenient sampling technique was used to select elderly patients who fulfill inclusion criteria during the study period.

4.6 Study variable

4.6.1 Dependent variable

- Anemia among elderly

4.6.3 Independent variable

- Socio-demographic and socio-economic characteristics: Age, sex, residence, educational level, occupational status, and monthly income
- Lifestyle characteristics: BMI, smoking cigerate, alcohol consumption, consumption of vegetables, and meats, drinking tea/coffee within 30 minutes after the meal

- Clinical factors: diabetic Mellitus (DM), chronic kidney disease (CKD), blood parasite {malaria}, hypertension, parasitic infection, and HIV/AIDS infection.

4.7 Data collection instrument and procedure

4.7.1 Socio-demographic and clinical data collection

Socio-demography characteristics and socio-economic status of study participants (age, sex, residence, educational level, occupational status and income) and potential risk factors (smoking cigerate, alcohol consumption, and drinking tea/coffee within 30 minutes after the meal, dietary habits- meats, and vegetables consumption) were collected via face-to-face interviews by using a pre-structured questionnaire (Annex III). The interview was conducted by one trained clinical nurse capable to read and write the local languages and the response of each participant to every question was recorded on the questionnaire as per the pre-determined instructions. The questionnaire was first prepared in English language and then translated into the local language (Af-somali) and into the 2nd language (Amharic). Clinical factors (such as the history of diabetic Mellitus (DM), chronic kidney disease (CKD), hypertension, malaria, HIV/AIDS, parasitic infections, and other illness) were obtained by reviewing clinical/medical records.

An analog sphygmomanometer (OMRON® HEALTH CARE, Japan) and anthropometric measures (weight scale and stadiometer) protocols were used, respectively, by competent persons (clinical nurses), to measure blood pressure (BP) and body mass index (BMI) (63,64).

All study participants had their anthropometric measurements taken, including their height and weight. BMI was calculated as the weight in kilograms divided by the square of their height in meters, and it was divided into four categories: underweight (BMI <18.5 kg/m2), normal weight (18.5-24.9 kg/m2), overweight (BMI 25-29.9 kg/m2), and obese (BMI 30 kg/m2) (65).

4.7.2 Laboratory sample collection and analysis

Consent from all study subjects was obtained by giving detailed information about the study and, then instructing the study subject about the procedure. The blood sample was collected at the median cubital vein (66). Four milliliters (4 ml) of venous blood was collected aseptically from the median cubital vein by syringe and needle from each study participant by trained laboratory technologists, and then the sample was poured into a tripotassium Ethylenediaminetetraacetic acid (K3EDTA) test tube for determination of total blood cell count. The sample is labeled with accurate participation information (Identification number of study) and gently mixed 8-10 times to allow the introduction of blood and anticoagulants to prevent the sample from clotting. Then the blood transported from the blood collection room to the central laboratory by trained individuals.

Complete blood counts (CBC) were examined at the JUSHYRH central laboratory using a Mindray hematology analyzer (Shenzhen Mindray™ Bio-medical electronics co., Ltd, China*).* Total of white blood cell (WBC) count, basophil, RBC count, and platelet (PLT) count were determined using the Electrical Impedance method; Hgb was determined by using the colorimetric method; and the four differential WBC counts (segmented neutrophil, lymphocyte, monocyte, eosinophil) were determined using flow cytometry (67).

Hgb level was used to determine the prevalence and severity of anemia. Anemia is defined according to the WHO criteria as Hgb concentration lower than 12.0 g/dL for women and 13.0 g/dL for men in the study population (3). Severity of anemia is graded as severe (Hgb < 7.0 g/dL), moderate (Hgb: 7.0–9.9 g/dL), and mild (Hgb: 11.0–12.9 g/dL for men and Hgb: 11.0–11.9 g/dL for women) (3).

Overall the total samples was prepared peripheral blood smear fixed with methanol and stained with wright stain was used for RBC morphological examination and malaria parasite. The morphological type of anemia was assessed based on the result of RBC morphology plus red blood cell indices from CBC. The reference values of red blood cell indices that were used in this study are MCV (80–100 fl), MCH (27–32 pg), and MCHC (32–36%)

Stool collection and examination: The elderly were provided with clean, leak-proof stool cup and clean wooden applicator stick for stool specimen collection. Elderly patients were informed to bring about 2mg stool sample of their own. The stool samples were processed for microscopic examination using wet mount method**.**

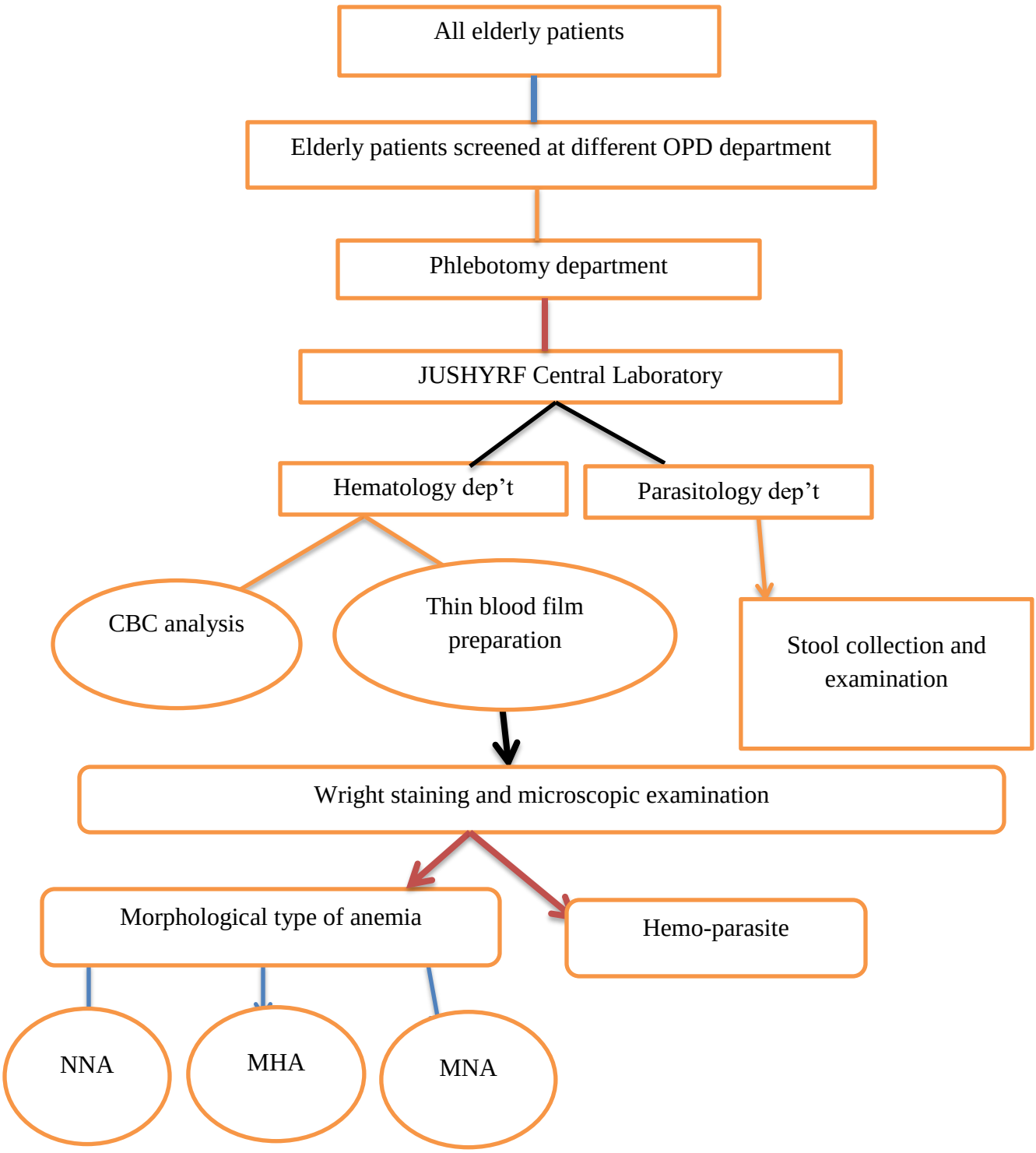

Figure 2:- Sampling and laboratory workflow for the determination of anemia and its associated factors among elderly patients at Jigjiga University Sheik Hassan Yabare Referral Hospital between June 20, and July 30, 2022.

4.8 Data quality assurances

In order to ensure the accuracy of the data, a number of quality control measures were put into place, including training data collectors prior to data collection, using standard operating procedures, checking reagents for expiration dates before any analysis, and regularly checking the completeness of each questionnaire while data collection was taking place.

During specimen collection and CBC analysis, standard operating protocols were followed to ensure the accuracy of the laboratory results. Thus, to avoid hemolysis after collection, blood was dispensed to the wall of the test tube and properly mixed by inverting the tube gently 8–10 times. Samples were checked to whether they are in the acceptable criteria like; hemolysis, clotting, adequate volume, and collection time (68). To avoid mix-up after collection, labeling was done on the sample and the questionaries' paper with the same identification number.

All laboratory testing was performed according to the manufacturer's instructions and standard operating procedures (SOPs). Background check: initial, repeated analysis of randomly selected specimens to see reproducibility. Randomly selected specimens (high, normal, and low), also verified by other similar hematology analyzers within the laboratory and as part of the laboratory routine for automated hematology analyzers in the hospital. The performance of hospital instruments is assessed using commercially available quality control materials of whole blood (low, normal, and high) (66,67).

Every day, Wright's stain was filtered using filter paper and kept in secured containers away from moisture and sunshine. The data collection, standard procedure implementation, and correctness of test results were all closely overseen by the principal investigator. The test results were kept confidential. Each laboratory test result was confirmed, reported and recorded properly.

4.9 Data analysis and interpretation

The data from both questioner and laboratory result were cleaned, edited, checked for its completeness a manually, and entered into EpiData version 4.6, and finally export into a statistical package for social sciences version 25 (SPSS, IBM, Chicago, IL, USA) for analysis. Before any analysis, the normal distribution of data was checked using the Kolmogorov-Smirnov.

Descriptive results was summarized by percentage and frequency, where also presented in tables and charts. Cross tabulation was done for describing the socio economic and socio-demographic data, to determine the prevalence of anemia. Binomial and multinomial logistic regressions were performed to assess the associated risk factors. Multinomial logistic regression were analyzed with a backward stepwise likelihood ratio when the p-value is less than or equal to 0.25 in binary logistic regression.

The association of the independent variable with the categorical outcome variable was measured by calculating the odds ratio at a 95% confidence interval. The model fitness of the final logistic regression model had been tested by using Hosmer and Leme-show test at a p-value greater than 0.05. Finally, p-value < 0.05 was considered statistically significant.

4.10 Operational definitions

Elderly: - individuals aged 60 years and above (69).

Anemia: - is defined as level of Hgb <13 g/dl for males and < 12 g/dl for women in elderly population (3).

A mild anemia:-Hgb level between 10-11.9 g/dl of Non-pregnant women (15 years of age and above), where Men (15 years of age and above) is 10-12.9g/dl (3).

A moderate anemia:-Hgb level between 7-9.9 g/d (3).

A severe anemia:-Hgb level between 7 g/dl (3)**.**

Alcohol abuse:

- For men, consuming more than 4 drinks on any day or more than 14 drinks per week
- For women, consuming more than 3 drinks on any day or more than 7 drinks per week

Trained health professionals: Data collectors and supervisors who receive two days of training on data collection processes and questionnaire completion techniques before the study begins by an experienced trainer

Illiterate: a person who cannot able to read and write

4.11 Ethical considerations

Ethical clearance was obtained from the institutional review board (IRB) of the Institute of Health, Jimma University (IHRPGY/830 27/05/2022), and a support letter also gained from Jimma University. Permission was gained from the Somali Regional Health Bureau, JUSHYRH Hospital administration, carried out the study. For those who wish to be confident of our acknowledgment, data collectors would have a letter to present. Patients who come in for usual OPD services was informed about the research's purpose, data collecting procedures and asked whether they would like to participate in the study.

Written informed consent was obtained from each elderly individual for participation in this study. Any information about the data were kept confidential and the result only be communicated to authorized concerned bodies.

Every study participant has the right to refuse to take part in the study and those with no willingness to participate not to be forced to be included in the study. Any abnormal test results of the participants were communicated to their attending physician immediately to make proper management and treatment.

4.12 Result dissemination plan

The results of this research will be presented and submitted to Jimma University, Institute of Health, school of medical laboratory sciences, Department of Clinical laboratory sciences especially hematology and immunohematology unit. The result will be also communicated through the Somali Regional Health Bureau, Jigjiga University Sheik Hassan Yabare Referral Hospital, and the source community via dialogues with focal individuals, direct mailings, reports, conferences, seminars, and local media-focused message delivery.

The study also kept at health facilities, colleges, public libraries, relevant government and non-governmental organization offices, and other locations as needed. A duplicate of this material also be kept at the Jimma University Library and Publications Office, College of Health Science, and the CBE Office. Finally, significant efforts are made to publish the study's findings in globally respected publication.

Chapter Five: Result

5.1 Socio-demographic characteristics of study participants

A total of 381 elderly patients participated in the study. Out of 381 study participants, 59.1% (225) were females; most of the elderly 61.4% (174) belong to the age group 60 to 69 years, with a mean ±SD age of 66.8(SD ± 7.2 years). Urban resident of the study participants was 63% (240) and 84.3% (325) of the study participants were married. More than half of the study participants 56.2% *(*214) were illiterate, and 38.3% *(*146) of the study participants were housewife/retired. Regarding the families' monthly income, which was based on a different survey done in Ethiopia (70), it showed that 33.1% (126) of the study participant earned less than 1000 Ethiopian Birr per month, 37.5% (143) earned between 1001 and 1999 ETB, and 29.4%(112) were earning more than 2000 ETB (Table 1).

Table 1: Socio-demographic characteristics of study participants among elderly patients attending JUSHYRH; June 20- July 30, 2022; Somali regional, Eastern Ethiopia

Variable	Categories	Frequency	Percent (%)
Age of patients	60-69	234	61.4
	70-79	100	26.2
	>80	47	12.3
Gender	Male	156	40.9
	Female	225	59.1
Place residence	Rural	141	37
	Urban	240	63
Marital status	Single	6	1.6
	Married	321	84.3
	Widowed	48	12.6
	divorced	6	1.6
Educational level	Illiterate	214	56.2
	primary school	92	24.1
	Secondary	13	3.4
	College and above	62	16.3

Occupational status	Farmer	66	17.3
	Daily laborer	55	14.4
	Private employer	43	11.3
	Governmental employer	71	18.6
	Housewife/retired	146	38.3
Monthly income in ETB*	<1000 ETB	126	33.1
	1001-1999 ETB	143	37.5
	>2000 ETB	112	29.4

5.2 Behavioral characteristic and nutritional status of study participants

Study participants who had a habit of consuming vegetables and meat less than once a week have 65.4% (249) and 54.1% (206), were also those who had a habit of consuming vegetables and meat at least once a week or more have 34.6% (132) and 45.9% (175) respectively. Furthermore, alcohol consumption, smoking cigarette, and drinking coffee and/or tea after 30 minutes a meal were 1.6% (6), 7.9% (30), and 6.8% (26) respectively. In terms of their nutritional status, 24.4% (93), 59.1% (225*)* and 15.5% (59) were underweight, normal weight, and overweight respectively (Table 2).

Table 2: Behavioral characteristic and nutritional status of study participants among elderly patients attending JUSHYRH; June 20- July 30, 2022; Somali regional, Eastern Ethiopia

Variable	category	Frequency	Percent (%)
Consumption vegetable	Less than once a week	249	65.4
	at least once a week or more	132	34.6
consumption of meat	Less than once a week	206	54.1
	at least once a week or more	175	45.9
drinking coffee or tea 30 minutes after a meal	Yes	26	6.8
	No	355	93.2
smoking Cigarette	Yes	30	7.9
	No	351	92.1
Amount of smoking cigarette	<10	29	7.6
	11-20	1	0.3

Alcohol consumption per week		14 drinks per week a men	5	1.3
		7 drinks per week a female	1	0.3
BMI		Malnutrition	4	1
		Underweight	93	24.4
		Normal	225	59.1
		Overweight	59	15.5

5.3 Clinical and other related characteristics of study participants

Intestinal parasites were detected in the stools of 22.8% (87) of the elderly patients, of which 7.1% (27) were Giardia-lambia, 12.1% (46) were E. histolytica, and 2.4% (9) were hook worms. Proportion of chronic renal disease, and malaria infection among the study participants was 3.4% and 1.3% (5) were diabetes mellitus. Rather than, 3.1% (12) had a systolic blood pressure of ≥140mmHg, and a diastolic blood pressure of ≥ 90mmHg (Table 3).

Table 3: clinical characteristic of the study participant among elderly patients attending JUSHYRH; June 20- July 30, 2022; Somali regional, Eastern Ethiopia

Variables	category	Frequency	Percent (%)
intestinal parasite	yes	87	22.8
	No	294	77.2
Type of parasite	A. lumbricoides	5	1.3
	G. lambia	27	7.1
	E. histolytica	46	12.1
	H. worms	9	2.4
Hypertension	Yes	12	3.1
	No	369	96.9
Diabetes Mellitus	Yes	5	1.3
	No	375	98.7
Chronic Kidney	Yes	13	3.4
Disease	No	368	96.6
Cancer	yes	2	0.5
	No	379	99.5
Malaria	Yes	13	3.4
	No	367	96.6

	Total	381	100

5.4 Prevalence, severity and types of anemia

5.4.1 Prevalence of anemia

The overall prevalence of anemia among elderly patients was 40.4% (154). Males were more likely to have anemia than female patients (21.3% & 19.2%). The range of Hgb levels was from 5 to 17.20 g/dl with mean Hgb levels of 12.6±SD 2.2 g/dl. The prevalence of anemia was 14.7% (56) among the 60-69 years age group, whereas 16.8% (64) among the 70-79 years age groups, and 8.9% (34) among the >80 years age groups. The proportion anemia of 25.2% (96) were housewives/retired elderly patients, 23.1% (88) were inhabitants of rural areas, 30.2% (115) were married people, 33.9% (129) were illiterates, and 28.6% were those earned <1000 ETB monthly income (Table 4).

Table 4: Distribution of anemia prevalence among elderly patients by socio-demographic factors at JUSHYRH, June 20- July 30, 2022; Somali regional, Eastern Ethiopia

Variables	Category	anemia patients	Percent (%)	Non-anemic patients	Percent (%)
Age of study participant	60-69	56	14.7	178	46.7
	70-79	64	16.8	36	9.4
	>80	34	8.9	13	3.4
Gender	Male	81	21.3	75	19.7
	Female	73	19.2	152	39.9
Place of residence	Rural	88	23.1	53	13.9
	urban	66	17.3	174	45.7
Marital status	Single	2	0.5	4	1
	Married	115	30.2	206	54.1
	Widowed	34	8.9	14	3.7
	Divorced	3	0.8	2	0.8
Educational status	Illiterate	129	33.9	85	22.3
	Primary school	18	4.7	74	19.4
	Secondary school	2	0.5	11	2.9
	college and above	5	1.3	57	15.0

Occupational status	farmer	35	9.2	31	8.1
	daily laborer	11	2.9	44	11.5
	private employer	6	1.6	37	9.7
	governmental employer	6	1.6	65	17.1
	housewife/retired	96	25.2	50	13.1
Monthly income	<1000 ETB	109	28.6	17	4.5
	1001-1999 ETB	39	10.2	104	27.3
	>2000 ETB	6	1.6	106	27.8
	Total	154	40.4	227	59.6

The study participants among elderly patients who consumed eating vegetables/&fruits and meat less than once a week had a high prevalence of anemia (33.9 and 36.2% respectively) than those who did once a week or more (6.6% and 4.2% respectively). The prevalence of anemia was 2.4% (9), and 0.5% (2), among participant who admitted to drinking coffee or tea within after meals and alcohol every week respectively. Distribution of anemia was 19.2% (73) among underweight study participants, 13.6% (52) among those who had an intestinal parasitic infection, 1.8% (7) among those who had chronic kidney diseases, 1.2% (4) and 1.3% (5) among who had a history of hypertension and diabetic mellitus, respectively (Table 5).

Table 5: Distribution of anemia prevalence among elderly patients by behavioral factors and clinical conditions at JUSHYRH, June 20- July 30, 2022; Somali regional, Eastern Ethiopia

Variables	Category	anemia patients	Percent (%)	Non-anemic patients	Percent (%)
Body mass index	Malnutrition	4	1	0	0
	Underweight	73	19.2	20	5.2
	Normal weight	68	17.8	157	41.2
	Overweight	9	2.4	60	13.1
	Obesity	0	0	0	0
Consumption of meat	Less than once a week	138	36.2	68	17.8
	At least once a week or more	16	4.2	159	41.7

Consumption vegetables	Less than once a week	129	33.9	120	31.5
	At least once a week or more	25	6.6	107	28.1
Smoking cigerate	Yes	11	2.9	17	4.5
	no	143	37.5	210	55.1
Amount of cigerate per day	<10	11	36.7	18	63.3
	10-20	0	0	1	3.3
Alcohol consumption	14 drinks per week a men	2	0.5	4	1
Drinking tea/coffee immediately after meal	Yes	9	2.4	17	4.5
	No	145	38.1	210	55.1
intestinal parasite	Yes	52	13.6	35	9.2
	No	102	26.8	192	50.4
Ova of parasite	A.lumbericoides	3	3.4	2	2.3
	G. lambia	16	18.4	11	12.6
	E. histolytica	26	29.9	20	23
	H. worms	7	8	2	2.3
Chronic Kidney Disease	Yes	7	1.8	6	1.6
	No	147	38.6	221	58
Cancer	yes	1	0.2	0	0
	No	153	40.4	227	59.4
Diabetes Mellitus	Yes	4	1.2	9	2.1
	No	150	39.4	219	57.8
Hypertension	Yes	5	1.3	7	1.8
	No	159	39.1	220	57.7
Malaria	Yes	7	1.8	6	1.6
	No	147	38.7	221	58
	Total	154	40.4	227	59.4

5.4.2 Severity of anemia among elderly patients

The anemia was graded according to WHO guidelines, the incidences of mild, moderate, and severe anemia among the elderly patients were 71.15% (109/154), 23.1%(36/154) and 5.8%(9/154) respectively. Figure 3

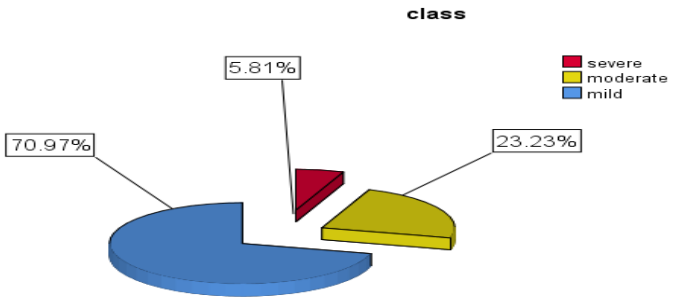

Figure 3: prevalence of severity of anemia among elderly patients attending JUSHYRH; June 20- July 30, 2022; Somali regional, Eastern Ethiopia

5.4.3 Morphological types of anemia among elderly patients

The commonest blood picture was normocytic normochromic anemia 55.13% (85/154), microcytic hypochromic anemia 37.18% (57/154) with few percent of target cell, ovalocytosis, and teardrop cells, and 7.69% (12/154) macrocytic normochromic anemia with macro-ovalocytes, hyper-segmentations and Howell-Jolly bodies. Figure 4

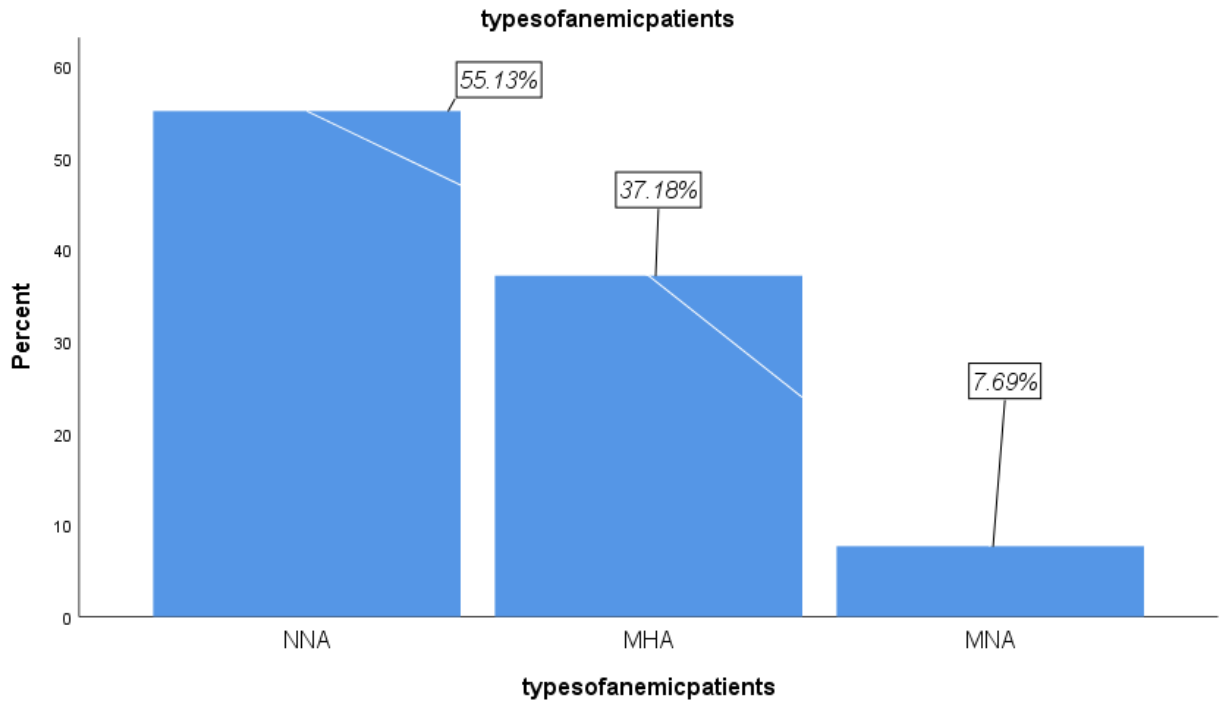

Figure 4: morphological type of anemia among elderly patients attending JUSHYRH; June 20-July 30, 2022; Somali regional, Eastern Ethiopia

5.5 Factors associated with anemia among elderly patients

According to the results of our study, socio-demographic and economic characteristics of older patients had an impact on the prevalence of anemia. In comparison, older adults (over 70) (25.7%), men (21.3%), and housewives/retired individuals (25.2%) all had higher prevalence of anemia than to their respective counterparts. Anemia affected a higher percentage of elderly patients who were illiterate (33.9%) compared to literate patients (6.5%). Similarly, elderly patients who earned less than 1000 ETB had higher prevalence of anemia (28.6%) those from high-income families (>2000 ETB), who had have 1.6% prevalence of anemia. In order to get the predictors of outcome variable, multivariate logistic regression analysis was performed after selected the candidate variables through binary logistic regression. Age, sex, place of residence, family income, occupation status, educational level, nutritional status, and habitual consumption of meat and vegetables were candidate of variable in multivariate logistic regression (Table 6)

Table 6: Binary logistic regression analysis of the factor associated with anemia among elderly patients at JUSHYRH, June 20- July 30, 2022; Somali regional, Eastern Ethiopia

Variables	Category	Anemic patients N (%)	Non-anemic patients N (%)	COR [95%CI]	p-value
Age of study participant	60-69	56(14.7)	178(46.7)	Ref(1)	
	70-79	64(16.8)	36(9.4)	9.929 (5.854, 16.83)	**<0.001**
	>80	34(8.9)	13(3.4)	0.663 (0.422, 1.043)	**<0.001**
Gender	Male	81 (21.3)	75(19.7)	0.445(0.292, 0.677)	**0.001**
	Female	73(19.2)	152(39.9)	Ref(1)	
Place of residence	Rural	88(23.1)	53(13.9)	0.228(0.147, 0.356)	**0.024**
	urban	66(17.3)	174(45.7)	Ref(1)	
Educational status	Illiterate	129(33.9)	85(22.3)	0.058(0.022, 0.150)	**<0.001**
	primary school	18(4.7)	74(19.4)	0.361(0.126, 1.030)	**0.057**
	secondary	2(0.5)	11(2.9)	0.482(0.083, 2.811)	0.418
	college and above	5(1.3)	57(15.0)	Ref(1)	
Occupational status	farmer	35(9.2)	31(8.1)	0.379(0.083, 1.728)	**0.210**
	daily laborer	11(2.9)	44(11.5)	0.588(0.121, 2.862)	0.510
	private employer	6(1.6)	37(9.7)	0.240(0.034, 1.713)	**0.155**
	governmental employer	6(1.6)	65(17.1)	0.318(0.038, 2.679)	0.292
	housewife/retired	96(25.2)	50(13.1)	Ref(1)	Ref(1)
Monthly income	<1000 ETB	109(28.6)	17(4.5)	0.009(0.003, 0.023)	**<0.001**
	1001-1999 ETB	39(10.2)	104(27.3)	0.151(0.061, 0.372)	**0.001**
	>2000 ETB	6(1.6)	106(27.8)	Ref(1)	
Body mass index	Underweight	73(19.2)	20(5.2)	0.049(0.049, 0.021)	**<0.001**
	Normal	68(17.8)	157(41.2)	0.416(0.193, 0.893)	**0.024**
	Overweight	9(2.4)	60(13.1)	Ref(1)	

Consumption of meat	Less than once a week	138(36.2)	68(17.8)	0.050(0.027, 0.089)	**<0.001**
	At least once a week or more	16(4.2)	159(41.7)	Ref(1)	
Consumption of fruit and vegetables	Less than once a week	129(33.9)	120(30.1)	0.217(0.132, 0.359)	**<0.001**
	At least once a week or more	29(7.6)	107(28.1)	Ref(1)	
Smoking cigerate	Yes	11(2.9)	17(4.5)	0.950(0.432, 2.089)	0.899
	No	143(37.5)	210(55.1)	Ref(1)	
Alcohol consumption	yes	2(0.5)	4(1)	0.734(0.133, 4.055)	0.722
	No	152(39.9)	223(58.5)	Ref(1)	
Drinking tea immediately after meal	Yes	9(2.4)	17(4.5)	1.304(0.566, 3.007)	0.533
	No	145(38.1)	210(55.1)	Ref(1)	
intestinal parasite	Yes	52(13.6)	35(9.2)	1.337(0.387, 2.265)	0.883
	No	102(26.8)	192(50.4)	Ref(1)	
0va of parasite	A. lumbricoides	3(3.4%)	2(2.3%)	1.556(0.165, 14.654)	0.699
	G. lambia	16(18.4%)	11(12.6%)	1.604(0.339, 7.597)	0.551
	E. histolytica	26(29.9%)	20(23%)	1.728(0.397, 7.524)	0.466
	H. worms	7(8%)	2(2.3%)	Ref(1)	
Chronic Kidney Disease	Yes	7(1.8)	6(1.6)	1.754(0.578, 5.323)	0.321
	No	147(38.6)	221(58.0)	Ref(1)	
Diabetes Mellitus	Yes	4(1.2)	9(2.1)	0.363(0.040, 3.277)	0.367
	No	150(39.4)	219(57.8)	Ref(1)	
Hypertension	Yes	5(1.3)	7(1.8)	1.055(0.329, 3.386)	0.929
	No	149(39.1)	220(57.6)	Ref(1)	
Malaria test	Yes	7(1.8%)	6(1.6%)	1.746(0.575, 5.299)	0.325
	No	147(38.7%)	221(58%)	Ref(1)	

5.6 Multivariate logistic regression analysis of anemia predictors

All variables having a $p \leq 0.25$ on binary analysis were added to the multiple logistic regression models to identify those that were independently risk factors of anemia since confounding variables might exist. In multivariate analysis, place of residence, marital status, occupational level, educational status and habit consumption vegetables/fruits less than a once a week were not retained in the final model. Age, sex, lower monthly income, and habit consumption of meat less than once a week were remained significantly associated risk factors of anemia.

Table 7:- Multivariate logistic regressions of selected factors associated with anemia among elderly patients at JUSHYRH, June 20- July 30, 2022; Somali region, Eastern Ethiopia

Variables	Category	COR [95%CI]	p-value	AOR* (95%CI)	p-value
Age of study participant	60-69	Ref(1)			
	70-79	9.929 (5.854, 16.83)	**<0.001**	2.620(1.265, 5.426)	**0.009**
	>80	0.663 (0.422, 1.043)	**<0.001**	1.372(0.594, 3.168)	0.458
Gender	Male	0.445(0.292, 0.677)	**0.001**	0.352 (0.174, 0.708)	**0.003**
	Female	Ref(1)		.	
Place of residence	Rural	0.228(0.147, 0.356)	**0.024**	1.057 (0.429, 2.608)	0.903
	urban	Ref(1)			
Educational status	Illiterate	0.058(0.022, 0.150)	**<0.001**	0.768 (0.115. 5.110)	5.110
	primary school	0.361(0.126, 1.030)	**0.057**	1.273 (0.208, 7.781)	7.781
	secondary	0.482(0.083, 2.811)	0.418	0.779 (0.080, 7.591)	7.591
	College/ above	Ref(1)			
Occupational status	farmer	0.379(0.083, 1.728)	**0.210**	1.221 (0.412, 3.620)	0.719
	daily laborer	0.588(0.121, 2.862)	0.510	1.844 (0.505, 6.738)	0.354
	private employer	0.240(0.034, 1.713)	**0.155**	0.813 (0.165, 4.007)	0.799
	governmental employer	0.318(0.038, 2.679)	0.292	1.057 (0.190, 5.894)	0.950
	housewife/retired	Ref(1)	Ref(1)		
Monthly income	<1000 ETB	0.009(0.003, 0.023)	**<0.001**	0.041 (0.011, 0.156)	**<0.001**
	1001-1999	0.151(0.061, 0.372)	**<0.001**	0.267(0.071, 1.004)	**0.05**

	ETB				
	>2000 ETB	Ref(1)			
Body mass Index	Underweight	0.049(0.049, 0.021)	**<0.001**	0.545 (0.165, 1.802)	0.320
	Normal	0.416(0.193, 0.893)	**0.024**	1.148 (0.409, 3.218)	0.793
	Overweight	Ref(1)		Ref(1)	
Habit of consumption of meat	Less than once a week	0.050(0.027, 0.089)	**<0.001**	0.301 (0.114, 0.793)	**0.015**
	At least once a week or more	Ref(1)		.	.
Habit of consumption of fruit & vegetable	Less than once a week	0.217(0.132, 0.359)	**<0.001**	1.851 (0.755, 4.542)	0.179
	At least once a week or more	Ref(1)			

Chapter six: Discussion

The overall prevalence of anemia among elderly patients in this study was 40.4% (154/381; 95%CI 0.69-0.91). According to WHO recommendations our findings showed a severe public health problem among elderly patients (3). Furthermore the incidence rate of mild, moderate, and severe anemia were 71.15% (109/154), 23.1% (36/154), and 5.81% (9/154), respectively. Age, sex, low monthly income, and meat consumption of less than once per week are all risk factors for anemia in elderly patients.

In our study, the overall prevalence of anemia is comparable to research by Pautas et al. in France, which found that 44.7% of the elderly patients (53), and Republic of Korea (37.3%) by Bryce *et al.*, (46).

However; our finding was higher than studies done in United state (13.6%) (24), Austria (21.1%) (48), and Egypt (17.5%) (58). This may be due to variations in the lifestyle of study participants, sample size, and different upper age limit. In addition to that it can be effect long term of low iron intake, and consumption of animal products.

Furthermore, our study anemia prevalence was lower than, reports of studies done in Tanzania (79.5%) by Chamba *et al.*, (57), France (53%) by Petrosyan *et al.*, (52), India (68.7%) by Pathania *et al.*, (54), Turkey (54.9%) by Sahin *et al.*,(49) and Ethiopia (54.5%) by Melku *et al*. The difference might be due to comorbidity of disease and difference in the study subjects, since elderly of this study was who sought medical intervention in the outpatient department, as opposed to the study subjects in the studies by Chamba et al., Pathania et al., and Sahin et al., who were institutionalized in long-term care facilities, and the study subjects by Petrosyan et al., who used a different study design than the current study.

Besides; our finding was lower than report of study done by Dunn et al. 77% (71) Mary Potter Hospice's in New-Zealand. This discrepancy can be attributed to the distinctive characteristics of the study participants since Dunn et al. included elderly patients who were admitted to the hospital for palliative care and higher percentages of hematological malignancy patients.

According to the RBCs morphological assessment, normocytic-normochromic anemia was most predominant type of anemia (54.8%). The reason could be because of the fact with chronic infection, chronic inflammatory disorders and also rises in hepcidin level, which can result a fall in Hb concentration in serum, while keeping the red cell indices within normal (72,73). This finding is concordant with the results of many studies conducted in different areas among elderly patients in Ecuador (87.4%) (47), France (82.1%) (52), Iran (75.17%) (74), and Gondar, Northwest Ethiopia (85.3%) (59).

Besides that; Hypochromic- microcytic anemia (37.4%) is second most abundant type of anemia in our study. The possible cause of this can be long term effect of low iron intake, decreased absorption of iron, chronic blood loss, GI bleeding, and increased circulating concentrations of hepcidin (75). This finding is in line with that studies conducted in Ecuador (7.6%) (47), France (9.5%) (52), Iran (20.29%) (74), and Northwest Ethiopia (9.20%) (59).

On the other hand, mild anemia was the common form of anemia in the current study, which accounted for 71.1%. This could be explained by the fact that our study was hospital-based; our population already had relatively progressed illnesses that required admission. However, it may also be possible that there is already a significant proportion of the elderly population. And when they acquire conditions that force them to seek medical care, the anaemia would have worsened and thus present with a moderate anaemia or severe anaemia. These findings concurred with those of research carried out among elderly patients in France (61.1%) (52), India (47.4%) (54), and Ethiopia (56.9%) (59).

Our study showed that the prevalence of anemia significantly with age (AOR=2.620 95%CI: 1.265, 5.426(p= 0.009). This finding is in line with reports of studies performed in United-state, Austria, Turkey, Iran, and Qatar (24,48,49,56). A few mechanisms can explain the correlation between the prevalence of anemia and advanced age. The first is the decreased capacity of renal hormone production leads to the development of anemia. In addition, pro-inflammatory cytokines are known to express themselves more frequently with age, which can result in EPO insensitivity. Therefore, hepcidin expression is induced (inflammation-related anemia) and cytokines limit the formation of erythriod colonies, which means that genetic variation in the expression of these cytokines can affect anemia development in older persons (14).

Regarding differences between the sexes, the prevalence of anemia was higher among males than women in the current study (AOR 0.352 95%CI 0.174, 0.708(p=0.003)). This was consistent with the findings of earlier studies conducted in Ecuador, Russia, and Egypt (21,51,58). The significant difference in the prevalence rates of anemia in men and women can be attributed to the substantial decline in free and bioavailable testosterone concentration in males after reaching middle age. This negatively impacts the enhanced metabolic processes of the bone marrow. As testosterone level decreases with aging, the rate of erythropoiesis tends to be declined and predispose men to increased risk of anemia (76,77).

Anemia was shown to be independently correlated with monthly income (<1000ETB) (AOR=0.041 95% CI: 0.011, 0.156(p=0.001)) and less than once a week of meat consumption (AOR=0.301 95% CI: 0.114, 0.793(p=0.015)). This correlation may be attributed to the long-term effects of low iron intake and micronutrient deficiencies. Our study in agreement with the study conducted in different areas like: Cuba and Dominican Republic (socio-economic status and meat intake) by Bryce *et al.*, (46), Russia (socio-economic status) by Bikbov *et al.*, (51), Iran (meat consumption) by Afag and co.,(74).

In this study, those who regularly consumed meat at least once a week or more had less anemic probabilities than those who did less frequent or less than once a week. There is evidence to suggest that cutting out meat and other animal products from your diet increases your chance of developing nutrient deficiencies (78,79). And also the risk of vitamin B12, iron, and other mineral shortages is therefore widespread in vegetarians, predisposing them to a higher risk of nutritional deficiency anemia (80). Along with severe undernutrition, which is a significant public health issue in many African nations due to poverty and limited access to essential social supports (81).

STRENGTH AND LIMMITATION OF THE STUDY

Strength of the study

- ✓ studying magnitude of anemia, severity, and morphological pattern of anemia
- ✓ determined associated risk factors of anemia among elderly patients

Limitation of the study

- ✓ Not performed inflammatory indicators (ESR, C-reactive protein)

Chapter seven: Conclusions and Recommendations

7.1 Conclusions

It is conclude that anemia is a severe significant health problem among elderly patients with the present prevalence of 40.7%. Majority of elderly patients were mildly anemic. More than half of the elderly patients presented with a blood picture of Normocytic normochromic anemic (54.8%) and the other types included 37.4% of Microcytic hypochromic anemic and 7.7% were Macrocytic normochromic.

The results of this study showed that the factors such as: age (advanced age), sex (men), socio economic status (low family income) and meat consumption (less frequent/ less than once at week were the factors contributing to the prevalence of anemia among elderly patients.

7.2 Recommendations

Based on the result obtained from this study the following recommendations were forwarded:

- Creating awareness of the elderly on nutrition, role of healthy diet and consequences of anemia among elderly.
- Designing for prevention of anemia in elderly patients, interventions for nutritional deficiencies should be considered by coordination of regional health bureau, and clinicians managing elderly patients.
- Further studies are needed to consider evaluation of iron indicators like serum ferritin, serum transferrin and also evaluation of serum folate and serum Vit-B12 etc.
- screening for anemia among elderly should be a part of their routine management

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Chapter Eight: annexes

8.1 Annexes I: Information sheet

English Version

- **Title of the project:** Prevalence of anemia and associated factors among elderly patients at Jigjiga University Sheikh Hassan Yabare Referral Hospital between June 20 and July 30, 2022.

Name of Principal Investigator: Niman Tayib

Organization: Jimma University (School of Medical Laboratory Science)

Name of sponsor: Jigjiga University

This information sheet is prepared for elderly patients attending Jigjiga University Sheikh Hassan Yabare Referral Hospital who involve in a project entitled above. We tell them about the whole processes that have been undertaken in the study and request them to participate voluntarily.

Description and Purpose of the study

- Anemia is a global public health problem that affects both developed and developing countries and has serious impacts on human health and social and economic development. Several factors are responsible for the development of anemia. The magnitude of anemia among the elderly and its associated factor is not well known in this study area. Therefore, this study was designed to determine the prevalence of anemia and

associated factors among elderly patients attending Jigjiga University Sheikh Hassan Yabare Referral Hospital between June 20 and July 30, 2022.

Aim of this study

- The objective of this research is to determine the prevalence of anemia and associated factors among elderly patients. If you agree to participate in the study, about 4ml of blood specimen were collected from you, as well as respond to the following questions voluntarily and it kept confidential.

Confidentiality

All the data obtained was kept strictly confidential and locking data, only study personnel have had access to the files. Anonymous testing were undertaken, which means samples was coded and results not be identified by names.

There is not be any payment or direct benefit for participating and you are not asked to pay for the laboratory examination. Your result is reported back to the physicians if it is found significant for further diagnosis and treatment.

Benefits: All the investigations done for participants of this study were free of charge.

Potential risks and Discomforts

During the collection of specimens from you, appropriate precaution would take and all samples were collected by trained health professionals. If anything happened, appropriate medical care was provided.

Compensation

You are not being directly compensated for your precious participation in this study but it may help indirectly other patients who may have the same problem as you.

Withdrawal:

Your participation in this study is purely voluntary, and you may stop the participation at any time or you may refuse to answer some of the questions if you feel uncomfortable. You are free to refuse to participate in the study or you can withdraw your consent at any time, without giving reasons and this will not involve any penalty or loss of benefits to which you are entitled such as proper care and treatment. Your access to treatment will not be dependent on your participation

in the study. If you are not comfortable please feel free to stop it at any level of the study. I appreciate your cooperation greatly. If you have questions regarding this study or would like to be informed of the results after its completion, please contact me through the following address.

Principal investigator: Niman Tayib TEL. 0948647413/0915058578 E-mail: nimantayibmohamoud@gmail.com Jimma University, Faculty of Health Science Department of medical laboratory science especially hematology and immunohematology

Lifaaqa I: Akhbaar guud oo loogu tala galay ka qayb galaha daraasaadkan.

Qaybta soomaliga ah

Ciwaanka borojeedka:- Heerka dhiigyaraanta iyo shayada Soboba ee dadka waaweyn ee yimaada dhakhtarka Jigjiga University Sultan Sheikh Hassan Yabare Referral Hospital

Magaca baadhaha daraasaadkan: Niman Tayib

Organization: Jimma University (School of Medical Laboratory Science)

Magaca maalgaliyaha: Jigjiga University

War bixinta waraaqdani waxaa loo diyaariyay dadka waaweyn ee soo buuqda dhakhtarka Jigjiga University Sultan Sheikh Hassan Yabare Referral Hospital kaas oo loogu talo galay inuu ka qayb qaato daraasaadkani kor aan ku xusnay. Waxaa loo sheegi dhamaan waxkasta oo ku saabsan daraasaadkani iyaga oo uga qayb qaadanaya six or ah.

Faahfaahin iyo ujeedooyinka daraasaadkani

Dhiig yaraantu waa mid balaadhan oo saamaysay caafimaadka dadka tasoo ku lug yeelatay wadamada horumaray iyo kuwa hada horumaraya, waxayna saamayn balaaran ugaysataa badqabka xaafimaadka qofka sidoo kale horumarka bulsho iyo dhaqaalaba.waxaa jira shayo(qodopo) badan oo sababi kara dhiigyaraanta. Heerka dhiigyaraan iyo shayda Soboba ee dadka waaweyn ee deegaankeena wali daraasaad lagama samaynin. Sidaa darted waxaan ugu talo galnay daraasaadkani in aan ku ogaano heerka dhiigyaraanta iyo shayada Soboba ee dadka waaweyn ee imaanaya amaba soo buuqanaya dhakhtarka.Jigjiga University Sultan Sheikh Hassan Yabare Referral Hospital from May 20 to June 20, 2022.

Ujeedada Daraasaadka:

ujeedada guud ee daraasadkani waa in la darso baahsanaanta dhiigyaraanta dadka waaweyn ee ka weyn 60 sano iyo sababaha ku keena dhiig yaraanta dadka waa weyn .

Hadii aad ogolaato kaqaybgalka daraasadkan, waxaa lagaa qaadi doonaa 5 ml oo dhiig ah, wax kaloo na siin doontaa jawaabaha- su;aalaha soo socda waxayna ahaan doonaan kuwo sir ah oo la ilaaliyay iyadoo lagu xusayn magacaaga.

Xog-dhawrka (sir-dhawrka).

Dhamaan akhbaarkasta oo dhankaaga laga helo waxay ahaan doontaa mid sir ah oo kaliya uu helikaro qofka daraasaadka samaynaya. Dhamaan xogtaada waxay ahaanayaan kuwo xidhan sidooklena number sir ah ayaa loosamayn doona marka tijaabooyinka lasamaynayo taas oo inaga caawinaysa wixii cilad caafimad ee jirta inaanan magac muuqda lagu garan Karin si fudud.

Wax lacag ah ood ka helaysaa daraasadkan nuuckasta oo ka qaybgal ah ha ahaado mid toos ah iyo mid dadbanba; habasaahaatee, dhamaan tijaabooyikaaga shaybaadhka waxaa laguugu fulinaya si ka madax banaan qiimo lacageed (bilaash) natiijooyinkaagana waxa dib loogu diridoonaa dhaqtarka si daawo aad u heshid iyadoo laga duulayo nuuca natiijada.

Faa'iidada: dhamaan baadhitaanada loo samayn doono ka qaybgalaha darasaadkan waxay unoqon doonaan kuwa bilaash ah

Magdhaw: daraasadkan si toos ah kalama kulmaysid wax magdhaw ah si toos ah balse waxay si dadban u caawin doontaa dad badan oo dhibaatadan nuucan oo kale ah kujira.

Joojin.

Ka qaybgalka daraasaadkani saafiyan waa mid iskaa ah oo aan qasab ahayn; waad iska joojinkarta ka qaybgalkaaga wakhti kasta waanad iska diidi kartaa ka jawaabida su'aalaha qaarkood hadii ayna kuqancin. Waad la noqonkartaa ogolaanshahaaga xiligay doontaba ha noqote mana jirayso wax ganaax ah oo kaaga iman doono. Daawayntaada iyo daryeelkaagu midna kuma xidhna joojinta ka qaybgalka daraasadkan. Hadii ay jiraan wax su aalo ah ood qabtid iyo hadii ay jiraan waxaad jeclaan lahyd in lagu ogaysiiyaba, igalasoo xidhiidh ciwaanadan hoos ku xusan fadlan.

Baadhaha koowaad: Niman tayib

Tel.no0948647413/0915057578. Email: nimantayibmohamoud@gmail.com

Jaamacada Jimma, Koolajka Caafimmadka Qaybta barashada dhiiga, noocyada kala duwan ee dhiiga iyo difaaca biniaadamka.

8.2 Annexes II: Consent form

English version

For the elderly individual, after being fully informed about the objectives and consequences of this study

I/we hereby provide assent for the participation of this study voluntarily.

a) Yes
b) No

For the elderly individual, after being verbally informed about the overall aim of the study, I/We hereby assent to voluntary participation in this study

Signature---------- Date: ----------

Lifaaqa II: Foomka Ogolaanshaha

Qaybta soomaliga ah

Ka qayb qaataha daraasaadka kabacdi markii si buuxda lanoo fahansiiyay (hadal ahaan iyo qoraal ba) ujeedka guud iyo natiijooyinka daraasadkan, waxaan/waxaanu halkan ku dhiibaynaa ogolaanshihii inuu ka qabgalo daraaasaadkan si tabarucaad ah.

b) Haa

t) Maya

Saxeex…….. Taariikhda…….

8.3 Annexes III: Questionnaire (data collection form).

English version

Questionnaires: Administered for the investigation to determine "Prevalence of anemia and its associated factors among elderly patients at Jigjiga Referral Hospital, Jigjiga, Ethiopia.
Card no: _____

Code: _____

Address: _____

General instruction

- ✓ for all questions that have a pre- coded response,
- ✓ Circle the responses that best match with your response

For open ended questions write your responses in blank space

Sn	Variables	Response	Skip to
Part i: socio-demographic characteristic			
1.1	Age in full year	----------	
1.2	Sex	0. Female 1. Male	
1.3	Residence	0. Urban 1. Rural	
1.4	marital status	0. Single 1. Married 2. Divorced 3. Widowed	
1.5	Educational status	0. Illiterate 1. Primary school 2. Secondary school 3. College to university	
1.6	Occupational status	0. farmer 1. daily laborer 2. private employee 3. governmental employee 4. housewife/retired	
1.7	Monthly income in Ethiopian birr	_____	

Part ii: dietary habits

2.1	Do you consume fruit and Vegetable?	0. No 1. Yes	If no skip to 2.3
2.2	If yes for q.2.1. How many days per week on average?	0. Less than once a week 1. At least once or more	
2.3	Do you consume meat?	0. No 1. Yes	If no skip to 2.5
2.4	If yes for q.2.3**.** How many days per week on average?	0. Less than once a week 1. At least once or more	
2.5	Do you have a habit of drinking coffee or tea immediately after the meal (within 30 minutes)	0. No 1. Yes	

Part iii: behavioral characteristics

3.1	Did you smoke a cigarette?	0. No 1. Yes	If no skip to 3.3
3.2	If yes to q.4.1, on average how many cigarettes do you smoke per day? (in number)	----------	
3.4	Habit of alcohol consumption	0. 14 drinks per weekly a men 1. 7 drinks per weekly a female	
3.5	Weight (to be measured by data Collectors)	_____kg	
3.6	Height (to be measured by data Collectors)	_____meter	

Part iii: clinical characteristic record

4.1	Did you have a known chronic illness like?	0. No 1. Yes	

4.2	If the q 4.1 says yes which one did you have the following once	
4.2	Diabetes mellitus	0. No **1.** Yes
4.3	Chronic kidney disease	0. No **1.** Yes
4.4	Chronic blood loss	0. No **1.** Yes
4.5	Parasitic infection	0. No **1.** Yes
4.8	Hypertension	0. No **1.** Yes
4.9	HIV/AIDS	0. No **1.** Yes

Lifaaqa iii: - su”aalaha daraasaadka
Qaybta soomaaliga ah
Nambarka kaarka: _____

Nambarka ka qaybqaataha: _____

Ciwaanka: _____

Warbixin guud

i. Dhamaan sulaahu waxay leeyihiin jawaaabo xadidan
ii. Goobo gali jawaabta ka qaybqaataha

Suaalaha furan ku qor booska banaan

Sn	Waydiimaha	Jawaabaha	Ka bood hadii maya tahay
Part i: qaybta koobaad ee ka qayb qaataha			
1.1	Da’da qofka	----------	
1.2	Jinsiga	0. Dhidig 1. Lab	
1.3	Deganaanshaha goobta	0. Magaalada 1. Miyiga	
1.4	Nolosha qofka	0. Kali 1. Guri leh 2. la soo furay 3. Lagadhintay	
1.5	Heerka waxbarasho	0. Waxba aan qorin waxna akhrin 1. Dugsi hoose 2. Dugsi sare 3. Ilaa heer jaamacadeed	
1.6	Nooca shaqada	0. Beero qodato 1. Iskii u shaqaysta 2. Dad ushaqeeya 3. Dowlada ushaqeeya 4. bilaa shaqo ah/guri joogto	
1.7	Meeqa lacag ah ayaa heshaa	_____	
Part ii: cuntooyinka			
2.1	Ma cuntaa khudaarta	0. Maya 1. Haa	Ka gudub haday maya tahay suaasha 2.2

2.2	Haday haa tahay suaasha 2.1 imisa jeer ayaad cuntaa todobaadkii	0. Hal mar kayar 1. Mar iyo in ka badan	
2.3	Maad cuntaa hilibka	2. Maya 3. Haa	Ka gudub haday maya tahay suaasha 2.4
2.4	Haday haa tahay suaasha 2.3 imisa jeer ayaad cuntaa todobaadkii	0. Hal mar kayar 1. Mar iyo in ka badan	
2.5	Ma cabtaa bunka amaba shaaha qadada kadip	0. Maya 1. Haa	

Part iii: qaybta sadexaad ee dabeecadaha qofka

3.1	Ma cabtaa sigaarka?	0. Maya 1. haa	Ka gudub haday maya tahay suaasha 3.2
3.2	Haday haa tahay suaasha 3.1 imisa jeer ayaad cabtaa maalintii	----------	
3.3	Ma isticmaasha khamriga?	0. 14 jeer isbuucii raga 1. 7 jeer isbuucii dumarka	Ka gudub haday maya tahay suaasha 3.4
3.5	Ma cuntaa jaadka	0. Maya 1. haa	
3.6	Cabiraada culayska	_____kg	
3.7	Cabiraada dhererka	_____meter	

Part iii: clinical characteristic record

4.1	Miyuu leeyahay xanuuno sida?	
4.2	Macaanka	0. Maya 1. Haa
4.3	Kalyo xanuun	0. Maya 1. haa
4.4	Dhiig bax	0. Maya 1. Haa
4.5	Gooryaanka caloosha	0. Maya

1. haa

4.6 Wadno xanuun

0. Maya
1. haa

4.7 Beer xanuun

0. Maya
1. haa

4.8 Dhiig kar

0. Maya
1. haa

4.9 Hiv/aids

0. Maya
1. Haa

8.4 Annexes IV: - Standard Operating Laboratory Procedure

A. Collection of Venous Blood Samples from Research Study Participants

After a brief explanation of the study participant will make draw venous blood collection for his arm hand.

The standard operating procedure (SOP) describes the procedures for the phlebotomist to perform the controlled act, performing a procedure on tissue below the dermis, by collecting venous blood samples from study participants.

EQUIPMENT

- 21 gauge needle for each participant with a closed vacutainer system
- Blood collection tube for each participant Ethylenediaminetetraacetic acid (K3EDTA) and sodium citrate test tube
- Tourniquet
- Box of nitrile/vinyl gloves (Do not use latex gloves due to allergies/sensitivities.)
- Alcohol wipes, Cotton balls/swabs, and Bandages
- Pillow/pad for raising an arm to comfortable elevation
- Disposable, single-use materials or equipment are to be used whenever possible

PROCEDURES/STUDY PROTOCOL

1. A phlebotomist performing a venous blood draw will examine the participant's arm for an appropriately sized vein. This is most commonly the medial carpal vein, although other veins on the forearm and back of the hand can be used.
2. After the blood drawer has selected the appropriate vein, the participant sits or lies down in a position where the sampler has access to a vein that is comfortable for the participant
3. Wipe the skin in the vein with an alcohol wipe.
4. A tourniquet will be applied 5-10 cm above the intended site of the venous puncture.
5. Collect a blood sample using a closed vacuum/syringe needle system. The gray rubber end of the vacutainer needle is opened and screwed into the vacutainer tube holder. The

needle is then released and inserted into a vein through the skin at an angle of 15-30 degrees.

6. A blood tube (k3EDTA) can then be inserted into the tube holder and holder. The vacuum in the tube draws up to 4ml of blood into the K3EDTA test tube
7. When the blood draw is complete, remove the tourniquet and quickly withdraw the needle from the vein.
8. Cotton is pressed into the venipuncture site.
9. Participants should sit still and apply pressure to stop bleeding and reduce the risk of bruising.
10. The phlebotomist will remove the needle directly from the holder into the sharp needle container.
11. Gently invert the blood sample after collection to mix the anticoagulant and mark the sample with participant information such as ours (identification number).
12. When the bleeding subsides or stop, cover the puncture site with a dressing and the participant should sit still for up to 10 minutes.
13. The hematologist informs you that the injection site may be bruised over the next several days to promote rapid healing, keep the injection site clean and dry and avoid lifting heavy objects for 24 hours to avoid further bruising.
14. Participants will be grateful for their participation.
15. Competitors are served with either apple or orange juice and a light snack (eg bagel, breakfast bar).

SAFEGUARDS/SAFETY PROCEDURES

PARTICIPANTS

- Universal precautions are being applied at all times. Refer to the Canadian Public Health Association universal precautions guidelines. See http://www.cpha.ca/uploads/portals/idp/19661e.pdf
- A new pair of disposable latex/vinyl gloves is used with each participant. Gloves are to be for single-procedure use only. Gloves should always be removed using a glove-to-glove or skin-to-skin technique which will prevent contaminating the hands.

- The use of gloves does not replace the need for hand hygiene. Hands should be properly washed before the gloves are put on and after the gloves are removed. Hand hygiene is also needed before and after the replacement of gloves during a procedure or in between tasks.
- Participants are reminded to do no heavy lifting for 24 hours.
- The phlebotomist is to have completed:

B. <u>Laboratory diagnosis</u>

After the blood collection, transportation and processing will perform laboratory tests to determine a hematological parameter for the study (CBC analysis)

<u>B. Complete Blood Count Mindray Bc-5380 Auto Hematology Analyzer</u>

COMPLETE BLOOD COUNT MINDRAY BC-5380 auto hematology analyzer is a quantitative, automated hematology analyzer and 5-part differential counter for in vitro diagnostic use in clinical laboratories. It provides 23 basic parameters, 4 parameters for research use, 3 histograms, and one scattergram of blood samples. It supports 2 measurement modes; CBC and CBC+DIFF

Principle

The MINDRAY BC-5380 auto hematology analyzer counts and sizes RBC, WBC, BASO, and PLT using the electronic resistance detection principle. This method is based on the measurement of change in electrical resistance produced by a particle suspended in conductive diluents as it passes through an aperture of a known dimension. An electrode is submerged in the liquid on both sides of the aperture to create an electrical pathway. As each particle passes through the aperture, a transitory change in the resistance between the electrodes occurs, producing a measurable electrical pulse. The number of pulses generated indicates the number of particles that pass through the aperture. The amplitude of each pulse is proportional to the particle volume. HGB is determined by the colorimetric method. Laser solution lyses RBCs and converts Hgb to Hgb complex whose absorbance is determined by analyzers at 525 nm. A LED is mounted on one side of the bath and emits a beam of monochromatic light, which passes through the sample and a 525nm filter, and then is measured by a photosensor that is mounted on

the opposite side. The signal is then amplified and the voltage is measured and compared to the blank reference reading. HGB is calculated per the following equation in g/L. HGB (g/L) =constant*log10 (blank photocurrent/sample photocurrent). Other parameters are derived as follows; MCV: based on the RBC histogram, the analyzer calculates the mean cell volume (MCV) and expresses the result in fL.

This analyzer calculates the HCT (%), MCH (pg), and MCHC (g/dL) as follows:

HCT%= RBC(x1012)*MCV (fL)/10

MCH= HGB/RBC(x1012)*10

MCHC=HGB/HCT*100

RDW-CV: Based on the RBC histograms, the analyzer calculates the CV (coefficients of variation) of the erythrocyte distribution width. RDW (%) =SD of MCV/mean MCV*100.

Reagents, Controls, and Calibrator

Because the analyzer, reagents (diluent, rinse, lyse, probe cleanser, and E-Z cleanser), controls, and calibrators are components of the system, the performance of the system depends on the combined integrity of all components. You should only use mindray specified reagents, which are formulated specifically for the fluidic system of your analyzer to provide optimal system performance. All references related to reagents in this manual refer to the reagent specifically formulated for this analyzer

Each reagent package must be examined before use. Inspect the package for a sign of leakage or moisture. Product integrity may be compromised in packages that have been damaged. If there is evidence of leakage or improper handling, do not use the reagent.

NOTE

- Store and use the reagent as instructed by instructions for the use of the reagent
- When you have to change the diluent, cleanser, or lyses run the background to see if the results meet the requirements

- Pay attention to the expiration date and open container stability days of all the reagents. Be sure not to use expired reagent
- After installing a new container of reagent, keep it still for a while before use

REAGENTS

M53D diluent

- It provides a stable environment for counting and sizing of blood cells.

M53 LEO (1) lyse

- It breaks down the red blood cell wall and cooperates with the M53 LEO (II) lyse to four differentiated white blood cells

M53 LEO (II) Lyse

- It cooperates with the M53 LEO (I) lyse to 4 differentiate WBCs, and dyes eosinophil's

M53 LH lyse

- It breaks down the red blood cell wall and converts hemoglobin to hemoglobin complex to determine hemoglobin. It 2 differentiates WBCs to basophils and other WBCs and determines WBC mount.

M53 Cleanser

- It is cleaning the solution formulated to prime and clean the fluidic channels and tubing and get rid of blood albumin and sediment

M53 P probe cleanser

- It's used to clean the analyzer regularly

Controls and calibrators

The controls and calibrators are used to verify the accurate operation of and calibrate the analyzer

The controls are commercially prepared whole blood products used to verify that the analyzer is functioning properly. They are available at a low, normal, and high levels. Daily use of all level verifies the operation of the analyzer and ensure reliable result are obtained

The calibration is a commercially prepared whole blood product used to calibrate the analyzer

Read and follow the instructions for using the controls and calibrating.

Specimen type: EDTA anticoagulated whole blood.

Startup and log in

Start the analyzer

1. Place the power switch on the left side of the analyzer in the ON position. The power indicator light will be on
2. Make sure the indicator light of the analyzer is on

- Start the external computer and the system software
    1. Start the external computer
    2. Turn on the display
    3. After entering the operational system double click the BC- 5380nauto hematology analyzer icon to run the software
    4. After starting the software the message box will pop up
    5. Enter the correct user name and password in the login message box
    6. Click the ok button to initiate the system

       Note: - before running the software, make sure the network cable of the external computer is connected to the analyzer properly.
    7. During the initiation, the startup information will be displayed in the operational/status information area at the bottom of the interface
    8. The whole process lasts 4 to 12 minutes. The time needed for the initiation of the system depends on how the analyzer was shut down previously.
    9. After the initiation process, you can enter the graph screen to check the background result.

Quality control: Initial daily background check initial, repeated analysis of randomly selected specimens to see reproducibility, randomly selected specimens (high, normal, and low) were checked by another similar hematology analyzer, and as a part of laboratory protocol whole blood quality control material (high, normal, and low) were performed to evaluate instrument performance.

Running the controls

- Selecting the whole blood mode
- Entering the 'L-J count' screen
- Be sure the system status area display is ready and the count's mode area displays whole
- Present a vial of control to the sample probe so that the tip is well into the vial, and press the aspirate key. The system status area will display running and the analyzer will start aspirating samples.
- When you hear the beep and the samples probe is out of the vials. The sample probe will retract into the analyzer and analysis progress will be displayed on the screen.
- When the analysis is finished the results will be displayed on the screen and the "NO. /Total" in the upper left corner of the screen will automatically increase by 1 and the sample probe will be repositioned. The analysis result is displayed on the screen.

OPERATING PROCEDURE

- Entering worklist information and select mode to enter the sample mode screen on the external computer
- Entering sample information like Id, name, age, sex, card number
- Enter button.
- At the count, the screen is sure the system status area display is ready and the count mode area display is whole.
- Presents the mixed samples to the sample probe so that the tip is well into the tube and presses the aspirate key. The system status area will display running and the analyzer will start aspirating samples.

- When you hear the beep and the sample probe is out of the tube, remove the sample tube. The sample will retract into the analyzer and the analysis progress will be displayed on the screen.
- When the analysis is finished, the result will be displayed on the screen and the sample ID will automatically increase by 1 and the sample probe repositioned. Press the print button, results will be automatically printed out.

Acceptable Background Counts

Parameter	Count
WBC	0.3 x 109/L or less
RBC	0.03x 1012/L or less
HCT	0.5% or less
HB	0.1 g/dL or less
PLT	10 x 109/L or less

- If the counts are unacceptable "Background Error" displays and the alarm sounds brief. Press [SELECT], Press Auto rinse. Repeat the Auto rinse

Source: MINDRAY BC-3000 plus operating manual.

C. <u>Preparation of thin blood film (Wedge method)</u>

Blood film morphological analysis is important in the investigation and management of anemia, infections, and other conditions that produce changes in the appearance of blood cells and differential white cell count.

Materials

- clean microscope slides
- Well-mixed EDTA blood sample
- Pipette

- Pencil
- Gloves
- Waste and sharps disposal containers

Procedures

- Use a glass slide that is free of dust, grease, and debris

  Note: It is essential to ensure slides are washed free from traces of detergent and the surface of the slide is completely clean and not greasy.
- Place a small drop of well-mixed K2EDTA blood (about 2-3 mm), 1.0 cm from the end of the glass slide, using either a plain capillary tube or another type of blood-dropping device.

  Note: When the blood is from an anemic patient larger drop of blood should be used. If using an anticoagulated blood sample, make sure the specimen is well-mixed. The blood drop should be placed (opposite from the frosted end, if that type of slide is provided) and in the middle of the slide.
- Place the spreading slide in front of the blood drop at an angle of about 30o - 40o to the slide and then move it back to make contact with the drop
- The drop spreads out quickly along the line of contact of the spreader with the slide. As the drop of blood spreads, be careful not to let it spread to both edges of the spreader
- Advance spreader slide with a smooth steady motion so that a thin film of blood is spread over the slide
- Allow the smear to air-dry

  Note: Do not blow on the smears as this can disrupt cellular morphology and causes the formation of unwanted artifacts, like target cells; also do not use heat for drying.
- Label the slide by the name of the patient and write the date or reference number on the head of the film using a lead pencil, a diamond marker.

Wright staining and examination

Principle: Wright's stain is a polychromatic stain consisting of a mixture of eosin and methylene

Blue. When applied to blood cells, the dyes produce multiple colors based on the ionic charge of the stain and the various components of the cell. The eosin ions are negatively charged and stain basic cell components an orange to pink color. The methylene blue ions are positively charged and stain the acid cell components in varying shades of blue. The neutral components of the cell are stained by both components of the dye producing variable colors.

Procedure

- Place the air-dried smear film side up on a staining rack
- Cover the smear with undiluted filtered stain and leave for 1 minute
- Add an equal volume of distilled water (i.e., the same number of drops as the stain)
- Mix by blowing until a metallic sheen appears.
- Allow the diluted stain to act for 3 minutes
- Wash off the stain with running tap water
- Wipe the back of the slide clean and stand it in a rack for the smear to dry.
- Examine gross morphology by 40x and use the 100x objective for studying the fine details of the cell morphology.

Interpretation: on microscopy, a normal red cell is compared to the size of the nucleus of a small lymphocyte. Five important features were studied for RBC morphology includes; shape, size, color, inclusions, and arrangement. The RBC has a diameter of 6-8 microns diameter on average with a central pallor area diameter of about 2-3 microns (one-third of size).

Microcytic hypochromic anemia: on microscopy, an average size of RBC is smaller than the size of the nucleus of a small lymphocyte. RBC has a diameter of less than 6 microns and MCV of less than 80fL and also has a central area of pallor of more than 3 microns in diameter. The complete blood counts showed an MCV value of less than 80 fL and an MCHC value of less than 32 g/dL.

Normocytic normochromic anemia: on microscopy; on average a normal red cell as compared to the size of the nucleus of a small lymphocyte. The complete blood counts showed MCV value between 80 fL and 100 fL and MCHC value between 32 g/dL and 36 g/dL

Macrocytic anemia: on microscopy, the average size of RBC is larger than the size of the nucleus of a small lymphocyte. RBC is approximately 9 microns or larger in diameter having an MCV of greater than 100 fl The complete blood counts showed an MCV value greater than 100fL.

Quality control: Wright stain was filtered every day by using filter paper and also stored in locked cabinets away from moisture and sunlight.

8.5 Annex V: Anthropometric and Blood pressure Measurement

Weight measurement: Before the study subjects' weight is measured, the weight scale will be placed on a firm, flat surface, turn to zero and the participant removes any coats, heavy sweaters, shoes, keys, or heavy pocket contents. Again, the participant will be asked to stand in the middle of the scale's platform with the bodyweight equally distributed on both feet. Weigh the participant in kilograms to the nearest 0.1 kg (100 grams).

Height measurement: Height will be measured using a height measure scale (Infiniti Med Lab Pvt. Ltd., India). Participants stand erect on the floorboard of the stadiometer with their back to the vertical backboard of the stadiometer. The heels of the foot are placed together with both heels touching the base of the vertical board. The foot will be pointed slightly outward at a 60-degree angle. The buttocks, scapulae, and head are positioned in contact with the vertical backboard. During the height measurement, the participant's shoes and hats will be removed. Recorded the height measurement to the nearest 0.1 cm.

BMI: After measuring the participant's height and weight, the BMI will be calculated by dividing weight in (kg) by height squared (m^2).

Blood Pressure (BP) measurement: BP will be measured by qualified personnel using an analog sphygmomanometer (OMRON HEALTH CARE Co., Ltd. Kyoto, Japan). The patient is seated upright with their upper arm positioned on the bench and excess clothing will be removed that might interfere with the BP cuff or constrict blood flow in the arm. Measurements will be taken from the upper arm with the hand at the heart level after the patient had been sitting for >5 minutes. Raised BP is defined as: systolic BP (SBP) >140 mmHg and/or diastolic BP (DBP) >90 mmHg.

8.6 Annexes VI: Laboratory result reporting format hematological parameter

ID _____Age_____ Sex_____

Test Result	result
RBC	----------x10^12/L
HGB	---------- g/dl
HCT	----------%
MCV	----------FL
MHC	----------PG
MCHC	----------%
RDW CV%	----------%
WBC	----------X 10^9/L
PLT	----------x10^3

Reporting form for peripheral morphology examination
ID_____ Age_____ Sex_____ Date _____
RBC series _____
WBC series _____
Platelet series_____
Possible conclusion _____

ANNEXES VII: DECLARATION

I, the undersigned, certify that this thesis paper is my original work and that it has never been submitted for a degree or other reason at Jimma University or any other institution of higher learning. I further declare that where other people's work was utilized, it was properly recognized and cited in accordance with the rules.

Name of the student: NIMAN TAYIB MOHAMOUD

Signature: _____ Date of submission: 05/01/2023

This proposal has been approved by the supervision of university advisors:

External examiner: Dr. Elsa T.

Signature: _____ Date of submission: 05/01/2023

Internal examiner: Mr. Girum Tesfaye

Signature: _____ Date of submission: 05/01/2023