ADDIS ABABA UNIVERSITY

COLLEGE OF HEALTH SCIENCES

DEPARTMENT OF MEDICAL LABORATORY SCIENCES

Evaluation of Hematological Profiles among *Helicobacter Pylori* Infected Adult Outpatients at Wolkite Health Center, Guraghe Zone, Southern Ethiopia

By: Yemeserach Moshago

Advisors: Aster Tsegaye (Professor, PhD)

Melatwork Tibebu (MSc, PhD candidate)

A Thesis Submitted to the Department of Medical Laboratory Sciences, College of Health Science, Addis Ababa University, in partial fulfillment of Master of Science Degree in Clinical Laboratory Sciences (Hematology and Immunohematology).

June, 2023

Addis Ababa, Ethiopia

Addis Ababa University

School of Graduate Studies

This is to certify that the thesis prepared by Yemeserach Moshago entitled:

Evaluation of Hematological Profiles among *Helicobacter Pylori* Infected Adult Outpatients at Wolkite Health Center, Guraghe Zone, Southern Ethiopia and submitted in partial fulfillment of the requirements for Master of Science degree in Clinical Laboratory Sciences (Hematology and Immunohematology) complies with the regulations of the University and meets the accepted standards with respect to originality and quality.

Signed by the Examining Committee:

Examiner_____ Signature _____Date _____

Examiner_____ Signature _____Date _____

Advisor_____ Signature _____Date _____

Advisor_____ Signature _____Date _____

_____

Chairman of the Department or Graduate Program Coordinator

Acknowledgement

I would like to express my deepest and sincere gratitude to my advisors Prof. Aster Tsegaye and Ms. Melatwork Tibebu for their constructive comments and encouragement throughout my thesis work.

I am indebted to Addis Ababa University, Department of Medical Laboratory Sciences for financing this study. Wolkite University is gratefully acknowledged for sponsoring my graduate study. My thanks also extended to all study participants for being voluntary to participate and wolkite health center laboratory staffs for their invaluable effort during data collection. Last but not least, my heartfelt thanks go to my families and friends for their financial and intellectual support during my study.

Table of Contents

List of Tables

List of Figures

List of Abbreviations

BMI	Body mass index
CBC	Complete blood count
EDTA	Ethylenediamine tetra acetic acid
H. pylori	*Helicobacter pylori*
HGB	Hemoglobin
IDA	Iron Deficiency Anemia
MALT	Mucosa-Associated Lymphoid Tissue
MCH	Mean Corpuscular Hemoglobin
MCHC	Mean Corpuscular Hemoglobin Concentration
MCV	Mean Corpuscular Volume
MPV	Mean Platelet Volume
PCT	Plateletcrit
Plt	Platelet count
QC	Quality Control
RBC	Red Blood Cell
RWD-CV	Red Cell Distribution Width Coefficient of Variation
SD	Standard Deviation
SOP	Standard Operational Procedure
TIBC	Total Iron-Binding Capacity
WBC	White Blood Cell

Abstract

Background: *Helicobacter pylori (H. pylori)* infection is the most prevalent gastrointestinal bacterial disease which can disturb hematological parameters of the host and results diverse complications such as anemia, thrombocytopenia and gastric lymphoma.

Objective: This study aimed to evaluate Hematological Profiles among *Helicobacter pylori* infected adult outpatients at Wolkite Health Center, from Jan-April 2023.

Methods: This study employed a comparative cross-sectional study design to recruit 340(170 each with age and sex matched) adult outpatients with and without *Helicobacter pylori* infection. Socio-demographic data was collected using questionnaire. A stool sample was obtained and examined for intestinal parasites and the *H. pylori* antigen. Blood was collected and analyzed for full blood count, using mindray BC 3000plus hematological analyzer and for screening hemo-parasites at wolkite health center laboratory. The entry and analysis of quantitative data were done using mean and standard deviation and were processed using SPSS version 22. Differences in mean values of hematological parameters between *H. pylori* positive and negative subjects were tested by independent sample T-test, at 95% confidence interval.

Results*:* Among 170 *H. pylori* infected adult outpatients 54% were females. The mean HGB (13.56±1.59 g/dl vs.14.01±1.33g/dl, p=0.005), hematocrit (40.98 ±4.81% vs 41.75±3.79%, p=0.029) and leukocytes (6.86 ±2.84 $x10^9$/l vs. 6.94±1.77 $x10^9$/l, p=0.003) were significantly decreased while percentage of lymphocyte was increased (31.89 ±11.29%vs. 29.56 ±8.60%, p<0.001), the mean difference count for platelets (242.62 ±68.82 x109/l vs 267.46 ±54.71 x109/l, p=0.054)was at the border of significant in *Helicobacter pylori* infected patients comparing with uninfected groups. Seventeen (10%) of the *H. pylori* infected patients were positive for intestinal parasites. However, only the MID population was significantly higher in the co-infected group, compared to *H. pylori* only infected patients (9.73 ±4.45 vs 9.64 ±3.00, p=0.021). Anemia was observed in 27/170 (15.8%) *H. pylori* positive patients (6 males and 21 females), 12/170(7.1%) and 20/170(11.8%) thrombocytopenia and leukopenia were observed in positive subjects respectively.

Conclusion*:* *H. pylori* can affect some hematological profiles of adult patients. Performing hematological test for *H. pylori* infected patients is recommended to manage this illness and further complications.

Key words: *Helicobacter pylori*, hematological profiles, intestinal parasites,

1. Introduction

1.1 Background of the Study

Helicobacter pylorus is coil formed gram-negative bacterium which exists and propagates in the coating of the stomach. *H. pylori* infection is known as the leading long-lasting bacteriological infection and the most significant factor of chronic gastritis(1).

Generally, *H. pylori* infection is the most recurrently observed illness for human being although different nations and population groupings have different prevalence of it. *H. pylori* infection is linked to a variety of stomach conditions, such as simple dyspepsia, heartburn, and gastric ulcer diseases, and is usually associated with upper gastrointestinal bleeding and, finally, it goes to the highest difficulty of gastric cancer unless it is treated with specific antibiotics properly(2).

Major transmission route of *H. pylori* bacterium is via feco-oral by consumption of the bacteria contaminated food and minor route is oral-oral transmission via infected saliva. Even though some motility illnesses can be recognized as signs of gastric *H. pylori* infection like Gastroesophageal reflux, esophagitis and delayed gastric emptying, majority of individuals infected with *H. pylori* do not develop clinical sign and symptoms; the host vulnerability and bacterial pathogenicity may be the causes of this deviation (2,3). Hematological abnormalities mainly anemia is among the most common clinical conditions in *H. pylori* infected individuals(4).

Two types of tests are currently being applied in the diagnosis of *H. pylori* infection: intensive and non-intensive test. Intensive tests are: gastrointestinal endoscopy, rapid urease test and culture and non-invasive tests: urea breath test, serological tests: for the detection of IgG and IgM antibodies of the bacterium, stool tests to detect the bacterium in feces and latest diagnostic technique is polymerase chain reaction that aims *H. pylori* species-specific gene with samples taken from different sources. The selection of diagnostic methods depends on population, occurrence of infection, affordability, variations in test performance, accessibility and clinical situation(2–4).

The complications related with *H. pylori* might be minimized through proper management. Prior to recently, the standard triple regimen using a proton pump inhibitor or ranitidine bismuth citrate, combined with clarithromycin and amoxicillin or metronidazole was treatment of choice

for *H. pylori* infection around the world. Nevertheless, increasing antibiotic resistances in many geographical areas has now made standard quadruple therapy (bismuth-based) or second-line therapy the preferred option(2,5).

Helicobacter pylori inhabit the gastric epithelium directly, causing gastritis, peptic ulcer disease, gastro duodenal ulcer, atrophic gastritis and cancer. Additionally, *H. pylori* infection was linked to extra gastrointestinal syndromes like lipid profile issues, iron deficiency anemia (IDA) and idiopathic thrombocytopenic purpura. The bacteria cause gastrointestinal bleedings which decrease the host's ability to absorb iron and increase of iron uptake by bacteria. Thus could result in IDA through several mechanisms. Ascorbic acid is transformed by hypochlorhydria into dehydroascorbic acid which inhibits iron absorption by converting the ferric to the ferrous form (6–8). Low platelet count can result from *H. pylori* infection by capture of host immunity over molecular imitation, where microbe molecules imitate host antigens and stimulate T lymphocytes to induce an immune reaction, this process might result in development of thrombocytopenia (7).

Gastric mucosa-associated lymphoid tissue (MALT) lymphoma is one of complications related with *H. pylori* infection and around 72-98 % of patients with gastric MALT lymphoma are with *H. pylori* infection. Besides, in 70 to 80 percent of cases, *H. pylori* removal alone causes the gastric MALT lymphoma to regress(9). Therefore, a better understanding of *H. pylori*'s effect on Hematological profiles will help to control those above mentioned complications early and help to improve patient outcome.

1.2 Statement of the problem

The most prevalent medical condition affecting more than half of the world's population is *Helicobacter pylori* infection(10). Although burden and association of hematological profile with *H. pylori* infection may vary in socio-demographic status(age, sex and residence) and presence of other infections affecting blood cells like intestinal helminthes (11–13), the infection can cause gastrointestinal disorders by impairing hematological profiles such as anemia by lowering hemoglobin synthesis and thrombocytopenia due to extra distraction of platelets(6–8).

H. pylori-related anemia that has not been treated may increase illness and death in females, interruption in the growth of a child, cognitive dysfunction, great potential for infection, and declining work capacity leads to lower productivity, which in turn places a heavy financial burden on the household and the community as a whole(14). Its frequency varies from 30% in developed nations to 80% in developing nations. The worldwide burden of *H. pylori* infection has shown that the highest occurrence of this bacterial infection was reported in Africa (70.1%). The prevalence in Northern America, Western Europe, Latin America, Asia and Australia were 37.1%, 34.3%, 63.4%, 54.7% and 24.6% respectively. While countrywide prevalence of *H. pylori* were in Portugal(86.4%), in Switzerland(18.9%) and 87.7% in Nigeria(10).

Infection with *H. pylori* affected 52.2% of the population in Ethiopia (15)**.** There are various investigations those showed the burden of *H. pylori* in Ethiopia. These includes 60.5% in Dessie (16), 36.8% in Addis Ababa (17), 83.3% in Hawassa (18) and 52.4% in Butajira (19).

Despite of the high burden of *H. pylori* infection in the world including Ethiopia, there is no consistent reports about the relationship between hematological profiles and *H. pylori* infection. some studies in different places reported that hematological parameters are significantly affected by *H. pylori* infection(20,21) while others reported hematological profiles are unaffected by *H. pylori* infection(22). On the other hand the association of *H. pylori* infection with hematological abnormalities mainly with anemia has also contradicted reports (23–25).

The link between hematological parameters and *H. pylori* infection should be evaluated in order to support efficient intervention actions to minimize its community health problem (14). Therefore, this study had aimed to evaluate hematological profiles among adult outpatients with *H. pylori* infection at wolkite health center, Guraghe zone, southern Ethiopia from January - April 2023.

1.3 Significance of the study

This study aimed to analyze the association between *H. pylori* infection and its influence on hematological parameters, in order to help close gaps between inconsistent findings in different places on the conceivable effect of *H. pylori* infection on hematological parameters of adult *H. pylori* Ag positive patients in comparison to non-infected control groups. Since few studies have been done in Ethiopia, our findings will help to understanding how this infection interrelates with hematological abnormalities. Thus, the information will serve as an input during treatment guideline revisions which helps to advance the clinical management of adult *helicobacter pylori* infected patients. The finding will also serve as reference for further study related to *H. pylori* infection.

2. Literature review

2.1 Hematological Profiles

A study conducted by Mwafy S *et al* in 2018 in Palestine revealed that when compared to controls, cases had significantly lower mean hemoglobin (HGB), red blood cell (RBC), white blood cell (WBC), and hematocrit (HCT), while cases had significantly higher red blood cell distribution width (RDW) than controls. However, there were no significant differences in mean blood cell volume, mean cell hemoglobin, or platelet counts. Iron, vitamin B12, and several hematological parameters had lower levels when *H. pylori* were present. As a result, it is connected to iron deficiency and may be a crucial marker and therapeutic tool for anemic individuals with gastritis (26).

A retroactive study conducted by Ahmad A. A (2022) in Saudi Arabia for assessment of hematological profiles in dyspepsia patient and *H. pylori* infection revealed that among 202 *H. pylori* Ag positive patients about 167 were non anemic and 35 were diagnosed as anemic. The values of RBCs, HGB, MCV, MCH, and serum ferritin between anemic and non-anemic patients infected with *H. pylori* were shown statistically significant difference ($p<0.05$). 17.3% of patients infected with *Helicobacter pylori* were anemic. In patients with *Helicobacter pylori* infection, the researchers also found no association between anemia and *Helicobacter pylori* infection , although there were significant differences in hematological parameters between those with and without anemia(27).

A retrospective cohort study shown by Ibrahim N *et al* in 2019 in Saudi Arabia reported that comparisons between hematological parameters of patients identified with *H. pylori* and those without it. The mean count of Red Blood Cells was slightly lower among patients who had positive *H. pylori* (4.70± .73 million) compared to the mean between persons with negative results (4.74± .96). However, there is no statistical significant difference ($P > .05$). Similar findings were also seen regarding the HGB and HCT values. No statistical significant associations were observed between the platelets counts, and white blood cell counts (WBCs) with the occurrence of *Helicobacter pylori* infection. There are several findings those reported the substantial association of *H. pylori* infection with some hematological parameters especially

HGB concentration, but this study concluded that no linkage was experiential between *H. pylori* and hematological profiles(22).

Cohort study conducted by Rahman YA *et al* in 2019 in Egypt to describe hematological impact of *Helicobacter pylori* infection revealed that HGB level, platelets count, serum iron level and vitamin B12 values, were highly reduced in patients with *H. pylori* infection than in non-infected individual. This study showed *H. pylori* can causes IDA, lowered vitamin B12, and decreased platelet counts, and therapy for *H. pylori* makes substantial increment of these parameters(28).

A case-control study conducted by Elamin E A *et al* in Sudan in 2018 shown that *H. pylori* had significant effect ($p < 0.001$) on majority of hematological parameters at the rate of 89.80%. RBC parameters (HGB, HCT, Mean Cell Volume, and Mean Cell Hemoglobin values) were decreased, but PLT and retic count were considerably higher in patients with gastritis than those without gastritis. On the other hand, no statistically substantial difference was found in some hematological parameters like total white blood cell counts, MCHC and total WBC differential count. This study suggested that *H. pylori* infection had statistically important influence on hematological parameters (29).

A case-control study conducted by Mohammed A *et al* in Sudan in 2021 showed that significantly lower mean RBC, HGB, HCT, MCV and neutrophil percentages in *H. pylori* – infected patients. The mean values were (4.2 (±0.7) × 10^{12}/ L vs. 4.8 (±0.3) × 10^{12}/ L; 12.04 (±1.84) g/dl vs. 13.4 (±1.4) g/dl; 34.2 (±6.1) l/l vs. 37.2 (±4.2) l/l; 83.1 (±6.7) fl vs. 86.6 (±4.9) fl; and 49.56±12.23% vs. 53.6 ±7.8% respectively). On the other hand, the mean lymphocyte % in *H. pylori* -infected patients was significantly higher than in controls (41.9 ±11.1% vs. 35.7 ±7.3%). Total white blood cell counts, platelet counts, monocyte counts, and eosinophil counts showed no meaningful variation between study subjects and healthy control groups(21).

Across-sectional study conducted by Baxendell K *et al* in central Ethiopia in 2019 that examined the association of young Ethiopian children with *H. pylori* and their platelet indices shown that both mean platelet volume and average platelet count were observed lower than non-infected children. Additionally, children with *H. pylori* infection had lesser RBC counts (adjusted mean difference: a '0.118×10 12 /L; 95% CI a '0.200 to a '0.036, p=0.005) compared with uninfected group (30). Thus, further understanding hematological parameters and their association with *H.*

pylori infection in a developing country including Ethiopia will offer further support for control and management of the disease and related complications.

A cross-sectional study conducted by Haile K *et al* in Hosanna, southern Ethiopia, in 2021 to evaluate the RBC parameters of adult patients infected by *Helicobacter pylori* shown that Mean value of HGB, RBC count and HCT ($p<0.001$). The mean value of MCV ($p=0.003$), MCH ($p=0.008$), and MCHC ($p=0.006$) were significantly lower in *H. pylori* -infected patients than in the control group. And patients with *H. pylori* infection had significantly higher mean RDW values ($p=0.003$). Lowered RBC count, HGB concentration, MCV, and HCT values were observed in7%, 13.3%, 18.2%, and 6.4%of patients with *H. pylori* -infection respectively. The study found that the mean value of RBC count, HGB, and other RBC indices differed statistically significantly between *H. pylori* -infected patients and controls. Thus, for appropriate diagnosis and management of patients with *H. pylori* infection, the values of hematological parameters ought be well-thought-out and performing complete blood count for *H. pylori* infected individuals was recommended (4).

2.2. Hematological Abnormality

A retroactive study conducted by Xu MY *et al* in china (2017) to explore the relationship of anemia with *Helicobacter pylori* disease revealed that the magnitude of anemia in *H. pylori* positive individual was meaningfully increased comparing with *H. pylori* negative control. The study showed that levels of most RBC parameters had significant change between *H. pylori* infected and non-infected control groups (values of RBC, MCV and MCHC had $p<0.05$) and the hemoglobin concentration was lower in *Helicobacter pylori* (+) subjects than in *Helicobacter pylori* (-) subjects. Thus the finding showed that infection of *Helicobacter pylori* might be correlated to anemia and hemoglobin level (31).

A cross-sectional study conducted by Shih H *et al* in 2013 in Taiwan to assess association of chronic *Helicobacter pylori* infection and IDA. The study reported that the demographic data between participants were shown no statistically significant deviations. The mean values of HGB were not changed between positive and negative groups, the value in positive group was 13.57 g/dL versus in the negative it was 13.65 g/dL ($P = 0.699$). Values of parameters related to serum iron deficiency anemia were estimated in positive group, but increased total iron binding capacity and reduced serum iron and ferritin were found. However, the deviation did not reached

statistical significance (iron $P = 0.824$, ferritin $P = 0.360$, and TIBC $P = 0.252$). Thus, IDA in their study participants was not due to chronic infection of *H. pylori*. After chronic *Helicobacter pylori* infection the values of TIBC, serum iron and ferritin and HGB concentrations were remain unchanged (7).

The study conducted by Erdem M E *et al* in 2014 in Cairo Egypt to evaluate the associations of Iron Deficiency Anemia and *Helicobacter pylori* infection shown that a statistically important reduction in cases when compared with controls concerning to RBC indices of hematological parameters such as hemoglobin value (9.2±1.7 vs. 13.7±0.88g/dL; P<0.001), HCT (28.6±3.41 vs. 40.7±3.31%; P<0.001), MCV (72.5±6.4 vs. 89.7±5.1fL; P<0.001)(24).

One of Kibru D et al. a study conducted in the southern Ethiopia in 2014 to assess the association between the extent of *Helicobacter pylori* and anemia in adult patients infected with *H. pylori* found that hematological abnormality such as anemia(30.9%) higher than shown in non-infected patients(22.5%), which was statistically significant. RBC parameters like HGB, MCV, MCH, MCHC, HCT, mean red blood cell count (SD) were statistically significant different between patients with and without Helicobacter pylori infection (19).

3. Objectives

3.1 General objective

To evaluate hematological profiles among adult outpatients with *Helicobacter pylori* at wolkite health center, Guraghe zone southern Ethiopia from January - April 2023.

3.2 Specific objectives

- To determine hematological Profiles of *H. pylori* antigen positive adult outpatients and apparently healthy adult individuals at wolkite health center from Jan - Apr 2023.
- To compare hematological abnormalities between *H. pylori* Ag positive and negative patients at wolkite health center from Jan - Apr 2023.

4. Hypothesis (Ho)

There is no distinction in hematological profiles among adult outpatients with *H. pylori* infection compared with apparently healthy individuals.

5. Materials and Methods

5.1 Study area

This study was conducted at Wolkite health center, found in wolkite town capital of Guraghe Zone, 158 km southwest of Addis Ababa, the capital of Ethiopia. The average annual temperature in Wolkite town is 18.6°C and the average rainfall is 1244 mm. The town has an altitude of 1910 to 1935 meters. Wolkite health center provides diagnosis and treatment for patients in the town of wolkite and nearby woreda such as Kibena, cheha, Abeshgea, oromia regions and others. Wolkite health center has a variety of professionals committed to offer health care services. The health center teams composed of general practitioner (GP) doctors, nurses, midwifes, laboratory technicians and technologists and others. The health center also has some administrative staff to support the health care activities(32).

5.2 Study Design and Period

A comparative cross-sectional study was conducted at Wolkite health center from Jan- Apr.2023 to evaluate the hematological profiles among adult outpatients with *H. pylori* infection comparing with apparently healthy individuals.

5.3 Population

5.3.1 Target Population

All patients who go to wolkite health center and are suspected of having *H. Pylori* infection

were target Population.

5.3.2 Study Population

Adult patients who visited Wolkite health center with symptoms and signs of *H. pylori* infection and positive for *H. pylori* feces antigen were included as study group and apparently healthy adult individuals without *H. pylori* infection (stool antigen negative) were included as control group. Participants as Control groups were Wolkite health center staff, Wolkite University students who were on practicum training and patient's families or caretakers.

5.4 Criteria for Eligibility

5.4.1 Inclusion Criteria

Adult out patients and adult healthy individuals (age greater than or equals to18 years old) who are voluntary to partake in the study and all patients having sign and symptoms with *H. pylori* infection and healthy individuals were included in the study. The participants of this study were age and sex matched within each groups.

5.4.2 Exclusion Criteria

Patients with *H. pylori* infections who received treatment within the previous 3months, those who have undergone prior stomach surgery, pregnant women, donate blood right away previous 3months, on anemia treatment, patients who had blood loss manifestations, and other chronic diseases(such as HIV, malaria, hematological malignancy) were excluded from the study.

5.5 Study variables

5.5.1 Dependent variable

- hematological parameters

5.5.2 Independent variables

- *H. pylori* infection
- Age
- Sex
- Residence
- Intestinal parasites
- Body Mass Index

5.6 Measurement and Data collection

5.6.1 Sample size determination

To determine sample size, hypothesis testing formula was used for two sample means. The level of significance was set to 95% confidence interval; corresponding Z-value is 1.96 and power of

the test set to 80%, σ= pooled standard deviation of the two groups Z-β = 0.84 for power of 80%, D = difference in means between two groups (m1-m2)

The mean of the groups was compared using similar previous study of RBC parameter that reported 4.63 and 0.59 mean & SD of cases, respectively and 4.83 and 0.72 mean &SD controls, respectively (19)**.**

$\sigma^2 = (S1^2 + S2^2)/2$

$\sigma^2 = (0.59^2 + 0.72^2)/2 = 0.8665/2 = 0.43325$

$$n = \frac{2\sigma^2 (1.96 + Z_{-\beta})^2}{D^2}$$

$n = 2*0.43325\ (1.96+0.84)^2 / (4.63-4.83)^2$

n=0.8665*7.84/0.04 n=169 so, the study needed at least 338 subjects to be able to conduct with 1: 1 ratio (169 for each group was age and sex matched) and **340**(170 from each groups were collected).

5.6.2 Sampling technique

This study employed convenient sampling technique for individuals who fulfill the criteria and recruited consecutively. In Wolkite health center approximately 10-15 *H. pylori* Ag/Abs tests were requested daily, among them on average 2-3 positive patients were found. In this study all eligible patients suggestive to *H. pylori* were tested and positive for stool antigen were taken as study participants until it reached 170.

5.6.3 Data collection procedure

Every professional involved in data gathering had single day data collection training. The training made clear the study's goals, how to select study members, data confidentiality, and safety measures to follow in collecting, shipping, examining and storage of blood and stool specimen. Demographic information was collected using pretested questionnaire by assigned personnel. About 3ml blood sample was collected following SOPs by a trained laboratory technologist into an EDTA anti-cougulated tube (purple cap). Then the blood was analyzed for full blood count, using **Mindray BC 3000plus** auto hematology analyzer and used for screening

hematological parasites. A wet mount test was performed to check for intestinal parasites and a one-step *H. pylori* feces test for *H. pylori* antigen from each participant in the study.

5.6.4 Laboratory analysis

The laboratory principles and techniques of hematological and stool sample analysis are described below;

Hematological analysis

An auto hematology analyzer made by Shenzhen Mindray Bio-Medical Electronics, China, called Mindray BC-3000plus was used to determined Hematological profiles. This quantitative, automated hematology analyzer is three-part white blood cell differential counter and employed for in vitro diagnosis. The BC-3000plus is used for the quantitative measurements of the 19 parameters (leukocytes(WBC), LYM#¸MID#, GRA#, LYM%, MID%-GRA% (three part leukocyte differential), hemoglobin (HGB), hematocrit(HCT), mean corpuscular volume(MCV), red cell distribution width (RDW-CV), red blood cell(RBC), mean corpuscular hemoglobin(MCH), mean corpuscular hemoglobin concentration(MCHC), platelet(PLT), mean platelet volume (MPV), plateletcrit (PCT) and platelet distribution width(PDW) and 3 histograms (RBC, leukocytes and PLT) of blood samples. this device is to identify normal patients using all normal parameters generated by the system, and to flag patient findings that require further investigations. The two separate measurement techniques this analyzer employs are: using the Coulter method to determine leukocytes, red cells and platelet data and colorimetric method to determine HGB. Samples are aspirated, diluted and mixed during every analysis cycle before the determination of each parameter is performed.

Blood sample was also used to screen blood parasites by preparing thick and thin blood film. Geimsa stain and ethanol alcohol were used for staining and fixing the blood film respectively.

Collection and Analysis of Stool Sample

After clarifying how to bring adequate volume of stool specimens, participants were given clean plastic cups. All participants provided about 2grams of feces, which were then taken and examined for intestinal parasites using a wet mount microscopic and for *H. pylori* by a one-step *H. pylori* stool Ag test (Guangzhou Wondfo Biotech, China). The principle of one-step *H. pylori* stool Ag test is detecting active *H. pylori* Ag using peroxidase-conjugated monoclonal antibody.

The development of two lines, the control (C) line and the test (T) line, shows *H. pylori* -positive test result, whereas the development of only the C line indicated a negative test result. In the occurrences where the T line was significantly fainter than the C line, the results would be interpreted and recorded as positive.

From each participant; weight and height were measured, and body mass index (BMI) was derived using a formula weight over height square. Individuals were grouped in to four based on their BMI: BMI<18.5 kg/m^2, between 18.5–24.99 kg/ m^2, between 25–29.99kg/m^2, and ≥30 kg/m^2 as underweight, healthy weight, overweight and obese respectively (4,30).

5.7 Data Quality Assurance

By collecting and processing blood and stool samples in accordance with standard operating procedures, the quality of the samples was guaranteed

Pre-analytic: Samples were inspected to see if they met the standards such as; hemolysis, coagulation, volume (for blood and stool), collection time and correct labeling (for blood and stool. specimen handling and Safety procedures were highly monitored, SOP and manufacturer instructions was firmly followed.

Analytical: Earlier to examination, blood samples were mixed by 10-15 inversions. The three levels of cell hematology controls (Normal, Low and High) were run to evaluate `effectiveness of automated hematology analyzer. The test strip for *H. pylori* stool Ag was checked by performing positive and negative controls before sample analysis.

Post analytical: The results of complete blood cell counts, stool analysis and *H. pylori* antigen test were registered on standardized recording format.

5.8 Statistical Analysis and Interpretation

Data were checked before analysis for their accuracy and consistency. The normal distribution of data was checked using skewness test. Cross tabulation and charts were used to illustrate the study finding and independent variables. The data were collected, sorted and analyzed using descriptive statistics (Mean, standard deviation, and percentage) and were processed using SPSS version 22 statistical package software. Differences in mean values of hematological parameters between *H. pylori* positive and negative subjects were tested by independent sample T-test. A 95% confidence interval was used in each case, and statistical significance was defined a P-value less than 0.05.

5.9 Ethical considerations

Ethical approval was attained from Addis Ababa University, College of Health Science, Department of Medical Laboratory Science Research and Ethical Review Committee (DRERC). The study has permission from Wolkite health center administration office and all concerned bodies at all levels. Patients' informed consent was obtained once the study's purpose, benefits, and ability to withdrawal at any time were made clear. The confidentiality of participant's records was maintained throughout the study, and samples were coded. In case of abnormal hematological profiles, presence of hemo parasite(malaria) and intestinal parasites of study participants, results were communicated with responsible healthcare workers for appropriate interventions.

5.10 Result Dissemination

The results of this investigation will be shared with and succumbed to Addis Ababa University, College of Health Science, Department of Medical Laboratory Sciences and to Wolkite health center. Outcomes of the study will be presented in relevant workshops, seminars and scientific conferences. Moreover, the outcome of this study will be published in nationwide or international reputable journals.

5.11. Operating definitions

Hematological profiles: states to quantifiable characters which comprise total leukocyte, PLT, RBC, HGB, WBC Differentials (GRAN, LYM and mid-sized cell (MID)), HCT, MCH, MCV, MCHC, RDW-CV

Adult patient: refers to patient whose age is greater than or equal to eighteen years.

Hematological abnormalities: refers to anemia (based on hemoglobin concentration in females and males as of HGB<12 g/dL and <13 g/dL respectively), Leukopenia total WBC<4.0 $x10^9$/L and Thrombocytopenia PLT<150 $x10^9$/L(33,34)

Apparently healthy: refers to the absence of disease based on clinical signs and symptoms and function normally by physical evaluation(35).

6. Results

6.1. Socio-Demographic Characteristics of Study Participants

A total of 340 Participants (170 *H. pylori* infected and 170 non infected groups) were involved in this study. Among them, 156 (45.9%) were male. The age of participants were categorized in four groups, the first group 18-27 years had the highest frequency 104(30.6%):52 *H. pylori* infected and 52 control groups of participants observed. The next highest frequency was in the age groups 28-37(92, 27.1%), followed by 38-47 (72, 21.2 %) and≥48, (72, 21.2%). Regarding marital status, most participants were married (70.3%). Mean body mass index of participants were 22.18 kg/m^2 among *H. pylori*-infected participants 3(1.8%), 155 (91.2%), and 12(7%) had under-weight, normal weight and overweight, respectively. Overall, 172(58.5%) of participants (97 *H. pylori* infected and 79 controls) were urban residents. Behavioral characteristics of study participants revealed that 126(37.1%), 56(16.5%) and 2(0.6%) had alcohol drinking, khat chewing and cigarette smoking habit respectively. Detailed analysis for cases and controls is shown in Table 1. Among *H. pylori* Ag positive groups intestinal parasites were seen in 17(8 male and 9 female) 10% participants.

Table 1: Socio-Demographic and Behavioral characteristics of *H. pylori* -Infected Patients and Controls at Wolkite health center, Guraghe zone southern Ethiopia from January-April 2023

Variable	Groups	*H. pylori*-infected group(n=170)		Controls Group(n=170)		
Sex		Males n=78(45.9%)	Females n=92(54.1%)	Males n=78(45.9%)	Females n=92(54.1%)	Total
Age in years	18-27	24	28	24	28	104
	28-37	24	22	24	22	92
	38-47	12	24	12	24	72
	≥48	12	24	12	24	72
Residency	Urban	45	51	47	58	
	Rural	33	41	32	33	139
	Total	78	92	79	91	340
Educational status	Cannot read and write	6	17	3	13	39
	Primary	23	31	24	23	101
	Secondary	23	30	21	33	107
	Higher level	26	14	30	23	93
	Total	78	92	78	92	340
Marital status	Single	27	13	28	17	85
	Married	55	73	41	69	238
	Divorced	0	1	3	1	5
	Widowed	0	5	2	5	12
Behavioral characteristics of study participants						
Alcohol drinking	Daily	0	1	0	0	1
	Every Weekend	13	1	11	1	26
	Occasionally	22	31	20	25	98
	Total	35	33	31	26	125
khat chewing	Daily	5	1	6	1	13
	Every Weekend	8	0	4	1	13
	Occasionally	17	3	9	3	31
	Total	30	4	19	5	58
Cigarette smoking	Yes	1	0	1	0	2
	No	77	92	77	92	338
	Total	78	92	78	92	340

6.2. Comparisons of Hematological Parameters between *Helicobacter* pylori infected patients and controls

The mean (SD) value of total white blood cells were (6.86 ±2.84 $x10^9$/l, vs. 6.94±1.77 $x10^9$/l, p=0.003), hemoglobin (13.56±1.59g/dl,vs.14.01±1.33g/dl, p=0.005) and hematocrit (40.98 ±4.81%,vs 41.75±3.79%, p=0.029) were significantly decreased in *H. pylori* positive patients comparing with negative control groups while among WBC differentials percentage of lymphocyte counts were considerably increased in patients infected by *H. pylori* than non-infected individuals which were (31.89 ±11.29%vs. 29.56 ±8.60%, p<0.001) respectively. In plt counts, MCV, MCH, MCHC, RBC, granulocytes, Mid-sized cells and PCT parameters the mean (SD) values (242.62 ±68.82 $x10^{9/}$l vs. 267.46 ±54.71 $x10^{9/}$l, 88.53 ±4.32fl vs.89.21±3.68fl, 29.30±2.00pg vs. 31.28 ±20.10pg, 33.10±1.13g/dl vs. 33.27±1.09g/dl, 4.63±0.53 $x10^{12}$ /l vs. 4.64±0.47 $x10^{12}$ /l) were observed as reduced in positive patients comparing with negative controls but their difference was not statistically significant. Other hematological parameters (RDW, PDW and MPV) showed non-significantly higher mean (SD) Values in infected-patients than non-infected individuals.

Table 2: Hematological Parameters of *H. pylori* -Ag Positive Patients v_s. Controls at Wolkite health center, Guraghe zone southern Ethiopia from January-April 2023

S. no	Parameter	*H. pylori* (+) Group Mean ±SD	Control Group Mean ±SD	p-value
1	**Hemoglobin (g/dl)**	**13.56 ±1.59**	**14.01 ±1.33**	**0.005**
2	RBC($x10^{12}$ /l)	4.63 ±0.53	4.64 ±0.47	0.568
3	**Hematocrit (%)**	**40.98 ±4.81**	**41.75±3.79**	**0.029**
4	MCV (fl)	88.53 ±4.32	89.21 ±3.68	0.152
5	MCH (pg)	29.30 ±2.00	31.28 ±20.10	0.209
6	MCHC (g/dl)	33.10 ±1.13	33.27 ±1.09	0.603
7	**WBC ($x10^9$/l)**	**6.86 ±2.84**	**6.94±1.77**	**0.003**
8	RDW%	13.30 ±1.14	13.16 ±0.94	**0.059**
9	GRAN %	58.46 ±12.11	65.08 ±55.52	0.565
10	**LYM %**	**31.89 ±11.29**	**29.56 ±8.60**	**<0.001**
11	MID (%)	9.65 ±3.17	9.44 ±3.15	0.348
12	PLT($x10^{9/}$l)	242.62 ±68.82	267.46 ±54.71	**0.054**
13	MPV(fl)	8.02 ±0.85	7.9 ±0.74	0.112
14	PDW (%)	15.23 ±1.07	15.16±0.74	0.928
15	PCT	0.19 ±0.05	0.21 ±0.04	0.419

To identify the impact of intestinal parasites on hematological parameters, all (170) *H. pylori* Ag positive individuals were selected and their hematological profiles computed with and without intestinal parasites. Among *H. pylori* stool Ag positive individuals 17(10%) (9 females and 8 males) were also infected by different intestinal parasites dominantly by *Giardia lamblia12*/17(70.6%), 3/17(16.5%) *Hook worm*, one *Teania* species and one *Strongyloides stercoralis*. Using independent sample t-test their hematological profiles were tested. Accordingly most hematological profiles (WBC, RBC, HGB, HCT, MCH, MCHC, MCV, NUE and PLT) were shown lower counts; however, there differences reached no statistically meaningful level (p=0.246,p= 0.878, p=0.833,p=0.892, P=0.609, p=0.368, P=0.511, p= 0.546, and p=0.855)respectively. Only mid-sized cells which was significantly higher in patient of *H. pylori* infected with intestinal parasites than only *H. pylori* infected individuals (9.73±4.45%vs. 9.64±3.00%, p=0.021) respectively.

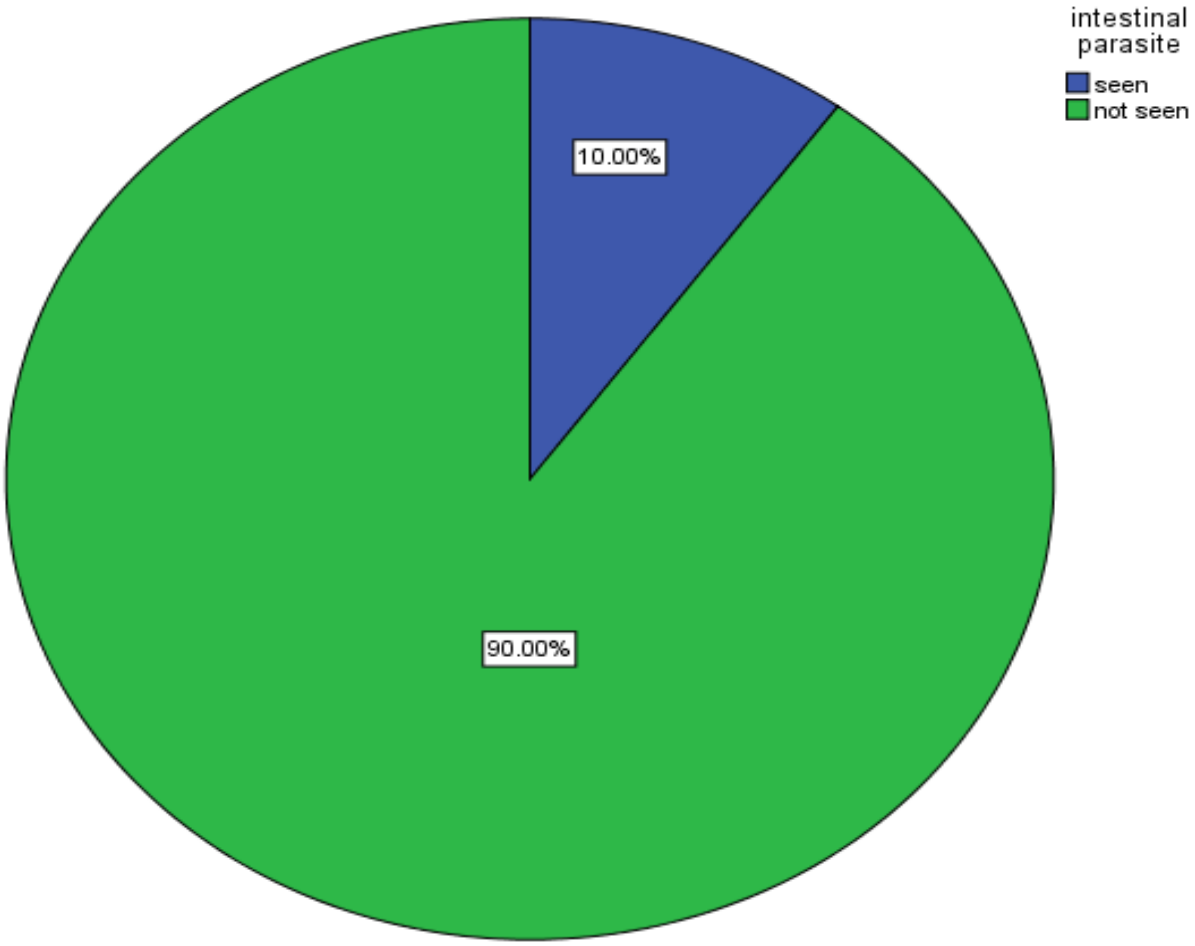

Figure 1: Frequency of Intestinal Parasites Seen in *H. pylori* Infected Patients at Wolkite health center, Guraghe zone southern Ethiopia from January-April 2023

Table 3: Hematological Parameters between *H. pylori* Infected Patients with and without Intestinal Parasites at Wolkite health center, Guraghe zone southern Ethiopia from January-April 2023

Parameters	*H. pylori* (+) &IP(+)Mean ±SD	*H. pylori* (+)&IP(-) Mean ±SD	p-value
WBC ($x10^9$/l)	5.74 ±1.80	6.98±2.92	0.246
RBC($x10^{12}$/l)	4.62±0.47	4.63 ±0.54	0.878
HGB (g/dl)	13.29 ±1.58	13.62 ±1.78	0.833
HCT (%)	40.63 ±4.37	41.02±4.87	0.892
MCV (fl)	88.05 ±3.8 0	88.58 ±4.37	0.511
MCH (pg)	28.87 ±1.68	29.35 ±2.03	0.609
MCHC (g/dl)	32.77 ±0.80	33.17 ±1.15	0.368
RDW%	13.34 ±1.12	13.29 ±1.14	0.697
GRAN %	56.41 ±13.28	58.68 ±12.00	0.546
LYM %	33.87 ±11.07	29.56 ±11.33	0.728
MID (%)	**9.73 ±4.45**	**9.64 ±3.00**	**0.0 21**
Plt(x109/l)	255.18 ±66.68	241.22 ±69.12	0.855
MPV(fl)	7.71 ±0.67	8.06±0.86	0.112
PDW (%)	15.38 ±0'35	15.21±1.12	0.378
PCT	0.20±0.05	0.19 ±0.05	0.755

Comparison of anemia between *H. pylori* Ag positive and negative patients

Anemia was observed in 29 overall participants. 27/170 *H. pylori* positive patients (6(1.8%) males and 21(6.8%) females), while 2/170(1.2%) females and no male participants from control groups were anemic according to the WHO cut-off (HGB<13g/dl) for males and (HGB<12g/dl) for females (34). The prevalence of anemia in *H. pylori* stool Ag positive patients were higher than in control groups (8.5%vs 1.2%) respectively. Anemia between gender and participant groups is indicated in the figure2 below.

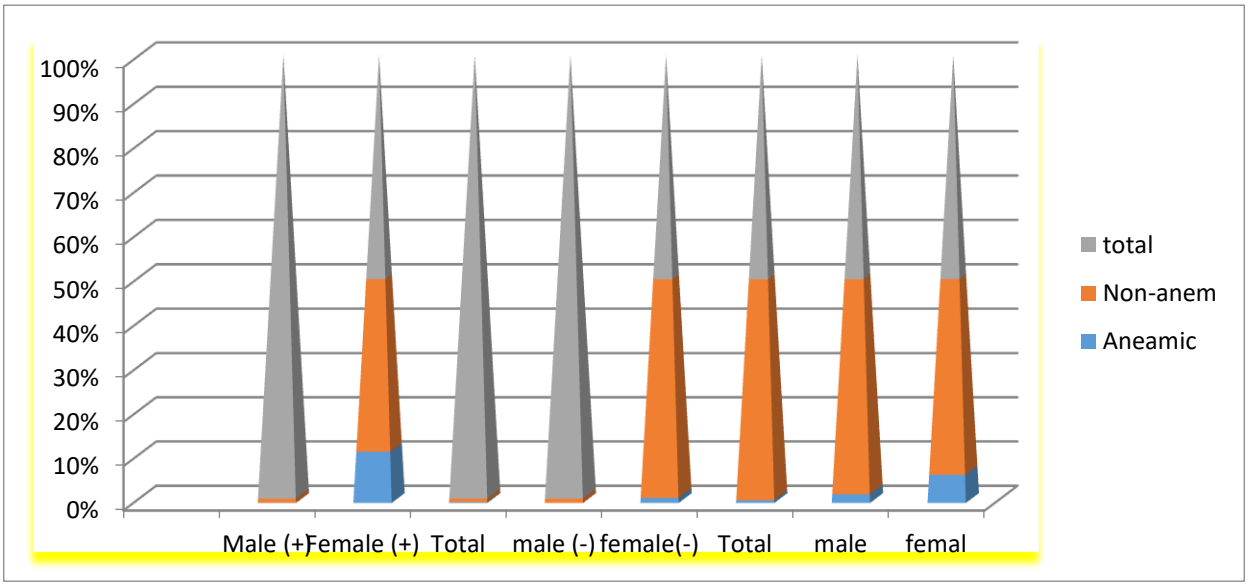

Figure 2: Comparison of Anemia between *H. pylori* Ag Positive and Negative Patients at Wolkite Health Center, Guraghe Zone Southern Ethiopia from Jan - Apr 2023.

Lower platelet counts (thrombocytopenia) were observed only in 12/170(7.1%) of *H. pylori* infected patients. Female participants were observed as twice higher than males to develop thrombocytopenia with *H. pylori* infection at Wolkite health center see figure3. In this study thrombocytopenia were not reported in control groups.

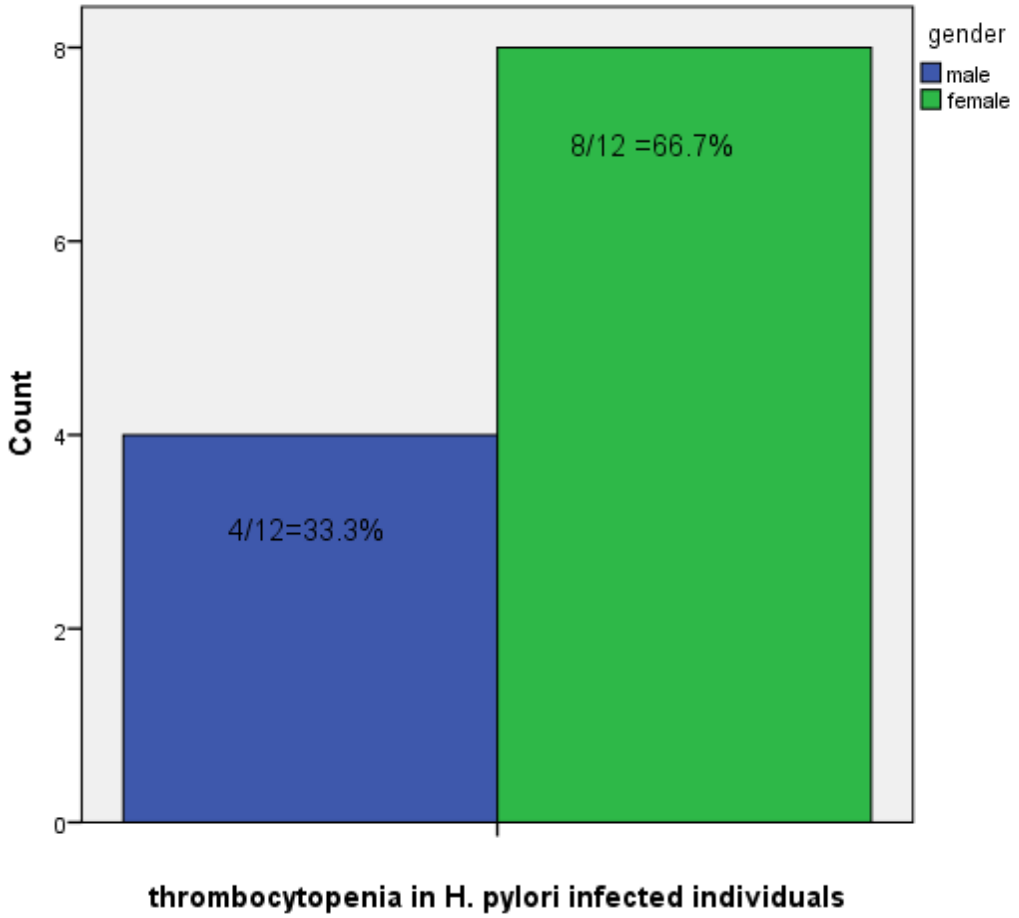

Figure 3: Thrombocytopenia between Males and Females of H. Pylori Ag Test Positive Groups at Wolkite Health Center, Guraghe Zone Southern Ethiopia from Jan-Apr. 2023

Comparison of leukopenia between *H. pylori* Ag positive and negative patients

Leukopenia (low leukocyte count) was observed in 26 participants (20/170(11.8%) cases and 6/170(3.5%) controls). Females were the higher to develop leukopenia overall 15/26(57.7%) prevalence in cases 14/20 (70%) than in control 1/6 (16.7%) while males were developed leukopenia overall 11/26(42.3%), in cases 6/20 (30%) and in controls 5/6(83.3%). Based on the participant age group distribution, the highest frequency of leukopenia was observed in the first age group 18-27years8/20 (40%) in patients with *H. pylori* while for control groups the highest frequency of leukopenia was seen in age group 28-37years4/6 (66.7%) see figure4&5 below for details.

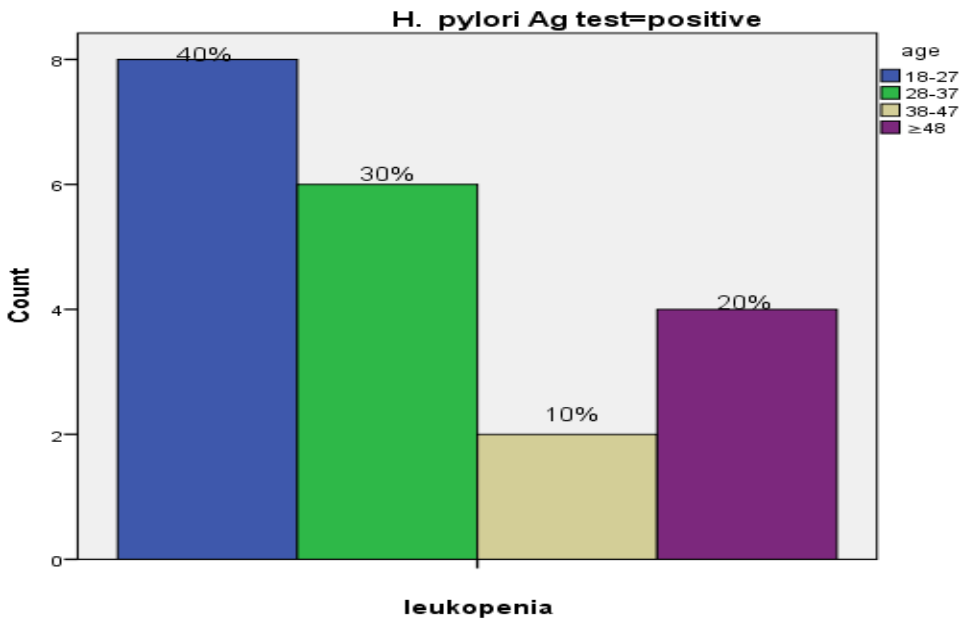

Figure 4: Age Group Distribution of Leukopenia among *H. pylori* Positive Patients at Wolkite Health Center,Guraghe Zone Southern Ethiopia from Jan-Apr. 2023

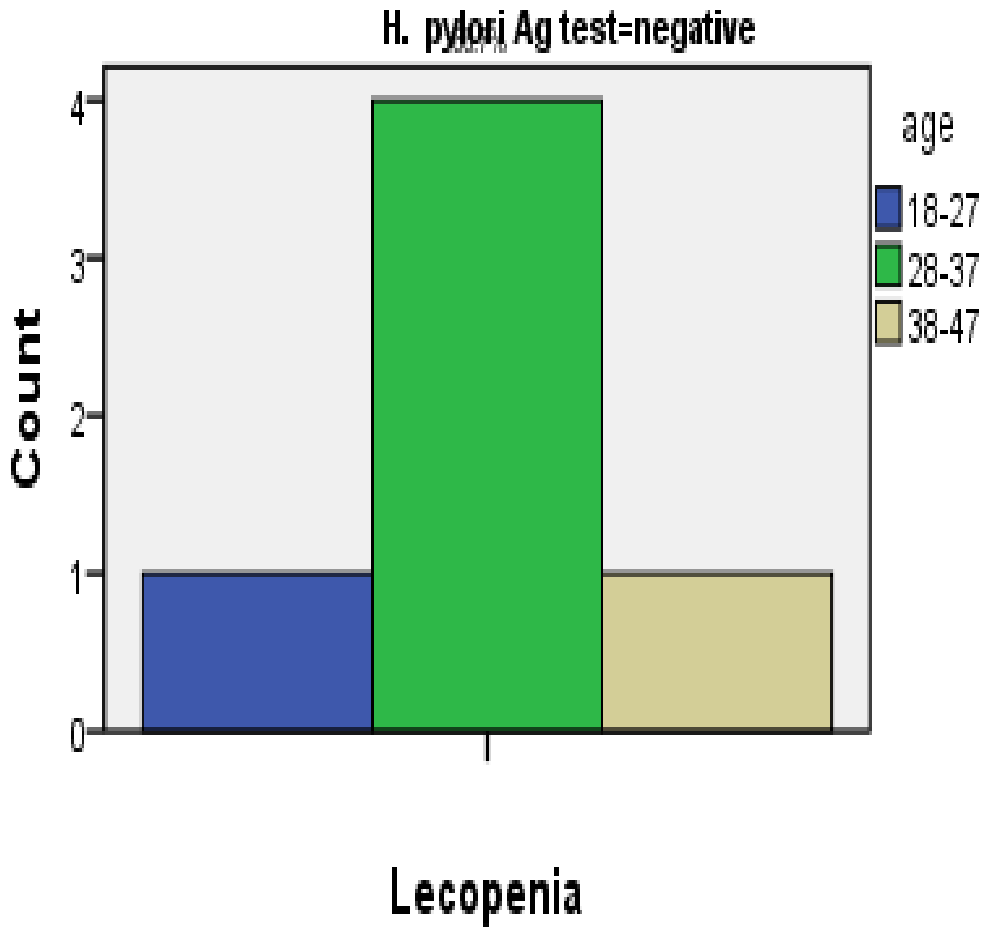

Figure 5: Age Group Distribution of Leukopenia among H. pylori negatives Patients at Wolkite Health Center, Guraghe Zone Southern Ethiopia from Jan-Apr. 2023

7. Discussion

Hematological profiles can change with different factors and underlined conditions such as genetic, environmental, diseases, nutritional status of the patients such as iron, and vitaminb12 intake which are important components in blood production especially for HGB synthesis. Lower HGB concentration is related with development of anemia (IDA). Anemia is reflected as one of difficulties of *Helicobacter pylori* infection(36).

This study was aimed to investigate the hematological profiles of adult patients infected by *H. pylori* in comparison with non-infected control groups. Accordingly, mean values of hemoglobin and hematocrit in *H. pylori* Ag positive adult out patients were significantly reduced compared with control groups which is regular with other investigations in Ethiopia, by Haile K *et al* in Hosanna(2021)(4) and Kibru D *et al* in Butajira(2014)(19). The results of the current research are also similar with result of study in Sudan by Erdem ME *et al* (24) and in Egypt by Rahman YA *et al*(28).

The mean ±SD values of platelet counts were reduced almost on the border of significance (p=0.05), but still not statistically significant when *H. pylori* infected compared with non-infected controls groups, which is similar to the result revealed by Mohamed A *et al* in Sudan (21). Low platelet count can result from *H. pylori* infection by catching the host's immunity over molecular imitation, where microbes molecule imitate host antigens and stimulate T lymphocytes to induce an immune reaction and *H. pylori*- induced antibodies highly destruct platelets (7). But some reports in contrast to this study showed platelet count was significantly increased in patients with *H. pylori* than without *H. pylori* Elamin E A *et al* in Sudan(29) and no statistically significant difference were found from Saudi by Ibrahim N *et al*(22).

The mean ±SD value of total WBC count and lymphocyte percentage were meaningfully decreased and increased respectively. The finding of elevated %lymphocyte were reported similarly by Mohammed A *et al* in Sudan(21). The statistically significant reduced leukocyte count difference between cases and controls in this study is equivalent to the report from Palestine by Mwafy S *et al* (37). contrariwise the reports of leukocytes and lymphocytes in this study are unlike with the findings of Elamin E A *et al* in Sudan(29). These inconsistent findings are possibly as a result of various study design.

In this study RBCs, MCH, MCV and MCHC showed no statistically important differences between positives and negative control groups (p=0.568, p=0.152.p=0.209,p=0.603)respectively.

These findings were in keeping with the results of Ibrahim N *et al* in Saudi Arabia(22) and another study in same country by Alshomar A. (27).But the current results are in contrast with the finding of Xu My *et al* in china(31) and Mwafy S *et al* in Palestine(37). The reason behind such different findings might be the difference by study design used and the nutritional status of participants(34).

This study computed the impact of intestinal parasites on hematological profiles of *H. pylori* infected participants. The result showed the only increased statistically significant difference was observed on mean (SD) of mid-sized cells (mixed count of eosinophil, monocytes and basophils) counts in the presence of intestinal parasite. Eosinophil and monocytes play an essential role in first protection beside parasites and in presence of parasites raised counts of eosinophil, monocytes and neutrophils are observed (38) which is in line with the current observation.

The development of anemia is the contribution of reduced hemoglobin value in *H. pylori* infection although how this infection exactly contribute is not well known(4) this study observed anemia in 8.5% of *H. pylori* infected groups while only 2(1.2%) in participants negative for *H. pylori* Ag tests. In the current study at Wolkite health center, being female and *H. pylori* infected have more possibility to develop anemia than non-infected controls. This finding is similar with finding of other research by Kibru D *et al* in Ethiopia(19) and Hou B *et al* in f China(39). But Alshomare A *et al* from Saudi Arabia reported that there was no relationship between anemia and *H. pylori* infection and similarly by Shih H *et al* in Taiwan(7,27). This difference might be because of varies factors which affect blood cells such as eligibility criteria, geographic characteristics of participants and malnutrition.

The current study revealed that 8/12(66.7%) thrombocytopenic participants were *H. pylori* infected females and the rest were infected males. Due to no thrombocytopenia observed in control groups of this study computing the results between positive and negative groups are impossible. But, the prevalence of thrombocytopenia in *H. pylori* infected patients suggests that this infection might alter with the patients platelet count.

Leukopenia is reduced leukocytes (<4 $x10^9$/l) mainly related with low neutrophil counts due to fungal or bacterial infections(40). In this study one of hematological abnormalities expected to observed with *H. pylori* infection was leukopenia which was reported in 26(20 case and 6 controls). The overall prevalence of leukopenia at Wolkite health center was 7.6% and the magnitude of leukopenia on patients with and without *H. pylori* infection was 11.8% and 1.8%

respectively. This result is slightly differed with the report of Getawa S *et al* in Gonder(33). The difference might be a result of different study population.

Finally, based on the results of this study the hypothesis of this research is not accepted even though some scholars revealed that there were no substantial hematological profile variance between individuals with and without *H. pylori*, statistically meaningful difference were observed on some hematological parameters. Such uneven findings could be due to differences in the technique used for identification of *H. pylor,i* discrepancies in age and nutritional status. Further remarkably, there were dispersal factors that influence levels of hematological parameters, and differences in the design of study between these studies (41). Genetic factors also could contribute significantly for variations between individual's hematological parameters. Therefore for well understanding exact cause and effect associations of *Helicobacter pylori* and hematological profiles, further research with large longitudinal study will be needed.

8. Strength and Limitation

8.1 Strength

The *Helicobacter pylori* detection was done using highly specific and sensitive one step stool antigen test which minimized the false positive and false negative results. Another strength of this study was in addition to *H. pylori* test; blood parasites and intestinal parasites were screened those may affect hematological profiles.

8.2. Limitation

The study is cross-sectional in nature; it doesn't indicate underlying association among *H. pylori* infection and the changes in hematological profiles. The serum iron level had not been measured in this study which might have strong relation with iron deficiency anemia and HGB synthesis. Additionally, because the study was only conducted on patients from one health center (wolkite health center, Guraghe zone, south Ethiopia), it is unbearable to generalize the results to whole of Ethiopia. But the reported information could provide an important aid to the existing evidence about *H. pylori* infection and its effect on hematological parameters.

9. Conclusion and Recommendation

9.1. Conclusion

According to this study, adult outpatients at Wolkite Health Center with *Helicobacter pylori* infections experienced substantial changes to various hematological parameters compared to uninfected control groups. This substantial variance between *H. pylori* Ag positive and negative participants should be in the considerations during management and control of *H. pylori* infection. Incorrect diagnosis and handling of this microorganism may lead to additional complications such as anemia and thrombocytopenia.

9.2. Recommendation

The current study found *H. pylori* infected patients had lower hematological parameters. Thus to manage additional complications of this microbe performing hematological tests for *H. pylori* infected individuals is recommended. In addition more research is recommended, mostly large longitudinal studies, to found cause-effect relationship.

10. References

1. Peter Katelaris, Richard Hunt, Franco Bazzoli, Henry Cohen, Kwong Ming Fock MGPM. World Gastroenterology Organisation Global Guidelines for Helicobacter pylori. 2021.
2. Rana R, Wang SL, Li J, Wang YX, Rao QW, Yang CQ. Helicobacter pylori infection : A recent approach to diagnosis and management. J Biomed. 2019;2:45–56.
3. Bagirova M, Allahverdiyev AM, Abamor ES, Aliyeva H. An overview of challenges to eradication of Helicobacter pylori infection and future prospects. Eur Rev Med Pharmacol Sci. 2017;21:2199–219.
4. Haile K, Timerga, Abebe. Evaluation of Hematological Parameters of Helicobacter pylori -Infected Adult Patients at Southern Ethiopia : A Comparative Cross-Sectional Study. J Blood Med. 2021;12:77–84.
5. Rokkas T. Treatment of H. pylori infection: Current recommendations. Ann Gastroenterol. 2005;18(2):119–26.
6. Sak ME, Özkul Ö, Evsen MS, Sak S, Evliyao O. The effects of gestational anemia on perinatal outcomes. Dicle Med J. 2017;1:39–42.
7. Shih H, Kuo F, Wang SSW, Liu Y, Wu M, Chang Y, et al. Helicobacter pylori Infection and Anemia in Taiwanese Adults. Gastroenterol Res Pract. 2013;2013:10–3.
8. Nasef WA, Shaker A, Alamodi HS, Alrefai AA, Nasif WA, Ali ASE, et al. Impact of Helicobacter pylori on hematological parameters among Saudi population. Saudi Med J. 2021;42:643–8.
9. Abdo-Francis JM. Helicobacter pylori infection. Rev Gastroenterol Mex. 2012;77:13–4.
10. Hooi JKY, Lai WY, Ng WK, Suen MMY, Underwood FE, Tanyingoh D, et al. Global Prevalence of Helicobacter pylori Infection: Systematic Review and Meta-Analysis. Gastroenterology. 2017;153(2):420–9.
11. Tsegaye A, Desta K, Taye B. Association of Helicobacter pylori infection with atopy and allergic disorders in Ziway , Central Ethiopia By : Mheret Tesfaye Gobena Addis Ababa University. 2017;
12. Ozbey G, Hanafiah A. Epidemiology, Diagnosis, and Risk Factors of Helicobacter pylori Infection in Children. Euroasian J Hepato-Gastroenterology. 2017;7(1):34–9.
13. Gokben Ozbey AH. Epidemiology , Diagnosis , and Risk Factors of Helicobacter pylori Infection in Children. Euroasian J Hepato-Gastroentero. 2015;7(1):34–9.
14. Kitila KT, Sori LM, Desalegn DM, Tullu KD. Burden of Helicobacter pylori Infections and Associated Risk Factors among Women of Child Bearing Age in Addis Ababa, Ethiopia . Int J Chronic Dis. 2018;2018:1–10.
15. Melese A, Genet C, Zeleke B, Andualem T. Helicobacter pylori infections in Ethiopia ; prevalence and associated factors : a systematic review and meta-analysis. BMC Gastroenterol. 2019;1–15.
16. Seid A, Demsiss W. Feco-prevalence and risk factors of Helicobacter pylori infection

among symptomatic patients at Dessie Referral Hospital, Ethiopia. BMC Infect Dis. 2018;18(1):260.

17. Shiferaw G, Abera D. Magnitude of Helicobacter pylori and associated risk factors among symptomatic patients attending at Jasmin internal medicine and pediatrics specialized private clinic in Addis Ababa city , Ethiopia. BMC Infect Dis. 2019;5:1–6.

18. Daka D, Tesfaye DY, Tadesse E, Daka D, Yemane D, Shimelis T. Seroprevalence of Helicobacter pylori infection and its related risk factors in symptomatic patients in southern Ethiopia. BMC Res Notes. 2014;7:834.

19. Kibru D, Gelaw B, Alemu A, Addis Z. Helicobacter pylori infection and its association with anemia among adult dyspeptic patients attending Butajira Hospital, Ethiopia. BMC Infect Dis. 2014;14(1):1–7.

20. Haile K, Timerga A. Evaluation of hematological parameters of Helicobacter pylori-infected adult patients at Southern Ethiopia: A comparative cross-sectional study. J Blood Med. 2021;12:77–84.

21. Mohammed A, Elkhalifa E, Agena AM, Tamomh AG, Hassan AFA, Albasheer FMH, et al. Complete Blood Counts among chronic patients of Helicobacter pylori infection. Majmaah J Heal Sci. 2021;9(2):12–22.

22. Ibrahim NK, Alsayes F. Helicobacter Pylori : Prevalence and Relationship to Hematological Parameters of Symptomatic Patients who Conducted Gastroscopy at King Abdulaziz Helicobacter Pylori : Prevalence and Relationship to Hematological Parameters of Symptomatic Patients who Con. J Adv Med Med Res. 2019;28(9):1–9.

23. Shih H, Kuo F, Wang SSW, Liu Y, Wu M, Chang Y, et al. Helicobacter pylori Infection and Anemia in Taiwanese Adults. Gastroenterol Res Pract. 2013;2013:10–3.

24. Erdem ME, Kecici S, Akin S, Cetin E, Kilic S, Kazan S, et al. Relationship Between Iron Deficiency Anemia and Helicobacter Pylori. Med J Okmeydani Train Res Hosp. 2014;30(2):82–5.

25. Xu M, Cao B, Yuan B, Yin J, Liu L, Lu Q. Association of anaemia with Helicobacter pylori infection : a retrospective study. Sci Rep [Internet]. 2017;1–8. Available from: http://dx.doi.org/10.1038/s41598-017-13955-3

26. Mwafy SN, Afana WM. Hematological parameters, serum iron and vitamin B12 levels in hospitalized Palestinian adult patients infected with Helicobacter pylori: a case–control study. Hematol Transfus Cell Ther. 2018;40(2):160–5.

27. Alshomar AA. Evaluation of hematological parameters in dyspepsia patients infected with Helicobacter pylori A retrospective study from the Central Region of Saudi Arabia. Saudi Med J. 2022;43(1):75–80.

28. Abdel Y, Abdel L, Mohamed RRR, Mohamed RRR, Ahmed M. Helicobacter pylori and its hematological effect. Egypt J Intern Med. 2019;31:332–42.

29. Elamin EAI, Suliman MA, Azoz ME, Ali EW, Olerile LD, Jiao Y, et al. Effect of Helicobacter pylori infection on haematological parameters in Kosti teaching hospital,

Sudan. Iran Red Crescent Med J. 2018;20(2).

30. Baxendell K, Walelign S, Tesfaye M, Wordofa M, Abera D, Mesfin A, et al. Association between infection with Helicobacter pylori and platelet indices among school-aged children in central Ethiopia: A cross-sectional study. BMJ Open. 2019;9(4):1–8.

31. Xu M, Cao B, Yuan B, Yin J, Liu L, Lu Q. Association of anaemia with Helicobacter pylori infection : a retrospective study. Sci Rep. 2017;791):1–8.

32. Solomon A, Kahase D, Alemayehu M. Trend of malaria prevalence in Wolkite health center : an implication towards the elimination of malaria in Ethiopia by 2030. Malar J. 2020;(2020)19:1–9.

33. Getawa SZGMM. Hematological Abnormalities and Associated Factors Among Undernourished Under-Five Children Attending University of Gondar Specialized Referral Hospital , Northwest Ethiopia. J Blood Med. 2020;11:465–78.

34. Khusun H, Yip R, Schultink W, Dillon DHS. Community and International Nutrition World Health Organization Hemoglobin Cut-Off Points for the Detection of Anemia Are Valid for an Indonesian Population 1. J Nutr. 1999;129:1669–74.

35. Story W. FAO/WHO expert consultation on human vitamin and mineral requirements. An Autom Irrig Syst Using Arduino Microcontroller. 2011;1908(January):2–6.

36. Wininger, Fred A. , Zeng R., Johnson, G.S., Katz, M.L., Johson G.C., Bush, W.W. , Jarboe, J.M., Coates JR. Case report. Can Fam Physician. 2020;47(10):788–9.

37. Mwafy SN, Afana WM. Hematological parameters, serum iron and vitamin B12 levels in hospitalized Palestinian adult patients infected with Helicobacter pylori. Hematol Transfus Cell Ther. 2018;40(2):160–5.

38. AHMED M. S. BAYOUMY, KHAIRY A. M. HASSAN, MORSY R. GENEIDY and AGSM. IMPACT OF INTESTINAL PARASITES ON HEMATOLOGICAL. J Egypt Soc Parasitol. 2018;48(1):157–64.

39. Hou B, Zhang M, Liu M, Dai W, Lin Y, Li Y, et al. Association of active Helicobacter pylori infection and anemia in elderly males. BMC Infect Dis. 2019;2019:1–9.

40. Dale DC. Overview of Leukopenias - Hematology and Oncology - MSD Manual Professional Edition. 2023.

41. Okada Y, Kamatani Y. Common genetic factors for hematological traits in Humans. J Hum Genet. 2012;57:161–9.

42. Shenzhen Mindray Bio-Medical Electronics Co. L matolog. Operation Manual. In: BC-3000 Plus Auto Hematology Analyzer Operation Manual. 2005.

Annexes

Annex I: Subject Information Sheet (For participants, English version)

Addis Ababa University

College of Health Sciences

Department of Medical Laboratory Sciences

Subject Information Sheet for participants whose blood is to be used for determination of hematological profiles

You are invited to participate in a study to be conducted by Masters Student at Addis Ababa, College of Health Sciences, Department of Medical Laboratory Science. Please read the following statements and ask any unclear points before you agree to participate (data collectors will read for those who cannot read).

Introduction

The topic of this study is Hematological Profile of adult outpatients with and without *H. pylori* at wolkite health center, guraghe zone, southern Ethiopia from January to April 2023. The aim of the study is to investigate hematological profiles of adult patients with *H. pylori* infection. And the attained values can be used as diagnostic and prognostic marker for certain complications in adult patients with *H. pylori* infection.

Participation in this study is exclusively voluntarily. If you are not interested to participate or if you once decide to participate and withdraw at any time, there will be no consequences and you will g*et al*l the services provided in the health center with no problems. If you decide to participate, you have to sign on the consent form and you may obtain a copy of this information sheet.

What is expected from me as a participant of the study?

As a participant of this study, you are expected to agree that about 2 gram of stool sample and 3-5mL venues blood will be collected from your hand. You need to know that your results might be discussed with other appropriate individual out of this health center. But your name, address and phone number will not be disclosed and rather than identification code will be used in such conditions.

How much time will I spent to participate in this study?

You will spend 20-25 minutes until the specimen is collected, the consent form is signed.

What are the risks of participating in this study?

The sample collection will pose minimal pain on you hand and the other thing you spend is just your time to sign consent form

How my information is to be kept in secret?

All information that you give and the results from your sample will be used for this study only, only limited numbers of professionals will have access to the information. All the information will be encoded in a computer and saved with password protection.

What are the benefits from participation?

Since this study is MSc student research, there will not be payments for participants. But your participation is important for determination of adult individuals' hematological profiles in the condition of *H. pylori* infection which is useful to improve the clinical management of adult patients with *H. pylori* with minimum cost.

What are my rights as a participant of this study?

You have the right to withdraw yourself from the study at any time and all the services provided in the health center will not be discontinued. You are also welcomed if you have any questions for further explanations about the study. You may also get the results of the analysis.

What can I do if I have a problem or a question?

Please direct any questions or problem you may encounter during this study to:

Yemeserach Moshago

Department of Medical Laboratory Sciences,

College of Health Sciences, Addis Ababa University

Mob: +251921646161

Email: yemeserachejamo@gmail.com

For additional information, please contact Department of Medical Laboratory Sciences, Addis Ababa University, Departmental Research and Ethics Review committee office;

P.O Box: 9086,

Addis Ababa, Ethiopia

Agree to participate? Yes ☐ No ☐

Annex II: Subject Information Sheet (For participants, Amharic Version)

አዲስ አበባ ዩኒቨርሲቲ
የጤና ሳይንስ ኮሌጅ
የሕክምና ላቦራቶሪ ሳይንስ ት/ክፍል
ከክንድ ላይ ደም ተወስዶ ለሚሰራው የአዋቂዎች አጠቃላይ የደም ምርመራ ውጤት ጥናት
ለጥናቱ ተሳታፊዎች የተዘጋጀ መረጃ

አዲስ አበባ ዩኒቨርሲቲ፤ የጤና ሳይንስ ኮሌጅ፤ የሕክምና ላቦራቶሪ ሳይንስ ት/ክፍል በማስተርስ ዲግሪ ተማሪ የመመረቂያ ጥናት ላይ እንዲሳተፉ ተጋብዘዋለው። እባክዎ በዚህ ጥናት ለመሳተፍ ከመስማማትዎ በፊት ከዚህ ቀጥሎ የሚገኘውን ምንባብ በጥሞና ያንብቡና ግልፅ ያልሆነልዎትን ማንኛውም ሃሳብ ይጠይቁ።

መግቢያ

የጥናቱ ርዕስ በክንድ ከተወሰደ የደም ናሙና ላይ የሚሰራ የአዋቂዎች አጠቃላይ ደም ምርመራ (CBC) ውጤት ነው።አላማውም የጨጓራ ባክትሪያ ከተገኘባቸው አዋቂዎች አጠቃላይ ደም ምርመራ ውጤቶች ዋጋ ማግኘት ሲሆን ጥቅሙም አዋቂዎች በጨጓራ ባክትሪያ ሲያዙ የሚኖራቸውን የእነዚህን አጠቃላይ ደም ምርመራ ውጤቶች ዋጋ በትክክል ለመተርጎም ይጠላል።

እርስዎ በዚህ ጥናት ላይ የሚኖርዎት ተሳትፎ ሙሉ በሙሉ በበጎ ፈቃደኝነት ላይ የተመሰረተ ሲሆን በዚህ ጥናት ዉስጥ ላለመሳተፍ ሆነ ለመሳተፍ ከወሰኑ በኋላ ለማቋረጥ የሚወስኑ ቢሆንም እንኳን በዚህ ጤና ጣቢያ ውስጥ የሚገኝ ማንኛውም አገልግሎት አይቋረጥም። በጥናቱ ለመሳተፉ ከፈለጉ የስምምነት ቅጹ ላይ በፅሁፍ ወይም በጣት ፊርማ ማረጋገጥ ይኖሩበታል። ከፈለጉም ይህን የመረጃ ቅፅ አንድ ቅጂ ለራስዎ መውሰድ ይችላሉ።

በጥናቱ ተሳታፊ በመሆኔ የሚጠበቅብኝ ምንድን ነው?

በዚህ ጥናት ላይ ለመሳተፍ የሚስማሙ ከሆነ በግምት 2ግራም ሰገራ እና 3-5ሚ.ሊ የደም ናሙና ከክንዶ ላይ እንደሚወሰድ እና ለጥናቱ እነደሚዉል መስማማት ይጠበቅቦታል። ከተወሰደው ናሙና ላይ የሚገኙ መረጃዎች ከዚህ ጤና ጣቢያውጭ ለሚገኙና ለስራዉ አግባብነት ላላቸው ሰዎች ቢነገር የማይቃወሙ መሆኑን መስማማት ይጠበቅቦታል። ስምም ሆነ የስልክ ቁጥር የመሳሰሉትን መረጃዎችን አይጨምርም። ይልቁንም ለዚህ ጥናት አገልግሎት ብቻ የሚዉል እርስዎን ለማወቅ የሚያስችል መለያ ቁጥር ጥቅም ላይ እንዲዉል ይደረጋል።

በዚህ ጥናት ስሳተፍ ምን ያህል ጊዜ ይወስድብኛል?

ናሙና እስኪወሰድና የስምምነት ቅጹ ላይ ለመፈረም ከ20-25ደቂቃ ያስፈልጋል።

በዚህ ጥናት መሳተፍ የሚያስከትላቸዉ ችግሮች አሉ?

ናሙና በሚወሰድበት ጊዜ የተወሰነ የህመም ስሜት ይኖረዋል ለላው የሚያጡት ነገር የስምምነት ቅጹ ላይ ለመፈረም የሚያጠፉት ጊዜ ነው።

የህክምና መረጃዬ በሚስጢር ተጠብቆ መቆየት የሚችለዉ እንዴት ነዉ?

ስለራስዎ የሰጡት ማንኛውም መረጃና ከተወሰደው ናሙና ላይ የተገኘው የላቦራቶሪ ውጤት የሚውለው ለጥናቱ አላማ ብቻ ነው። ይህን ማህደር ሊያገኙ የሚችሉት የተወሰኑ የጥናቱ ተባባሪ ሰራተኞች ብቻ ናቸው፡ ከዚያም በላይ ስለእርስዎ ያለውን ማንኛውም መረጃ የተለየ የይለፍ ቃል ባለው ኮምፒዩተር የመረጃ ማህደር ውስጥ እንዲቀመጥ ይደረጋል።

በዚህ ጥናት ላይ መሳተፍ የሚያስገኛቸው ጥቅሞች ምንድን ናቸው?

ይህ ጥናት የማስተርስ ዲግሪ ተማሪ መመረቂያ እንደመሆኑ መጠን ለተሳታፊዎች ገንዘብ አይከፈልም፤ ነገር ግን የእርስዎ ተሳትፎ በደም ካንሰር የሚጠቁ ህፃናትን ለመርዳትና በህፃናቱ ላይ የተገኘውን የአጠቃላይ ደም ምርመራ ውጤቶችን ለመተርጎም ይጠቅማል።

በዚህ ጥናት ተሳታፊ በመሆኔ መብቶቼ ምንድን ናቸው?

በጥናት ውስጥ ያልዎትን ተሳትፎ በማንኛውም ጊዜ የማቋረጥሙሉ መብትዎ የተጠበቀ ከመሆኑም በላይ ራስዎ ከጥናቱ በማግለልዎ ምክንያት ምንም አይነት የጤና ጣቢያአገልግሎት አይቋረጥብዎትም። ከዚህም በተጨማሪ ጥናቱን በተመለከተ ማንኛውም ጥያቄ የመጠየቅና ገለፃ የማግኘት መብት አሉዎት።የላቦራቶሪ ምርምራ ዉጤቱንም በነፃ ማግኘት ይችላሉ።

ጥያቄ ካለኝ ወይም ችግር ቢያጋጥመኝ ምን ማድረግ ይገባል?

ይህን ጥናት በተመለከተ ወይም ከዚህ ጥናት ጋር በተዛመደ መልኩ ስለሚያጋጥሙ ድንገተኛ አደጋዎች ወይም ጥያቄ ካልዎት በሚከተለው አድራሻ ይጠቀሙ።

<u>የምስራች ሞሸጎ</u>

የህክምና ላቦራቶሪ ሳይንስ ዲፓርትመንት የጤና ሳይንስ ኮሌጅ፤ አዲስ አበባ ዩኒቨርሲቲ

ሞባይል፡ +251921646161 ኢሜይል፡ yemeserachejamo@gmail.com

ለተጨማሪ መረጃ የአዲስ አበባ ዩኒቨርሲቲ ህክምና ፋከልቲ ዲፓርትመንታል ሪሰርች ኤንድ ኢቲካል ሪቭው ኮሚቴ ይጠይቁ። ፖ.ሳ.ቁ፡ 9086፣ አዲስ አበባ፤ ኢትዮጵያ

ለመሳተፍ ይስማማሉ?

እስማማለሁ ☐ አልስማማም ☐

Annex III: Consent form (for participants, English version)

Code No._____

I have been informed about the study which is aimed to determine hematological parameters of blood sample. The aim of the study was explained to me. I have been given the opportunity to ask questions and my questions have been answered to my satisfaction. In addition; I have also been informed that the benefit of the participation is to get the results of the analysis measured for free via the counselor. It is therefore, with full understanding of the situation I voluntarily consent that I would participate and I gave the informed consent voluntarily to the researcher to collect stool and blood sample and be a participant in this study and understand that I have the right to withdraw myself from the study at any time.

Name of participant_____ Date____ /____ /____ (dd/mm/yy) signature_____

Name of witness (if illiterate) _____Date____/____/_____ (dd/mm/yy) signature _____

Name of councilor_____ Date ____/____/_____ (dd/mm/yy) signature _____

Please direct any questions or problem you may encounter during this study to:

Principal investigator; Yemeserach Moshago

Mob: +251921646161 Email: yemeserachejamo@gmail.com

For additional information, please contact Department of Medical Laboratory Sciences, Addis Ababa University, Departmental Research and Ethics Review committee office;

P.O Box: 9086, Addis Ababa, Ethiopia

Annex IV: Consent Form (for participants, Amharic Version)

የስምምነት ቅጽ (ለጥናቱ ተሳታፊዎች)

የምስጢር ቁጥር_____

እኔ ስሜ ከላይ የተጠቀሰው ተሳታፊ በክንድ ላይ ተወስዶ ስለሚሰራው የአጠቃላይ ደም ምርመራ (CBC) ውጤት ጥናት በቂ ገለፃ ተደርጎልኛል። ለጥናቱም ጥቂት ሰገራና ከክንድ የተወሰደ የደም ናሙና እንደሚያስፈልግ ተገለፆልኛል። የጥናቱን አላማዎችንም ተረድቻለው።በጥናቱም የሚገኙ መረጃዎች በሙሉ በምስጥር የተጠበቁ እንደሚሆኑ ተነግሮኛል። በጥናቱ ላይ ያለመሳተፍና ማንኛውም መረጃ ያለመስጠት እንዲሁም በማንኛውም ጊዜ ከጥናቱ እራሴን የማግለል መብቴ የተጠበቀ መሆኑን ተገለፆልኛል። ስለዚህ ለዚህ ጥናት የስምምነት ቃሌን የሰጠሁት በአጠቃላይ ሁኔታውን በመረዳትና በፍፁም ፈቃድኝነት ነው። ከክንድ ላይ የሚወሰደዉ ናሙና የእኔን ጤና ሁኔታ ማወቅ እና ለምርምር እነደሚውልም ተረድቻለው። በተጨማሪም ጥያቄ እንድጠይቅ ተፈቅዶልኝ ለማወቅ የፈለጉትን ማብራሪያ አግኝቻለሁ። የዚህ ጥናት ተሳታፊ በመሆኔ የላቦራቶሪ ምርምራ በነፃ ማግኘት እንደሆነ ተረድቻለው።

የተሳታፊው/ዋ ፊርማ/ የጣት አሻራ_____ቀን_____

የምስክር ስም_____ፊርማ_____ ቀን_____

(የስምምነት ቅጹን ማንበብ ለማይችሉ ተሳታፊዎች)

የአማካሪ ስም_____ ፊርማ_____ ቀን_____

ይህን ጥናት በተመለከተ ወይም ከዚህ ጥናት ጋር በተዛመደ መልኩ ስለሚያጋጥሙድንገተኛ አደጋዎች ወይም ጥያቄ ካልዎት በሚከተለው አድራሻ ይጠቀሙ።

የምስራች ሞሻ

የህክምና ላቦራቶሪ ሳይንስ ዲፓርትመንት የጤና ሳይንስ ኮሌጅ፤ አዲስ አበባ ዩኒቨርሲቲ

ሞባይል፡ +251921646161 ኢሜይል፡ yemeserachejamo@gmail.com

ለተጨማሪ መረጃ የአዲስ አበባ ዩኒቨርሲቲ ህክምና ፋከልቲ ዲፓርትመንታል ሪሰርች ኤንድ ኢቲካል ሪቭው ኮሚቴ ይጠይቁ። ፖ.ሳ.ቁ፡ 9086፣ አዲስ አበባ፤ ኢትዮጵያ

Annex V: Standard Operating Procedure (SOP)

Procedures for whole Blood Collection

1. Firstly ensure that the patient should be in the comfortable position.
2. Assemble syringe with needle and EDTA-anticoagulated tube(purple cap)
3. Place the patient's arm on the table.
4. Apply the tourniquet and select patient's prominent vein.
5. Disinfect the selected site for puncture using the Alcohol swab.
6. Draw 3-5 ml of blood into the syringe.
7. Then, carefully pull the syringe with the needle off and by opening the tube transfer the blood with care into test-tube carefully and mix by inverting gently 8-10times.
8. Explain the patient to relax and open the fist and remove the tourniquet
9. Immediately apply manual pressure over the venipuncture site with the cotton swab, remaining the patient's arm straight.
10. Tell the patient to apply the pressure to the wound until the blood ceases.
11. When bleeding stops, apply a fresh bandage, gauze or tape.
12. Dispose of the vacutainer unit as a unit into an appropriate sharps container.

SOP for Mindray BC-3000 plus auto hematology analyzer

1. Purpose: To determine complete blood count using BC-3000plus automated hematology analyzer

2. Principle: electrical impedance method for counting and cyanide free method for hemoglobin

The Mindray BC-3000 plus is a quantitative automated hematology analyzer for in vitro diagnostic use for determining 19 hematological parameters and 3histograms (WBC, RBC and PLT). Examination of the numerical findings of the complete blood count are useful in diagnosis of disease states such as anemia, leukemia, allergic reactions, viral, bacterial, and parasitic infections. The BC-3000plus hematology analyzer uses two independent measurement method principles to count cell populations. 1. Coulter method to determine white blood cells, Red blood cells and platelet data (this method is based on measuring the change in electrical impedance occurred by a blood cell, suspended in a conductive diluent as it passes through an aperture of known sizes.) 2. colorimetric method to determine HGB (The WBC/HGB dilution is delivered

to the WBC bath where it is bubble mixed with a certain amount of lyse, which converts hemoglobin to a hemoglobin complex that is measurable at 525 nm).Samples are aspirated, diluted and mixed during every analysis cycle before the determination of each parameter is performed. The Mindray BC-3000plus analyzer directly measures the WBC, RBC, HGB, HCT, and PLT, LYM #, MID #and NEUT #. The remaining parameters are calculated or derived MCV, MCH, MCHC, MPV, RDW-CV, RDW-SD, PDW and PCT and differential percentages LYM%, MID%, NEUT%. The resulting WBC histogram is discriminated into lymphocyte, neutrophil and mid-sized cell populations. The mid-sized cell population contains monocytes, basophils and eosinophil.

1. **Materials**

Reagent, Control and Calibrator

Because the analyzer, calibrator, reagent, and controls are components of a system, performance of the system depends on the combined integrity of all components.The fluidic system of the machine is formulated to use Mindray-specified reagents. Therefore, perform the tests using only allowed reagents for best performance.

Reagents:

- Diluent, M-30D DILUENT
- Rinse, M-30R RINSE
- Lyse; 0.5 mL/analysis M-30CFL LYSE
- Probe Cleanser M-30P PROBE CLEANSER
- E-Z Cleanser M-30E E-Z CLEANSER

Reagent stability and storage:

- Leave the reagent at room temperature (15 - 30°C) for at least 24 hours before using
- Use reagents within the expiration date
- CELLPACK expires after 60 days of opening

Supplies

- Disposable gloves
- EDTA vacationer tubes (purple top)
- Thermal paper

4. Sample

- Type: whole blood collected according to the SOP.
- Transport and storage: Ambient temperature (15-25 0C)
- Stability: within 8 hours of sample collection

5. Special safety precautions:

- Wear protective clothing
- Follow infection prevention principle during sample handling
- Wear gloves for handling blood or serum
- Decontaminate working area with 0.5% bleach solution
- Change gloves when they become contaminated
- Wash hands after handling specimens
- CELLCLEAN is a strong alkaline detergent; take care not to have it adhere to the skin or clothes. If the skin or clothes should come in touch with it, flush it away using plenty of water. Otherwise, it can damage the skin or clothes

6. Quality control and Calibrators

The control reagents and calibrators are used to verify accurate operation of and calibrate the analyzer. The controls and calibrators are commercially prepared whole-blood products used to verify the function of the analyzer is proper and to calibrate the analyzer respectively. controls are available in low, normal, and high levels. Daily use of all levels verifies the operation of the analyzer and ensures reliable results are obtained.

7. Procedures for analysis

Initial Checks (Inspecting)

Apply the following inspections before turning on the analyzer.

1. Inspect the waste container; and be sure the container is empty.

2. Checking tubing and power connections ;

   ✓ Check and make sure the diluent, rinse and waste tubes are properly connected and not bent;

- ✓ Check and make sure the power cord of the analyzer is properly plugged into an electrical outlet.

3. Inspect the printer

Check and be sure required amount of printer is installed. Inspect and be sure the power cord of the printer is properly plugged into an electrical outlet. Inspect and be sure the printer cable is properly connected to the analyzer.

4. Check keyboard connection.

Inspect and be sure the keyboard is properly connected to the keyboard interface (marked KB) of the analyzer.

Power-on

At back of the analyzer place the power switch in the ON position (1) to turn on the analyzer. The power indicator light will be illuminated and you will see on the screen "**Initializing…**".

Analyzer initialize files one by one, and the whole initializing process lasts 3 to 5 minutes, based on how the analyzer was last time shut down.

If any error occurs during the initialization, the analyzer will display the error messages in the screen. Fix any error or unaccepted background count before running samples.

Acceptable background count

- WBC $\leq 0.3 \times 10^9$ / L
- RBC $\leq 0.03 \times 10^{12}$ / L
- HGB ≤ 1 g / L
- HCT ≤ 0.5 %
- PLT $\leq 10 \times 10^9$ / L

Aspiration

Whole blood samples and prediluted blood samples are processed by analyzer.

whole blood sample are simply presented to the sample probe and press the aspirate key, the analyzer aspirate 13µL of the sample.

If you need to test a capillary blood sample, you must first manually dilute the sample (20 μL of capillary sample needs to be diluted by 0.7mL of diluent) and then present the pre-diluted sample to the sample probe and press the aspirate key to aspirate 0.3ml of the sample into the analyzer

Inputting Sample/ID number

At the "**Count**" screen, press [ID] and edit window opens,

- Entering ID

ENTER the ID number in the "**ID**" box..

- choosing gender of patient

Choose the wanted item ***from the "Gender" pull-down list***

- Entering the patient age

You can enter patient age in 3ways– in days, in months and in years. The 1st way is for the neonatal patients less than 28 days; the 2nd for the infant patients 1month - 1year; the 3rd is designed for the adult or pediatric patients older than 1year. You can select only one of the 3 ways to enter the patient age.

"**Enter**" key

After entering the all the required information, ***CLICK*** the "**Enter**" button to save the changes and back to the "**Count**" screen.

Analyzing samples

1. At the "**Count**" screen, make sure the **System Status** region displays "**Ready**" and **Count Mode** area displays "**Whole**".
2. provide the mixed sample to the sample probe and the tip is well into the tube, then press the aspirate key. The **System Status** area will display "**Running**" and the analyzer will start aspirating sample.
3. When you hear the beep and the sample probe is out of the tube, remove the sample tube. The sample probe will retract into the analyzer and the analysis progress will be displayed on the screen.
4. While the analysis is completed, the result will be displayed on the screen And if the auto print function is on, the analysis result will be automatically printed out.
5. Repeat the above steps on other samples.

Shutdown

Perform the "**Shutdown** "procedure to shut down the analyzer daily.

1. Press [MENU] to enter the system menu and ***SELECT*** "**Shutdown**"

2. A message box will open to ask for conformation of shutdown
3. ***CLICK*** "***Enter***"and a window will pop up to instruct you how to shut down the analyzer,
4. Present the E-Z cleanser to the sample probe and press the aspirate key. The analyzer will aspirate the E-Z cleanser and automatically clean the fluidic lines and the baths. You will observe the cleaning progress on the screen.
5. When the cleaning is finished, place the switch at the back of the analyzer to OFF (O) to turn off the analyzer;
6. Empty the waste container.

8. Limitations

- Each time the results are outside the normal limits, it is recommended that the laboratory following whatever written procedure is in place for validating results.
- If an error occurs, the analyzer displays the corresponding error message In case of errors related to the fluidic system (such as clogging or bubbles), it is recommended that you re-run the sample after removing the error.
- If the PLT value is less than 100×10^9 / L, it is recommended the result be verified by a microscope.

9. Interpretations

- If an"H" or "L" displayed with result, it means the analysis result has exceeded the upper or lower limit of the reference range.
- If you see *** as opposed to the result, it means the result is either unreliable or out of the operating range.
- If the WBC result is less than 0.5×10^9/L, this analyzer will not perform the differential analysis and all the related parameter values will be non-numeric (***).
- Low level of HGB seen in patient with anemia and the different red cell parameters give for the possible type of etiology.
- Elevated white blood cell count may indicate infection.
- Neutrophils may be increased due to an acute bacterial infection or hematological malignancies such as Myeloid Leukemia.
- Parasitic infection or an allergic Reaction will increase in eosinophils counts.
- An increase in lymphocytes may be due to viral infections or chronic infections(42)

Standard operational procedure for *H. pylori* one step stool Ag test

Purpose of *H. pylori* antigen rapid test is to detect active *H. pylori* infection using one step rapid test strip (Guangzhou Wondfo Biotech, China).

Principle of test: detecting active *H. pylori* antigen using peroxidase-conjugated monoclonal antibody.

Procedure;

1. Explain to the participants how to collect adequate and representative specimen and provide clear cupped container
2. Take a small amount of feces and homogenize it with buffer solution
3. Add two drops of the stool/buffer to the test well.
4. After 15 min, the test was read.

Result Interpretations The development of two lines, the control (C) line and the test (T) line, shows an *H. pylori* -positive test result, whereas the development of only the C line indicated a negative test result. In the occurrences where the T line was significantly fainter than the C line, the results would be interpreted and recorded as positive.

Annex VI: Questionnaire (For Participants, English Version)

Addis Ababa University

College of Health Sciences

Department of Medical Laboratory Sciences

Questionnaire for Data Collection from participants whose whole Blood is to be used for hematological profile determination

1. . Introduction

   Subject identification code_____ MRN _____

   Age (in years) _____ Sex: 1. Male 2. Female

   Residential Place _____ 1. Urban 2. Rural

   Tel: _____

2. Body mass index (BMI) _____ 1. Height_____ 2. Weight _____
3. Educational level

   ☐ Illiterate ☐ Primary (1-8) ☐ Secondary (9-12)

   ☐Higher level (diploma, degree and above)

4. Marital status

   ☐Single ☐Married ☐Divorced ☐Widowed

5. Do you drink alcohol? ☐Yes ☐ No
6. If your answer for question 5 is ‘yes’, how often do you drink alcohol?

   ☐Daily ☐Every weekend ☐ occasionally

7. Do you smoke cigarettes? ☐Yes ☐ No
8. Do you chew chat? ☐Yes ☐No
9. If your answer for question 7 is ‘yes’, how often do you chew chat?

☐Daily ☐Every weekend ☐occasionally

10. Have you been sick any chronic disease for the last 3 months? ☐Yes ☐ No

If yes, when ---------- describe illness ----------

11. Are you taking any prescribed medication? ☐Yes ☐No

If yes, specify the name? ----------

12. Are you donating blood within last 3 months? ☐Yes ☐No

End of interview

Thank you!

Annex VII: Questionnaire (For Participants, Amharic Version)

አዲስ አበባ ዩኒቨርሲቲ

የጤና ሳይንስ ኮሌጅ

የሕክምና ላቦራቶሪ ሳይንስ ት/ክፍል

ከክነድ ላይ ደም ተወስዶ ለሚሰራው አጠቃላይ የደም ምርመራ ሁኔታ ጥናት ለሚሳተፉ ተሳታፊዎች የተዘጋጀ መጠይቅ

1. መግቢያ

መለያ ቁጥር_____ ካርድ ቁጥር_____

እድሜ (በአመት)_____ ጾታ: 1. ወንድ 2. ሴት

መኖሪያ አድራሻ _____

2. የትምህርት ደረጃ

ሀ. ማንበብና መፃፍ የማይችል/ትችል (ያልተማሩ) ሐ. ሁለትኛ ደረጃ (9-12ኛ)

ለ. መጀመሪያ ደረጃ (1-8ኛ) መ. ኮሌጅ ሰርቲፊኬት/ዲፕሎማ/ድግሪ እና ከዛ በላይ

3. የትዳር ሁኔታ

ሀ. ያገባ ለ. ያላገባ ሐ. የፈታ መ. የሞተባት

4. አልኮል ይጠጣሉ?

ሀ. እጠጣለሁ ለ. አልጠጣም

5. ለ 4ኛው ጥያቄ መልሰዎ እጠጣለሁ ከሆነ በየስንት ጊዜዉ ይጠጣሉ?

ሀ. በየቀኑ ለ. በሳምንቱ መጨረሻ ቀናት ሐ. አልፎ አልፎ

6. ሲጋራ ያጨሳሉ?

ሀ. አጨሳለሁ ለ. አላጨስም

7. ጫት ይቅማሉ?

ሀ. እቅማለሁ ለ. አልቅምም

8. ለ7ኛው ጥያቄ መልስዎ እቅማለሁ ከሆነ በየስንት ጊዜዉ ይቅማሉ?

ሀ. በየቀኑ ለ. በሳምንቱ መጨረሻ ቀናት ሐ. አልፎ አልፎ

9. በአለፈው 3ወራት ዉስጥ ታመው ነበር/ የቆየ ህመም አለቦት?

ሀ.አዎ ለ. አልታመምኩም

አዎ ከሆነ መልስዎ መች?----------ህመሙ ምን እንደነበር ይግለጹ----------

10. መድሐኒት በመውሰድ ላይ ነዎት?

ሀ. አዎ ለ. አይደለም

ከወሰዱ የመድሐኒቱን ስም? ----------

11. በአለፈው 3ወራት ዉስጥ ደም ለግሰው ነበር?

ሀ. አዎ ለ. አይደለም

መጠይቁን ጨርሰዋል

አመሰግናለሁ!

Annex VIII: Data collection format

I. Demography

1. Code:_____
2. Address:_____
3. Age:_____(Year)
4. Sex: 1. Male 2. Female

II. Participant group

1. Study 2. Control

III. Laboratory screening test results

1) *H. pylori* Ag test: 1. Positive 2. Negative
2) Hemo-parasite: 1. Seen (specify _____)2. Not seen
3) Intestinal parasites 1. Seen (specify_____) 2. Not seen

5. Laboratory (at presentation)

CBC	Count in units
Total WBC count(cell /uL	
RBC Count(cell /uL)	
Hemoglobin level (g/dl)	
Hematocrit (%)	
MCV (fL)	
MCH (Pg)	
MCHC (g/dl)	
RDW (%)	
Platelets((cell /uL))	
MPV (fL)	
PDW (%)	
PCT (%)	
NEU (%)	
LYM (%)	
MID (%)	
#NEU	
#LYM	
#MID	

Lab tech initial name and sign _____date_____ (G.C)

PI sign _____date_____ (G.C)

Declaration

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

M.Sc. candidate: **Yemeserach Moshago (B.Sc.)**

Signature: _____

Date of submission: _____

This proposal has been submitted with our approval as advisors.

Advisor: **Aster Tsegaye (PhD, Prof.)**

Signature: _____

Date: _____

Place: Addis Ababa, Ethiopia.

Advisor: **Melatwork Tebebu (MSc, PhD candidate)**

Signature: _____

Date: _____

Place: Addis Ababa, Ethiopia.