ANEMIA AND ITS ASSIOCIATION WITH *HELICOBACTER PYLORI* INFECTION AMONG ADULT DYSPEPTIC PATIENTS ATTENDING WACHEMO UNIVERSITY NIGIST ELEIN MOHAMMAD MEMORIAL REFERRAL HOSPITAL: HOSANNA, SOUTHWEST ETHIOPIA

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A research thesis submitted to School of Medical Laboratory Sciences Faculty of Health Sciences, Institute of Health , Jimma University in partial fulfillment of the requirements for the degree of Masters of Science in Clinical Laboratory Science specialty in Hematology and Immunohematiology

JIMMA UNIVERSITY

INSTITUTE OF HEALTH

FACULTY OF HEALTH SCIENCES

SCHOOL OF MEDICAL LABORATORY SCIENCES

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Abstract

Background: Anemia is a public health problem worldwide and also associated with *H. pylori* infection. Determining prevalence of anemia and its association with *H. pylori* infection is important to develop evidence based decision and intervention strategies. The magnitude of anemia and its association with *H. pylori* infection is not well known in this study area.

Objectives: To determine prevalence of anemia and its association with *H. pylori* infection among adult dyspeptic patients attending Wachemo University Nigist Elein Mohammad Memorial Referral Hospital from January 8,2019 to April 1,2019; Hosanna, southwest Ethiopia.

Method: A cross sectional study was conducted involving 362 consecutive adult dyspeptic patients who came to the Hospital during the study period. Socio-demographic, clinical and other related data were collected by structured questionnaire. Four milliliter of venous blood sample was collected for hematological parameters analysis and blood film preparation. Stool sample was collected to detect *H. pylori* antigen and intestinal parasite. Data were analyzed by SPSS version 21. Frequency table, graph and descriptive summaries were used to describe variables. Independent sample T-test, bivarate and multivariate logistic regression analysis were performed. In this study $p<0.05$ was considered as statistically significant.

Results: The overall prevalence of anemia among dyspeptic patients was 24.3% (95%CI: 19.9, 28.7). Among *H. pylori* infected participants 29.2% were anemic, of which 69.2% had mild anemia and 63.5% had normocytic normochromic anemia. Rural residence (AOR: 1.9, 95%CI: (1.1, 3.3), *H. pylori* infection (AOR: 1.77, 95%CI: (1.05, 2.98) and intestinal parasitic infection (AOR: 2.14, 95%CI: (1.14, 4.03) were significantly associated with anemia. The mean (SD) values of HGB, RBC, HCT, MCV, MCH, MCHC and RDW were significantly different between *H. pylori* positive and negative participants.

Conclusion: The prevalence of anemia in this study indicated that it is a moderate public health problem. Rural residence, *H. pylori* and intestinal parasitic infection were significantly associated with anemia. The findings of this study should be taken into account for prevention and control of anemia among dyspeptic adults.

Key words: Anemia, *H. pylori* infection, dyspeptic, Hosanna, southwest, Ethiopia.

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Acronyms and abbreviation

AOR	Adjusted Odds Ratio
BMI	Body mass index
CBC	Complete blood count
CI	Confidence interval
COR	Crude Odds Ratio
EDHS	Ethiopian Demographic and Health Survey
EDTA	Ethylenediaminetetraacetic acid
fL	Femtoliters
g/dL	Grams per deciliter
GI	Gastrointestinal
HCT	Hematocrit
Hgb	Hemoglobin
ID	Iron deficiency
IDA	Iron deficiency anemia
MCH	Mean cell hemoglobin
MCHC	Mean cell hemoglobin concentration
MCV	Mean cell volume
RBC	Red blood cell count
RDW	Red cell distribution width
SD	Standard deviation
SNNPR	Southern Nations Nationalities and People's Region
SOPs	Standard operating procedures
SPSS	Statistical Package for Social Science
TIBC	Total iron binding capacity
WHO	World Health Origination
WUNEMMRH	Wachemo University Nigist Elein Mohammed Memorial Referral Hospital

Chapter one: Introduction

1.1. Background

Anemia is defined as a decreased blood cell hemoglobin concentration; it is a common manifestation of various etiologies, for example, iron-deficiency (ID), vitamin B12 deficiency, folic acid deficiency, gastrointestinal bleeding, acute and chronic infections (1,2).

Anemia can be classified as microcytic hypochromic, normocytic normochromic and macrocytic based on morphological criteria of red cells in thin blood film, and also on red cells indices; mean cell volume (MCV), mean cell hemoglobin (MCH) and mean cell hemoglobin concentration (MCHC) (3). Whereas based on hemoglobin concentration (Hgb) it can be categorized as mild, moderate and severe anemia (4).

Anemia has multi-factorial underlying causes and associated with socio-demographic factors, parasitic infection, chronic disease, nutritional status and dietary habit (5–7). Despite this, *H. pylori* infection is a highly prevalent microbial infection (8), has been found more frequently in dyspeptic patients (9).

Helicobacter pylori (*H. pylori*) infection is one of the most common bacterial infections in humans and infecting more than 50% of the world's population (10). It causes gastritis, peptic ulcer disease, gastro duodenal ulcer, atrophic gastritis and dyspeptic symptom (11,12). Furthermore, it has been implicated in some hematological manifestations such as anemia, ID and vitamin B12 deficiency (13,14).

H. pylori infection is associated with anemia by impairing iron absorption as a result of chronic gastritis which causes gastric hypochlorhydria, leading to impair reduction of the dietary iron from the ferric to ferrous form (15,16). Because most dietary iron is in the ferric form, and an acidic intra-gastric pH and ascorbic acid is needed to reduce it to the ferrous form for absorption (17). Hence, *H. pylori* is a major causes of chronic superficial gastritis leading to atrophy of gastric glands , resulting in reducing gastric acid secretion (18).

Iron uptake by *H. pylori* is enhanced during their growth, can compete with the host (19) , and increased hepcidin production secondary to *H. pylori* infection decreases the release of iron from macrophages and entrecotes (20). Because hepcidin, act as a acute phase reactant in response to the inflammation produced in the gastric mucosa, resulting in anemia of inflammation or chronic disease (16). Other possible mechanism includes, increased iron loss due to hemorrhagic gastritis and active bleeding peptic ulcers (21).

In another hand, it has been implicated that *H. pylori* infection cause deficiency of vitamin B12 by producing chronic gastritis and atrophic gastritis (22); a mechanism that has been proposed to explain this association is *H. pylori* infection causes hypochlorhydria, leads to increased bacterial colonization. Bacteria compete with the host for vitamin B12. Gastric acid in the stomach and pepsin was required to release protein bounded vitamin B12 from food; thereby vitamin B12 cannot bind to haptocorrin (R-protien) and then with intrinsic factor, lead to malabsorption of vitamin B12. In addition, decreased production of intrinsic factor due to atrophic gastritis which may results in vitamin B12 deficiency and anemia (20,23,24).

1.2. Statements of problems

Anemia is a global public health problem affecting both developed and developing countries with major consequences for human health as well as social and economic development (25).

According to World Health Organization (WHO) report, anemia affects around 1.62 billion people globally, which was estimated 24.8% of the global population. It affects 45.7%-65.5% of individuals in South-East Asia and 47.5%–67.6% in Africa. The highest prevalence of 47.4% is in preschool-age children and the lowest prevalence of 12.7% is in men (26). In addition, the global prevalence of anemia for all women of reproductive age was 29.4%. Four hundred ninety six million women of reproductive age, 32 million pregnant women, and 273 million children were anemic in 2011(25). But in 2016, globally 578 million women of reproductive age were anemic. Burden of anemia were highest in Africa and South-East Asia (27).

Anemia is one of the most common public health problems in Ethiopia, according to the Ethiopian Demographic and Health Survey (EDHS) report in 2016, 24% of women and 15% of men were anemic (28). Burden of anemia among women of reproductive age declined from 27% in 2005 (29) to 17% in 2011 (30), but increased to 24% in 2016 (28).

H. pylori infection is a worldwide public health problem, affecting both developed and developing countries; globally 48.6% of adults suffering from this infection (8). In Ethiopia it affects 83.3% adults in southern Ethiopia (31), 72.2% in northern Ethiopia (9) and 71% in Somali region (32). In addition it has been reported that *H. pylori* infected individuals are at higher risk of anemia, ID and vitamin B12 deficiency compared to *H. pylori*-negative individuals(33–36). Different studies also suggest that anemia is prevalent among *H. pylori* infected patients, a study showed that 91.8% of *H. pylori* infected individuals were anemic and HGB had significantly reduced among infected individuals relative to uninfected (37), and another study showed that magnitude of anemia among *H. pylori* infected adults were 63.6% (38). In Butajira, Ethiopia a study showed that 30.9% of *H. pylori* infected individuals were anemic (39).

Anemia has serious implications for the health, as well as social and economic development (40). anemia reduces individuals wellbeing, cause fatigue, weakens, and impairs physical capacity and work performance (1). In addition it results in negative impacts in adults, increases susceptibility to different kinds of infections, increased hospitalization and mortality (19,41). Adult is one of the economic main powers for developments of country, however, anemia results in millions of adults to impaired health and quality of life, which lead communities and nations to impaired economic productivity and development (25,27,42).

A systematic analysis using national and sub national survey data estimated 32.9% worldwide burden of anemia in all ages combined, Sub-Saharan Africa has the highest burden of anemia (43). Burden of anemia became continued as a public health problem in Ethiopia (28). However, WHO targeted a 50% reduction of anemia burden especially in women of reproductive age until 2025 (44). In order to achieve WHO target it requires an integrated approach, so identifying possible contributing factors is important in order to reduce anemia burden. *H. pylori* infection is one of the possible contributing factors for burden of anemia and prevalent among adult (32,45).

Identifying possible contributing factors at different set ups is important in order to combat anemia burden and for proper management of anemic patients.

Many epidemiological study revealed high magnitude of *H. pylori* infection in developing countries including in Ethiopia (31,46). Previously one study conducted in Ethiopia showed high prevalence of anemia among *H. pylori* infected adults (39). However, this study had not determined type and severity of anemia among infected individuals and cannot control some of the potential confounding variables. Availability of updated information on magnitude of anemia and its association with *H. pylori* infection, and determining type and severity of anemia has a major role in the management and controlling of anemia, which is not well known in this study area. Therefore, this study was done to determine prevalence of anemia and its association with *H. pylori* infection among adult dyspeptic patients and also to determine type and severity of anemia among infected individuals.

1.3. Significance of the study

H. pylori infection is a worldwide problem and the overall prevalence is high in developing countries. It has been found more frequently in dyspeptic patients and result in anemia and micronutrients deficiency like ID and vitamin B12.

Strategies for prevention and control of anemia focused on its underlying causes and associated factors. So, disclosing the prevalence of anemia and its association with *H. pylori* infection among adult patients in the study area will help to develop evidence based decision and intervention strategies to improve the health status of the adults. So, intervention measures are planned and improved based on available information.

On top of this, knowing the burden of anemia and its association with *H. pylori* infection in adult population in Ethiopia can provide information for health policy makers as an input to design effective management and control of anemia and *H. pylori* infection. Also finding may serve as base line data for the future research on the area.

Chapter two: Literature review

Anemia is a common manifestation of various etiologies such as ID, vitamin B12 deficiency, folic acid deficiency, gastrointestinal bleeding, acute and chronic infections (2,26). Different study has been reported that *H. pylori* infection plays a role in magnitude of anemia.

A retrospective study conducted at Kutahya, Turkey in between 2011-2015 among 1408 dyspeptic patients showed that 53.2 % of the patients were anemic and 64.4% had *H. pylori* infection. Among infected individuals, 91.8% were anemic and Hgb was significantly reduced among individuals infected with *H. pylori* relative to uninfected patients (37).

Another retrospective study performed in between 2012-2016 in Beijing, China revealed that 5.3% had anemia and 43.9% had *H. pylori* infection. Among *H. pylori* infected 5.5% were anemic; of which 4.2% and 1.3% had mild and moderate to severe anemia respectively. *H. pylori* infection was significantly associated with anemia after adjusting for age, sex, underlying chronic diseases and body mass index (BMI). Significant differences in the rates of anemia were found in age, sex, underlying chronic diseases and BMI (47).

A cross-sectional study conducted in Central Plateau of Haiti on 132 adults in 2009 showed 63.6% overall prevalence of anemia and 50.8% had *H. pylori* infection. The prevalence of anemia was 55.2 % in *H. pylori* infected individuals. Among anemic adults 53.8%, 8.3%, 1.3% had mild, moderate and severe anemia, respectively. This study revealed that *H. pylori* infection was not associated with anemia (38).

However, another cross-sectional study conducted in General Hospital of Chinese PLA among 646 subjects in 2015 revealed that *H. pylori* infection was significantly associated with anemia. Compared to the *H. pylori* negative group, *H. pylori* positive group was 2.53 times more likely to be anemic and prevalence of anemia in the *H. pylori* positive group was 5.3% (35).

The overall prevalence of anemia was 25.2% in a cross-sectional study conducted in General Medicine ward Jinnah Postgraduate Medical Centre, Karachi in 2017 among 115 adult patients presenting with *H. pylori* gastritis (48).

Another cross-sectional study conducted in 2007 among 1,117 adults in 31 primary health care units in Pelotas, southern Brazil 20.6 % showed overall prevalence of anemia and *H. pylori* infection was 70.7%. The magnitude of anemia was 20.9 % in the individuals with *H. pylori* infection and this study revealed absence of association between *H. pylori* infection and anemia among adults attending primary health care units (49).

However, in Iraq a cross sectional study carried out on 115 patients attending primary health care canter showed that *H. pylori* infection was associated with higher prevalence of anemia and overall prevalence of anemia and *H. pylori* infection were 53.04%, 60%, respectively (50).

Another study conducted in United States among 7,462 participants in 2005 showed that *H. pylori* infection was associated with anemia, IDA and ID (34).

Mean value of HGB, HCT and RBCs count showed significant differences between cases and controls in a prospective case control study conducted in Khartoum, Sudan on 60 *H. pylori* infected and 60 *H. pylori* uninfected controls. This study showed association between *H. pylori* infection and HGB, HCT and RBC (51).

In Palestine, a case–control study performed in 2016 among 150 adult patients infected with *H. pylori* and 150 uninfected adults showed mean levels of HGB, RBC and HCT were significantly lower in cases compared to control. *H. pylori* positive individuals are at 4.25 times and 3.5 times higher risk of having low levels of serum vitamin B12 and iron, respectively compared to *H. pylori* negative individuals (33).

In 2014 retrospective study conducted in Turkey among 117 individuals, 69 study and 48 control groups showed that the mean levels of RBC, HGB, HCT and MCV in the study group were lower than those in the control group and there was no association between IDA and *H. pylori* infection (52).

However, study conducted in Baghdad, Iraq among 78 *H. pylori* infected patients and 22 uninfected subjects as control showed that 30.7% of the patients had ID and *H. pylori* infection was associated with ID and subsequently IDA (53).

A cross-sectional study performed in Cuba among 391 women in 2014 showed prevalence of anemia and *H. pylori* infection was 24.6% and 47.1%, respectively. Out of anemic study participants; 62.5% had mild and 37.5% had moderate anemia respectively. *H. pylori* infection was not associated with ID and anemia(54).

Recent systematic review and meta-analysis on the association between *H. pylori* infection and IDA reveled; as compared to uninfected individuals, *H.pylori*-infected individuals showed 1.15 times more likelihood of anemic, 1.72 more times likelihood of IDA, and 1.33 times more likelihood of ID. This study indicates increased likelihood of depleted iron stores in relation to *H. pylori* infection and also suggested that *H. pylori* eradication therapy, added to iron therapy might be beneficial in improvements of hematological parameter (55).

Another systematic review and meta-analysis performed in 2010 from 15 observational epidemiological studies on the association between *H. pylori* infection and IDA revealed *H.pylori*-infected individuals were 2.22 times more likely to be anemic compared to their non-infected counterparts(36).

The mean (SD) values of HGB, MCV, MCH, MCHC, HCT and RBC count was significantly different between *H. pylori* infected and uninfected patients in a cross-sectional study conducted in Butajira Hospital in 2013 among 401 dyspeptic patients and the overall prevalence of anemia and *H. pylori* infection was 26.9% and 52.4% , respectively, among infected patients prevalence of anemia were 30.9%. This study showed absence of association between anemia and *H. pylori* infection (39).

Anemia in adults related to different factors linked to socio-demographic factors (5). Increased age, sex, rural residence, illiteracy and low socio-economic classes were found to risk factors associated with anemia in different studies(6,56–58). The highest percentage of anemia was seen in the female (64.5%) and in a study participants within a age group of 25-34 years (76%) in a cross-sectional study conducted in Central Plateau (38). Whereas distribution of anemia was higher in the age group of 18 to 24 years (25%), female (21.5%), low socioeconomic class (20.7%) and study participants who attends primary educational level (22.2%) in a cross-

sectional study done in southern Brazil (49). Another cross-sectional study conducted among adults in Tamil Nadu, India revealed rural residence, increased age, meat consumption, tea (sugar) consumption were associated with anemia. The larger proportion of anemia were seen in illiterate (51%) and labourer as primary occupation (61.9%) (57).

Anemia has been associated with nutritional status, underlying chronic diseases, infectious diseases and heavy blood loss as a result of menstruation, or parasitic infections (7,47,58). Anemia was significantly associated with intestinal parasite infections and BMI in a cross-sectional study conducted in India (7) and Uganda (6). Proportion of anemia among study participants were 18.8% on who had history of bleeding (hemorrhoids) in a cross-sectional study done in southern Brazil (49). Low consumption of meat and vegetables showed associations with anemia and ID in a study done in Cuba (54). Another cross-sectional study carried out at Creek General Hospital, Karachi showed that infectious diseases, nutritional deficiency and gastrointestinal blood loss were the most common identified causes of anemia (59), and also anemia was associated with advanced age and presence of chronic diseases in a study conducted in Portugal (58) and Sao Paulo (60). Anemia was linked to some behavioral characteristics like smoking and alcohol consumption, 18.3% and 20.2% of study participants were anemic who had habit of smoking and alcohol consumption, respectively (49).

Many epidemiological and clinical studies were conducted to determine association between anemia and *H. pylori* infection; however, results from different areas and countries are not consistent and majority of them not identified type and severity of anemia among *H. pylori* infected patients. Most of them preformed with relatively small sample sizes and cannot consider strength and direction of the associations. Thus no studies conducted to investigate prevalence of anemia and its association with *H. pylori* infection and also determined type and severity of anemia in infected individuals in this study area.

Chapter three: Objectives

3.1. General objective

To determine prevalence of anemia and its association with *H. pylori* infection among adult dyspeptic patients attending Wachemo University Nigist Elein Mohammad Memorial Referral Hospital from January 8,2019-April 1, 2019.

3.2. Specific objectives

- To determine prevalence, severity and types of anemia among adult dyspeptic patients
- To identify associated risk factors with anemia among adult dyspeptic patients

Chapter four: Methods and materials

4.1. Study area

The study was conducted at Wachemo University Nigist Elein Mohammed Memorial Referral Hospital (WUNEMMRH) which is located in Hosanna town, Haddiya Zone, Southern Nations Nationalities, and People's Region (SNNPR). The town is 232 km far from the capital-city Addis Ababa, to southwest and 157km from regional city Hawassa. The town lies on the average at 2,177 m above the sea level.

This hospital was established in 1976 EC and its catchment area population is estimated around 3,200,000. It contains 201 beds giving service in four major departments. In addition to other services, the hospital has surgical, Gyne& obstetric, internal medicine and pediatric departments. Each department has their own inpatient, outpatient and referral clinics. The study was conducted in central laboratory which provides parasitological, hematologic, microbiological, clinical chemistry and serological service.

4.2. Study design and period

Facility based cross sectional study design was employed from January 8, 2019-April 1, 2019.

4.3. Population

4.3.1 Source population

All adult patients attending WUNEMMRH

4.3.2 Study population

All identified adult dyspeptic patients attending WUNEMMRH during study periods.

4.4. Sample size determination and sampling technique

The sample size was calculated by using the single population proportion formula taking 30.9 % prevalence of anemia (39) and by considering 95% confidence interval (CI) and 5% margin of error.

$$n = \frac{(Z)^2 * P(q)}{d^2}$$

$$n = (1.96)^2 * 0.309(0.691) / (0.05)^2$$

Where n=Sample size

Z= Confidence level 95%

P= prevalence of anemia 30.9 %

q= 1-p. 1-0.309 =0.691

d= Margin of error 5%

Final sample size after adding (10%) non response rate was =362.

Sampling Technique

All consecutively identified adults who fulfilled the inclusion criteria were enrolled in the study.

4.5. Variables

4.5.1. Dependent variables

- Anemia

4.5.2. Independent variable

- Socio-demographic characteristic (age, sex, residence, marital status ,educational status, monthly income and occupational status)
- *H. pylori* infection
- Intestinal parasitic infection
- Chronic disease
- History of malaria infection
- History of blood loss
- BMI
- Dietary habit
- Behavioural characteristic (cigarette smoking and alcohol consumption)

4.6. Eligibility criteria

4.6.1 Inclusion criteria

All adult patients (≥18year) who have dyspeptic complaints were included in the study.

4.6.2 Exclusion criteria

Patients who took treatment for *H. pylori* infection within the last three month (61), who had previous stomach or small bowel surgery, donate blood within last three month and on a treatment for anemia prior to data collection, pregnant women and severally ill patients were excluded.

4.7. Data Collection

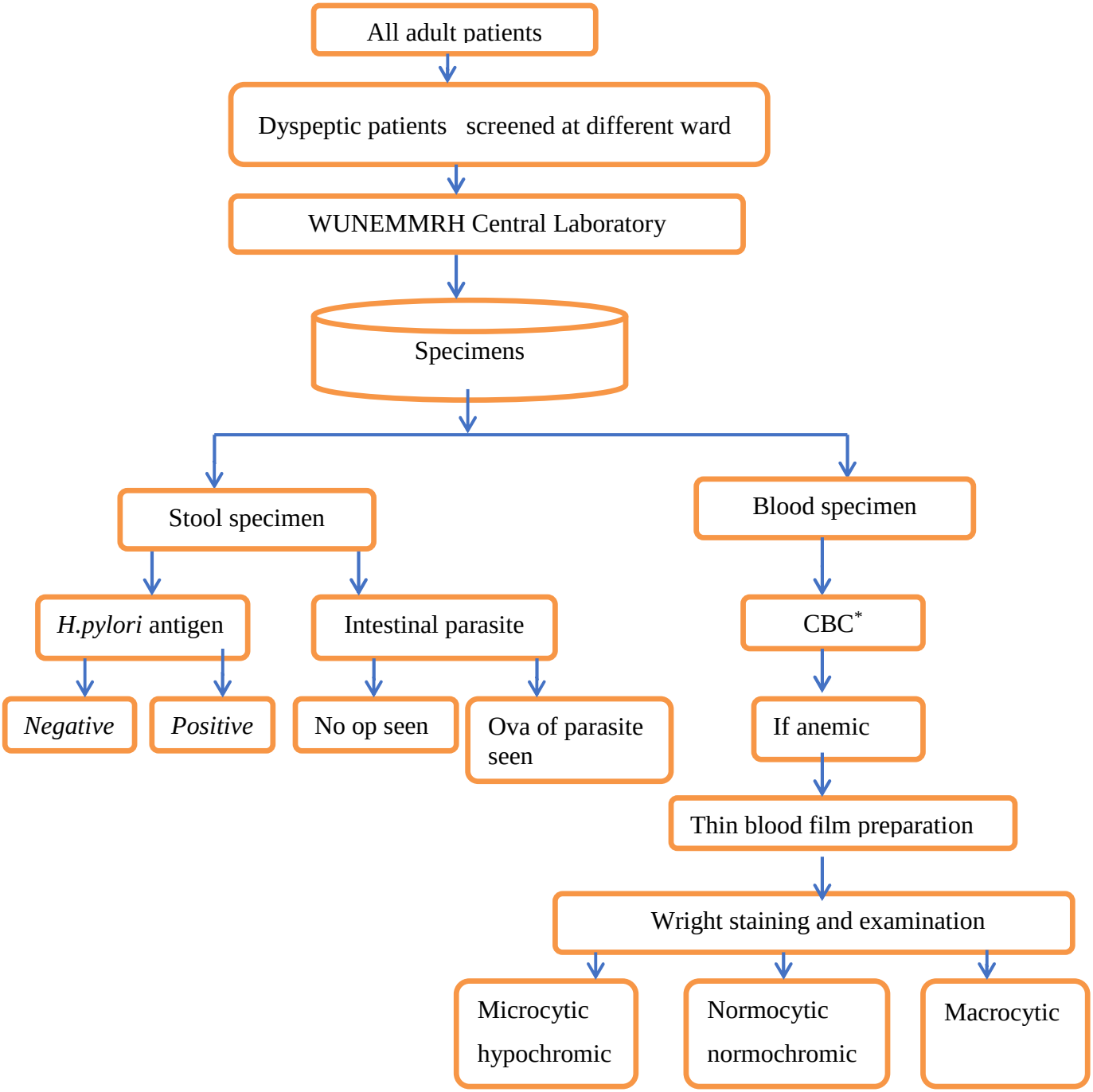
4.7.1. Socio-demographic and related clinical data

Data on socio-demographic characteristics, clinical and other related factors were collected using a structured questionnaire (**Annex III**). The questionnaire was adapted from other similar studies and prepared in English. It was translated into Amharic and Haddiyissa. Finally it was translated back into English to check for consistency.

4.7.2 Study participant recruitment and Laboratory procedures

Study participants who have dyspeptic complaints were screened in outpatients, inpatients and emergency ward and sent to central laboratory with request form. Eligible participants were requested for their consent after explaining the purpose, benefit and risk of the study by data collector (**Annex I**). Consented participants were interviewed using structured questionnaires about socio-demographic, clinical and other related factors. For laboratory data, 4ml of venous blood sample were collected in ethylenediaminetetraacetic acid (EDTA) tubes by laboratory technologist from each study participants for hematological parameter analysis and blood film preparation. All sample received in the laboratory were checked for quality, and labeled by code numbers. Hematological parameters; HGB, RBC, HCT, MCV, MCH, MCHC and RDW were determined using automated blood analyzer MINDRAY BC-3000 PLUS (*Shenzhen mindray Bio-Medical Electronics Co.,Ltd, China).* Thin blood films were prepared, air-dried, labeled and then stained by wrights stain to evaluate RBC morphology of anemic study participants (**Annex IV)**.

In addition, after explaining how to collect representative stool specimen clean cupped plastic container was given to the participants. Approximately five gram of stool specimen was collected from each study participant and checked for the presence of *H. pylori* antigen by wondfo one step *H. pylori* feces test *(Guangzhou Wondfo Biotech Co.,Ltd, China)* and intestinal parasites were detected from remaining sample by wet mount techniques (**Annex IV)**. Anthropometric measurements (height and weight) were measured from all study participants and BMI was computed as weight in kilogram divided by the square of height in meter and categorized in a four groups; BMI<18.5 kg/m^2 as underweight, BMI = 18.5–24.9 kg/m^2 as normal weight, BMI = 25-29.9 kg/m^2 as overweight, and BMI ≥ 30 kg/m^2 as obese (62).



CBC*, complete blood count

Figure 1 Sampling and laboratory work flow

4.8. Quality assurance

To ensure the quality of data different quality control activities were involved, including giving training to data collectors prior to data collection, standardization of procedures, checking reagents and test kits for their expiry date before any analysis started and completeness of each questionnaire was checked regularly during data collection.

All laboratory tests were done by following the standard operation procedures (SOPs) and manufacturer instructions (**Annex IV**). For automated hematology analyzer, backgrounds check initial, repeated analysis of randomly selected specimens to see reproducibility, randomly selected specimens (high, normal, and low) were checked by other similar hematology analyzer and as a part of laboratory protocol Hospital laboratory evaluates instrument performance by commercial available quality control material.

Wright stain was filtered every day by using filter paper and also stored at locked cabinets away from moisture and sunlight. The quality of *H. pylori* kits were checked while every new batch was opened by using known positive and negative samples and checked as recommended by manufacturer's instruction. The data collections, application of standard procedure, accuracy of test results were supervised by principal investigator strictly.

4.9. Operational definition

Dyspeptic Patients: those patients who have suggestive sign and symptom for *H. pylori* infection.

Anemia: defined according to WHO cutoff value as a HGB concentration of less than 12 g/dL in women and less than 13 g/dL in men, mild anemia: HGB concentration lies between 11-12.9 g/dL in men and 11-11.9 g/dL in women, moderate anemia: HGB concentration lies between 8-10.9 g/dL in men and women, severe anemia: HGB concentration less than 8 g/dL in men and women (4).

Normocytic normochromic anemia: MCV value lies between 80 fL and 100 fL and MCHC value lies between 32 g/dL and 36 g/dL.

Microcytic hypochromic anemia: MCV value less than 80 fL and MCHC value less than 32 g/dL.

Macrocytic anemia: MCV value greater than 100 fL.

Heavy menstruation: history of prolonged menstruating for longer than 7 days.

Accidents: car, motor and other occasion which result in blood loss.

Immediately after meal: a study participant who took tea or coffee 30 minute after meal.

Alcohol abuse: is consumption of >40ml alcohol per day.

4.10. Data processing and analysis

After checking the data for completeness, missing values, and coding of the questionnaires, the data were entered into Epi-Data version 3.1(Epi-Data, Odense, Denmark), exported to Statistical Package for Social Science (SPSS) version 21(SPSS, Chicago, USA) statistical software for analysis. Frequency table, graph and descriptive summaries were used to describe the study variables. Association between anemia and *H. pylori* infection was assessed by logistic regression.

Logistic regression was used to determine the effect of independent variables on the prevalence of anemia. Bivarate analysis was performed for each independent variable to select variables candidate's for multivariate analysis. Variables in bivarate analysis with P value <0.25 were taken as candidates for multivariate analysis. Multiple logistic regression analysis was used to control the effect of confounding variables and to identify associated risk factors for prevalence of anemia. A 95% CI was used and in all case P-value <0.05 was considered as statistically significant.

The difference in the mean (SD) values of RBC parameters between *H. pylori* positive and negative individuals were explored using independent sample T-test. Data were tested for the normality of its distribution by Kolmogorov–Smirnov test. Age, monthly income and food consumption day peer week was categorized based on previous study (39,49). HGB concentration was adjusted for altitudes and smoking as per recommended by WHO standard guide line (4).

4.11. Ethical clearance

Ethical clearance was obtained from the Institutional review Board of Health Sciences faculty, Institute of Health, Jimma University. Letter of cooperation was written to WUNEMMRH. Written letter of permission was obtained from WUNEMMRH. Written informed consent was obtained from each study participants after explaining purpose and procedures of study before enrolling in the study (**Annex I)** and those willing to participate were included. The entire study groups were informed that, their response will be kept confidential. To ensure confidentiality of data, study participants were identified using codes and unauthorized persons not able to access the collected data. In addition, the clinical specimens collected during the study periods were used for the stated objectives only. The study participant results were reported to the physician for proper management.

4.12. Dissemination and utilization of results

The findings will be presented to School of Medical Laboratory Science Faculty of Health Science Institute of Health, Jimma University as a partial fulfillment of MSc. thesis. It will also be disseminated through publication on peer reviewed international journals and presented for WUNEMMRH. The copy of the result will be submitted to Faculty of Health Science.

Chapter five: Results

5.1. Socio-demographic characteristics of study participants

A total of 362 adult dyspeptic patients; 58 %(n=210) females and 42 %(n=152) males were included in the study. The mean age of the study participants were 31.1 with a standard deviation of ±7.5 years. About 57.7% (n=209) of the study participants were rural area residents. Majority 60.2 %(n= 218) of participants were married (Table1).

Table 1 Socio-demographic characteristics of adult dyspeptic patients attending WUNEMMH; Hosanna; Southwest Ethiopia, January 8– April 1, 2019

Variable	Categories	Frequency (%)
Age in year	18-24	85(23.5)
	25-29	91(25.1)
	30-34	59(16.3)
	35-39	56(15.5)
	40-44	54(14.9)
	45-49	17(4.7)
Gender	Female	210(58)
	Male	152(42)
Residence	Urban	153(42.3)
	Rural	209(57.7)
Marital status	Single	136(37.6)
	Married	218(60.2)
	Divorced	4(1.1)
	Widowed	4(1.1)
Educational level	Illiterate	99(27.3)
	Primary	50(13.8)
	Secondary	115(31.8)
	Higher	98(27.1)

Monthly income in ETB*	<776	69(19.1)
	776-1552	97(26.8)
	>1552	196(54.1)
	Farmer	94(26)
Occupational status	Daily laborer	55(15.2)
	Employee	80 (22.1)
	Students	69(19.1)
	Merchants	57(15.7)
	self employee	7(1.9)

ETB* *Ethiopian birr*, Employee+, *governmental employee*

5.2. Clinical and other related characteristics of study participants

H. pylori infection and intestinal parasites were detected in 49.2% (n= 178) and 16.3% (n= 59) of the study participants, respectively. Among the study participants, 2.5% (n= 9) had known chronic disease (diabetes mellitus) and 3% (n= 11) had history of bleedings and reasons for bleedings were accidents in a 45.4 %(n= 5), hemorrhoids in a 27.3 % (n=3) and heavy menstruation in a 27.3 %(n= 3) of participants. As to the BMI level, 83.7% (n= 303) of participants had normal weight. Majority 70.4 %(n= 157) participants had habit of eating fruit and vegetable 1-3 day per week. During time of the data collection, 34% (n=123) of participants had habit of consumption of red meat and 50.3 %(n=182) had habit of drinking coffee or tea immediately after meal (Table2).

Table 2 Clinical and other related characteristics of adult dyspeptic patients attending WUNEMMH; Hosanna; Southwest Ethiopia, January 8– April 1, 2019

Variables	Categories	Frequency (%)
***H. pylori* antigen test**	Negative	184(50.8)
	Positive	178(49.2)
Intestinal parasite	No	303(83.7)
	Yes	59(16.3)
	A. lumbericoides	17(28.8)
	G. lambilia	23(39)
	E. histolytica	15(25.4)
	H. worms	4(6.8)
Having known chronic disease	No	353(97.5)
	Yes	9(2.5)
History of malaria infection	No	357(98.6)
	Yes	5(1.4)
History of bleedings	No	351(97)
	Yes	11(3)
Reason for bleeding	Accidents	5(45.4)
	Hemorrhoids	3(27.3)
	Heavy menstruation	3(27.3)
BMI in kg/m^2	Underweight	11(3)
	Normal weight	303(83.7)
	Overweight	48(13.3)
Habit of consumption of fruit & vegetable	No	139(38.4)
	Yes	223(61.6)
Consumption of fruit &vegetable per week	1-3	157(70.4)
	≥4	66(29.6)
Habit of consumption of red meat	No	239(66)
	Yes	123(34)

Consumption of red meat per week	1-3	123(100)
drinking coffee or tea immediately after meal	No	180(49.7)
	Yes	182(50.3)
Cigarette smoking	No	354(95.3)
	Yes	17(4.7)
Number of cigarette per day	<10	15(88.2)
	10-20	2(11.8)
Alcohol consumption	No	344(95)
	Yes	18(5)
Volume of alcohols consumed per day	300ml	3(16.7)
	500ml	8(44.4)
	>500ml	7(38.9)

5.3. Prevalence, severity and types of anemia among study participants

The mean (SD) HGB concentration of the study participants were 13.5(1.93) g/dL and 14.1(1.55) g/dL in females and males respectively.

The overall prevalence of anemia was 24.3% (n=88) with 95%CI (19.9, 28.7). Of which 71.6 %(n=63) had mild and 28.4% (n=25) had moderate anemia and there was no severe anemia identified and also 70.5% (n=62) had normocytic normochromic anemia, 27.3% (n=24) had microcytic hypochromic anemia and 2.3% (n=2) had macrocytic types of anemia respectively.

The prevalence of anemia among *H. pylori* infected participant was 29.2 %(n=52), of which 69.2% (n=36) had mild anemia and 30.8 % (n=16) had moderate anemia, respectively. Among *H. pylori* infected study participants 63.5% (n=33) had normocytic normochromic anemia, 34.6% (n=18) had microcytic hypochromic anemia and 1.9 %(n= 1) had macrocytic anemia, respectively.

Among the study participant proportion of anemia was 25.9% (n= 22) with in age group of 18-24 years, 29.7% (n=62) in rural area residents, 26.1% (57) in a married and 31.3% (n=31) in illiterate. Distribution of anemia was 35.6% (n=21) among study participant who had intestinal parasitic infection, 44.4 % (n=4) who had chronic diseases, 45.5% (n=5) who had history of bleeding and 45.5% (n=5) who had underweight, respectively (Table3).

5.4. Factors associated with anemia

In bivarate analysis, rural residence (COR (95% CI) = 2.06 (1.23, 3.45), illiteracy (COR (95% CI) = 1.89 (0.98, 3.65) and primary educational level (COR (95% CI) = 1.78 (0.81, 3.9) were identified candidate variables to be tested for association with anemia in multivariate analysis

In addition, *H. pylori* infection (COR (95% CI) = 1.69 (1.04, 2.76), intestinal parasitic infection (COR (95% CI) = 1.94(1.07, 3.54), chronic disease (COR (95% CI) = 2.56 (0.67, 9.7), history of bleedings (COR (95% CI) = 2.69(0.8,9.04) and underweight (COR (95% CI) = 3.16(0.8,12.5) were identified candidate variables to be tested for association with anemia in multivariate analysis (Table 3).

Table 3 Bivarate analysis of the association between anemia and socio-demographic, clinical and other related factors among adult dyspeptic patients attending WUNEMMH; Hosanna; Southwest Ethiopia, January 8 – April 1, 2019

Variables	Categories	Anemia		COR(95%CI)	p-value
		No (%)	Yes (%)		
	18-24	63(74.1)	22(25.9)	1.13(0.33,3.84)	0.83
	25-29	68(74.7)	23(25.3)	1.09(0.32,3.71)	0.879
Age in year	30-34	44(74.6)	15(25.4)	1.10(0.31,3.92)	0.874
	35-39	42(75)	14(25)	1.08(0.30,3.87)	0.90
	40-44	44(81.5)	10(18.5)	0.73(0.19,2.74)	0.65
	45-49	13(76.5)	4(23.5)	1.00	
Gender	Female	155(73.8)	55(26.2)	0.78(0.47,1.28)	0.32
	Male	119(78.3)	33(21.7)	1.00	
Residence	Urban	127(83)	26(17)	1.00	
	Rural	147(70.3)	62(29.7)	2.06(1.23,3.45)	0.006*
Educational level	Illiterate	68(68.7)	31(31.3)	1.89(0.98,3.65)	0.056*
	Primary	35(70)	15(30)	1.78(0.81,3.9)	0.149*
	Secondary	92(80)	23(20)	1.03(0.52,2.04)	0.91
	Higher	79(80.6)	19(19.4)	1.00	
Monthly income in	<776	52(75.4)	17(24.6)	1.03(0.54,1.96)	0.913
ETB	776-1552	73(75.3)	24(24.7)	1.04(0.59,1.83)	0.88
	>1552	149(76)	47(24)	1.00	
***H. pylori* antigen test**	Negative	148(80.4)	36(19.6)	1.00	
	Positive	126(70.8)	52(29.2)	1.69(1.04,2.76)	0.033*
Intestinal parasite	No	236(77.9)	67(22.1)	1.00	
	Yes	38(64.4)	21(35.6)	1.94(1.07,3.54)	0.029*
Having known chronic disease	No	269(76.2)	84(23.8)	1.00	
	Yes	5(55.6)	4(44.4)	2.56(0.67,9.7)	0.16*
History of bleedings	No	268(76.4)	83(23.6)	1.00	
	Yes	6(54.5)	5(45.5)	2.69(0.8,9.04)	0.109*
	Underweight	6(54.5)	5(45.5)	3.16(0.8,12.5)	0.1*
BMI in kg/m^2	Normal	230(75.9)	73(24.1)	1.2(0.57,2.54)	0.62
	Overweight	38(79.2)	10(20.8)	1.00	

Consumption of fruit & vegetable	No	101(72.7)	38(27.3)	1.3(0.79,2.1)	0.29
	Yes	173(77.6)	50(22.4)	1.00	
Consumption of red meat	No	178(74.5)	61(25.5)	1.2(0.72,2.04)	0.45
	Yes	96(78)	27(22)	1.00	
Drinking coffee or tea immediately after meal	No	136(75.6)	44(24.4)	1.00	
	Yes	138(75.8)	44(24.2)	0.98(0.61,1.59)	0.95
Cigarette smoking	No	262(75.9)	83(24.1)	1.00	
	Yes	12(70.6)	5(29.4)	1.3(0.45,3.8)	0.6
Alcohol consumption	No	262(76.2)	82(23.8)	1.00	
	Yes	12(66.7)	6(33.3)	1.59(0.58,4.39)	0.36

*1.00 = Referent category, *candidate variables for multivariate analysis p-value <0.25*

5.5. Multivariate logistic regression analysis of anemia predictors

Candidate variables for multivariate logistic regression were selected by considering p<0.25 from bivarate model. Accordingly, rural residence, *H. pylori* infection and intestinal parasitic infection were significantly associated factors with anemia (Table 4).

Table 4 Factors associated with anemia among adult dyspeptic patients attending WUNEMMH; Hosanna; Southwest Ethiopia, January 8 – April 1, 2019

Variables	Categories	Anemia		COR(95%CI)	AOR(95%CI)	p-value
		No (%)	Yes (%)			
Residence	Urban	127(83)	26(17)	1.00	1.00	
	Rural	147(70.3)	62(29.7)	2.06(1.23,3.45)	1.9(1.1,3.3)	0.02**
Educational level	Illiterate	68(68.7)	31(31.3)	1.89(0.98,3.65)	1.66(0.82,3.3)	0.15
	Primary	35(70)	15(30)	1.78(0.81,3.9)	1.54(0.67,3.5)	0.3
	Secondary	92(80)	23(20)	1.03(0.52,2.04)	1.12(0.55,2.27)	0.74
	Higher	79(80.6)	19(19.4)	1.00	1.00	
***H. pylori* antigen test**	Negative	148(80.4)	36(19.6)	1.00	1.00	
	Positive	126(70.8)	52(29.2)	1.69(1.04,2.76)	1.77(1.05,2.98)	0.03**
Intestinal parasite	No	236(77.9)	67(22.1)	1.00	1.00	
	Yes	38(64.4)	21(35.6)	1.94(1.07,3.54)	2.14(1.14,4.03)	0.018**
BMI in kg/m^2	Underweight	6(54.5)	5(45.5)	3.16(0.8,12.5)	2.88(0.68,12.1)	0.15
	Normal	230(75.9)	73(24.1)	1.2(0.57,2.54)	1.29(0.59,2.8)	0.5
	Overweight	38(79.2)	10(20.8)	1.00	1.00	
Known chronic disease	No	269(76.2)	84(23.8)	1.00	1.00	
	Yes	5(55.6)	4(44.4)	2.56(0.67,9.7)	1.87(0.46,7.59)	0.37
History of bleedings	No	268(76.4)	83(23.6)	1.00	1.00	
	Yes	6(54.5)	5(45.5)	2.69(0.8,9.04)	3(0.84,10.76)	0.09

*1.00 = Referent category, **statistically associated p-value <0.05*

5.6. Association between RBC parameter with *H. pylori* infection

The mean (SD) of the parameters related to red blood cell were also compared between *H. pylori* positive and *H. pylori* negative study participants. Accordingly statistically significant differences were observed in mean value of HGB, RBC, HCT, MCV, MCH, MCHC and RDW, respectively (Table 5).

Table 5 Association between RBC parameter with *H. pylori* infection among adult dyspeptic patients attending WUNEMMH; Hosanna; Southwest Ethiopia, January 8– April 1, 2019

RBC Parameter	***H. pylori* negative mean (SD)**	***H. pylori* positive mean (SD)**	**p-value**
HGB g/dL	14.2(1.88)	13.3(1.6)	<0.001
RBCx10^6/μL	4.79(0.6)	4.47(0.39)	<0.001
HCT (%)	45.6(5.3)	40.3(4.1)	<0.001
MCV(fL)	90.4(4.9)	88.8(5.4)	0.003
MCH(pg)	28.9(2.2)	28.2(2.1)	0.001
MCHC g/dL	32.3(1.45)	31.9(1.3)	0.004
RDW (%)	13.9(1.12)	14.2(1.47)	0.01

P-value, *independent sample T-test*

Chapter six: Discussion

Anemia is continued to be a public health concern worldwide with major consequences for human health as well as social and economic development (27). Anemia in adults is associated with negative impacts, including impairs the work productivity, increases susceptibility to different kinds of infections, increased hospitalization and mortality (19). *H. pylori* infection is one of the possible contributing factors for burden of anemia and prevalent among adult (32,45).

The current study attempted to asses anemia prevalence and its association with *H. pylori* infection among adult dyspeptic patients. The overall prevalence of anemia among adult dyspeptic patients was 24.3%. According to WHO classification of the public health importance of anemia (4), anemia prevalence in this study indicated a moderate public health problem.

The overall prevalence of anemia obtained in this study was consistent with study done in Butajira (26.9%) (39), Cuba (24.6%) (54), Karachi (25.2%) (48) and Southern Brazil 20.6% (49). However, the findings of this study was lower than reports from Kutahya, Turkey (53.2%) (37) and Central Plateau (63.6%) (38). The lower prevalence of anemia in the current study might be due to differences in cutoff values of HGB to define anemia and difference in sample size. For example, in Kutahya, Turkey cutoff values of HGB to define anemia for male was less than 14 g/dL which is different as compared to our study (less than 13g/dL for male).

The current study finding revealed higher prevalence of anemia compared to studies conducted in China (5.3%) (47). This might be due to methodological variation and presence of intestinal parasitic infection in our study (16.3%). For instance a study conducted in China was retrospective and exclude study participants without results of either *H. pylori* infection status or hematological parameter.

The prevalence of anemia among *H. pylori* infected participants obtained in this study was 29.2 %, which was consistent with the study finding in Butajira 30.9 % (39) while higher magnitudes of anemia among *H. pylori* infected were reported from Kutahya, Turkey (91.8%) (37) and Central Plateau (55.2%) (38). This might be due to difference in *H. pylori* diagnostic method.

According to WHO classification for degree of anemia based on HGB concentration (4), in this study mild anemia was common (69.2%) followed by moderate anemia (30.8%) among *H. pylori* infected study participant. Similar findings were reported in studies done in China (47) and Cuba (54).

Considering the morphological classification of anemia, normocytic-normochromic anemia (63.5%) was the predominant types of anemia among *H. pylori* infected study participants followed by microcytic hypochromic anemia (34.6%) in this study. This might be due to the reason that blood loss secondary to chronic erosive gastritis, decreased iron absorption secondary to chronic gastritis and hypochlorhydra, and also rises in hepcidin level after *H. pylori* infection which might contribute in anemia (13,16).

H. pylori infection is a worldwide public health problem, globally 48.6% of adults were suffered from this infection (8). Moreover, it has been associated with different hematological manifestations (33,53). The association between anemia and *H. pylori* infection has been explored by number of previous studies (37–39,47,49,54).

The current study revealed that there is statistically significant association between anemia and *H. pylori* infection. *H. pylori*-infected individuals were 1.77 times more likely to be anemic compared to their non-infected counterparts. This findings were in agreement with previous studies conducted in China (35,47) and USA (34).

Recent systematic review and meta-analysis showed association between *H. pylori* infection and anemia with pooled OR (95% CI) = 1.15(1.00–1.32), with IDA pooled OR (95% CI) = 1.72 (1.23–2.42) and ID with pooled OR (95% CI) =1.33(1.15–1.54) (55). In addition, different meta-analyses had reported similar results(36,45,63).

The possible mechanism that might explain the association between anemia and *H. pylori* infection may include; consumption of iron by the organism itself (64), gastrointestinal blood loss due to *H. pylori*-induced gastrointestinal lesions (16), and gastritis increased levels of neutrophil-derived lactoferrin, and since *H. pylori* has a lactoferrin-binding protein receptor, the infection may result in increased iron losses related to bacterial turnover. Since these bacteria

have a high turnover rate, a large amount of iron may be lost in stools in the form of dead bacteria (13,23). In addition, *H. pylori* chronic gastritis can change the physiology of the stomach, inducing reductions in gastric acid secretion, while acidic intragastric PH was essential for the absorption of dietary iron ; thereby inhibiting dietary iron absorption (12).

In the current study mean level of HGB, RBC, HCT, MCV, MCH, MCHC and RDW was showed statistically significant difference between *H. pylori* positive and *H. pylori* negative study participants. Similar observation was reported from Butajira (39), Turkey (52) and Sudan (51). Change in hematological parameters might be due to *H. pylori* infection. different studies also reported that eradication of *H. pylori* infection shows improvements in hematological parameters (36,63).

Gastrointestinal (GI) blood loss is one of the most important underlying cause of anemia in adults (59,65). In this study anemia was significantly associated with intestinal parasitic infection. Study participants who had intestinal parasitic infection were 2.14 times more likely to be anemic compared to their non-infected counterparts. Similar observation was reported from Uganda (6) and India (7). This might be due to gastrointestinal blood loss which may contribute in anemia. The worm in the intestine may cause intestinal necrosis and blood loss as a result of the attachment to the intestinal mucosa and chronic infections may lead to iron deficiency and anemia resulting from the excessive loss of iron (59,66).

Socio-demographic factors can play role in determining anemia (5). In current study anemia was significantly associated with rural residence. This finding is in agreements with study done in India (57). This might be likely related to lack of information about adequate nutrition, inaccessibility of health care centers and way of life. Thus, they lack information on factors causing anemia and possible strategies to prevent the risk factors of anemia. For example in current study (26%) was farmers as primary occupation which may predispose them for infection with soil-transmitted helminthes and also (27%) study participants were illiterate.

Limitation of the study

- The cross sectional nature of the study design prohibited to establish causal links between anemia and factors which are significantly associated with anemia.
- Micronutrient (serum iron, foliate and vitamin-B12) levels were not assessed.
- Intestinal parasites were not detected by concentration techniques
- As anemia is multifactorial, some of the variables cannot be addressed due to constraints of time and budget which may hinder the results.

Chapter seven: Conclusion and recommendation

7.1. Conclusion

The prevalence of anemia in this study indicated that it is a moderate public health problem. Higher prevalence of anemia was observed in study participants having intestinal parasitic infection; *H. pylori* infection and reside in rural area. Rural residence, *H. pylori* and intestinal parasitic infection were significantly associated with anemia.

7.2. Recommendations

This study demonstrates anemia prevalence and its association with *H. pylori* infection among adult dyspeptic patients. It is evident that anemia in adults results in reduce individuals wellbeing, cause fatigue and lethargy, and impair physical capacity and work performance. Based on the finding of this study the following points are recommended; developing intervention based strategies on identified factors mainly on; prevention and control of *H. pylori* and intestinal parasitic infection, routine screening and treatments of *H. pylori* infection, routine screening and deworming of intestinal parasitic infection and performing large longitudinal community based studies.

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Annexes

Annex I: Information Sheet

English Version

Title of the project: Prevalence of anemia and its association with *H. pylori* infection among adult dyspeptic patients attending Wachemo University Nigist Elein Mohammad Memorial Referral Hospital, Hosanna, south west Ethiopia.

Name of Principal Investigator: Kassahun Haile

Organization: Jimma University (School of Medical Laboratory Science)

Name of sponsor: Jimma University

This information sheet was prepared for adult dyspeptic patients attending Wachemo University Nigist Elein Mohammad Memorial Referral Hospital who were involved in project entitled above. We told them about the whole processes that have been undertaken in the study and requesting them to participate voluntarily.

Description and Purpose of the study

Anemia is a global public health problem affecting both developed and developing countries with major consequences for human health as well as social and economic development. Multiple factors are responsible for the occurrence of anemia. The magnitude of anemia and its association with *H. pylori* infection is not well known in this study area. Therefore, this study was designed to determine prevalence of anemia and its association with *H. pylori* infection among adult dyspeptic patients attending Wachemo University Nigist Elein Mohammad Memorial Referral Hospital from January 8, 2019-April 1, 2019.

Procedures

In order to undertake the above-mentioned study, some questions related with the topic; socio-demographic, clinical and other related characteristics and specimens were taken from each study participants for laboratory investigation. Informed consent was obtained from each study participants and they were kindly asked to give required samples and information related with the study. The collected sample was processed in Wachemo University Nigist Elein Mohammad Memorial Referral Hospital Central Laboratory.

Risks and discomforts

There was no any possible risk but there is little pain and discomfort during venous blood sample collection. All samples were collected following standard operational procedures (SOPS).

Benefits and Compensation

They were informed that no direct financial benefit for participating in this study. Based on the laboratory result they were referred to the physician for further care and treatment.

Confidentiality

The information obtained during this study was remained confidential. Records were remained confidential. To maintain confidentiality, records were kept in locked cabinets and the results of the tests were coded to prevent identification of the volunteers. Collected sample was not used for other research purposes and safely disposed of after the completion of the study.

Right to refuse or withdraw: they were informed that free to withdraw from the study at any time and not discriminated in any form of health services due to refusal

Whom to Contact: the following contact addresses were given to contact investigators at any time

Mr Kassahun Haile, Phone No +251-926-074374, Email: kassahaile07@gmail.com

Dr TilahunYemane Phone No +251-917-804067, Email: tilaunye@gmail.com

Mr GirumTesfaye, Phone No +251-920-274035, Email: girumtesfaye12@gmail.com

Hadiyissa version

Sorophii qoxoi woshi; Wachaami Yunvursitena Nigiste Ellinii Mohammad Matasabeei Hospitalena qorri mannanene yo'oi xiggi hofechi qaxomaa *H. pylori* jabbine yokii mateyoma laimma.

Wonii sarayanchi: Kassahun Hayle

Haramuki kitaphii mini: Jimmi Yunvursitee/Labratoorii Losanni Mine

Kuhi woshii kitabi gudukoki hanani kuramuki sorrobane bikkokik manina ihukuyyaa lulomanemii kahi sorrobimane hasisokii luwuwwaa kuriminate . kaahi sorobane bikokii mani hundemi ixxi hasenina ihoisa chakinsammo.

Sorophii woni woshii ; xiqqi hofechi qaxomaa *H. pylori* jabine yoii mateyommma qorri manene wachaami unvursitena nigiste ellinii mohammad matasabeei hospitalena laimaa.

Sorophii fintouwaa

Sorrobane bikokimina itamuki manisi woroni yokii woshuwaa xigga shumaa masinomisa kullamo.

- Fayyommi mazigabaa monomissa
- Oddimi 10 dakikina xamicha xaminomisa
- xigga oddimii shumaa masina monomisa.

Kuhi wixuki xiggi oddimii shummii wachaami yunvursitena nigisti ellinii mohammad matasabeei hospitalena lamebanchi labratorena baxamo.

Sorophii dangoii daffii

Xigga massakamare hofi qaxii xissi machesamoisa kullamo.xigga oddimii shumma xoxolakosine masinamo.

Sidesena xanokii luwuwwa

Kahi sorobane bikamichine sidamoki luwuwuwi birochomi ihuki muli luwi beisa xabii kullamo. Kahi sorobane bikokii manene xiqqi hofechi oddimi *H. pylori* jabbi sidamulas errii fayaomina hakimichii beyo maramoisa isinomo.

Sorophii maxamii woshuwaa

Ayyi kaa sorobane sidamukii woshuwii daphakosine disinomo.Ayyi kaa sorobane sidamukii woshuwii ka sorrobina yakaa uwamukii anan xiggina afurohani ihukuyaa kaha soroba baxokii maninse mulli kenii la'ena xanoyoo. kaa sorophi bikaneka sidamukii woshaa bikkanchi furrmane'e itamukissa kurubellasesnse chakinsomoyo. kuhi sorobi sansawe'e woshii ihukisam ku kitabii erri higgi chakishanehe bikaneka manomato kuroni firoissa kuramakoo.

Sabimmik oddimi ae'imi urmii xanomisa kurimaa

kaa sorobane bikoki manii hundimi hundomissinemi ixii amanene hassa ittaha bikoissa kullamo. sorobanse hasukii amanene itukii belasii firim xanamoisa kullamo. kaa sorobane bikimaa sabimine illagene mahi luwamii hogobeisa fayomii quxone mahi dangoimi affoo beisaa kuramakoo

xammichi yollassi

ka sorobane ayyi xamichi yolassi kanii woronii yooki silki quxurene teimi emailena xamimaa xanamoisa chakinisamoo

Kasahun Hayle:quxuri +251-926-074374, imalii :kassahaile07@gmail.com

Dr. Tilaahun Yamana:quxuri +251-917-804067, imalii: tilaunye@gmail.com

Abachii Giruumi Tasfayee: quxuri +251-920-274035: imalii: girumtesfaye12@gmail.com

Amharic version

የጥናቱ ርዕስ፡ ባዋቸሞ ዩንቫርስት ንግስት ኢሊን መሃማዲ ማታሳቢያ ሪፈራል ሆሲፒትል ባሚመጡ ኣዋቅ ሳዎች ላይ ያለዉን ያዳም ማናስ ክስተት ማጣንና ካኣቺፕይሎር ባሽታ ጋር ያላውን ታዛማዥናት ማወቅ.

የዋና ተመረማሪ ስም: ካሳሁን ሃይሌ

የድርጅቱ ስም፡- ጅማ ዩኒቨርሲቲ / የህክምና ላቦራቶሪ ሳይንስ ትምህርት ክፍል

ይህ የመረጃ ቅጽ የተዘጋጀዉ ከላይ በተጠቀሰው ጥናት ለሚሳተፉ ተሳታፊዎች ሲሆን በአጠቃላይ በጥናቱ ውስጥ ልናካሂዳቸው ስለፈለግናቸው ጉዳዮች እና ስለ ጥናቱ ጠቅላላ ማብራርያ ለመስጠት ነበር። በመሆኑ በጥናቱ የሚሳተፉት በራስዎ ፍላጐት ብቻ መሆኑን በትህትና እንገልፃለን።

ያጥናቱ ኣላማ፡ ያዳም ማናሲ ክስተት ማጣንና ካኣቺፕይሎር ባሽታ ጋር ያላውን ታዛማዥናት ባኣዋቅ ሳዎች ላይ ባዋቻሞ ዩንቫርሲት ንግስት ኢልን ሞሃማዲ ማታሳቢያ ሪፈራል ሆሲፒትል ማወቅ።

የጥናቱ ሂደት ዝርዝር

በጥናቱ ለመሳተፍ ከተስማሙ የሚከተሉትን መረጀዎችና ናሙና እንደምንወስድ ገልፀንላቸዋል፡ የ መሀባራዊ ጉዳይ ጥየቄ እንዳ ምጣየቁ ፤የህክምና መዝገባቸዉ እንደምታይ: እንዲሁምየ10 ደቂቃ ቃለ መጠየቅ እንደም ደረግላቸዉ። ናሙና ይወሰዳል ምርመራም እንደምደረግ።ያተሰበሰበዉ ናሙና ባዋቻሞ ዩንቫርሲት ንግስት ኢልን ሞሃማዲ ማታሳቢያ ሪፈራል ሆሲፒትል ማኢካላዊ ላቦራቶሪ ላይ ይሳራል።

ስጋትና ጉዳት

በአጠቃላይ ከላይ የተጠቀሰዉን ናሙና በሚወሰድበት ጊዜ ሊያጋጥም የሚችል አደጋ እንደማይኖር ግን ያዳም ናሙና ሲዎሳድ ትንሽ ያህማም ስሜት ኢንዳምኖር ተነግሮዋቸዋል።

ሊያስገኛቸው የሚችሉት ጥቅሞች

በዚህ ጥናት ውስጥ በመሳተፋቸዉ በጥሬ ገንዘብ የሚከፈል የካሳ ክፍያ እንደማይኖር ተነግሮዋቸዋል። የጥናቱ ተሳታፊዎች በምርመራ ዉጤት ላይ በመማርኮዝ ለበለጠ ህክምና እንክብካቤ ወደ ሃክማችው እንድሄዱ ይደርጋል።

የጥናቱ ምስጢራዊነት

ማንኛዉም በጥናቱ የተገኙ መረጃዎች ምስጢራዊነቱ የተጠበቀ ነዉ:: የጥናቱ መረጃዎች በሙሉ የተቀመጡት ለጥናቱ ተብሎ በሚሰጠው ስውር ቁጥር ሲሆን ጥናቱን ከሚያስከሄዱት ባለሙያዎች በስተቀር ማንም ሊያውቅ አይችልም።

ያለ መቀበል ወይም ጥሎ የመውጣት መብት

በዚህ ጥናት ዉስጥ የሚኖረዉ ተሳትፎ ሙሉ በሙሉ ፈቃደኝነት ላይ የተመሰረተ እንደሆነ የተገለፀላቸዉ ሲሆን በማንኛውም ጊዜ ይህንን ጥናት የማቋረጥ መብታቸዉ ሙሉ በሙሉ የተጠበቀ እንደሆነ ተገልፆላቸዋል:: በጥናቱ ባለመሳተፋቸዉ ወይም ከጥናት በመገለላቸዉ ምክንያት በአሁኑ ወይም የወደፊት የህክምና እርዳታ ላይ ተፅዕኖ እንደማይኖረዉ በግልፅ ተነግሮዋቸዋል፡

ጥያቄ ካለወት

ስለ ጥናቱ ማንኛዉም ጥያቄ ወይም ቅሬታ ስኖራቸዉ የሚከተሉትን ስልኮች ወይም ኢሜል አድራሻ በመጠቀም የጥናቱን ባለቤቶች ማነጋገር እንደሚችሉ ተገልፆላቸዋል።

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Annex II: Consent form

English Version

Participants name _____

I am informed fully in the language I understand about the aim of above mentioned research. I understood the purpose of the study entitled with “prevalence of anemia and its association with *H. pylori* infection among adult dyspeptic patients attending Wachemo University Nigist Elein Mohammad Memorial Referral Hospital” I have been informed specimen was taken and there will be interview. In addition they have been told all the information collected throughout the research process will be kept confidential. I understood my current and future medical services will not be affected if I refused to participate or with draw from the study.

Agree_____ Not agree_____

Therefore I give my consent freely for my participation in this study.

Name of participant: _____Signature:_____ Date_____

Name of researcher: _____Signature_____ Date_____

Haddiyissa version consent form

Bikanchi anani xiggi _____

Bikanchi summi_____

Anni hanane summi chakisumi bikanchi wachaami yunvursitena nigiste ellinii mohammad matasabeei hospitalena qorri mannanene yo'oi xiggi hofechi qaxomaa *H. pylori* jabbine yokii mateyoma laimmina gudukii sorrobane anii laomanine machesomi saggarine ihokii woshuwaa kuramako. Fayaaomii woshuwaa xammkamisa xiggaa shumaa mahi dangimi afonii masakamisa chakisamako. Oddimii masakamii woshuwaa hundami maxamisane amdakamisa chakisamako. Kaa sorobane bikimm urimaa shigigumlas ayii amanenem sabimaa xanomisa kuramakoo kaka isimina mahi luwamii hoggomi beisaa danamisa chakisamko.

Itaamomo_____ittamumoyyo _____

Bikanchi summi _____furma'i---------- balii ----------

Sorobanchi summi ---------- furma'i ---------- balii ----------

Amharic version consent form

የተሳታፊው ልዩ መለያ ቁጥር _____

የተሳታፊው ስም _____

እኔ ስሜ ከላይ የተጠቀሰው ተሳታፊ ባዋቸሞ ዩንቨርስት ንግስት ኢሌን ሞሃማዲ ማታሳቢያ ሪፈራል ሆሲፒትል ባኣዋቅ ሳዎች ላይ ያዳም ማናሲ ክስተት ማጣንና ካአቺፕይሎር ባሽታ ጋር ያላውን ታዛማዥናት ለማወቅ በታሰበው ምርምር ላይ በሚገባኝ ቋንቋ በቂ መረጃ አግኝቻለሁ። የህክምና መረጃና ናሙና ምንም አይነት ጉዳት በማያደርስ መልኩ እንደሚወሰድ ተረድቻለሁ። በተጨማሪም የሚወሰዱ ማናቸውም መረጃዎች በሚስጢር እንደሚያዙ ተነግሮኛል። እንድሁም የሚጠየቀውን መረጃ ያለመስጠትና በጥናቱ ያለመሳተፍ መብት እንዳለኝ እንድሁም ከጥናቱ በማንቸውም ወቅት ራሴን ማግለል እንደምችል የተገለፀልኝ ሲሆን ይህንንም በማድረጌ ወደ ፊት ምሆንና አሁን የማገኛቸው የህክምና ግልጋሎቶች እንደማይጓደሉብኝ ተነግሮኛል።

እስማማለሁ _____አልስማማም _____

የታካሚ/ የተሳታፊ ስም _____ፊርማ ---------- ቀን ----------

የተመራማሪ ስም ----------ፊርማ ---------- ቀን ----------

Annex III: questionnaire

English version

Card no: _____

Code: _____

Address: _____

General instruction

i. 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 characteristics			
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 level	_____	
1.6	Monthly income in Ethiopian birr	_____	
1.7	Occupational status	0. Farmer 1. Daily labourer 2. Governmental employee 3. Students 4. Merchants	

		5. Other specify_____	
	Part II: clinical character		
2.1	Do you have known chronic illness like?		If No skip to 2.2
	Diabetes mellitus	0. No 1. Yes	
	chronic kidney disease	0. No 1. Yes	
	TB	0. No 1. Yes	
	Hepatitis	0. No 1. Yes	
	If other Specify	_____	
2.2	Do you have history of malaria infection?	0. No 1. Yes	If No skip to 2.4
2.3	If yes to Q. 2.2 when?	Specify _____	
2.4	Do you have history of bleedings?	0. No 1. Yes	If No skip to 3.1
2.5	If yes to Q.2.4. What is reason for bleeding?	0. accidents 1. haemorrhoids 2. heavy menstruation 3. other specify _____	
Part III : Dietary habits			
3.1	Do you consume fruit and vegetable?	0. No 1. Yes	If No skip to 3.3
3.2	If yes for Q.3.1. How many days per week in average?	0. 1 1. 2 2. 3 3. other specify _____	
3.3	Do you consume red meat?	0. No 1. Yes	If No skip to 3.5
3.4	If yes for Q.3.3**.** How many days per week in average?	0. 1 1. 2 2. 3 3. other specify _____	
3.5	Do you have habit of drinking coffee or tea immediately after	0. No 1. Yes	

	meal?		
Part IV: behavioural characteristic			
4.1	Do you smoke cigarette?	0. No 1. Yes	If No skip to 4.3
4.2	If yes to Q.4.1, in average how many cigarettes do you smoke per day? (in number)	_____	
4.3	Do you drink alcohol?	0. No 1. Yes	If No skip to 4.5
4.4	If yes to Q.4.3. How much do you drink per day in average?	0. 330ml 1. 500ml 2. >500ml	
4.5	Weight (to be measured by data collectors)	_____kg	
4.6	Height (to be measured by data collectors)	_____meter	

Haddiyisaa version

Caridik xiggi:_____

Anani quxurii:_____

Beyyii :_____

Hundemi xamichuwina dabachi yohani ihubikina ihokii dabachi kahi √ sagara kitabehe.

Baboo ihuki xamichuwaa kinuwiina agukisinehe dabacha kitabehe

X.K	varaabiluwa	dabachuwaaa	Higgae ehanone
shootii I: hechii kankikuwi xammichuwaa			
1.1	Ummur womi hinchone	_____	
1.2	alibacha	0. Landicho 1. gooncho	
1.3	Hechi beyi	0. gandisa 1. gaxaraa	
1.4	Mini isata?	0. isumoyoo 1. issamo 2. isaa tiramo 3. isea/ixi lehako	
1.5	Losani gaballi?	_____	
1.6	agganane sidohik ethiopea birrane me'oo?	_____	
1.7	Baxi shotoi?	0. abulancho 1. balli baxanancho 2. adili baxancho 3. losancho 4. dadarancho 5. mulaniihulas chakisa___	
	Shotti II: fayaommi xammichuwaa		
2.1	Ka illage hixe'a amanina jabbo jabbita lako'o .eyaa ihulas dabach hink jabbo amadukoki?		aea'e ihula X2.2 higgae.
	sukali jaboo	0. aea'e 1. eya'a	
	mulie jabboo	0. aea'e 1. eya'a	
	suko'i jabo	0. aea'e 1. eya'a	

	afali'i jabbo	0. aea'e 1. eya'a	
	mullek yolassi chakisehe/he	_____	
2.2	Ka illage kachisi jaboo xisata lakoo?	0. aea'e 1. eya'a	aea'e ihula X2.4 higgae.
2.3	Eya ihulas dabach X .2.2.hinkane amane ?	chakisee _____	
2.4	ka illage xiggi dunamaha laqonhe	0. aea'e 1. eya'a	aea'e ihula X3.1 higgae.
2.5	Eya'a ihulas dabach ka X 2.4. mahine	0. gabayatonehe 1. kintarotinena 2. higgi manistrashinenete 3. mulleki yollas chakise	
Shotti III : hurbat itimi amalii xamichuwaa			
3.1	Misha oddimi shano mutani itaha laqo?	0. aea'e 1. eya'a	aea'e ihula X3.3 higgae.
3.2	Eyi ihulas X .3.1.dabachi saantanhe me'are itooho?	0. 1 1. 2 2. 3 3. Mulleka chakisea ___	
3.3	Kashari mara itooho?	0. aea'e 1. eya'a	aea'e ihula X3.5 higgae.
3.4	Eyi ehulas X .3.5.dabachi saantanhe me'are itohi?	0. 1 1. 2 2. 3 3. Mullekachakisea __	
3.5	Hurbata itahaa lasage buna oddim shayee agaa laqo?	0. aea'e 1. eya'a	
Shootti IV: ammalii xammichuwa			
4.1	Sijjaraa ago	0. aea'e 1. eya'a	aea'e ihula X4.3 higgae.
4.2	eyii ihulas X.4.1 dabachi ballane meai sijjara agohi?(xigine kure)	_____	
4.3	Haraqee agohonehe?	0. aea'e 1. eya'a	aea'e ihula X4.6 higgae.
4.4	eyii ihulas X.4.3. ballanehe hinkane aggotiki?	0. 330ml 1. 500ml 2. >500ml	
4.5	Guratii	_____kg	
4.6	kerralloma	_____metera	

Amharic Version

ካርድ ቁጥር: _____

ኮድ: _____

አድራሻ: _____

ለሁሉም ጥያቄዎች አማራጮች ያሉት ሲሆን እርሶ ተገቢ ነዉ በሚሉት ላይ ያክብቡ ወይም √ ምልክት ያስቀምጡ ለክፍት ጥያቄዎች በራስዎ ቃል፤ሐረግ ወይም ዐረፍ ተነገር ይግለፁ።

ተቁ	መጠይቆች	ምላሾች	ይዝለሉ
ክፍል I:: ማህበራዊናአከባብያዊኑሮባህሪያት			
1.1	ዕድሜ በአመት	_____	
1.2	ፆታ	0. ሴት 1. ወንድ	
1.3	ሚኖሩባት ቦታ	0. ገጠር 1. ከተማ	
1.4	ትዳር ሁኔታ	0. አላገበሁም 1. ባለትዳርነኝ 2. ፈትቼሃለሁ 3. ባሌ ሞቶዋል/ምስቴ ሞታለች	
1.5	ትምህርት ዳራጃ	_____	
1.6	ያወር ጋብ ባኢትዮጵያ ብር	_____	
1.7	ስራ ማስክ	0. ቀን ሳራታኛ 1. ገባሬ 2. መንግስት ሳራታኛ 3. ተመር 4. ሌላ ካሆና ግልጽ_____	
	ክፍል II:: ያባሽታዋች ባህሪያት		
2.1	በህክምና የተረጋገጠ ስር ሰዳጅ በሽታ (chronic illness) አለብዎት?ለምሳሌ		የለም ካሆና ወደ2.2 ጥያቄ ይዝለሉ
	ሱካር በሽታ	0. የለም 1. አዎን	
	ኩላሊት በሽታ	0. የለም 1. አዎን	
	ሳንባ ናቃርሳ በሽታ	0. የለም 1. አዎን	
	ጉባት በሽታ	0. የለም 1. አዎን	
	ሌላ ካላ ግላጽ	_____	

2.2	ወባ ተመህ/ሽ ታቃላ?	0. የለም 1. አዎን	የለም ካሆና ወደ ጥያቄ 2.4 ይዝለሉ
2.3	አዎን ካሆና ላጥይቀቁ.2.2ማች?	ግላጽ _____	
2.4	ዳም ፋሶብ/ሽ ታቃላህ/ሽ?	0. የለም 1. አዎን	የለም ካሆና ወደ ጥያቄ 3.1 ይዝለሉ
2.5	አዎን ካሆና ላጥይቀ ቁ.2.4 ምክንያቱ ምን ናባር?	0. ኣዳጋ 1. ክንታሮቲ 2. ካባድ ያወራ ኣባባ 3. ሌላ ካላ ግላጽ _____	
ክፍል III:: የአማጋጋብ ባህር			
3.1	አሮንጋደ ቅጣላ ቅጣል ትማጋባላህ/ሽ?	0. የለም 1. አዎን	የለም ካሆና ወደ ጥያቄ3.3 ይዝለሉ
3.2	አዎን ካሆና ላጥይቀቁ.12 ባሳምንት ባ አማካይ ስንት ግዜ?	0. 1 1. 2 2. 3 3. ሌላካላግላጽ	
3.3	ጥሬ ስጋ ትማጋባላህ/ሽ? ?	0. የለም 1. አዎን	የለም ካሆና ወደ ጥያቄ3.5 ይዝለሉ
3.4	አዎን ካሆና ላጥይቀቁ.3.3 ባሳምንት ባ አማካይ ስንት ግዜ?	0. 1 1. 2 2. 3 3. ሌላካላግላጽ	
3.5	ምግብ ኢዳታማጋብክ/ሽ ቶሎ ሻይ ዋይም ቡና የማጣጣት ልማድ ኣላብሂ/ሽ?	0. የለም 0. አዎን	
ክፍል IV:: የባህር			
4.1	ስጋራ ታጨሳለህ?	0. የለም 1. አዎን	የለም ካሆና ወደ ጥያቄ 4.3 ይዝለሉ
4.2	ለጥያቄ 4.1.መልስዎ አዎን ከሆነ ባ አማካይ በቀን በቁጥር ስንት ስጋራ ያጨሳሉ?	_____	
4.3	አልኮሆል ይጠጣሉ?	0. የለም 1. አዎን	የለም ካሆና ወደ ጥያቄ 4.6 ይዝለሉ
4.4	ለጥያቄ 4.3 መልስዎ አዎን ከሆነ በቀን በአማካይ ምን ያህል አልኮሆል ይጠጣሉ?	0. 330ml 1. 500ml 2. >500ml	
4.6	ክብደት (በመረጃ ሰብሳብዎች ይለካል)	_____kg	
4.7	ቁመት (በመረጃ ሰብሳብዎች ይለካል)	_____meter	

Annex IV: Laboratory procedure

A. Specimen collection

- **Venous blood collection**

Materials

- Glove
- 70% alcohol
- Tourniquet
- test tube with EDTA anticoagulant
- Gauze pads or cotton
- Marker
- Rack
- Sharp container
- Band Aid

- **Procedure**

✓ Assemble the necessary materials and equipment

✓ Identify the right patient and allow him/her to sit comfortably preferably in an armchair stretching his/her arm.

✓ Apply the tourniquet

✓ select vein puncture site

✓ Cleanse site with a gauze pad or cotton moistened with 70% alcohol.

✓ pick up needle and remove cover and inspect

✓ anchor vein with thumb

✓ insert the needle into the vein with the bevel faced up and collect sample , release the tourniquet

✓ withdraw needle

✓ disposal of the needle

✓ fill test tubes, mix tubes well

✓ labeling test tubes with ID number

B. Complete Blood Count by *MINDRAY BC-3000 Plus*

- **Principle**

The *MINDRAY BC-3000 Plus* counts and sizes red blood cells (RBC) using 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 known dimension. An electrode is submerged in the liquid on both side of the aperture to create electrical pathway. As each particle pass 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. Lyzer solution lyses RBCs and converts HGB to a 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 light, which passes through the sample and a 525nm filter, and then is measured by a photo-sensor 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*$\log_{10}$ (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($x10^{12}$)*MCV (fL)/10
MCH= HGB/RBC($x10^{12}$)*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.

***MINDRAY BC-3000 plus* Reagents**

- **diluents(M-30 DILUENT)**

  The diluents are formulated to meet the following requirements:

- to dilute the blood sample
- to provide the blood cells with an environments similar to the blood plasma
- To maintain the cell volume of each RBC and platelet during the count and sizing portion of the measurement cycle.
- To provide a conductive medium for impedance counting of WBC, RBC and platelets.

- **Lyse (M-30CFLLYSE)**

Lyzer is formulated to meet the following requirements:

- To rapidly break down RBC walls, release the haemoglobin from the cell, and reduce the size of cellular debris to a level that does not interface with white blood cell counting.
- Lyses RBCs for accurate measurements of HGB and convert haemoglobin to a complex whose absorbance is determined by analyzers at 525 nm.

- **Rinse(M-30R RINSE)**

The rinse is formulated to rinse the baths and metering tubes and

- To provides proper meniscus formation in the metering tubes and maintain it during each measurement cycle.

- **E-Z cleanser (M-E E-Z CLEANSER**) – is an enzymatic-based isotonic, cleaning solution and wetting agents formulated to clean the fluidic lines and baths.
- **Probe cleanser (M-30P PROBE CLEANSER):** is an alkaline cleaning solution formulated to clean the fluidic lines, apertures and baths.
- **Controls and calibrators:** are used to verify accurate of operation and calibrate the analyzer.
- Controls are commercially prepared whole blood products used to verify that the analyzer is functioning properly. They are available in low, normal and high levels.

- Calibrators are commercially prepared whole-blood products used to calibrates the analyzer.

Specimen type: EDTA anticoagulated whole blood.

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 other 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 ready and the counts 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 sample.
- 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 increased by 1 and the sample probe will be repositioned. The analysis result is displayed on the screen.

OPERATING PROCEDURE

- Press menu and select mode to enter the sample mode screen
- Entering sample information like Id, name, age, sex, card number
- Enter button
- At the count screen be sure the system status area display ready and the count mode area display whole.

- Presents the mixed samples to the sample probe so that the tip is well in to the tube and press the aspirate key. The system status area will display running and the analyzer will start aspirating sample.
- 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 increased by 1 and the sample probe repositioned. Press print button , results will be automatically printed out.

Acceptable Background Counts

Parameter	Count
WBC	0.3 x 10^9/L or less
RBC	0.03x 10^{12}/L or less
HGB	0.1 g/dL or less
HCT	0.5% or less
PLT	10 x 10^9/L or less

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

Source: MINDRAY BC-3000 plus operating manual.

Wondfo one step *H. pylori* feces test

Principle

Wondfo one step *H. pylori* feces test is a qualitative test based immunoassay for the detection of *H. pylori* antigen in human fecal specimen. In this test procedure, *H. pylori* antibodies are immobilized in the test line region of the device.

When the specimen is added into the test strip, the specimen is absorbed in to the device by capillary action, mixes with the antibody-dye binding conjugate and flows across the pre-coated membrane.

Materials

- 25 individual sealed pouches, each containing 25test strip 25 specimen collection tubes with 1.5 ml buffer solution(0.85% saline solution)
- Dust bean

Storage and stability:

- Store at 4^{o}c-30^{0}c in the sealed pouch within the expiration date.
- Keep away from sunlight, moisture and heat.
- Do not freeze.

Stool sample collection

- ✓ Assemble the necessary materials and equipment
- ✓ Explain sample collection producers
- ✓ After explaining how to collect stool specimen, give clean, dry, water-proof, wide-mouth container
- ✓ Ask the patient to pass the stool sample directly into a container without contamination of water and urine. On average 5 grams of the well-formed stool
- ✓ Label with respective ID and address

Test procedure

- Collect stool sample by using the sample collection tube provided. First, unscrew the cap of the sample collection tube, take out the sample stick.
- Insert the sample stick in to stool sample at 6 different sites
- Put the sampling stick back to the sample collection tube and screw tightly, mix well.
- Remove the test device from the foil pouch by tearing at the notch and place it on a level surface
- Holding a sample collector upright , carefully break off the tips of the collector at break point
- Squeeze 3 drops (about 80µl) of sample solution to the sample pad below the mark line

Interpretation of results

- **Positive:** rose –pink band are visible both in the control line region and the test region.
- **Negative:** rose –pink band are visible in the control line region. No colour band appears in the test region.
- **Invalid:** no visible band at all or there is a visible band only in the test region but not in the control region.

Quality control: though there is internal procedural control line in the test device and checked by positive and negative sample when every new butch opened.

Source: Guangzhou Wondfo Biotech manual

Stool Examination

- **Principle**: wet mount made from patient stool specimens and can be examined under low and high power for the presence of parasites.
- **Reagents and equipment:**

✓ Cover slips
✓ Glass slides

- ✓ Gloves
- ✓ Microscopes
- ✓ Normal Saline
- ✓ Pipettes

Microscopic examination

- **Procedure:**

1. Apply the patient's sample to a small area on a clean microscope slide.
2. Immediately before the specimen dries, add 2 drops of saline with a pipette. Mix with a pipette tip.
3. Cover the specimen with a cover slip.
4. Examine the specimen with the low power objective
5. Examine the entire cover slip
6. Ova, cysts, trophozoites and adult worms can be identified as per their characteristic features.

- **Quality control:** Check the saline. It should be clear with no visible signs of contamination.

Preparation of thin blood film

Materials

- Clean microscope slides
- Well-mixed EDTA blood sample
- Pipette
- pencil
- Gloves
- Waste and sharps disposal containers

Procedures

- Place a small drop of well mixed EDTA blood at the end of the glass slide

- The spreading slide is placed in front of the drop of blood at an angle of about 40^{o} to the slide and then is moved back to make contact with the drop
- The drop will spread out quickly along the line of contact of the spreader with the slide
- The spreader is advanced with a smooth steady motion so that a thin film of blood is spread over the slide
- Allow the smear to air-dry
- Label with patients ID and address

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 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 micron diameter on an average with central pallor area diameter of about 2-3 micron (one third of size).

Microcytic hypochromic anemia: on microscopy, on average size of RBC is smaller than size of the nucleus of a small lymphocyte. RBC having diameter less than 6 micron and MCV of less than 80fL and also having central area of pallor more than 3 micron in diameter. The complete blood counts showed MCV value less than 80 fL and MCHC value 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, on average size of RBC is larger than size of the nucleus of a small lymphocyte. RBC are approximately 9 micron or larger in diameter having MCV of greater than 100 fL. The complete blood counts showed MCV value greater than 100fL.

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

Annex V- Laboratory Result Reporting Format

Laboratory Request Form for hematological parameter

ID _____Age_____ Sex_____ Physician Name _____

Test	Result
RBC	
HGB	
HCT	
MCV	
MCH	
MCHC	
RDW	
WBC	
PLT	

Laboratory Request Form for *H. pylori* and intestinal parasite

ID _____ Age_____ Sex_____ Date _____ Physician Name _____

Test Required

Test	RESULT
H. pylori Antigen test	
Stool examination	

Reporting form for peripheral morphology examination

ID_____ Age_____ Sex_____ Date _____

Physician Name _____

RBC series _____

WBC series _____

Platelet series_____

Possible conclusion _____

Annex VI: - Declaration form

ASSURANCE OF PRINCIPAL INVESTIGATOR

I, the undersigned, hereby declare that this MSc. thesis is my original work, and has never been presented for any degree in Jimma University or any other institutions of higher learning in Ethiopia. I also declare the duly acknowledgement of all material sources used for this thesis work.

Name of the student: Kassahun Haile (BSc.)

Signature: _____ Date of submission: _____/_____/_____

APPROVAL OF THE ADVISORS

This thesis has been approved by the supervision of University advisors:

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Examiner

Name: _____ Signature: _____

Name of School head: _____

Signature: _____ Date of submission: _____/_____/_____