ADDIS ABABA UNIVERSITY
COLLEGE OF HEALTH SCIENCE
DEPARTMENT OF MEDICAL LABORATORY SCIENCE

TITLE: MAGNITUDE OF *HELICOBACTER PYLORI* INFECTION AMONG PEPTIC ULCER DISEASES PATIENT OF TWO PRIVATE HEALTH FACILITES OF ADDIS ABABA, ETHIOPIA : EVIDENCE OF STOOL ANTIGEN TEST AND STAINING OF BIOPSY MATERIALS

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This is to certify that the thesis prepared by Tewodros Kassahun entitled "MAGNITUDE OF *HELICOBACTER PYLORI* INFECTION AMONG PEPTIC ULCER DISEASES PATIENT OF TWO PRIVATE HEALTH FACILITES OF ADDIS ABABA, ETHIOPIA: EVIDENCE OF STOOL ANTIGEN TEST AND STAINING OF BIOPSY MATERIALS" and submmitted in partial fulfillment of the requirements for Master of Science degree in Clinical Laboratory Science (Diagnostic and Public Health Microbiology) complies with the regulations of the University and meets the accepted standards with respect to originality and quality.

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List of abbreviation

Ag- Antigen

CD4- Cluster of Differential

DREC -Department of Research and ethics review Committee

DU: Duodenal ulcer

GU: Gastric ulcer

HP -Helicobacter pylori

HPSA -Helicobacter pylori stool Antigen

MALT- Mucosa Associated Lymphoid Tissue

NUD -Non Ulcer Dyspepsia

OPD- Out Patient Department

OR -Odds Ratio

PPI- Proton Pump Inhibitors

SPSS- Software Package for Social Study

TASRH- Tikur Anbessa Specialized Referral Hospital

UGI- Upper Gastro-Intestinal

WHO- World Health Organization

ABSTRACT

BACKGROUND: *Helicobacter pylori* infection is one of the most common chronic bacterial infections of humans and has a worldwide distribution. Epidemiological studies strongly suggested that more than 50% of the world's populations are colonized by *H. pylori*. Peptic ulcer disease (PUD) is the most common illness in the Ethiopian population visiting outpatient department of health facilities, and it has also been associated with *H.pylori* infection. The aim of this study was to assess the magnitude of *H.pylori* and its associated factors among peptic ulcer disease patients who visited at landmark general hospital and adera internal medical speciality center outpatient department. Around 70–90% of peptic ulcer disease (PUD) is due to *helicobacter pylori* and requires treatment with antimicrobials to which these bacteria are susceptible.

OBJECTIVE: To assess the magnitude of *helicobacter pylori* infection among peptic ulcer diseases pateints of two private health facilites Addis Ababa, Ethiopia: evedence of stool antigen test and staining of biopsy materials

METHODS: An institutional-based cross-sectional study was conducted on 150 peptic ulcer patients. Non-probability convenient sampling techniques were used to select study participants. Data were collected by using structured questionnaire via face-to-face interview. *H.pylori* infection was diagnosed using stool antigen test method. The data were entered into Epi info version 3.5.3 and transferred to Statistical Package for Social Sciences version 20. Both Bivariable and multivariable binary logistic regression analyses were performed to see the effect of independent variables on the dependent variable.

RESULTS: Of the total study participants, 45(41%), 52(58%) and 36(43%) were married, rural residents and male, respectively. All peptic ulcer disease patients had abnormal histopathology findings. The overall magnitude of *H.pylori* infection was 52 %. In bivariable logistic regression analysis, sex and marital status were significantly associated with *H.pylori* infection. The sensitivity and specificity of *H.pylori* stool antigen test is 70.5 % while the sensitivity of giemsa and gram stain was 88.5 %. However, all methods have 100 % specificity.

CONCLUSION: The magnitude of *H.pylori* infection is 52%. This is high indicating that it is a public health problem in the study area. According to this study, sex and marital status were

significantly associated with *H.pylori* infection. Hence, effective preventive, control and screening strategies need to be designed to reduce the burden of the disease.

Key words: PUD, Endoscopy, *Helicobacter pylori stool antigen, sensitivity, specificity, Staining method*

1. INTRODUCTION

1.1 BACKGROUND

H.Pylori are a spiral, curved, gram-negative, micro-aerophilic with two to six lophotrichious flagella that gives it the motility to withstand rhythmic gastric contractions and penetrate the mucosa. The principal reservoir for *H. pylori* infection appears to be the stomach especially antrum(1).

It was established in 1982 by Robin Warren and Barry Marshall as the causative agent of gastritis and peptic ulcer, a discovery that revolutionized gastroenterology (2).

Helicobacter pylori infection is well known to be the most common human infection worldwide on the basis of the fact that approximately 50% of the world's populations are infected and that human beings are the main reservoir (3, 4). The pattern of infection is an early childhood acquisition of *H. pylori* (30%-50%) that reaches over 90% during adulthood in developing countries (5). This has been attributed to the poor socioeconomic status and overcrowded conditions (6–7). Infection in developed countries is less common in young children and reaches up to 60% in older ages (3, 8, and 9). In the United States, a 20% infection rate among adolescents is being reported, (10) and recently an overall prevalence of 36% was reported, suggesting rapidly improving socioeconomic conditions (11). While the prevalence of infection has decreased significantly in many parts of North America and Western Europe, no such decline has been noted in the majority of the developing world (10). Studies from many African countries reported similar prevalence rates of 91.7% in Egypt (12), 97% in Gambia (13), and 75.4%.in Ghana (14). In Ethiopia overall prevalence of the infection in adult dyspeptic patients 90% (15).

Helicobacter pylori were the first formally recognized bacterial carcinogen. It has been etiologically associated with gastritis, peptic ulcer disease, gastric adenocarcinoma, and primary gastric lymphoma (16, 17).

H. pylori cause acute and chronic gastritis, and can cause duodenal and gastric ulcers. Although these significant diseases are typically found among adults, there are clear parallels with gastro duodenal disease in children. In particular, Peptic ulceration, abdominal pain in the absence of peptic ulceration and Gastro-esophageal reflux diseases disorders has been associated with *H .pylori* (18).

Although some infected individuals harbor the organisms throughout their lives with no overt clinical symptoms, in 80-90% while approximately 10- 20% of infected individuals manifest one of many different outcomes, such as peptic ulcer disease, including gastric ulcer and duodenal ulcer, gastritis, non-ulcer dyspepsia, gastric cancer, and mucosa-associated lymphoid tissue lymphoma(MALT)(19, 20).

Various diagnostic tests for *H. pylori* have been developed and they can be broadly classified into invasive and non-invasive tests (21). Invasive tests utilize endoscopic biopsy samples for histology, culture, and rapid urease test (RUT) and polymerase chain reaction. All these tests have been found to have sensitivity and specificity that are well above 90% (22).

The non-invasive tests do not require endoscopy. These include urea breath test (UBT), immunoglobulin G and M serology, stool antigen test, saliva antibody test and urinary antibody test; in Ethiopia the invasive tests are not widely available. Among the non-invasive tests stool antigen, urea breathe test (UBT) and immunoglobulin IgG test are available (21).

According to the World Gastroenterology Organization report, the prevalence of *H. Pylori* in developed countries ranges from 20–50%. The infection has a higher prevalence rate in developing countries (80–95%). In Ethiopia, the prevalence is as high as 48% in children aged 2–4 year, 80% at the age of 6 year, and above 95% in adults (23).

1.2 STATEMENT OF THE PROBLEM

50% the world total population and 80% of people living in the developing countries are chronically infected with *H. pylori* (24, 25).

Approximately 10-20% those infected develops symptomatic disease like dyspepsia, gastric and duodenal ulcer. An additional 1-2% develops gastric malignancy (adenocarcinoma, MALT) and pancreatic cancer. Of the population in developed and developing countries are known to be infected with *H. pylori*. And this has been attributed to many factors like poor socioeconomic status, hygienic practice, overcrowding, etc. The infection is more prevalent in adult population than children (26).

The public health impact of *Helicobacter pylori* infection is gradually becoming obvious, the bacterium now being implicated as an etiologic agent in a variety of peptic ulcer diseases.The overall prevalence is high in developing countries and lower in developed countries and within areas of different countries. There may be similarly wide variations in the prevalence between more affluent urban populations and rural populations. The principal reasons for these variations involve socioeconomic differences between populations.

In Ethiopia (WGO) the prevalence of *helicobacter pylori* were 48 % in age between 2- 4, 80% at the age of 6 and were 95%in adult's population (27).

The prevalence of *Helicobacter pylori* and the relative contribution of the various routes hence it is the commonest chronic bacterial infection of mankind with significance health consequence of transmission in humans in Ethiopia have not been adequately studied. So this study is designed to assess the magnitude of *helicobacter pylori* infection using stool Ag test and comparing with endoscopic and biopsy finding in patients suspected to have peptic ulcer disease in landmark general hospital and adera internal medical specialty center.

1.3 SIGNIFICANCE OF THE STUDY

- The study aims to generate prevalence data that were relevance to clinicians to diagnose the patient suspected with peptic ulcer disease (PUD) and policy makers for patient care and preventive measures.
- On this study sex and marital status were significantly associated with *H.pylori* infection, effective therapeutic control to be designed to reduce the burden of the disease.
- The study serves as base line data and plays a major role in adding valuable information for interested researchers and academicians for further analysis in the sector.

2. LITERATURE REVIEW

Before the twentieth century, gastric ulceration constituted the bulk of peptic ulcer disease and duodenal ulcers were quite rare. The incidence of duodenal ulcers increased progressively, reaching a peak in the 1950s. The cause of this rise is unclear, because *H. pylori* are thought to have been ubiquitous in the human population for thousands of years. The prevalence of gastric and duodenal ulceration has decreased in Western Europe and the USA over recent decades, following a decrease in the prevalence of *H. pylori* (28).

H. pylori infects about 40% of adults in developed countries as shown in (Table 1.) and is strongly associated with greater age and with markers of overcrowding and poor hygiene in childhood. These associations arise because *H.pylori* infected progressively fewer children during the second half of the 20th century. In developing countries on the other hand, the prevalence of *H. pylori* infection is usually more than 80%, and immigrant populations from developing to developed countries (28).

Table 1: Worldwide prevalence of *Helicobacter pylori* in the mid-1990s as shown in this table.

Country	**Prevalence (%)**
United States and Canada	30-40
Mexico and Central/South America	70-90
Western Europe	30-50
Eastern Europe	70
Africa	70-90
Asia	70-80
Australia	20

Study conducted in Pakistan Chronic gastritis was the commonest endoscopic diagnosis.Out of 93 patients with gastritis 78 (84.78%) patients had chronic gastritis associated with *H. Pylori* infection prevalence of *H pylori* infection in this study was 85% in case of chronic gastritis and about 100% in case of duodenal ulcer and duodenitis (29).

The study conducted in Nigeria showed gastritis was the commonest endoscopic finding (60.5%), serious gastro duodenal pathology (gastric ulcer, duodenal ulcer and gastric cancer) were documented in only 12 (14%) patients. Thirty three (63.5%) of the 55 patients with gastritis had *H. pylori* infection while 7(58.3%) of the 12 patients with serious gastro duodenal lesions

had the infection. Thirteen (72.2%) of the 18 patients that had normal endoscopic findings were *H. pylori* positive.The prevalence of *H. pylori* among dyspeptics using biopsy based methods is 63.5% in the South-Western part of Nigeria (30).
It is therefore important to test and treat *H. pylori* among Nigerians with dyspepsia (31).

Study conducted in Gondar by Desie Kasewet.al an institutional-based cross-sectional study was conducted on 354 dyspeptic patients. *H.pylori* infection was diagnosed using stool antigen test method. Of the total study participants, 201(56.8%), 195(55.1%) and 182(51.4%) were married, urban residents and females, respectively. The overall magnitude of *H.pylori* infection was 37.6%. In bivariable logistic regression analysis, sex and marital status were significantly associated with *H.pylori* infection, but in multivariable logistic regression analysis only marital status was significantly associated with *H.pylori* infection. Finally they conclude the magnitude of *H.pylori* infection is high indicating that it is a public health problem in the study area (32).

A comparative cross-sectional study was conducted among dyspepsia and non-dyspepsia adults from March 2015 to October 2015 at Assosa General Hospital in Ethiopia. The presence of stool antigen of *H. pylori* was determined against anti-*Helicobacter pylori* antibody conjugated with colloid gold nitrocellulose membrane strip and a structured face-to-face interview was also administered to assess risk factors for *H. pylori* infection. Among a total of 230(115 dyspeptic and 115 non-dyspeptic) study participants, overall 112(48.7%) antigens of *H. pylori* were detected. The prevalence of *H. pylori* was significantly associated with which gender in both dyspepsia [AOR=2.33, 95% CI: 1.13-5.86), p=0.023] and non-dyspepsia adults [AOR=1.07, 95% CI: 1.01- 3.83, p=0.035] (33).

A case control study was conducted between December 2010 to February 2011 on a total of 106 patients at Hawassa Teaching and Referral Hospital, South Ethiopia. Of the total 106 participants, 54 (51%) were male and 52(49%) female with mean age 32 years, range 18-75 years. Of these the seropositivity for *Helicobacter pylori* infection was found in 37(70%) of 53 dyspeptic patients (95% CI, 55.7% - 81.7%) and 29(54%) of 53 non dyspeptic participants (95% CI, 40.4% - 68.4%) p >0.05). The seroprevalence in participants that have family size > 5 was 71.4 %(45/63) and 48.8 %(21/43) for family size < 5 (AOR=2.6 (3.97- 7.127) p<0.05) (34).

3. OBJECTIVES

3.1 GENERAL OBJECTIVE

- To assess the magnitude of *helicobacter pylori* infection among peptic ulcer diseases pateints of two private health facilites : evedence of stool antigen test and staining of biopsy materials.

3.2 SPECIFIC OBJECTIVE

- To determine the magnitude of *H. pylori* infection using different methods (endoscopic method, stool *H.Pylori* antigen test and different staining methods).
- To compare the yield of different technique or effective of *Helicobacter Pylori* infection.
- To assess the associated factors for *H. pylori* infection.

4. HYPOTHESES OF THE STUDY

- *H.pylori* infection is prevalent in patients having upper gastrointestinal endoscopy for clinically suspected PUD in the study area.

5. MATERIAL AND METHOD

5.1 STUDY AREA

The study was conducted in Landmark General Hospital, it is a private General hospital located at the center of Addis Ababa, Kirkos sub-city, woreda 06, and House no.355.Landmark General Hospital is owned by Landmark Plc. The hospital was officially inaugurated on Sene 8, 2000 E.C. / 15th June 2008 of the Gregorian/western calendar. At present Landmark general Hospital is providing health care services with full capacity in various general and specialty areas. It is located near to genet hotel kirkos sub-city wereda, 06 house no 355. Secondly Adera Internal medical specialty center, it is private health center located at Bole road, kirkos sub-city, wereda 09, and house no. new. Adera internal medical speciality center was officially inagurated on Tir 5,2001 E.C./ 13th January ,2009 of the Gregorian/western calander.At present Adera internal medical speciality center is providing health care service with full capacity in various general and speciality areas. It is located near to Flamingo area Kirkos sub-city, wereda 09.

5.2. STUDY DESIGN AND PERIOD

This was a cross-sectional study carried out from January, 2019 to October, 2019.

5.3. STUDY POPULATION

All patients with upper Gastro-Intestinal (UGI) symptoms who were visit the hospital during the study period.

5.4. INCLUSION AND EXCLUSION CRITERIA

5.4.1 INCLUSION CRITERIA

The study includes patients with suspected peptic ulcer disease. Inclusion was based on having symptoms and signs of peptic ulcer disease as judged by the attending physician and consent to participate in the study. These symptoms included burning pain in the epigastrium with or without any of the following: bloating, nausea, dark or black stool, vomiting blood, or weight loss. The patients were individually approached to obtain informed consent (or assent with legal guardian consent) and consecutively recruited into the study. A predesigned data collection questionnaire form will be used to collect both demographic and clinical data.

5.4.2 EXCLUSION CRITERIA

- Patients with documented *H.pylori* infection, history of taking triple antibiotic treatment for *H.Pylori* infection within two months before the study period.
- Patients who took antibiotics for other reasons with in past one month.
- Patients who took PPI in the prior one week.

5.5 STUDY VARIABLES

5.5.1 DEPENDENT VARIABLES:

Magnitude of *helicobacter pylori* infection among peptic ulcer diseases patients of two private health facilities: evidence of stool antigen test and staining of biopsy materials.

5.5.2 INDEPENDENT VARIABLES:

Socio demographic factors; Age, Sex, educational status, hygiene practice like hand washing, marital status, alcohol intake, family members environmental conditions (latrine, water source etc.)

5.6. SAMPLE SIZE CALCULATION AND SAMPLING METHODS

5.6.1 SAMPLE SIZE CALCULATION

According to World Gastroenterology Organization Global Guidelines, 2010 a previous study of prevalence (P) of *H. pylori* infection in Ethiopian were 90% in adult (16).Therefore the minimum sample size based on the previous studies is calculated using single proportion formula by assuming that prevalence of *H.pylori* is 90% in adults who is clinically diagnosed for pain.

$$N = \frac{Z^2 P (1-P)}{D^2}$$

Where Z= 90% confidence interval (1.96)

P = Estimated prevalence rate (90%) = (0.90)

D = Marginal of sampling error 0.05

N = minimum sample size

$$= \frac{(1.96)2 \times 0.90(1-0.90)}{(0.05)^2}$$

$$= \frac{3.8 \times 0.90\ (0.10)}{0.0025}$$

$$= 137$$

Therefore by adding 10% non-response rates, a total of 150 study subjects will be included in the study.

5.6.2 SAMPLING METHODS

Non-probability convenient sampling techniques were used. Consecutive patients having complained of upper gastrointestinal pain because of a suspicion of *H. pylori* infection or any other disease was asked to participate in this prospective study.

5.7. MEASUREMENT AND DATA COLLECTION

5.7.1. DATA AND SAMPLE COLLECTION

Data on socio-economic, demographic and behavioral characteristics of the study participants were collected using structured pre-tested questionnaire via face-to-face interview. Approximately 2 gram of stool sample was collected from each participant in a clean container. *H. pylori* stool antigen was detected by Intec one step *H.pylori* feces test kit (Guangzhou Wondfo Biotech, China) the test was processed in the laboratory within 1 hours of sample collection.

Gastric biopsy samples were taken from tissue antrum of patients referred for endoscopy that had not been on any antibiotic or eradication therapy for the past four months. Endoscopies were performed by Gastroenterologists using standard endoscopy procedures. The tissue samples collected were kept in a plastic universal bottle containing 10% formalin for histopathological examination and staining as described more in the diagnostic methods described below.

5.7.2. DIAGNOSTIC METHODS

5.7.2.1. ENDOSCOPIC BIOPSY

Principle: Gastric mucosal biopsy were taken as per the Sydney protocol, i.e. 5 pieces (1 from the antrum 2-3 cm from the pylorus, lesser curvature, 1 from the greater curvature, 1 from the

corpus 8 cm from the cardia lesser curvature, 1 from the corpus 8 cm from the cardia greater curvature, 1 from the angularis) in the diagnosis of *H.pylori* infection via histologic examination. For prevention of bacterial contamination from other sites and false positive results, the forceps and endoscope used were high level disinfected (soaked with enzymatic solution, clean with distilled water and then cleaned with CIDEXOPA before next use.)

Interpretation of Endoscopy

- Esophagitis: is infection of the esophagus.
- Gastritis: is infection of the stomach.
- Peptic ulcer: endoscopically perceptible mucosal defect with or without complications.

5.7.2.2 *HELICOBACTER PYLORI* STOOL ANTIGEN

Principle: It is a chromatographic immunoassay for the qualitative determination of *H.pylori* antigen in human feces sample. When faces sample is added to sample pad, it moves through the conjugate pad and mobilizes gold anti-*H.pylori* conjugate that is coated on the conjugate pad. The mixture moves along the membrane by capillary action and reacts with anti-*H.pylori* that is coated on the test region. If *H.pylori* is present, the result is the formation of acolored band in the test region. If there is no *H.pylori* in the sample the area will remain colorless. The sample continues to move to the control area and forms a pink color, indicates the test is working and the result is valid.

Stool samples requested from each participating was collected in airtight containers at the time of the encounter, at any time. Analysis of *H. pylori* stool antigen test, Intec one step *H.pylori* feces test kit (Guangzhou Wondfo Biotech, China), is performed as per the manufacturer's instructions. A standard positive control test was run after every 20 tests, all of them being verified as positive. The Intec one step *H.pylori* feces test kit (Guangzhou Wondfo Biotech, China) HpSA strip is a rapid lateral flow chromatographic immunoassay that utilizes a monoclonal anti-*H.pylori* antibody as the capture and detector antibody. Approximately 1-2gmof stool was transferred into the sample diluents vial and vortexes for 15 seconds. Three drops of the specimen was applied to the test and the result was read after 15 minutes. The results were reported as positive or negative based on the manufacturer's instruction.

5.7.2.3. GIEMSA STAIN

Principle: The "neutral" dyes combining the basic dye methylene blue and the acid dye eosin, give a wide color range when staining.Methylene blue is a metachromatic dye and therefore many structures are dyed purple and not blue.

Steps of Giemsa stain

Deparaffinize the section using xylene stain with stock giemsa stain for 15 minute then differentiate the section with 95 % ethanol alcohol for 10seconds, clear the section with xylene and mount with DPX cover the section with cover slide see the *H.pylori* bacteria on 40x and 10x objective.

Result interpretation

Helicobacter pylori	-dark-blue
Mast cells	-purple-violet
Tissue elements shades of	-blue and pink

5.7.2.4. GRAM STAIN

Principle:

Bacteria's, positive and negative, cell wall is composed of peptidoglycan, (the gram-positive has a thicker wall) and both will take up the crystal violet. The gram-negative has a layer of lipopolysaccharide external to the peptidoglycan wall, which is disrupted in the acetone rinse, allowing the crystal violet to be differentiated out. This allows the gram-negative bacteria to take up the basic fuchsine stain.

Steps of Gram stain

Deparaffinize the section using xylene add crystal violet and lugol's iodine for one minute, then add acetone alcohol for 30 seconds and saffranine for 3mintes clear the section with xylene see the *H.pylori* bacteria on 100x objective.

Result interpretation

Gram-positive bacteria	-blue
Gram-negative bacteria	-red
Nuclei	-red
Background	-yellow

5.8. DATA QUALITY CONTROL

The stool samples were tested according to the manufactures instruction. And all quality issue will maintained by using standard operating procedure in detection of *H.pylori* Ag in stool sample during pre-analytical, analytical and post analytical stages.

In order to ensure quality of the data, proper training was given to data collectors. Each of the Questionnaires will check whether the necessary information will be properly filled.

The test result was examined independently with two experienced laboratory technologist and finally checked by the principal investigator.

5.9. DATA ANALYSIS, INTERPRETATION AND WRITE UP

Information from the laboratory analysis was entered into SPSS, version.20. Statistical test were used to estimate odds ratio (ORs) with 95% confidence interval (CI) of positive responses to the different risk factors. Comparison between groups was compared and a *P*-value of < 0.05 will considered significant.Findings will be tabulated, calculated, described using graphs and written up for publication and presentation. The combination of the three tests, namely, *Helicobacter pylori* stool antigen test, gram stain and giemsa stain is used as a gold standard. If one of the three tests is positive, the test is considered as positive for *Helicobacter pylori*, if all the tests are negative at the same time, it is considered as negative. Based on this findings, the sensitivity, specificity, predictive values of individual tests were determined.

5.10. OPERATIONAL DEFINITION

Gastroenteritis- means inflammation of the stomach and intestine categorized as vomiting with or without diarrhea, or diarrhea alone.

Dyspepsia-Defined as pain associated with the stomach or upper abdomen. Ulcers are one cause of dyspepsia, but many patients have no evidence of gastric damage.

***Helicobacter pylori*-** Bacterium that causes stomach inflammation (gastritis) and ulcers in the stomach and duodenum.

Peptic ulcer disease-Upper abdominal pain or discomfort is the most prominent symptom in patients with peptic ulcer.

5.11. ETHICAL CLEARANCE

An ethical review committee of the Department of Clinical Laboratory Sciences, School of Allied Health Sciences, College of Health Science and Addis Ababa University was approved

this study with an ethical letter. A written permission was obtained from Landmark General Hospital and Adera internal medical specialty center. Names and any other sensitive personal information of individual study subject was not record during sample collection. Moreover the sample collectors were laboratory professional working in the laboratory department of the Landmark General Hospital and Adera internal medical specialty center Addis Ababa Ethiopia and were being monitored daily by the principal investigator. Sample was collected after getting consent/assent from the participant. The confidentiality of the test result of the study was kept by investigator.

5.12. DISSIMINATION OF THE RESULT

This study only completion could serve as reference material to researcher, experts, or policy maker for intervention. To reach this body the finalized paper were submitted to college of health science department of medical laboratory AddisAbaba University. So it can serve as reference library in addition the copy of this material was given to landmark general hospital.

The result will also be disseminated through publication in peer-reviewed local and internal journals presenting in relevant workshops in the seminar.

6. RESULT S

6.1. Socio-demographic characteristics of study participants:

A total of 150 peptic ulcer patients were included in the study. About 84 (56.0%), 89(59.3%), and 111(74.0%), of the participants were male, urban residents and married respectively. About 50(33.3%) and 41(27.3%) of the study participants were 46-60 years old and in the age ranges of 61-80 years respectively. Of the total participants, 38(25.3) and 70(46.7%) were read and write and above secondary school respectively. With regard to the family size, 84(56.0%) of them were living within the family having 1-4 members (Table 2).

Table 2: Socio-demographic characteristics of Peptic Ulcer patients attending at Landmark General Hospital and Adera internal medical speciality center outpatient Department, January, 2019-October, 2019.

Variables		**Frequency**	**Percent**
Sex	Male	84	56.0%
	Female	66	44.0%
Age	18-25	22	14.7%
	26-45	37	24.7%
	46-60	50	33.3%
	61-80	41	27.3%
Residence	Urban	89	59.3
	Rural	61	40.7%
Marital status	Married	111	74%
	Unmarried	39	26%
Educational status	Unable to read & write	17	11.3%
	Read and write	38	25.3%
	Primary & secondary school	25	16.7%
	Above secondary school	70	46.7%
Family size	1-4	84	56%
	5-6	58	38.7%
	>6	8	5.3%

6.2. Behavioral and hygiene practices of study participants:

Considering lifestyle and hygiene, 78(52.0%) of the participants had been drinking alcohol; of those only 77(51.3%) had been drinking more than three times per week. Nearly 126(84.0%) of the subjects had habit of a hand washing both before meal and after visiting toilet, while the remaining 24(16.0%) were practicing either before meal or after visiting toilet (Table 3).

Table 3: Life style and hygiene related factors of peptic ulcer patients attending at Landmark General Hospital and Adera internal medical speciality center outpatient Department, from January, 2019-October, 2019.

Variables		**Frequency**	**Percent**
Alcohol drinking habit	Yes	78	52.0%
	No	72	48.0%
Frequency of alcohol intake	Not drunk	73	48.7%
	1-3 times/week	77	51.3%
Type of alcohol	Not drunk	72	48.0%
	Local alcohol	30	20%
	Fabricated alcohol	36	24.0%
	Both local & fabricated	12	8.0%
Hand washing habit	Either before meal or after toilet	24	16.0%
	Both before meal & after toilet	126	84.0%

6.3. Magnitude of *H.pylori* infection with respect to socio-demographic characteristics

The study showed that high magnitude of *H.Pylori* infection was found in study participants who were male, 36(43%), 22(44%) of participant with age 46-60 years were positive for *H.pylori*, age group of 46-60 years, 52(58%) of participant were *H.pylori* positive were live in rural and 45(41%) of participant were *H.pylori* positive were married. Lower magnitude was observed among participants aged 26-45 years (Table 4).

Table 4: Magnitude of *H.pylori* with respect to socio-demographic characteristics of study participants attending at Landmark General Hospital and Adera internal medical speciality center outpatient Department, from January, 2019-October, 2019.

Variables		***H. pylori* status**		**Total (%)**	**P value**
		Infected%	**Non infected%**		
Sex	Male	36(43%)	48(57%)	84(56%)	0.001
	Female	25(38%)	41(62%)	66(44%)	
Age	18-25	14(64%)	8(36%)	22(15%)	
	26-45	10(27%)	27(73%)	37(25%)	0.042
	46-60	22(44%)	28(56%)	50(33%)	0.002
	61-80	15(37%)	26(63%)	41(27%)	
Residence	Urban	37(61%)	24(39%)	61(41%)	
	Rural	52(58%)	37(42%)	89(59%)	0.002
Marital status	Married	45(41%)	66(59%)	111(74%)	0.003
	Unmarried	16(41%)	23(59%)	39(26%)	
Educational status	Unable to read & write	8(47%)	9(53%)	17(11%)	0.014
	Read and write	15(39%)	23(61%)	38(25%)	0.014
	Primary & secondary school	15(60%)	10(40%)	25(17%)	0.014
	Above 2ndschool	23(46%)	47(94%)	50(33%)	
Family size	1-4	31(37%)	53(63%)	84(56%)	
	5-6	27(47%)	31(53%)	58(39%)	0.014
	>6	3(38%)	5(63%)	8(5%)	0.014

6.4. Magnitude of *H.pylori* with respect to hygiene practices of study participants

The magnitude of the infection was higher in participants who take alcohol 35(45%), and the frequency of alcohol intake 1-3 times per week 35(45%). (Table 5)

Table 5: Magnitude of *H.pylori* with respect to socio-demographic characteristics of study participants attending at Landmark General Hospital and Adera internal medical speciality center outpatient Department, from January, 2019-October, 2019.

Variables		*H. pylori* status		Total (%)	P value
		Infected%	Non infected%		
Alcohol drinking habit	Yes	35(45%)	43(55%)	78(52%)	0.089
	No	26(36%)	46(64%)	72(48%)	1
Type of alcohol	Not drunk	26(36%)	46(64%)	72(48%)	
	Local alcohol	10(33%)	20(67%)	30(20%)	0.775
	Fabricated alcohol	21(58%)	15(42%)	36(24%)	1.000
	Both local & fabricated	4(33%)	8(67%)	12(8%)	2.800
Frequency of drinking	Not drunk	26(36%)	47(64%)	73(49%)	
	1-3 times/week	35(45%)	42(55%)	77(51%)	0.000
Hand washing habit	Either before meal or after toilet	12(50%)	12(50%)	24(16%)	
	Both before meal & after toilet	49(39%)	77(61%)	126(84%)	1.703

Out of 150 peptic ulcer patients, histological examination showed, chronic Gastritis in 24 (43.6%) patients and all were positive for *H. pylori* infection. Among 22 patients with adenocarcinoma, 14(25.5 %) were *H. pylori* stool antigen positive. and the association between presence of adenocarcinoma and *H. pylori* infection was statistically significant ($P < 0.001$). However, *H. pylori* infection was not significantly associated with gastritis ($P > 0.005$). According to the endoscopic examination 42 of them (76.4%) had chronic gastritis and *H.pylori* stool antigen positive ($P< 0.005$).

Based on gram and giemsa staining methods among 45(81.8%) peptic ulcer disease patients were positive for *H.pylori* infection as demonstrated by the presence of gram negative bacteria (Table 6).

Table 6: Yield of *H.pylori* infection with different diagnostic techniques of study participants attending at Landmark General Hospital and Adera internal medical speciality center outpatient Department, from January, 2019-October, 2019.

Pathology report	*H.pylori* status		Total N%	P- value
	Positive(55)	Negative(95)		
Adenocarcinoma	14(25.5%)	8(8.4%)	22(14.7%)	0.001
Chronic gastritis	24(43.6%)	26(27.4%)	50(33.3%)	0.002
Gastritis	6(10.9%)	24(25.3%)	30(20.0%)	0.035
Duodenitis	11(20.0%)	37(38.9%)	48(32.0%)	0.001
Endoscopy report				
Chronic Gastritis	42(76.4%)	51(53.7%)	93(62.0%)	0.003
Duodenitis	12(21.8)	36(37.9%)	48(32.0%)	0.002
Malignancy	1(1.8%)	8(8.4%)	9(6.0%)	0.024
Gram stain result				
Positive	45(81.8%)	24(25.3%)	69(46.0%)	0.002
Negative	10(18.2%)	71(74.7%)	81(54.0%)	0.001
Giemsa stain result				
Positive	45(81.8%)	24(25.3%)	69(46.0%)	0.002
Negative	10(18.2%)	71(74.7%)	81(54.0%)	0.001

From the combined methods, stool antigen test has sensitivity and specificity of 70.5% and 100% respectively and gram stain and giemsa stain each has sensitivity and specificity of 88.5% and 100 % respectively. (Table7).

Table 7: Summary of combined methods of *H.pylori* infection with different diagnostic techniques of study participants attending at the two private health facilities outpatient Department, from January, 2019-October, 2019.

Methods		Positive	Negative	**Sensitivity (%)**	**Specificity (%)**	**PPV (%)**	**NPV (%)**	**TE (total error) (%)**
Stool antigen test	Positive Negative	55 23	0 72	70.5%	100%	100%	75.8%	84.7%
Gram / Giemsa stain	Positive Negative	69 9	0 72	88.5%	100%	100%	88.9%	94%
Total		78	72					

7. DISCUSSION

The overall prevalence of *H. pylori* infection among suspected peptic ulcer patients attending the outpatient department of Landmark General Hospital and Adera internal medical specialty center was 52%, this finding was lower than other results reported in Ethiopia and elsewhere; 55- 70% in Bair Dar (35), Gondar (36); 66.1% in Cameron (37); 66.1% in south Africa (38) and 53-64.8% in Iran (39). 83.3% in hawassa (40); 81-89% in Addis Ababa (41, 42); 85.6% in Gondar (43) and elsewhere in the world, 66% in Kenya (44).

Further, the present finding was found to be higher than the figures reported in Greenland 43% (45) and Canada 29.4% (46).The difference in prevalence of *H. pylori* infection may be attributed to differences in study area, subjects, sample size, personal hygienic condition and variations in the socio-economic status of the study subjects as well as difference in the sensitivity and specificity of testing methods.

In the current finding, a significant association was observed between *H. pylori* infection and rural residence compared to urban which was in line with previous studies in Ethiopia (43) and Turkey (47).Higher prevalence of *H. pylori* infection in rural residents may be attributed to factors related to the lack of safe water supply and hygiene condition in the rural part of the country. The lower the family income was the higher significantly associated with prevalence of *H. pylori* observed in the current study contrary to a study in China (48) and Benin (49).

The prevalence *H. pylori* was associated with marital status of participants ($p< 0.05$) which is in line with other studies in Ethiopia (43,50) and China (48), but different from other study in Northwest Ethiopia (51), in these case marital status was associated with prevalence of *H. pylori*. There was no statistically significant difference in the prevalence of *H. pylori* with respect to number of family in the household which is parallel to other studies in Ethiopia (50, 51), Brazil (52) and Benin (49), but this study is different from other studies elsewhere (44, 53).

According to this study, marital status statistically associated with *H. pylori* infection, which is comparable with studies done in Gondar, Northwest Ethiopia (42), Ontario, Canada (53) and Yangzhong city, China (48). Contrary to our study, a study done in Lanyu Island, Taiwan (49) revealed that married individuals had shown significantly higher odds of *H.pylori* infection than

unmarried ones. Evidences showed that marital status has shown to have an impact on transmission of *H.pylori*. These evidences suggested that interfamilial transmission plays an important role in spread of *H.pylori* particularly in developing countries (53, 54). Authors described that individuals who were married to families with gastric ulcer and lived with infected partners increased the risk of *H.pylori* infection (55).

There are contrasting reports on the association between alcohol consumption and prevalence of *H. pylori*. In this study there was no statistical association between alcohol consumption and *H. pylori* infection ($p>0.05$) which is similar to other studies in Thailand (56), South Africa (57) China (58) and Ethiopia (59). The absence of association in this study might be due to less number of alcohol users, the type and amount of alcohol consumed has effect on the association. But this study is inconsistent with other studies done in Ethiopia (44, 61).The reason for this contradictory result might be due to the difference in the type of alcoholic beverages consumed and the life time history of alcohol consumption.

There was no statistically significant difference in the prevalence of *H. pylori* with respect to number of family in the household which is parallel to other studies in Ethiopia (61, 59), Brazil (62) and Benin (63), but this study is different from other studies elsewhere (64, 65).

In this study, chronic gastritis and duodenitis were found to be the most common endoscopic abnormalities. Moreover, this study also demonstrated the association between *H. pylori* infection and chronic gastritis and duodenitis is statistically significant as also reported by (66). There is ample evidence regarding the beneficial role of *H. pylori* eradication therapy in patients with peptic ulcer disease and gastritis (67, 68).

Thus, clinicians should test and treat for the infection if resources are available. In resource-poor settings where confirmatory test is not available or may not be cost-effective, empirical therapy is recommended (World Gastroenterology Guidelines, 2010) (69).

In the present study, among 150 peptic ulcer patients with chronic gastritis with variable degree of activity, we found 42 (76.4%) positive results for *helicobacter pylori* and we found 45 (81.8%) positive results for *helicobacter pylori* by Giemsa stain and Gram stain.

From the combined method stool antigen test the sensitivity and specificity, 70.5% and 100% respectively and on gram stain and giemsa stain the sensitivity and specificity, 88.5% and 100% respectively in the diagnosis of *H. pylori* infection.Gram stain and giemsa stain had a significantly higher sensitivity than stool antigen with sensitivity of 88.5%, However the specificity of Gram stain giemsa stain and stool antigen 100%. On both tests were false negative result observed.

8. STRENGTH AND LIMITATION OF THE STUDY

8.1 STRENGTH

We have included both endoscopic and histopathology examination of the gastric biopsy materials which is rarely included in *H. pylori* research.

8.2 LIMITATION

The limitation of this study is that it is a hospital-based study. Since the infection is mostly symptomless, patients who did not visit the hospital, not willing full to diagnose on endoscopy and difficult to get endoscopy sample for gram stain and giemsa stain.

Only an immunological stool antigen test method was used for the detection of *H. pylori*, which probably underestimated the prevalence of *H.Pylori.*

9. CONCLUSION AND RECOMMENDATION

9.1. CONCLUSION

- The magnitude of *H.pylori* infection is 52%.This is high indicating that it is a public health problem in the study area. Community-based studies should also be conducted in the general population to understand the burden of the disease since the infection is usually asymptomatic.
- Our results indicated that a majority of the peptic ulcer patients were older people of age 46-60years.
- In conclusion the high positive predictive value of 100% (P value = 0.002) of the modified Giemsa stain and Gram stain should be recommended in studying *H.pylori* infection.

9.2. RECOMMENDATIONS

- Mostly stomach ulcer is caused by *H.pylori* bacteria to know the cause of ulcer whether bacterial or not, both giemsa and gram stain test is parallel to histopathological examination seems vital.
- These findings strengthen the action to implement the control and prevention of *H.pylori* infection more effectively to prevent gastric cancer and other related complications in Ethiopia.
- Although the trend of infection showed a decreasing pattern; appropriate use of eradication therapy, health education primarily to improve knowledge and awareness on the transmission dynamics of the bacteria, behavioral changes, adequate sanitation, population screening and diagnosis using multiple tests are required to reduce *H.pylori* infections.
- Giemsa stain and Gram stain parallel ordered with histopathological examination because it is easy to use, inexpensive, and provides consistent results.

10. REFERENCES

1. Brown LM. *Helicobacter pylori*: epidemiology and routes of transmission. Epidemiologic reviews. 2000;22(2):283–297.

2. Marshall BJ, Warren JR. Unidenified curved bacilli on gastric epithelium in active chronic gastritis.Lancet.1983; i: 1273.

3. Go MF. Review article: natural history and epidemiology of *Helicobacter pylori* infection. Aliment PharmacolTher. 2002;16:3–15.

4. Brown LM. *Helicobacter pylori* epidemiology and routes of transmission. Epidemiol Rev. 2000;22:283–97.

5. Megraud F. Epidemiology of *Helicobacter pylori* infection. In: Rathbone BJ, Heatley RV, editors. *Helicobacter pylori* and gastrointestinal disease. Oxford: Blackwell Scientific; 1992. 107–23.

6. Bardhan PK. Epidemiological features of *Helicobacter pylori* infection in developing countries. Clin Infect Dis. 1997;25:973–8.

7. Cheng H, Hu F, Zhang L, Yang G, Ma J, Hu J, et al. Prevalence of *Helicobacter pylori* infection and identification of risk factors in rural and urban Beijing, China. Helicobacter.2009;14:128–33.

8. Axon AT. The transmission of *Helicobacter pylori*: which theory fits the facts? Euro J GastroenterolHepatol.1996;8:1–2.

9. Frenck RW, Jr, Clemens J. Helicobacter in the developing world. Microbes Infect. 2003;5:705–13.

10. Smith JG, Li W, Rosson RS. Prevalence, clinical and endoscopic predictors of *Helicobacter pylori* infection in an urban population.Conn Med. 2009;73:133–7.

11.T. N. A. Archampong, R. H. Asmah, E. K. Wiredu, R. K. Gyasi, K. N. Nkrumah, and K. Rajakumar, et al. “Epidemiology of *Helicobacter pylori* infection in dyspeptic Ghanian patients,” Pan African Medical Journal,2015; 20:178.

12. El Dine SS, Mubarak M, Salama R, El Raziky M, El Sherbily E, Zakzria S, *et al*. Low seroprevalence of anti-CagA antibodies in spite of high seroprevalence of anti-*H pylori* antibodies in rural Egyptian community. Res J Med MedSci 2008;3:118-23.

13. Secka O, Antonio M, Tapgun M, Berg DE, Bottomley C, Thomas V, *et al.* PCR-based genotyping of *Helicobacter pylori* of Gambian children and adults directly from biopsy specimens and bacterial cultures. Gut Pathog 2011; 3:5.

14. Baako BN, Darko R. Incidence of *Helicobacter pylori* infection in Ghanaian patients with dyspeptic symptoms referred for upper gastrointestinal endoscopy. West Afr J Med 1996; 15:223-7.

15.Asrat D, Nilsson I, Mengistu Y, Ashenafi S, Ayenew K,. Prevalence of *Helicobacter pylori* infection among adult dyspeptic patients in Ethiopia. Ann Trop Med Parasitol, 2004; 98(2): 181-189.

16. J. G. Kusters, A. H. M. Van Vliet, and E. J. Kuipers, "Pathogenesis of Helicobacter pylori infection," Clinical Microbiology Reviews,2006; 19,3: 449–490.

17. M. Safavi, R. Sabourian, and A. Foroumadi, "Treatment of Helicobacter pylori infection: current and future insights," World Journal of Clinical Cases,2016; 4:1: 5–19.

18.Dr. David I Campbell. Gastric cancer and Helicobacter pylori: a combined analysis of 12 case control studies nested within prospective cohorts. Gut 2001; 49:347 53.

19. Mitipat N, Siripermpool P, Jadwattanakul T, Chaunthongkum S. The prevalence of *Helicobacter pylori* infection in patients with gastrointestinal symptoms in Chon Buri, Thailand. *Southeast Asian J Trop Med Public Health* 2005; 36(2):341-346.

20. Chey WD, Wong BCY. American College of Gastroenterology Guideline on the Management of *Helicobacter pylori* infection. *Am J Gastroenterol* 2007;102:1808-1825.

21. Malfertheiner P, Megraud F, O'Morain C, Bazzoli F, El-Omar E, Graham D, et al. Current concepts in the management of Helicobacter pylori infection: the Maastricht III Consensus Report. Gut. 2007; 56(6):772-781.

22. Graham DY[1], Lew GM, Malaty HM, Evans DG, Evans DJ Jr, Klein PD,et.al. Factors influencing the eradication of *Helicobacter pylori* with triple therapy. 1992 Feb;102(2):493-6.

23. Hooi J.K.Y, Lai W.Y., Ng W.K., "Global prevalence of Helicobacter pylori infection: systematic review and meta-analysis," Gastroenterology, 2017; 153: 2: 420–429.

24. Go MF. Review article: natural history and epidemiology of *Helicobacter pylori* infection. Aliment PharmacolTher. 2002;16:3–15.

25. Brown LM. *Helicobacter pylori* epidemiology and routes of transmission. Epidemiol Rev. 2000; 22:283–97.

26. Niknam R, Seddigh M, Fattahi MR, Dehghanian A, Mahmoudi L. Prevalence of Helicobacter pylori in Patients With Dyspepsia, Jundishapur J Microbiol. 2014; 7(10):131-137.
27. Hunt RH, Xiao SD, *Helicobacter pylori* in developing countries, world gastroenterology organization global guideline. J Gastrointestinal Liver Dis. 2011; 20(3):299–304.
28. Debabrata Majumdar, James Bebb, John Atherton. *Helicobacter pylori* infection and peptic ulcers, Gastroenterology/General Medicine at Nottingham University Hospitals NHS Trust, UK, 2007; 35:204-209.
29. Abiodun Christopher Jemilohun, Jesse A Otegbayo, Abideen O Oluwasola, Samuel Ola. Pan African Medical Journal 6(1) · September 2010 *with* 490 Reads.
30.Abiodun Christopher Jemilohun[1,&,] Jesse Abiodun Otegbayo[2], Samuel Olawale Ola, Olayiwola Abideen Oluwasola[3], Adegboyega Akere[2], Prevalence of helicobacter pylori among Nigerian patients with dyspepsia in Ibadan. Volume 34 (September - December 2019).
31.Qureshi H, Ahmed W, Syed S. *Helicobacter pylori* clearance and its eradication in duodenal ulcer patients. J Pak Med Assoc 1995;45:2-5.
32. Bashir MT, Ali BU. Peptic ulcer disease and *Helicobacter pylori* infection at Kano, Nigeria. Internet J Gastroenterol 2009;8:1-3.
33.Tebelay Dilnessa1, Muluwas Amentie, Prevalence of Helicobacter pylori and risk factors among dyspepsia and non-dyspepsia adults at Assosa General Hospital, West Ethiopia: A comparative study. October,2017;31(1).
34.Alem Alemayehu, Seroprevalence of Helicobacter pylori infection and its risk factors among adult patients with dyspepsia in Hawassa Teaching and Referral Hospital, South Ethiopia, june,2011.
35. Desie Kasew,[1,]Ashenafi Abebe,[1]Ufaysa Munea,[1]Tekalign Deressa,[2] and Yalewayker Tegegne,. Ethiopia journal of health science, 2017 Nov; 27(6): 571–580.
36.Tadege T, Mengistu Y, Desta K, Asrat D. Seroprevalence of *Helicobacter pylori* Infection in and its Relationship with ABO Blood Groups. *Ethiop. J. Health Dev*2005;19(1):55-59.
37.Mathewos B, Moges B, Dagnew M. Sero-prevalence and trend of *Helicobacter pylori* infection in Gondar University Hospital among dyspeptic patients, Gondar, northwest Ethiopia. *BMC Research Notes* 2013;6:346.

38. Ndip RN, Malange TAE, Ojongokpoko JEA,. *Helicobacter pylori* isolates recovered from gastric biopsies of patients with gastro-duodenal pathologies in Cameroon: current status of antibiogram. Trop Med Int Health 2008;13(6):848-854.

39.Dube C, Nkosi TC, Clarke AM, Mkwetshana N, Green E, Ndip RN, et al. *Helicobacter pylori* in an asymptomatic population of Eastern Cape Province, South Africa: Public health implication. Rev Environ Health 2009;24 (3):249-255.

40.Jafarzadeh A, Ahmedi-Kahanali J, Bahrami M, TaghipourZ. Sero-prevalence of anti-*Helicobacter pylori* and anti-CagA antibodies among healthy children according to age, sex, ABO blood groups and Rh status in southeast of Iran. *Turk J Gastroenterol*2007;18(3):165-171.

41.Tadesse E, Daka D, Yemane D, Shimelis T. Sero-prevalence of *Helicobacter pylori* infection and its related risk factors in symptomatic patients in southern Ethiopia. *BMC Research Notes* 2014;7:834.

42.Asrat D, Nilsson I, Mengistu Y, Ashenafi S, Ayenew K, Al-Soud WA, et al. Prevalence of *Helicobacter pylori* infection among adult dyspeptic patients in Ethiopia. *Ann Trop Med Parasitol*2004;98(2):181-189.

43. Desta K, Asrat D, Derbie F. Sero-prevalence of *Helicobactor pylori* infection among health blood donors in Addis Ababa, Ethiopia. *Can J Gastroenterol*2007;21:501-506.

44.Moges F, Kassu A, Mengistu G, Adugna S, Andualem B, Nishikawa T, et al. Sero-prevalence of *Helicobacter pylori* in dyspeptic patients and its relationship with HIV infection, ABO blood groups and life style in a university hospital, Northwest Ethiopia. *World J Gastroenterol*2006;12(12):1957-1961.

45.Shmuely H, Obure S, Passaro DJ, Abuksis G, Yahav J, Fraser G, et al. Dyspepsia Symptoms and *Helicobacter pylori* Infection, Nakuru, Kenya. *Emerging Infectious Diseases* 2003;9(9):1103-7.

46.Koch A, Krause TG, Krogfelt K, Olsen OR, Fischer TK, Melbye M, et al. Sero-prevalence and Risk Factors for *Helicobacter pylori* Infection in Greenlanders. *Helicobactor,* 2005;10(5): 433–342.

47. Naja F, Kreiger N, Sullivan T. *Helicobacter pylori* infection in Ontario: Prevalence and risk factors. *Can J Gastroenterol*2007;21 (8):501-506.

48. Seyda T, Derya C, Fusun A, Meliha K. The relationship of *Helicobacter pylori* positivity with age, sex and ABO/Rhesus blood groups in patients with gastrointestinal complaints in Turkey. *Helicobacte*r 2007;12(3):244-250.

49.Shi R, Xu S, Zhang H, Ding Y, Sun G, Huang X, et al. Prevalence and Risk Factors for *Helicobacter pylori* Infection in Chinese Populations.*Helicobacter*2008;13:157-165.

50. Aguemon DB, Struelens JM, Massougbodji A, Ouendo ME. Prevalence and risk-factors for *Helicobacter pylori* infection in urban and rural Beninese populations. *ClinMicrobiol Infect* 2005; 11: 611-617.

51. Kebede W, Mathewos B and Abebe G. *Helicobacter pylori* Prevalence and its Effect on CD4+ Lymphocyte Count in Active Pulmonary Tuberculosis Patients at Hospitals in Jimma, Southwest Ethiopia. *International Journal of Immunology*2015;3(1):7-13.

52. Abebaw W, Kibret M, Abera B. Prevalence and Risk factors of *H. pylori* from Dyspeptic patients in Northwest Ethiopia: A Hospital Based Cross-sectional Study. *Asian Pac J Cancer Prev* 2014;15 (11):4459-4463.

53.Lyra CA, Santana G, Santana N, Silvany-Neto A, Magalhaes E and Pereira ME, et al. Sero-prevalence and Risk Factors associated with *Helicobacter pylori* Infection in Blood Donors in Salvador, Northeast-Brazil. *Brazilian Journal of Infectious Diseases* 2003;7(5):339-345.

54. Rodrigues MN, Queiroz DM, BezerraFilho JG, Pontes LK, Rodrigues RT, Braga LL, et al. Prevalence of *Helicobacter pylori* infection in children from an urban community in North-east Brazil and risk factors for infection. *Eur J Gastro enterolHepatol*2004;16(2):201-205.

55. Nurgalieva ZZ, Malaty HM, Graham DY, Almuchambetova R, Machmudova A, Kapsultanova D, et al. *Helicobacter pylori* infection in Kazakhstan: Effect of water source and household hygiene. *Am. J. Trop. Med. Hyg*2002;67(2):201-206.

56. Klein PD, Graham DY, Gaillour A, Opekun AR, Smith EO. Water source as risk factor for *Helicobacter pylori* infection in Peruvian children.*Lancet*1991;337:1503-1506.

57. Mitipat N, Siripermpool P, Jadwattanakul T, Chaunthongkum S. Prevalence of *Helicobacter pylori* infection in patients with gastrointestinal symptoms in Chon Buri, Thailand. Southeast Asian J Trop Med Public Health 2005; 36(2):341-346.

58. Tanih NF, Okeleye BI, Ndip LM, Clarke AM, Naidoo N, Mkwetshana N, et al. *Helicobacter pylori* prevalence in dyspeptic patients in the Eastern Cape province- race and disease status. S Afr Med J 2010;100:734-737.

59. Shi R, Xu S, Zhang H, Ding Y, Sun G, Huang X, et al. Prevalence and Risk Factors for *Helicobacter pylori* Infection in Chinese Populations. Helicobacter 2008;13:157-165.

60. Kebede W, Mathewos B and Abebe G. *Helicobacter pylori* Prevalence and its Effect on CD4+ Lymphocyte Count in Active Pulmonary Tuberculosis Patients at Hospitals in Jimma, Southwest Ethiopia. International Journal of Immunology2015;3(1):7-13

61. Abebaw W, Kibret M, Abera B. Prevalence and Risk factors of *H. pylori* from Dyspeptic patients in Northwest Ethiopia: A Hospital Based Cross sectional Study. Asian Pac J Cancer Prev 2014;15 (11):4459-4463.

62. Lyra CA, Santana G, Santana N, Silvany-Neto A, Magalhaes E and Pereira ME, et al. Sero-prevalence and Risk Factors associated with *Helicobacter pylori* Infection in Blood Donors in Salvador, Northeast Brazil. Brazilian Journal of Infectious Diseases 2003;7(5):339-345.

63. Aguemon DB, Struelens JM, Massougbodji A, Ouendo ME. Prevalence and risk-factors for *Helicobacter pylori* infection in urban and rural Beninese populations. ClinMicrobiol Infect 2005; 11: 611-617.

64. Shmuely H, Obure S, Passaro DJ, Abuksis G, Yahav J, Fraser G, et al. Dyspepsia Symptoms and Helicobacter pylori Infection, Nakuru, Kenya. Emerging Infectious Diseases 2003;9(9):1103-7.

65. Rodrigues MN, Queiroz DM, BezerraFilho JG, Pontes LK, Rodrigues RT, Braga LL. Prevalence of Helicobacter pylori infection in children from an urban community in North-east Brazil and risk factors for infection. Eur J Gastro enterolHepatol 2004;16(2):201-205.

66. Ratnakar C. Prevalence of *helicobacter pylori* infection in disorders of the upper gastrointestinal tract in South India. Natl Med J India.1998;11:5–8.

67. Hopkins RJ, Girardi LS, Turney EA. Relationships between H.pylori eradication and reduced duodenal and gastric ulcer recurrence: A review. Gastroenterology.1996;110:1244–52.

68. Lee Y, Chen T, Chiu H, Shun C, Chiang H, Liu T, et al. The benefit of mass eradication of *Helicobacter pylori* infection: A community-based study of gastric cancer prevention. Gut.2012;62:676–82.

69. Hunt RH, Xiao SD, Megraud F, Leon-Barua R, Bazzoli F, van der Merwe S,et al. *Helicobacter pylori* in developing countries. World Gastroenterology Organization Global Guideline. 2011 Sep;20(3):299-304.

11. ANNEX LIST

I. Information sheet and Consent form (English version)

My name is Tewodros Kassahun and I am MSc student in Medical Laboratory technology at AAU. I am doing a research entitled Magnitude of *Helicobacter Pylori* infection among peptic ulcer disease pateints of two private health facilites of Addis Ababa, Ethiopia : Evedence of stool antigen test and staining of biopsy materials.

The objective of the study is to to assess the magnitude of *helicobacter pylori* infection using different testing technique among patients with suspected peptic ulcer disase .

If you agree to participate in the study, you will allow us to use the sample that you will give for your medical examination and you will be interviewed. All the data obtained will be kept strictly confidential by using only code numbers and locking the data, so that only study personnel will have access to the files. Samples will be coded and positive result will not be identified by names. Participation and not participation has no influence on the service you seek to get in the health facilities.

Participant's response: I am well informed of the study aims and procedures and that I am free to decline to be in this study, or to withdraw from it at any point and also to jump a question that feels me discomfort. My decision as to whether or not to participate in this study will have no influence on my present or future medical service.

Principal Investigator Address: Tewodros Kassahun : Department of Medical Laboratory Sciences, Collage of health sciences, Addis Ababa University, Addis Ababa, Ethiopia

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ANNEXII: ENGLISH VERSION QUESTIONNAIRE

STUDY TOOL

Questionnaire for "Magnitude of *Helicobacter Pylori* infection among peptic ulcer disease pateints of two private health facilites of : Evedence of stool antigen test and staining of biopsy materials, at Landmark General Hospital and Adera internal medical speciality center addis ababa,Ethiopia.2019"

Interviewer Name _____Questionnaire Number_____

Date of interview_____Participant Code _____

Name of Health Centre_____

The questionnaire is designed to obtain demographic information such as the age and sex of the patient, family structure, hygiene, food and feeding practices, history on chronic illnesses and antimicrobial treatment in the 5 months prior to sample collection. To describe household characteristics, number of rooms, bedrooms and toilets were described. The concept of domestic crowding will relate to the number of people in a household and sharing of a bed and bedroom. Food and feeding habits and practices will be investigated.

I socio-demographic related questions

1. Your age in year _____

2. What is your gender?

A. Male B. Female

3. What is your marital status?

A. Married B. Unmarried

4. Where do you live?

A. Urban B. Rural

5. How much is your monthly income?

A. 1000-3000 B. 4000-6000 C. 7000-9000D. 10000 and above

6. How many bedrooms do you have in the house?

A. 1-2 B. >3

7. How many family members live within the house?

A. 1–4 B. 5-6 C. >6

8. Do you smoke cigarette?

A. Yes B. No

9. Do you have alcohol drinking habit?

A. Yes B. No

10. What type of alcohol you drink?

A. Does not drunk B. Local alcohol C. Fabricated alcohol D. Both

11. How many times do you drink alcohol?

A. Do not drunk B. 1-3 times per week C. >3 times per week

12. What is your hand washing habit?

A. Either before meal or after toilet B. Both before meal and after toilet

13. What is your level of education?

A. Illiterate B. Read and write C. Primary school D. Secondary School

E. Above secondary school

14. What is your water Source for drinking?

A. Tap water B. Bottled water C. Boiled tap water

15. What type of toilet used in your house?

A. Pit latrine B. flush toilet

16. Do you have a habit of washing your hands after using toilet?

A. Yes B. No

17. Symptoms?

A. Epigastria pain B. Vomiting C. Bloating D. Weight loss

18. Endoscopic conclusion

A. Duodenal ulcer or Gastric ulcer B. Gastritis C. Duodenitis D. Malignancy

19. *H.pylori* stool Antigen test result

A. Positive B. Negative

20. Biopsy (Histology) index inflammation

A. Adenocarcinoma B. Chronic gastritis (gastric ulcer) C. Gastritis D. Duodenitis (Duodenal ulcer)

21. Gram stain A. Positive B. Negative

22. Giemsa stain A. Positive B. Negative

ANNEX III. AMHARIC VERSION CONSENT FORM

የስምምነት ፎርም

ለሁለተኛ ዲግሪ/ማስተርሰ የጨጋራ ኤችፓይሮሊ ባክተሪያ አንቲጅን ስለሚያስከትለው ካንሰር ላይ የሚደረግ ምርምር

ተመራማሪው፡- ቴዎድሮስ ካሳሁን

የምርመራ ናሙና የሚወሰድበት፡- ላንድማርክ አጠቃላይ ሆስፒታል ፤አደራ የውስጥ ደዌ ልዩ ማዕከል

የምርምሩ አስፈላጊነት፡- የጨጋራ ካንሰርን ለመከላከል

ለምርምሩ ትብብር የሚያደርጉ ታካሚዎች ሚስጥር ይጠበቃል። ታካሚዎች ለህክምና የሚሰጡት ናሙና ለምርምር እንዲውል ፈቃዳቸው ይጠየቃል ሲስማሙ የሚከተለው መረጃይሰጣሉ።

1. ቃለ መጠይቅ አድራጊው/ዋ----------

2. የቃለ መጠይ ቁከድ/ቁጥር----------

3. ቀን----------

4. የተጠያቂው/ዋ ኮድቁጥር----------

5.የጤና ተቃሞች ላንድማርክአጠቃላይ ሆስፒታል፤አደራ የውስጥ ደዌ ልዩ ማዕከልና

ANNEX IV: AMHARICVERSION OF QUESTIONNAIRE

የጨጋራ ህመሞች ምክንያት ዩሆነውን የኤችፓይሮሊ ባክተሪያ አንቲጅን በታካሚዎች ላይ ስላለው ስርጭትና የመተላለፊያ መንገዶችንና ተዛማጅ ችግሮችን ለይቶ ለማወቅ የተዘጋጀ ቃለ መጠይቅ።

1.እድሜዎት ስንት ነው_____

2. ጾታ ሀ. ወንድ ለ.ሴት

3. የጋብቻ ሁኔታ ሀ. ያገባ ለ. ያላገባ

4. የትነው ሚኖሩት ሀ. ከተማ ለ. ገጠር

5. ወርኃዊ ገቢዎ ስንት ነው ሀ. 1ሺ-3ሺ ለ.4ሺ-6ሺ ሐ.7ሺ-9ሺ መ.ከ10ሺበላይ

6. በቤት ውስጥ ስንት መኝታ ክፍሎች አሉ ሀ.1-2 ለ. ከ 3 በላይ

7.በቤት ውስጥ ስንት የቤተሰብ አባላት አሉ ሀ.1-4 ለ.5-6 ሐ.ከ 6 በላይ

8. ስጋራ ያጨሳሉ ሀ. አዎ ለ. አይ

9. አልኮል የመጠጣት ልምድ አሎት ሀ. አዎ ለ. አይ

10. ምንዓይነት አልኮል ይጠቀማሉ ሀ. አልጠቀምም ለ. ባህላዊ ሐ.ዘመናዊ መ.ሁለቱንም

11. መጠጥ የመጠጣት ልምድዎ

ሀ. አልጠቀምም ለ. በሳምንትከ1-3ግዜ ሐ.ከ 3 ግዜበላይ

12. እጅን የመታጠብ ልምድዎ ምን ያህ ነው

ሀ. ከምግብ በፊት ወይም ከሽንት ቤት በኃላ ለ.ከምግብ በፊት እና ከሽንት ቤት በኃላ

13.የትምህርት ደረጃዎ እንዴት ነው

ሀ. ያልተማረ ለ.ማንበብና መጻፍ ሐ. የመጀመሪያ ደረጃ ትምህርት

መ. ሁለተኛ ደረጃ ትምህርት ሠ. ከሁለተኛ ደረጃ ትምህርት በላይ

14.ለመጠጥ የሚጠቀሙት ውሀ ምን ዓይነት ነው

ሀ. የባንባ ውሀ ለ. የታሸገ ውሀ ሐ.ፈልቶ የቀዘቀዘ የባንባ ውሀ

15. በቤት ውስጥ ምንዓይነት ሽንት ቤት ነው ሚጠቀሙት ሀ.የጉድጋድ ሽንት ቤት ለ. ዘመናዊ ሽንት ቤት

16. ሽንት ቤት ከተጠቀሙ በኃላ እጅ የመታጠብ ልምድ አለዎት ሀ. አዎ ለ. አይ

12. TEST PROCEDURES

TEST PROCEDURE FOR *HELICOBACTER PYLORI* STOOL ANTIGEN

1. Bring all reagents and sample.do not open punches until ready to perform the assay.
2. Remove the applicator stick from the diluent tubes/sample collectors. Insert and turn the stick into faces at different sites.
3. Re-insert the applicator stick into the diluent tubes/sample collectors, screw the cap and shake the tube vigorously to mix the sample well.
4. Remove the test card from the foil pouch and place on a clean dry surface.
5. Rotating clockwise to loosen the lid of diluent tubes/sample collectors cap.
6. Dispense 2 drops (100µl) of sample or control into the circular sample well on the card.
7. Interpret the test results at 15-20 minutes. Do not interpret the results after 20 minutes.

TEST PROCEDURE FOR GIEMSA STAIN

1. Deparaffinize the section using xylene (BioClear).
2. Rinse the section using distilled/demineralized water for 10 seconds.
3. Stain the section with the Giemsa solution by immersing it for 10-15 min or until an optimal level of staining is achieved. Note: Use undiluted Giemsa solution.
4. Differentiate the section using the Ethanol alcohol 95% solution for 10 seconds.
5. Rinse the section using distilled/demineralized water for 10 seconds.
6. Clear the section through with xylene and for 3 minutes.
7. Mount with appropriate medium. BioMount DPX, Cover the section with a cover glass.
8. See on 40x and 10x objective

TEST PROCEDURE FOR GRAM STAIN

1. Deparaffinize and hydrate to distilled water.
2. Place slides on staining rack, drop crystal violet stain onto tissue section, stain for 1 minute.
3. Wash in tap water.
4. Lugol's iodine, 1 minute.
5. Wash in tap water.
6. Blot sections, breath on section then quickly pour acetone over section until no color runs off.
7. Wash in tap water.
8. Place slides on staining rack, drop Safranine on tissue sections and stain 3 minutes.
9. Wash in tap water, blot gently but not completely dry.

10. Air dry; dip into xylene.
11. Drop oil and see on 100x objective.

PROCEDURE FOR ENDOSCOPY

1. The patient has an empty stomach for this test so you should not have anything to eat or drink.
2. The patient sign a consent form indicating your agreement to proceed with the test.
3. After signing the consent form you will put on a hospital gown and will remove any glasses, contacts, and dentures.
4. An IV needle will be placed into a vein in your arm or hand. Fluids and medications will be administered through this IV.
5. You will be taken into a special room for the procedure and asked to lie on your right side.
6. Monitoring devices will be placed on your skin to measure blood pressure, heart rate and blood oxygen during the procedure.
7. After you are sleepy, the doctor will place the thin flexible tube (endoscope) through the mouth guard. When you swallow he will gently advance the scope down the esophagus. A small video camera on the tip of the scope allows the doctor to see.
8. The endoscope is advanced through the pylorus (the opening between the stomach and duodenum). You may feel some slight pressure here but should not experience any pain. The first portion of your small intestine (duodenum) is then carefully examined for any abnormalities.
9. After this, any diagnostic or therapeutic maneuvers will be performed and the scope will be gently withdrawn from your mouth. The entire procedure takes 10–30 minutes.

Deceleration

The undersigned declares that this proposal complies with the regulations of the University and meets the accepted standards with respect to originality and quality. PI also agrees to accept responsibility for the scientific ethical and technical conduct of the research project and for provision of required progress reports.

M.Sc. Candidate: Tewodros Kassahun (B.Sc.)

Signature: _____

Date of submission: _____

This proposal has been submitted with our approval as advisors.

Advisor: Kassu Desta(MSc, Phd Fellow, Associate Professor)

Signature: _____

Date: _____

Place: Addis Ababa, Ethiopia.