ADDIS ABABA UNIVERSITY

COLLEGE OF HEALTH SCIENCES

DEPARTMENT OF MEDICAL LABORATORY SCIENCES

Magnitude of *Helicobacter Pylori* infection among patients with Esophageal cancer and apparently healthy individuals, at Tikur Anbesa Specialized Hospital, Addis Ababa, Ethiopia; a comparative cross-sectional study.

By: Yismu Eshetu (BSc)

Advisors: Kassu Desta (MSc, PhD candidate)
Professor Aster Tsegaye (MSc, PhD)
Dessie Abera (BSc, MSc,)
Professor Birhanu Nega (MD, Cardiothoracic surgeon)
Dr Siyasebwe Mamo (MD, Cardiothoracic surgeon)

A research thesis submitted to the Department of Medical Laboratory Sciences, College of Health Science, Addis Ababa University, in partial fulfillment of Master of Science Degree in Clinical Laboratory Sciences (Diagnostic and Public Health Microbiology).

June 2023

Addis Ababa, Ethiopia

Addis Ababa University

School of Graduate Studies

This is to certify that the thesis prepared by Yismu Eshetu entitled: "Magnitude of *Helicobacter Pylori* infection among patients with Esophageal cancer and apparently healthy individuals, at Tikur Anbesa Specialized Hospital, Addis Ababa, Ethiopia; a comparative cross-sectional study" and submitted in partial fulfillment of the requirements for Master of Science degree in Clinical Laboratory Sciences (Diagnostic and Public Health Microbiology) complies with the regulations of the University and meets the accepted standards with respect to originality and quality.

Signed by the Examining Committee:

Examiner_____ Signature _____Date _____

Examiner_____ Signature _____Date _____

Advisor_____ Signature _____Date _____

Advisor_____ Signature _____Date _____

Advisor_____ Signature _____Date _____

_____

Chairman of the Department or Graduate Program Coordinator

Acknowledgements

First, I would like to thank Almighty God and Virgin Merry for giving me the strength to face and pass the challenges in my life. Secondly, I would like to Acknowledge Department of Medical Laboratory Sciences, College of Health Sciences, and Addis Ababa University for financially supporting this study. I would like to express my deepest gratitude to my Advisors Professor Aster Tsegaye, Mr. Kassu Desta and Mr. Dessie Abera for their unreserved advice and follow-up of this research thesis development. I would also like to give my acknowledgment to Professor Birhanu Nega and Dr Siyasebwe at Black lion Hospital for their guidance through this process and discussion for the question I ask to prepare this thesis. I would like to extend my sincere thanks to Addis Ababa University College of Health Science librarian. I would especially like to thank my amazing family for the love, support, and constant encouragement. I am very much grateful for our study participants and staffs who helped a lot in data collection in general.

Contents

List of Tables

List of Figures

Abbreviation

BE	Barrettes esophagus
CagA	Cytotoxin-associated gene A
DRERC	Departmental Research and Ethics Review Committee
EC	Esophageal Cancer
EAC	Esophageal Adenocarcinoma
ESCC	Esophageal Squamous Cell carcinoma
EIA	Enzyme Immune Assay
GI	Gastrointestinal
GERD	Gastroesophageal Reflux Disease
GIT	Gastrointestinal tract
HP	*Helicobacter Pylori*
HPV	Human papilloma virus
OR	Odds ratio
UBT	Urea Breath Taste
RUT	Rapid Urease Test
TASH	Tikur Anbesa Specialized Hospital

Abstract

Background: *Helicobacter pylori* are Gram-negative bacterium that can cause diverse types of gastrointestinal tract malignancies including esophageal cancer (EC), though evidences regarding EC are conflicting. This study aimed to determine the magnitude of *H. pylori* among EC as compared to Healthy individuals.

Objective: To investigate the magnitude of *Helicobacter Pylori* infection among patients with esophageal cancer as compared to apparently healthy individuals.

Methods: a health facility-based comparative investigation was done from May 2021 to August 2022 cross-sectionally at Tikur Anbesa Specialized Hospital. Convenient sampling was used to recruit 302 (112 cases and 190 (healthy individuals) participants. Cases of EC were clinically confirmed as well as by Histological Examination. Apparently healthy individuals (staff and attendant) were taken as a comparative group. *H. pylori* stool antigen as well as serum antibody were measured following standard methods. Data analysis was done using SPSS Version 22. Odds ratio was calculated with 95% confidence interval to describe the association between *H. pylori* and esophageal cancer with level of significance set at $p<0.05$.

Results: Out of 302 study participants, 52% (157/302) were males and 91.1% (102) were residing outside Addis Ababa. Majority, 74.1 % of EC cases had squamous type tumor and 37.4% were in stage II. The magnitude of *H. pylori* among Esophageal Cancer Patient were 40.2% (45/112) and 4.2% (8/190) in Healthy individuals. H. pylori infection is more infecting the esophageal adenocarcinoma (EAC) type 48.3% (14/29) compared to ESCC which is 37.4% (31/83); highest prevalence was noted in Stage IV patients (57.1 %). In the multivariable logistic regression analysis, being in the age group 40 years and above (AOR=12.23, 95%CI=4.09-36.53), living outside Addis Ababa (AOR=18.57, 95%CI=5.76-59.8), attaining primary level education and below (AOR=17.24, 95%CI=5.31-55.96), and being positive for *H. pylori* stool antigen (AOR=20.13, 95%CI=4.79- 84.62) were significantly associated with EC after controlling for throat illness, taking alcohol, family history of throat illness and consuming hot food. **Conclusion;** Magnitude of *Helicobacter pylori* was remarkably high among Esophageal Cancer patients compared to Healthy individuals. Further study is needed and moreover, advocacy of patients on the beneficiary effect of identified factors is one strategy to prevent EC.

Key words: *H. pylori*, EC, Adenocarcinoma, SCC, Magnitude, Addis Ababa, Ethiopia.

1. Introduction

1.1. Background

Helicobacter pylori are helical shaped, gram negative bacteria that have been implicated as etiological agent in various diseases [1]. *Helicobacter pylori* bacteria were related with diverse types of benign and malignant gastrointestinal tract (GIT) diseases [2]. These bacteria can be a causative agent for cancer and is classified as a "group I carcinogen" by the International Agency for Research on Cancer [3]. All types of *H. pylori* strain produce an enzyme urease to convert urea to ammonia as well as carbon dioxide. This can change the acidic environment in to alkaline for a short time as a result the bacteria can be protected [3].

Negative relationship between *H. pylori* infection and esophageal adenocarcinoma has been reported. This could be as a result of lowered acidity through induction of atrophic gastritis and production of ammonia [4]. On the other hand, *H. pylori* infection has been shown to increase the risk of esophageal squamous cell carcinoma through inducing the synthesis of nitrosamines [4]. Esophageal cancer (EC) is considered among the most critical malignancies of the GIT [5]. It has two histological types. Esophageal squamous cell carcinoma (ESCC), is commonly related to environmental causes like smoking and alcohol intake). And the second type is esophageal adenocarcinoma (EAC), which is found near the gastroesophageal junction [5.6]. The incidence rate of Esophageal adenocarcinoma is increasing. Besides, it is associated to poor prognosis as well. As a result, EAC is gaining increased attention in recent years. Endoscopy or chest computed tomography are helpful in locating ESCC at times of narrowing of the esophageal lumen with the mass [7].

H.pylori colonizes the lower part of the esophagus. Therefore, the bacterium may exacerbate esophageal mucosal damage and be responsible for the rise of incidence of Barrettes esophagus (BE) and EAC [5]. However, the role of *H. pylori* in the development of esophageal adenocarcinoma has not been clearly established [8]. *H. pylori* infection could be a risk in causing gastroesophageal reflux disease (GERD). This process could be responsible for the occurrence of BE. Once the precancerous lesion is formed as a result of BE, esophageal cancer may ultimately occur [5,9]. These sequence of events in the pathogenesis of EAC through GERD → BE → dysplasia as well as the role played by *H. pylori* in each of the events towards EAC, in certain population groups, has been supported by evidences. Longstanding GERD leads to BE,

which a precursor lesion to EAC [6]. The cytotoxin-associated gene A (cagA) is a vital and well investigated virulence factor. In contrary to several bacterial pathogens, *H. pylori* are a life-long infection unless the patient is treated. Treatment consisted of esomeprazole 40 mg orally, amoxicillin 1g orally twice in a day, and clarithromycin 500 mg orally twice daily for up to 7–14 days [2].

1.2. Statement of the problem

Approximately 4.4 billion individuals, almost half of the global population, had been infected with *H. pylori* in the year 2015 [6]. The bacterium remains to be a common cause of morbidity as well as death. The prevalence of *H. pylori* infection varies greatly in different regions. For example, an increased prevalence is observed in Latin American countries (83%), while a low prevalence has been reported in Japan (39.6%) and the US (17.1%) compared to other countries. [2, 11]. Esophageal cancer is a global challenge. In 2012, about 398,000 esophageal squamous cell carcinoma (ESCC) and 52,000 esophageal adenocarcinomas (EAC) were detected at a global level [12]. Based on a meta-analysis in 2013, it ranked 8th among cancers with 481,645 newly diagnosed cases and 6th in terms of mortality with 406,533 deaths. [3].

According to a nested case-control study in China, a significant risk of esophageal squamous cell carcinoma was related to the detection of serum CagA antibodies which was found in infection, particularly with CagA-positive strains [4]. Esophageal cancer still prevalent in men, and the worldwide epidemiology, showed ESCC is two to three times higher in men than in women. In general, this male dominance is even more pronounced in the histological subtype EAC [13]. According to various studies, a protective role of *H. pylori* infection is suggested as evidenced by decreased ESCC risk in Eastern populations as well as reduced EAC risk in the general population [1,3,4]. This observation led to the hypothesis that *H. Pylori* may have a protective role with regards to esophageal squamous cell carcinoma in Eastern and EAC in total population. However, other researchers reported that infection of the esophageal mucosa with the bacterium aggravates inflammation of the lower part of the esophagus and induces intestinal metaplasia or even adenocarcinoma [5].

In Africa there is also evidence that shows low prevalence of esophageal adenocarcinoma, and Barrettes esophagus. This was used as argument for *H. pylori* to have a protective role for esophageal cancer. The mechanism was suggested the bacteria may have a potential to reduce gastric acid secretion and elevation of the PH [14]. The role of *H. pylori* for extra gastric cancer such as esophageal cancer in Africa is still a controversial issue. A study in South Africa reported that there were 30 H. pylori positive patient out of 59 ESCC and the prevalence become 51% [15].

H. pylori is part of the 16 “high-priority pathogen that pose greatest risk to human health list by WHO” prioritized for research and development of new and effective treatments [16]. Ethiopia was considered as high-risk corridor for ESCC in Africa. At the same, the infection rate of *H. pylori* is more than 50%. There are favorable conditions for *H. pylori* like low socioeconomic status, hygiene condition, people living in a crowded environment, contaminated food and water and general poverty. Ethnic background could also play a role. It was reported that *H. pylori* detection was 55%. Besides, cagA gene was detected in 20 out of 34 positive specimens [16,17]. Based on findings from biopsies samples the bacterium was reported as common and much higher in esophageal cancer in Ethiopia. However, *H. pylori* stool antigen positivity in esophageal cancer is not studied in our country. Existing evidence whether *H. pylori* is protective or harmful is inconclusive warranting additional studies from elsewhere [17].

As a result, lack of enough information and controversial ideas on the prevalence of *H. pylori* in Esophageal and apparently healthy individuals in our country Ethiopia leads to conduct another study. Therefore, this study was planned to provide additional evidence from esophageal patients from Ethiopia.

1.3. Significance of the Study

The study will be beneficial for initial screening of *H.pylori* on patients suspected for esophageal cancer. This helps patients to get the appropriate treatment and prevent other related diseases caused by the bacteria. It also opens an opportunity for other researchers to study and understand the reason why Helicobacter is prevalent or not in esophageal cancer or apparently healthy individuals. It will also be important for other researchers to know whether *H.pylori* eradication may cause esophageal cancer after long period of time. In addition to these policy makers may take these researches finding as an input to revise the National Guideline for EC screening, diagnosis, and treatment and hence *H. pylori* test become a routine diagnostic tool for esophageal cancer patients.

2. Literature Review

2.1. Epidemiology of Esophageal Cancer

Esophageal cancer is a major challenge worldwide. In 2012, nearly 398,000 esophageal squamous cell carcinomas and 52,000 esophageal adenocarcinomas were diagnosed globally [12]. Esophageal squamous cell carcinoma is one of the most frequent esophageal diseases in developing countries [18]. More than 450,000 people worldwide are affected by esophageal cancer. Incidence is increasing and SCC predominates compared to the AC form. Based on the study in China, ESCC pathogenesis is multifactorial such as multiple environmental factors. Risk factors for esophageal squamous cell carcinoma included tobacco smoking and alcohol consumption [7]. The highest risk for esophageal cancer was reported for people between the ages of 45 and 70. This cancer affects more men than women. The incidence is three times higher in black Africans than in whites [19].

In Malawi, ESCC accounts for 94% of EC cases and is the second most common cancer in this population. A significant female surplus was observed only in Sudan, while males predominated in eastern and southern Africa [20]. In Kenya, late stage diagnosis is common and accounts 70-80% of cancer cases due to ignorance among patients and medical staff, poor access to health facilities and inadequate diagnostic capabilities. For this reason, EC is unique in Kenya with its high proportion of young cases (< 30 years) [21].

In Ethiopia it was reported that EC is more common among khat users and ESCC was the most common one in our country [17]. As Solomon S and Mulugeta W Studied, in Ethiopia, esophageal cancer ranked 3rd in men following bone and soft tissue cancer (16.5%), and colorectal cancer (12.2%). Esophageal cancer account 9.1%. In these articles, esophageal cancer was referred as a leading cause of death in males in Kenya [22]. Another prospective hospital-based study by Gashaw Messele *et al*, reported that from a total of 500 study subject in Gondar 3.0 % were Esophageal cancer positive. The majority of patients are under 35 years old. Only 15% had a history of upper GI bleeding. Surgical treatment was indicated in 11% and Helicobacter pylori eradication therapy was affected in 27.2% of the study cases (younger age groups) in the study area of both esophageal and gastric carcinoma [23]

2.2. Magnitude and Association of H. Pylori in Esophageal cancer

H. pylori infection is usually asymptomatic and affects nearly 50-75% of the world's population. Its extent varies from country to country [24]. The infection rate also remains high (> 50%) in many parts of the world, although infection rates are falling in some developed countries [2]. When we look the association with esophageal cancer, based on a study in Turkey *H. pylori* infection was observed in 39 (68.4%) of 57 ESCC patients and 128 (85%) of 151 dyspeptic controls. They found a significantly lower rate of H. pylori in patients with ESCC compared to dyspeptic subjects. According to these studies, patients with esophageal squamous cell carcinoma had a significantly lower prevalence of *H. pylori* compared to the healthy population (p 0.001) [18]

Reciprocally, some case control and cohort studies suggest that H. pylori infection might protect against gastroesophageal reflux disease [25]. There is also a report in UK by Mustard RA, et al, and *H. pylori* was present in 663 from a total of 1485 (45%) patients and in 120 from a total of 312 (38%) patients with esophagitis. Antibody was found in (78%) *H. pylori* positive patients. Similarly, anti-CagA antibody was found in (81%) patients with a normal esophagus finally the study confirms the risk of severe esophagitis was significantly decreased for patients infected with cagA+ *H. pylori* patients [26] Most epidemiological studies indicate that reflux symptoms, mucosal diseases and esophageal cancer occur less frequently in patients with *H. pylori* infection [27].

Another investigation shows absence of significant association between *H. pylori* infection and the risk of esophageal squamous cell carcinoma (ESCC) (OR = 0.97, 95% CI: 0.76-1.24) in the total population and that there is a significant association between *H. pylori* infection and risk of ESCC was found in eastern subjects (OR = 0.66, 95% CI: 0.43-0.89), with a protective effect. For esophageal adenocarcinoma (EAC), the overall OR for *H. pylori* infection is 0.59 (95% CI: 0.51–0.68) [3] According to a 2014 study in the United States, in a precancerous stage of esophageal adenocarcinoma, the occurrence of *H. pylori* in a predominantly male population was observed to be inversely associated with BE, and this inverse association was limited to subjects who have characteristics that reduce stomach acid production, such as corpus atrophy or the weekly intake of antisecretory drugs. It is estimated that *H. pylori*-positive patients are about half as likely to develop BE as non-*H. pylori*-infected individuals [28]. According to a study by Wu,

I.C *et al*. H pylori seropositivity in those with esophageal squamous cell carcinoma was 35.3%, lower than controls (40.5%-59.3%) [29]. A meta-analysis study showed a negative relationship between being positive for *H. pylori* and the risk of EAC in western society. With regard to ESCC risk, no significant relationship was noted in western subjects [3].

As Ming Hu H studied in Tawin with newly collected ESCC subjects and controls, they found the protective effect of *H. pylori* in ESCC, which means the bacteria is low prevalent in ESCC and its was found to be more common in non-esophageal cancer [7]. In contrast in our country *H. pylori* was frequently identified in EC biopsies (55%)/ Most of them had the pro-inflammatory gene cagA [17]. The decline in *H. pylori* colonization may result in an increase in EAC incidence rates. The study shows that with an OR of 0.5, even with a complete disappearance of *H. pylori*, EAC incidence could double. As a result, the absence of *H. pylori* had an impact on the development of esophageal cancer and the presence of the bacteria might have a protective effect on EC [1].

In Ethiopia, the prevalence of *H. pylori* infection was 52.2% (95% CI: 45.8,58.6) in a reviewed study of 37 studies involving a total of 18,890 subjects. Regional difference within the country was noted where highest rate was recorded in Somali (71%; 95). % CI: 32.5,92.6) and the smallest in Oromia (39.9%; 95% CI: 17.3,67.7). Lack of washing hands after toilet (OR = 1.8, 95% CI; 1,19,2.72), alcohol use (OR = 1.34, 95% CI; 1.03,1.74), and gastrointestinal (GI) symptoms (OR = 2 .23, 95% CI; 1,59.3.14) have been associated with *H. pylori* infection [16]. Another study also done by Leon ME and Kassa E *et al*, from a total of 73 cases, the bacterium was detected in the biopsy specimens 55% (95% CI: 42–68%) [17].

2.3. Transmission and pathogenesis

H. pylori infection is acquired through oral ingestion of the bacterium. It is mainly transmitted within families in early childhood [25]. Besides, carcinogenic pathogens were suggested as a risk factor for EC. Poor oral hygiene, malnutrition, and viral and bacterial infections such as hepatitis B have been shown to be strong risk factors for certain types of cancer [30].

H.pylori was reported to cause more serious disease of the stomach such as peptic cancer, stomach cancer and duodenal cancer [9]. Its invasion of the lower esophagus, as in the stomach, could trigger a pathological process and favor the development of BE and even EAC. However, based on the study conducted on animals, there was enhanced cellular expansion and apoptosis

in the esophagus of mixed reflux rats resulting from the infection. The imbalance between the two (cell proliferation versus apoptosis) might be responsible for *H. pylori*-induced esophageal malignancy [5]. In addition, bacterial growth and duodenal reflux are possible consequences of atrophy that may contribute to the disease pathogenesis. Quantitative and qualitative changes in the gut microbiota due to hypochlorhydria could result in enhanced N-nitrosation responses. This in turn, could result in carcinogenesis on the esophageal mucosa [31]. CagA translocation into host cells has been indicated to activate several oncogenic pathways in them [32].

2.4. Socioeconomic Factors

As Jansson C *et al.* study, poor socioeconomic status is associated with an increased risk of esophageal squamous cell carcinoma (SCC). But the association with adenocarcinoma is uncertain. Based on their study, unskilled manual workers (OR 3.7; 95% CI 1.7 to 7.7) and self-employed (OR 3.7; 95% CI 1.7 to 8.1) were around fourfold significantly increased risk of esophageal adenocarcinoma compared to professionals [33]. On the other hand, there is also few reports that shows possible association between *Helicobacter pylori* and ESCC, although most have been based on serology. Cigarette smoking excessive alcohol consumption was linked to an increased risk of ESCC development. H. *pylori* is considered among the most important human carcinogens of the upper GIT and stomach [18].

Low socioeconomic condition, living in a crowded place, and unsatisfactory sanitation have also been identified as risk factors for *H. pylori* infection. And most people in Africa became infected by the age of 10 and 80% increase in young adults [14]. According to a study by Khademian A and co-workers the risk factors for esophageal cancer were different in developed and developing countries. Based on several pieces of evidences, unlike in developed nations, cigarette smoking and alcohol consumption are not the most important factors in the developing world. Infections contribute more to cancer in developing countries as opposed to their developed counterparts (about 7% versus 26%). For example, papillomavirus, hepatitis B and C virus, and *H. pylori* were among the microbial agents indicated in Iran [34]. Smoking is singled out as important risk factor for both AC and SCC. Alcohol is also a major contributor to SCC. A combined use of alcohol and tobacco leads to a significant rise in the risk of esophageal cancer and genetic factors, dietary modification/food preparation and consumption of hot food or beverages are useful attributes for EC in Kenya [19, 21].

2.5. Diagnosis of H. pylori

There are invasive (endoscopic gastric biopsy and serology) and non-invasive (urea breath test, stool antigen test) diagnostic types [2.25]. According to Lopes AL *et al.* from 2014, the stool antigen test is among non-invasive techniques for detecting *H. pylori* and is usually recommended when the UBT is not available. The two types of stool antigen tests are the EIA and an immunochromatography-based test. A global sensitivity of 94% (95% CI: 93-95) and specificity of 97% (95% CI: 96-98) is reported following a meta-analysis of existing publications. Serology was one of the first methods to diagnose *H. pylori* infection.

The bacterium induces both a local and systemic antibody response composed of IgM, IgA and IgG antibodies. The IgG antibody test appears to be the most sensitive and specific method. Serology is currently recommended for screening, which requires further confirmation by histology and/or culture before treatment [35]. Endoscopic method used by assessing gastric biopsy using histological analysis and culture are used in the diagnosis and the stool antigen test is indicated as highly sensitive (80-100%) compared to urea breath test [10]. Another study reported commercial RUTs having specificity of 95%-100%. Yet, they had slightly lower sensitivity of 85%-95% [35]. The reliability of the urea breath test depends on the age of children. It is reliable in children aged above six years. Further validation is required to use it in younger children [25].

3. Objective

3.1. General objective

To investigate the magnitude of *Helicobacter Pylori* infection and associated factors of esophageal cancer among esophageal cancer patients at Tikur Anebesa specialized Hospital, Addis Ababa, Ethiopia from May 2021 to August 2022 GC.

3.2. Specific objective

- To determine the magnitude of *Helicobacter pylo*ri infection among patients with EC as compared to apparently healthy individuals.
- To identify the associated factors of esophageal cancer.

4. Research Hypothesis

There is No difference on magnitude of H.pylori among Healthy individual's compared to esophageal cancer patients.

5. Material and method

5.1. Study Area

Tikur Anbessa Specialized Hospital (TASH) as selected as a site for this study since it is the main referral center for EC patients. TASH is the largest teaching and referral hospital in Ethiopia which was initially under the Federal Ministry of Health but later transferred to Addis Ababa University in 1998. With its capacity of about 700 beds, it serves as a tertiary level referral hospital for the whole nation and a teaching center for both diverse specialties and subspecialties clinical training as well as preclinical training of most health disciplines (like nurses, midwives, anesthesia, radiography, medical laboratory and pharmacy). The hospital has 929 academic, 825 nurses ,55 medical laboratory, 74 pharmacies, 69 midwife, 39 anesthesia, 14 physiotherapy, 37 radiology, 15 biomedical, 6 environmental health, and 15 administrative staff dedicated to providing health care services. The hospital also has about 950 permanent and contract administrative staff to support the hospital activities. Moreover, the hospitals in Addis Ababa (both regional and federal) are affiliated to the School of Medicine as clinical services and teaching sites. In cardiothoracic referral clinic patients were visited 3 days per week (Monday, Wednesday, and Friday). At the Surgical referral clinic, a total of at least 25-30 charts are viewed per day, resulting in 4-5 new cases of esophageal cancer patients per day, as well as 12 cases per week and 48 cases per month.

5.2. Study design and Period

Cross-sectional comparative study carried out to determine the magnitude of *H. pylori* infection in esophageal and apparently healthy individuals and the study was conducted between May 2021 to August 2022 GC.

5.3. Population

5.3.1. Source of population

Those patients who visit the referral clinic and suspected for esophageal cancer during the study period were the source for cases and all apparently healthy individuals (Care givers, Attendants, and Staff) in the Hospital were used as comparative group.

5.3.2. Study population

Cases were those patients who were suspected for the esophageal cancer at surgical referral clinic and confirmed positive for esophageal cancer and fulfill the inclusion criteria.

Comparative group were all apparently healthy individuals that fulfill the inclusion criteria.

5.4. Inclusion and Exclusion criteria

5.4.1. Inclusion criteria

For cases; volunteering esophageal Cancer patient who are confirmed by tissue histology to have esophageal cancer were included.

For Comparative group; apparently healthy volunteers who don't have a disease.

5.4.2. Exclusion criteria

For cases: those Esophageal cancer patients who have taken previous treatment of *H. pylori* for one week and above as well as seriously ill patients were excluded.

For Comparative Group: those who have previous history of esophageal cancer, throat related disease were excluded.

5.5. Study Variables

5.5.1. Dependent variable

The magnitude of *H. Pylori* in esophageal and apparently healthy individuals was the dependent variables.

5.5.2. Independent variable

Age, Sex, Residence, Educational level, Occupation, Alcohol, Smoking, Hot food, Hot Drink, Family member with throat cancer, Vegetable, Illness related to throat.

5.6. Measurement and Data collection

5.6.1. Sample size determination

Sample size was calculated from a similar study conducted in our country and OR was taken as an input to calculate the sample size. The calculation was by using Epinfo version 7.0 and by using the exposure status for the case and control, making the ratio of case to control 1:1.5, power 80%, the sample size can be calculated. From a study in Tikur Anbesa ("Prevalence of Helicobacter pylori in esophageal and gastroesophageal junction cancer biopsies from a case–control study in Ethiopia"), the exposure status of the case was 13% and the control was 7% [16] from these to increase the precision Using the formula $(Z\ \alpha/2 + Z\ \beta)^2 * (p1\ (1-p1) + p2\ (1-p2)) / (p1 - p2)^2$, the sample size was become 200 for case and 300 for Comparative group but due to esophageal cancer patient swallowing problem and fail to give stool sample for *H. pylori* Stool

Antigen test 25 patient were excluded. As a result, the expected sample size was not taken. Finally **112** cases and **190** Comparative group (Healthy individuals), total of 302 study participant included in the study. This sample size was much higher than the previous published study.

5.6.2. Data collection procedure

Convenient sampling was used for the case and apparently healthy individuals. Esophageal cancer patients were confirmed by senior doctors at surgical referral clinic (SRC) clinically, and by other diagnostic tools such as CT scan, Endoscopy and Tissue Histology. Healthy individual's attendant, staffs that do not have disease were included in study. After patient was identified at the clinic those patients who fulfill the criteria were taken and based on informed consent those who agree were included in the study. Stool sample by stool cup and blood sample by serum separator tube (SST) were taken by principal investigator at the site of sample collection from both group of the study participant. During sampling all sample was labeled with unique identification code and the result was registered based on the ID given during sample collection. The sample from the case and the comparative group was differentiated by giving unique ID for the two groups. After sample was collected, it was transported to the laboratory and processed based on the standard procedures. H. pylori Stool Antigen test were tested from stool sample and H. pylori antibody test from serum after centrifugation of the clotted sample. The result of the laboratory record using result log Book. Consent form, Questioners and Data collection format had been used to record all information required for the study participant as well as their response to the interviewed questions of each study participant. In addition to this for each study participant in the case and control their age, sex as well as the type of sample taken, the result after the identification of the bacteria in the lab was recorded daily. The type of the esophageal cancer (ESCC or EAC) was included in the data collection format.

5.6.3. Principles of Laboratory analysis

H. pylori Stool Antigen Test

It is a noninvasive method that is more economical than endoscopy to confirm eradication. It is based on the qualitative immunochromatographic principle. The test device has a sample hole made of the material that allows for the passage of the reagent. . Helicobacter pylori antibody takes part in the end section buffer membrane contenting the golden resultant. In case the bacteria antigen is present in patients' stool, it dissolves in the solution included in the sampling bottle then precedes by using the mixture form of chromatographically antigen -antibody-antigen golden particles towards test space(T) in order to form a visible line. A positive result is confirmed when a line in the T region is clearly seen. Absence of this line confirms a Negative result. The control space (C) shall reveal a visible colored line, which is a procedural indicator. It sufficient volume is used, sample expands in the test properly and reagent is in good condition.

Serology Testing; The *H. pylori* antibody test is a qualitative membrane-based immunoassay for the detection of *H. pylori* antibodies in whole blood, serum or plasma. Micro titer plates coated with *H. pylori* antigens, combined with a secondary antibody, and are used to detect *H. pylori*-specific IgG. In this test procedure, anti-human IgG is immobilized in the test line region of the test. After specimen is added to the specimen well of the device, it reacts with *H. pylori* antigen coated particles in the test. This mixture migrates chromatographically along the length of the test and interacts with the immobilized anti-human IgG. If the specimen contains *H. pylori* antibodies, a colored line will appear in the test line region indicating a positive result. If the specimen does not contain *H. pylori* antibodies, a colored line will not appear in this region indicating a negative result. To serve as a procedural control, a colored line will always appear in the control line region, indicating that proper volume of specimen has been added and membrane wicking has occurred.

5.7. Data Quality Assurance:

The data collection form was checked for its completeness and accuracy before the data was entered. The *H. pylori* stool antigen and serum antibody result for both the case and apparently healthy individuals was recorded carefully before entry to SPSS. Furthermore, data cleaning and double-data entry was applied in order to assure quality of the data. From patient identification up to the test is going to be processed all required recording age, sex, ID (code) for patient, was

documented on the sample collection format and sample was labeled based on the ID given in the format. Based on that the result was recorded accordingly. During sample collection the patient was asked for previous treatment to prevent false negative result. During testing all procedure was followed based on the principle of the test. There was timer to ensure the accuracy of the result during reporting.

5.8. Data analysis and Interpretation

Data which was gathered from the data collection form was entered into Epi Info Version 3.5.3. Data was analyzed using SPSS Version 20. Magnitude of H. pylori and OR ratio was calculated to look the association of the case and the healthy individuals with the bacteria. Mean and Standard deviation was used to describe continuous variables. Logistic regression model was used to estimate relative risks in the form of odds ratios (ORs) with 95% confidence intervals (CIs). Those variables with $p<0.25$ in the bivariate analysis were entered into the multivariable logistic regression analysis model and the final significance was determined based on the 95% confidence interval at $p<0.05$. The exposure status of the case and the healthy individual's control was analyzed. The Magnitude of *H. pylori* was seen in terms of the two types of esophageal cancer (esophageal adenocarcinoma and Esophageal squamous cell carcinoma).

5.9. Ethical considerations

Prior to conducting the study, the departmental research and ethics review committee (DRERC) of the Medical Laboratory Sciences, College of Health Sciences; Addis Ababa University reviewed and ethically approved the study protocol. Permission letter was obtained from TASH medical laboratory department the hospital. Patients were well informed about the study including their right not to participate or decline from the study at any of the steps. Their test results were shared specific to the attending clinicians only. All information collected in this study was given code numbers and no name was recorded. Confidentiality of patients result were kept by recording the result in computer system and the attending clinicians only has access for that to look their patients result using their user name and password. The key to this code numbers was kept in a locked file and accessible to the authorized staff.

5.10. Dissemination of Result

The result which was produced from this study will be submitted to the Department of Medical Laboratory Sciences, Addis Ababa University and defended in public. The results will be shared to other concerned bodies through presenting on conferences, publication on reputable journals and copies will be availed in the library.

5.11. Operational Definitions

Cases; - Patient confirmed as esophageal cancer.

Apparently healthy individuals: staffs, attendants, free from disease.

Esophageal cancer: malignant tumor of the esophagus which is diagnosed clinically, and by other diagnostic tools such as CT scan, Endoscopy and Tissue Histology.

***H. pylori* positive;-** patient that we tested for *H. pylori* Stool Antigen and become positive.

***H. Pylori* negative;** patient who were tested for *H. pylori* Stool Antigen and become negative.

6. Results

6.1. Socio Demographic characteristics of study population

A total of 302 study participant was included in this study. They were classified in two groups. EC patients (n=112) as cases group and Healthy Individual (n=190) as comparative group. The median age was 32 years [IQR=25-44] in the control groups and 50 years [IQR=42-60] in the cases. Most of the esophageal cases were Female 72 (64.3 %) and majority of healthy individuals were Male 105 (55.3%). More than two third of the study participants were above the age of 40 (84.8%). Majority of cases group are farmers 47 (42.4%) and control group 78 (41.0%) work in private. More than 90% of the esophageal cancer patients were from outside of Addis Ababa. In addition, 71.4% of esophageal cancer patients were uneducated, 6.3% had throat related illness, 25% drink alcohol, and 5.4% had family member with throat cancer. (Table1).

Table 1. Sociodemographic characteristics of EC patients and Healthy individuals at TASH, Addis Ababa, Ethiopia, from May 2021 to August 2022 GC, (n=302).

		Esophageal cancer (n=112)		**Healthy individuals (n=190)**	
Variables	Category	Frequency	%	Frequency	%
Age	<40	17	15.2	133	70.0
	40 and above	95	84.8	57	30.0
Sex	M	40	35.7	105	55.3
	F	72	64.3	85	44.7
Occupation	Government	8	7.2	84	44.2
	House wife	44	39.6	3	1.6
	Private	10	9.0	78	41.0
	Student	2	1.8	17	9.0
	Farmer	47	42.4	8	4.2
Residency	Addis Ababa	10	8.9	131	68.9
	Out of Addis Ababa	102	91.1	59	31.1
Educational levels	Uneducated	80	71.4	0	0.0
	Primary	20	17.8	58	30.5
	Secondary	5	4.5	57	30.0
	Diploma and above	7	6.3	75	39.5
throat related illness	Yes	7	6.3	9	4.7
	No	105	93.7	181	95.3
Alcohol	Yes	28	25.0	57	30.0
	No	84	75.0	133	70.0
Vegetable	Usually,	56	50	58	30.5
	Rarely	11	9.8	128	67.4
	None	45	40.2	4	2.1
Family member with throat Ca	Yes	6	5.4	10	5.3
	No	106	94.6	180	94.7

When data was disaggregated by cancer type, 74.1 % of cases had squamous type of EC and majority of them (37.4 %) were in stage II (Table 2).

Table 2. Characteristics of participant with Esophageal Cancer; Tikur Anbesa Specialized hospital Addis Abba Ethiopia, from May 2021 to August 2022 GC, (n=112).

Variable	Category	Frequency (%)
Type of EC	Squamous	83 (74.1%)
	Adenocarcinoma	29 (25.9 %)
Stage of EC	Stage I	21 (18.8%)
	Stage II	42 (37.4%)
	Stage III	28 (25.0%)
	Stage IV	21 (18.8%)

6.2. Magnitude of H pylori among EC patients and apparently healthy individuals.

The Magnitude of *H.Pylori* is higher among EC cases than healthy individuals. As shown in the table, 40.2% (45/112) of cases and 4.2% (8/190) of controls were positive for stool antigen. Antibody tests indicate passive infection of H.pylori and antigen test indicates active infection of H.pylori.

Table 3. Comparison of *H. pylori* with EC Cases and Healthy individuals at Tikur Anbesa Specialized hospital Addis Ababa, Ethiopia. From May 2021 to August 2022 GC, (n=302).

Variables	**Category**	**Cases (n=112)**	**Healthy (n=190)**
		Frequency (%)	**Frequency (%)**
***H. pylori* Antigen**	Negative	67(60 %)	182(96 %)
	Positive	45(40.2%)	8(4.2%)
***H. pylori* Antibody**	Negative	71(63%)	144(76%)
	Positive	41(37%)	46(24%)

Figure 1 and 2 also described the *H. pylori* status with EC types and disease stages. *H. pylori* infection was more infecting the EAC type 48.3% (14/29) compared to ESCC which is 37.4% (31/83) (Figure 1). Stage IV esophageal cancer patients are more infected by H. pylori compared to others which is (57.1 %). Next to this stage II (40.5%) and Stage III (35.7%) were infected by H. Pylori and Stage I (28.6 %) esophageal cancer patient were less infected.

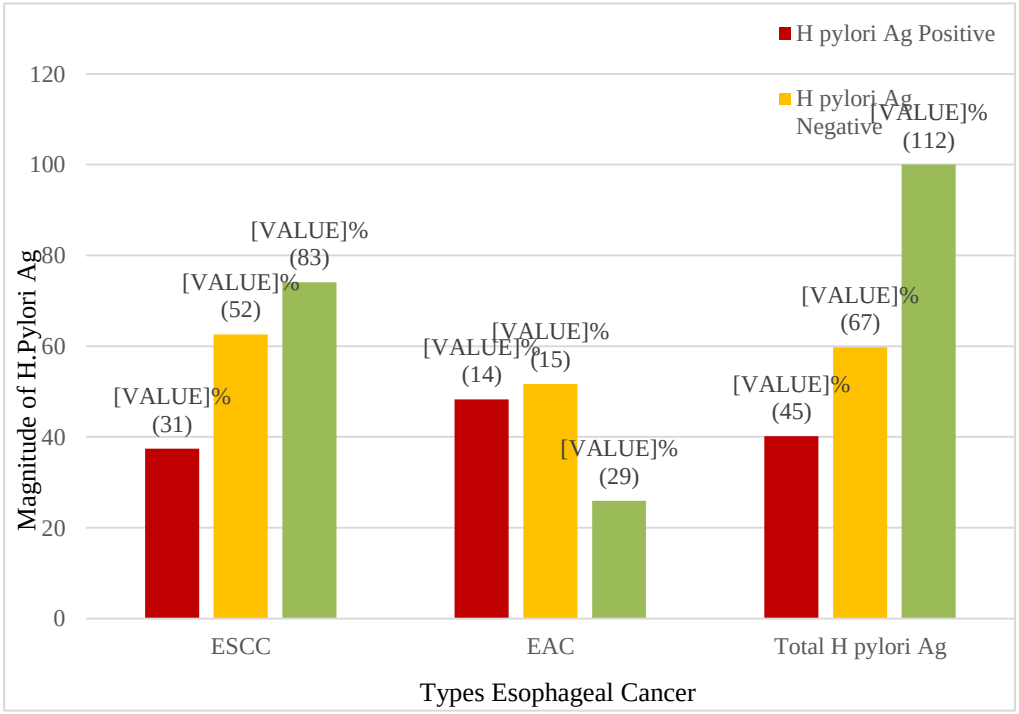

Figure 1. Comparison of *H. pylori* infections among ESCC and EAC types of esophageal cancer. at Tikur Anbesa Specialized hospital Addis Ababa, Ethiopia. From April 2021 – May 2023 (n=112).

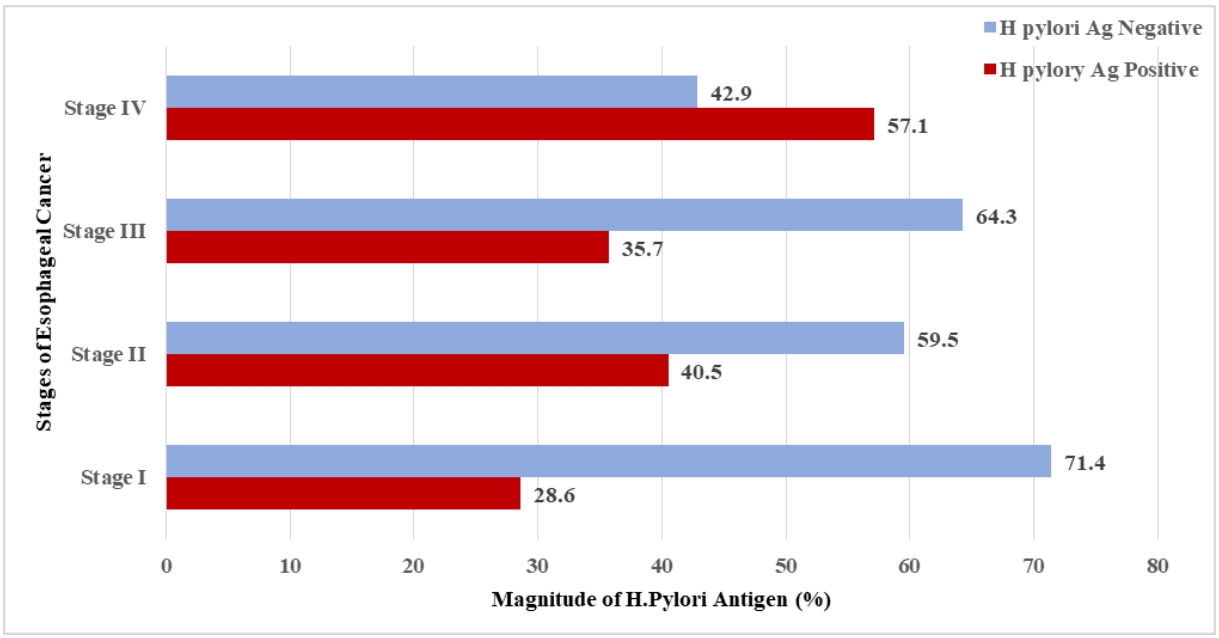

Figure 2. Comparison of *H. pylori* Antigen by Esophageal Cancer Stages. at Tikur Anbesa Specialized hospital Addis Ababa, Ethiopia. From May 2021 to August 2022 GC, (n=112).

As shown in the table below, those variables with p<0.25 in the bivariate analysis were entered into the multivariable logistic regression analysis model. In the multivariable logistic regression analysis, being in the age group 40 years and above (AOR=12.23, 95%CI=4.09-36.53, p=0.0001), living outside Addis Ababa (AOR=18.57, 95%CI=5.76-59.83, p=0.0001), attaining primary level education and below (AOR=17.24, 95%CI=5.31-55.96, p=0.0001), and being positive for *H. pylori* stool antigen (AOR=20.13, 95%CI=4.79- 84.62, p=0.0001) were more likely to be associated with EC after controlling for throat illness, taking alcohol, family history of throat illness and having hot food as shown in Table 4. On the other hand, patients who had vegetable diet rarely or never were 77% less likely to have EC (AOR=0.23, 95% CI=0.08- 0.66, p=0.006).

Table 4. Characterization of associated factors with esophageal cancer and apparently healthy individuals; Tikur Anbesa Specialized hospital Addis Ababa Ethiopia, From May 2021 to August 2022 GC, ((n=302).

Variables	Category	Esophageal cancer		COR [95% CI]	P value	AOR [95% CI]	P value
		Yes Frequency (%)	**No** Frequency (%)				
Sex	M	40(35.7)	105(55.3)	1		1	
	F	72(64.3)	85(44.7)	2.22[1.37, 3.60]	0.001	2.34[0.76, 7.26]	0.139
Age (Years)	<40	17(15.2)	133(70.0)	1		1	
	40 and above	95(84.8)	57(30.0)	13.04[7.14, 23.81]	0.0001	12.23[4.09,36.53]	**0.0001**
Occupation	Employed	65(58.6)	170(89.5)	1		1	
	Unemployed	46(41.4)	20(10.5)	6.02[3.31, 10.94]	0.0001	2.32[0.64, 8.42]	0.201
Residency	Addis Ababa	10(8.9)	131(68.9)	1		1	
	Out of Addis Ababa	102(91.1)	59(31.1)	22.65[11.04,46.46]	0.0001	18.57[5.76,59.83]	**0.0001**
Educational levels	Primary and below	100(89.3)	58(30.5)	18.96[9.67, 37.20]	0.0001	17.24[5.31,55.96]	**0.0001**
	Secondary and above	12(10.7)	132(69.5)	1		1	
Throat related illness	Yes	7(6.3)	9(4.7)	1.34[0.49, 3.71]	0.572		
	No	105(93.7)	181(95.3)	1			
Alcohol	Yes	28(25.0)	57(30.0)	0.78[0.46, 1.32]	0.351		
	No	84(75.0)	133(70.0)	1			
Vegetable	Usually,	56(50.0)	58(30.5)	1		1	
	Rarly/Never	56(50.0)	132(69.5)	0.44[0.27, 0.71]	0.001	0.23[0.08, 0.66]	**0.006**
Family member throat Ca	Yes	6(5.4)	10(5.3)	1.02[0.36, 2.88]	0.972		
	No	106(94.6)	180(94.7)	1			
Smoking	Yes	16(14.3)	18(9.5)	1.59[0.78, 3.27]	0.204	3.04[0.75, 12.27]	0.119
	No	96(85.7)	172(90.5)	1		1	
Hot food	Yes	69(61.6)	125(65.8)	0.83[0.51, 1.35]	0.464		
	No	43(38.4)	65(34.2)	1			
Hot drink	Yes	98(87.5)	175(92.1)	0.60[0.28, 1.29]	0.193	0.49[0.09, 2.52]	0.390
	No	14(12.5)	15(7.9)	1		1	
H. pylori stool Ag	Yes	45(40.2)	8(4.2)	15.30[6.85, 34.09]	0.0001	20.13[4.79,84.62]	**0.0001**
	No	67(59.8)	182(95.8)	1		1	

7. Discussion

The current research, aimed to investigate the magnitude of the *H. pylori* Bacterium in esophageal cancer patients at TASH. It also tried to describe other factors associated with EC. Infection with *H. pylori* has been reported to be linked to esophageal cancer although reports against this were also available in the literature. In this study there is a remarkably high difference in terms of *H. pylori* stool antigen positivity between esophageal cancer patients and healthy indviduals. In the present study we found high magnitude of H. pylori (40.2%) among Esophageal cancer [AOR=20.13, 95% CI = 4.79-84.62] compared to Healthy individuals and this was consistent in the former work done at Tikur Anbesa specialized hospital Ethiopia and in other African countries, and also in symptomatic patients screened using stool specimens [17, 50]. The reason for the difference were the bacterium may exacerbate esophageal mucosal damage and be responsible for the rise of incidence of Barrettes esophagus (BE) and following this it causes EAC [5].

On the other hand Xie. FJ et al ,[1] found that the overall positive rate of H. pylori infection in EAC was 35.96% (479/1332), which was significantly lower than that in normal healthy individuals 44.00% (2070/4705. There is also another supporting finding showing no significant associations between H. pylori infection and ESCC risk were found in Eastern Asian subjects and cytotoxin-associated gene-A (CagA) positive patients had decreased risk of ESCC [3]. The reason behind was Helicobacter pylori might play a protective role due to the ability of the bacterium to degrade ammonia and reduce acidity of the environment as well [2,4].

Studies based on stool antigen positivity to determine H pylori infection among EC are limited in Ethiopia, as a result the present finding were compared with studies using different method (molecular techniques) and this finding were concordance with the present study with magnitude of 55% (34/62) [17]. In this study, most of the cases were squamous cell carcinoma type accounting 74.1% (83/112) compared to Esophageal Adenocarcinoma types 25.9% (29/112) which is in concordance with the global reports and in some East African countries [1,38].

In this study magnitude of *H. pylori* antibody was 24% (46/190) among healthy and 37% (41/112) among Cases which is different from a study from Sweden that reported magnitude of 19 % among esophageal cancer cases and 40 % among healthy group [4]. Likewise, cancer types, 74 % SCC and 43 % EAC cases had *H. pylori* infection compared to 48.3 % of EAC and

37.4 % of ESCC cases in the current study. The result is similar with the current study even large sample size difference especially in the control group in Sweden study than ours. Moreover, the sociodemographic factors and living style in the two countries are not comparable. In addition to this study the magnitude of *H. pylori* antibody was also more prevalent among ESCC 39.7% (33/83) and lower among EAC cases, 27.6% (8/29).

In this work all stages of tumors with rate of 18.8 % (n=21) stage I, 37.4 % (n=42) stage II, 25 % (n=28), stage III and 18.8% (n=21) stage IV which is in accordance with the global report [41,42] However, it is in contrast with a previous study in Ethiopia [43] that reported 47.4% patients had staged IV and 68 patients (34.7%) had metastases (stage IVB) and lower rate of stage of II (7.1 %) and III (9.7 %) [43]. Stage of cancer could be affected by duration of the diseases and weather the patients are on treatment or not. Patients of the current study might have presented to the health facility early because of the relatively better attention given to awareness campaigns as compared to the earlier times.

In this study older age people had higher risk for acquiring EC [AOR = 12.23, 95% CI=4.09-36.53] which is similar with former study in our country [17]) and studies in Asia, and USA [45, 46]. Moreover, females accounted the highest percentages of Esophageal cancer (64.3 %, n=72) as compared to males (35.7%, n=40) and this was similar with the previous study in Ethiopia that esophageal cancer was more common among females 54% (n=15) compared to males 46% (n=13) [17]. Furthermore, most of the esophageal cancer patients were from outside of Addis Ababa as compared to patient in Addis Ababa, and the majority of patients coming from outside Addis Ababa reside in Oromia region. Similarly Dessalegn.B, Enqueselassie .F et al, and Leon ME, Kassa E et al. [17,44] reported that rural residents were found to be at a higher risk of developing esophageal cancer than urban residents. On the other hand, patients who had vegetable diet rarely or never were 77% less likely to have EC (AOR=0.23, 95% CI=0.08- 0.66, p=0.006).

The healthy controls belong to the rarely category was merged with the none consuming category since number of non-consumers in the healthy group was small and when overall consumption was analyzed 45/112 (40.2%) of patients compared to only 4/190 (2.1%) reported they do not consume fruits and vegetables. This more frequent use among control group than cases which may play a protective role is consistent with former study in Ethiopia [44]. In this

study alcohol consumption Were not found to be a risk factor for EC which is opposite with other reports Xu W, et al and Dessalegn B, Enqueselassie F et al. [30, 44].

In this study occupation was not found to be a risk factors for EC [OR=2.32 95% CI=0.64, 8.42]. However, smoking, and consumption of hot food and drink also were not identified as risk factors. Finally in the logistic regression analysis, the association between *H. pylori* and EC was significant (p=0.0001) while other behavioral, sociodemographic and clinical factors were being included in the model. On the other hand, family history of throat infection was not significantly associated with EC unlike a report from elsewhere [49]. Further investigations of risk factors are required to explain the controversial findings.

8. Strength and Limitation

The strength of this study was both antigen and antibody were tested for all patients and also confirmed esophageal cases were taken.

The limitation was the study was cross sectional. Since it's not a strong case control study rather its comparative it needs another study to support the information given by the present study. In addition to this some patients had swallowing problem and critically ill and it was difficult to get sample and the required sample size was not collected.

9. Conclusions and Recommendation

We have shown that the magnitude of *H. pylori* is high among EC cases than the healthy controls. It is also more prevalent in those with ESCC type carcinoma and in the advanced stage (Stage IV). Importantly, stage I, II, III and, stage IV tumors were reported in the current study which underscore EC is one of the major types of cancer in our study area. Hence it is important to design a large scale case control study and selective screening strategy to unravel the interaction of *H.pylori* and esophageal cancer in Ethiopia. Moreover, it is very advisable that people should be advised on diet and to avoid smoking and alcohol consumption as this could aggravate the incidence of EC.

10. References

1. Xie FJ, Zhang YP, Zheng QQ, Jin HC, Wang FL, Chen M. et al. Helicobacter pylori infection and esophageal cancer risk: An updated meta-analysis. World Journal of Gastroenterology.2013;19(36);6098–6107. https://doi.org/10.3748/wjg.v19.i36.6098
2. Islami F and Farin Kamangar K. Helicobacter pylori and Esophageal Cancer Risk a Meta Analysis. *Bone*, 2008;*23*(1),1–7. https://doi.org/10.1158/1940-6207.CAPR-08-0109
3. Testerman TL, Morris J. Beyond the stomach: An updated view of Helicobacter pylori pathogenesis, diagnosis, and treatment. World Journal of Gastroenterology, 2014;*20*(36), 12781–12808. https://doi.org/10.3748/wjg.v20.i36.12781
4. Ye W, Held M, Lagergren J, Engstrand L, Blot WJ, McLaughlin JK. Helicobacter pylori infection and gastric atrophy: Risk of adenocarcinoma and squamous-cell carcinoma of the esophagus and adenocarcinoma of the gastric cardia. Journal of the National Cancer Institute, 2004;*96*(5), 388–396. https://doi.org/10.1093/jnci/djh057
5. Chu YX, Wang WH, Dai Y, Teng GG, Wang SJ. Esophageal Helicobacter pylori colonization aggravates esophageal injury caused by reflux. World Journal of Gastroenterology, 2014;*20*(42), 15715–15726.
6. Polyzos SA, Zeglinas C, Artemaki F, Doulberis M, Kazakos E, Katsinelos P. *et al.* Helicobacter pylori infection and esophageal adenocarcinoma: A review and a personal view. Annals of Gastroenterology, 2018;*31*(1), 8–13.
7. Hu HM, Kuo CH, Lee CH., Wu IC, Lee KW, Lee JM, et al. Polymorphism in COX-2 modifies the inverse association between Helicobacter pylori seropositivity and esophageal squamous cell carcinoma risk in Taiwan: A case control study. BMC Gastroenterology. 2009;(*9)*;1–8. https://doi.org/10.1186/1471-230X-9-37
8. Yang L, Chaudhary N, Baghdadi J, Pei Z. Microbiome in reflux disorders and esophageal adenocarcinoma. Cancer Journal ,2014;*20*(3), 207–210.
9. Tomasello G, Giordano F, Mazzola M, Jurjus R, Jurjus A, Damiani P, *et al* .Helicobacter pylori and Barrett's esophagus: A protective factor or a real cause? Journal of Biological Regulators and Homeostatic Agents.2017;*31*(1), 9–15.
10. Murphy MS. Helicobacter pylori related disease. Archives of Disease in Childhood, 2001;*84*(6), 525.

11. Kao CY, Sheu BS, Wu JJ. Helicobacter pylori infection: An overview of bacterial virulence factors and pathogenesis. Biomedical Journal, 2016;*39*(1), 14–23.
12. Ajayi TA, Cantrell S, Spann A, Garman KS. Barrett's esophagus and esophageal cancer: Links to microbes and the microbiome. PLoS Pathogens, 2018;*14*(12), 1–9.
13. Abbas G, Krasna M. Overview of esophageal cancer. *Annals of Cardiothoracic Surgery*, 2017;*6*(2), 131–136.
14. Segal I, Ally R, Mitchell H. Helicobacter pylori-An African perspective. QJM - Monthly Journal of the Association of Physicians, 2001;*94*(10), 561–565.
15. Kogomo M, Elngar AA, Mokoena J, Jeske C, and Naegel GJ. Prevalence of Helicobacter infection in patient with squamous cell of esophageous. A descriptive case series study.Gournal of gasterointestinal cancer.2016; 47:396-398.
16. Melese A, Genet C, Zeleke B, Andualem T. Helicobacter pylori infections in Ethiopia; Prevalence and associated factors: A systematic review and meta-analysis. BMC Gastroenterology, 2019;19(1), 1–15.
17. Leon ME, Kassa E, Bane A, Gemechu T, Tilahun Y, Endalafer N et al. Prevalence of human papillomavirus and Helicobacter pylori in esophageal and gastroesophageal junction cancer biopsies from a case-control study in Ethiopia. Infectious Agents and Cancer, 2019;14(1), 1–10. https://doi.org/10.1186/s13027-019-0233-x
18. Poyrazoglu OB, Dulger AC, Gultepe BS. Helicobacter pylori infection in patients with esophageal squamous cell carcinoma. Clinics, 2017;72(3), 150–153. https://doi.org/10.6061/clinics/2017(03)04
19. Hull R, Mbele M, Makhafola T, Hicks C, Wang SM, Reis R. et al. A multinational review: Oesophageal cancer in low to middle-income countries (Review). Oncology Letters, 2020;20(4), 1–14. https://doi.org/10.3892/ol.2020.11902
20. Middleton DRS, Bouaoun L, Hanisch R, Bray F, Dzamalala C, Chasimpha S. Esophageal cancer male to female incidence ratios in Africa: A systematic review and meta-analysis of geographic, time and age trends. Cancer Epidemiology, 2018;119–128. https://doi.org/10.1016/j.canep.2018.01.020
21. Odera JO, Odera E, Githang'a J, Walong EO, Li F, Xiong Z, Chen XL. Esophageal cancer in Kenya. American Journal of Digestive Disease,2017; 4(3), 23–33. http://www.ncbi.nlm.nih.gov/pubmed/29082268%0A.

22. Solomon S, Mulugeta W. Diagnosis and Risk Factors of Advanced Cancers in Ethiopia. Journal of Cancer Prevention, 2019;24(3), 163–172.
23. Getahun GM, Yassin MO, Adurahman Z. Analysis of Endoscopic Findings Among Gastrointestinal Patients. Ethiop. J. Health Biomed Sci,2020,0–4.
24. Ofori EG, Adinortey CA, Bockarie AS, Kyei F, Tagoe EA, Adinortey MB. . Helicobacter pylori Infection, Virulence Genes' Distribution and Accompanying Clinical Outcomes: The West Africa Situation. BioMed Research International, 2019. https://doi.org/10.1155/2019/7312908
25. Suerbaum S, Michetti PHelicobacter pylori infection. The new England journal of medicine. 2002;347(15), 1175–1186.
26. Mustard RA. The significance of cagA+ Helicobacter pylori in reflux oesophagitis. Canadian Medical Association Journal, 1957;76(10), 811–822.
27. Liu L, Gao H, Wang H, Zhu K, Yu W, Zhang Y, et al. Comparison of esophageal function tests to investigate the effect of helicobacter pylori infection on gastroesophageal reflux disease (Gerd). Medical Science Monitor, 2018;24, 4791–4797.
28. Fischbach LA, Graham DY, Kramer JR, Rugge M, Verstovsek G, Parente P, et al. Association between Helicobacter pylori and Barrett's Esophagus: A case-control study. American Journal of Gastroenterology, 2014;109(3), 357–368.
29. Wu IC, Wu DC, Yu FJ, Wang JY, Kuo CH, Yang SF, et al. Association between Helicobacter pylori seropositivity and digestive tract cancers. World Journal of Gastroenterology, 2009;15(43), 5465–5471. https://doi.org/10.3748/wjg.15.5465
30. Xu W, Liu Z, Bao Q, Qian Z. Viruses, Other Pathogenic Microorganisms and Esophageal Cancer. GastrointestinalTumors, 2015;2(1),2–13.
31. Otsuka F, Vorpahl M, Nakano M, Foerst J, Newell JB, Sakakura K, et al. Serum pepsinogens and Helicobacter pylori in relation to the risk of esophageal squamous cell carcinoma in the ATBC Study *Bone*. 2012;*23*(1);1–7.
32. Sgouras DN, Trang TTH, and Yamaoka Y, Pathogenesis of Helicobacter pylori infection. Physiology & Behavior, 2017;176(3);139–148.

33. Jansson C, Johansson ALV, Nyrén O, Lagergren J. Socioeconomic factors and risk of esophageal adenocarcinoma: A nationwide Swedish case-control study. Cancer Epidemiology Biomarkers and Prevention, 2005;14(7), 1754–1761.
34. Khademian A, Rajabnia R, Ferdosi-Shahandashti E, Khafri S, Shokri Shirvani J, Sadeghi F, et al. Presence of the Helicobacter pylori in Esophageal Squamous Cell Carcinoma Samples. International Journal of Enteric Pathogens, 2016;4(3), 7–37902.
35. Lopes AI, Vale FF, Oleastro M. Helicobacter pylori infection - Recent developments in diagnosis. World Journal of Gastroenterology, 2014;20(28), 9299–9313.
36. Gisbert JP, Calvet X. Helicobacter pylori "test-and-treat" strategy for management of dyspepsia: A comprehensive review. Clinical and Translational Gastroenterology. 2013;4(3), 32-17.
37. Uhlenhopp D.J, Then E.O, Sunkara T and Gaduputi V. Epidemiology of esophageal cancer: update in global trends, etiology and risk factors. clinical journal of Gastroenterology; 2020 (13)1010–1021
38. Wondimagegnehu A, Hirpa S, Abaya SW, Gizaw S, Getachew S, Ayele W *et al.* Oesophageal cancer magnitude and presentation in Ethiopia 2012–2017. PLOS One 2020;15(12).
39. Kahase D, and Haile K. Helicobacter pylori Infection and Predictors Among Dyspeptic Adult Patients in Southwest Ethiopia: Cross-Sectional Study. Research and Reports in Tropical Medicine. 2020:11 141–147
40. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al. Global Cancer Statistics 2020, GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. CA Cancer J Clin. 2020;71(3):209–49.
41. Talsma. K, van Hagen .P, Grotenhuis BA, Steyerberg .EW, Tilanus .HW, van Lanschot .JJB, et al. Comparison of the 6th and 7th Editions of the UICC-AJCC TNM Classification for Esophageal Cancer. Ann Surg Oncol.2012;19(7):2142–8. 4.
42. Rice TW, Patil DT, Blackstone EH. 8th edition AJCC/UICC staging of cancers of the esophagus and esophagogastric junction: application to clinical practice. Ann Cardiothorac Surg. 2017;6(2):119– 30.
43. Feyisa JD, Adisse A, Kantelhardt EJ, Zingeta GT, Eyoel N, Wondimagegnewu. A ,*et al* .Clinical feature, treatment pattern and survival of Esophageal cancer at Tikur Anbessa

Specialized Hospital, Ethiopia: a prospective cohort study. BMJ Yale 2020; doi: https://doi.org/10.1101/2023.02.14.23285932.

44. Dessalegn B, Enqueselassie F, Kaba M, Assefa M and Addissie A. Risk factors of oesophageal cancer at health facilities in Addis Ababa, Ethiopia: Unmatched case control study. Cancer Prevention, Frontiers in oncology.2022;(1-9).
45. Tramacere I, La Vecchia C, Negri E. Tobacco smoking and esophageal and gastric cardia adenocarcinoma: a meta- analysis. Epidemiology 2011; 22:344-9.
46. Ribeiro A, DeVault KR, Wolfe JT, Stark ME. Alendronate-associated esophagitis: endoscopic and pathologic features. Gastrointest Endosc 2008; 47:525.
47. Lagergren J, Bergström R, Lindgren A, Nyrén O. Symptomatic gastroesophageal reflux as a risk factor for esophageal adenocarcinoma. N Engl J Med 1999; 340:825.
48. Bulcha GG , Leon ME, Gwen M, Abnet CC, Sime A, Pritchett NR et al. Epidemiology of Esophageal Cancer (EC) In Oromia Region, Ethiopia. Journal of global oncology ,2016; 4
49. Chen T, Cheng H, Chen X, Yuan Z, Yang X, Zhuang M, et al. Family history of esophageal cancer increases the risk of esophageal squamous cell carcinoma. Sci Rep. 2015 Nov 3; 5:16038. doi: 10.1038/srep16038.
50. Kgomo M, Elnagar AA, Mokoena T, Jeske C, Nagel GJ. Prevalence of helicobacter pylori infection in patients with squamous cell carcinoma of the Oesophagus. A descriptive case series study. J Gastrointest Cancer. 2016;47(4):396–8.

Annex

Annex I. Information sheet

Title of the Research Project: Magnitude of Helicobacter Pylori infection among patients with esophageal cancer and apparently healthy control at Tikur Anbesa Specialized Hospital visiting oncology Clinic. a case-control study" Addis Ababa Ethiopia.

Principal Investigator: Yismu Eshetu (BSc, MSc candidate)

Name of the Organization: Addis Ababa University Tikur Anbesa Specialized Hospital, College of Health Sciences School of Medicine Department Surgical referral clinic.

Introduction: You are invited to participate as a study subject in a research conducted by MSc candidate, from Addis Ababa University. Your participation is voluntarily. The research teams will include one principal investigator, three advisors; one from Tikur Anbesa specialized Hospital department of cardiothoracic surgery and two School of Laboratory. Please take as much time as you need to read or listen in the information sheet.

Purpose of the Research Project: -We are asking you to take part in this study because we will try to Know the Magnitude of H. pylori in esophageal and apparently healthy individuals.

Procedures and the expected participation

If you are willing to participate, you need to understand the purpose of the study and give your consent. Not only this but also specimen collected from you will be used for the research purpose, and the results of your sample will be exposed to some concerned professional staffs as it is needed. The required clinical sample will be collected by Nurses of surgical referral clinic department. Then, you are requested to give your consent to the sample collector. After consent, blood sample will be collected from your arm and you will give stool. Moreover, there will be a face-to-face interview for additional questions.

Potential risks and Discomfort During collection of specimens from you, appropriate precaution will be taken and all samples will be collected by trained health professionals. If anything happened, appropriate medical care will be provided to you.

Confidentiality

We respect your privacy and confidentiality. Any information that identifies you will not be shared with anyone else outside the study team. The information we will collect from you as part of the study will be kept in a locked file cabinet, or be protected by a password on the computer only accessible to personnel involved in the study. There is no sensitive issue that you will be asked related with your social desirability but any information that is obtained in connection with this study and that can be identified with you will remain confidential.

Potential benefits

You will not receive any payment for your participation in this research study as compensation. However, based on the diagnosis result you will be treated in view of that. In addition, the result of the study will be beneficial for the detection and managing of Esophageal cancer. Hence, you are indirectly benefiting other patients and the society in this respect.

Participation and Withdrawal from the Study

The participation is voluntary and you have the right not to participate in this study. You may withdraw at any time and place without consequences of any kind. You may also reject to give any sample. You can ask any questions regarding to this study and you have a right to get a laboratory diagnosis result free.

Contact information

If you have any questions about this study you can contact the following principal investigators and advisors for further information.

Name; -Yismu Eshetu, Mobile: 0941314614 **E-mail:** yismueshetu2021@gmail.com

Annex II. Information sheet (Amaharic version)

በአዲስ አበባ ዩኒቨርሲቲ ጤና ሳይንስ ኮሌጅ የሕክምና ላቦራቶሪ ሳይንስ ት/ክፍል በማስተርስ ድግሪ ተማሪ የመመረቂያ ጥናት ላይ እዲሳተፉ ተጋብዘዋል፡፡እባክዎ በዚህ ጥናት ለመሳተፍ ከመስማማትዎ በፊት ከዚህ ቀጥሎ የሚገኘዉን ምንባብ በጥሞና ያንብቡና ግልጽ ያልሆነልዎትን ማንኛዉም ሃሳብ ይጠይቁ፡፡

መግቢያ

የጥናቱ ርዕስ: Magnitude of Helicobacter Pylori infection among patients with esophageal cancer and apparently healthy individuals at Tikur Anbesa Specialized Hospital visiting oncology Clinic, Addis Ababa Ethiopia.

የእርስዎ በዚህ ጥናት ላይ የሚኖርዎት ተሳትፎ ሙሉ በሙሉ በበጎ ፈቃደኝነት ላይ የተመሰረተ ነዉ፡፡ በዚህ ጥናት ዉስጥ ላለመሳተፍ ወይም ለመሳተፍ ከወሰኑ በጎላ ለማቋረጥ የሚወስኑ ቢሆንም እንኩዋ በዚህ ሆስፒታል የሚሰጠዉ ማንኛዉም አገልግሎት አይቋረጥም፡፡በጥናቱ ለመሳተፍ የሚስማሙ ከሆነ የስምምነት ቅጹ ላይ በጹሁፍ ወይም በጣት ፊርማ ማስቀመጥ ይጠበቅዎታል፡፡

የጥናቱ ተሳታፊ ለመሆን የሚጠበቅበዎት ምንድን ነው?

በዚህ ጥናት ለመሳተፍ የሚስማሙ ከሆነ ናሙናዎ ለጥናቱ እንዲሚዉል መስማማት ይጠበቅብዎታል፡፡ከተወሰደዉ ናሙና ላይ የሚገኙ መረጃዎች ከዚህ ሆስፒታል ዉጭ ለሚገኙና ለስራዉ አግባብነት ላላቸዉ ሰዎች ቢነገር የማይቃወሙ መሆኑን መስማማት ይጠበቅብዎታል፡፡ይሁን እንጅ ይህ አይነቱ መረጃ የርስዎን ማንነት የሚገልጡ መረጃዎችን ማለትም ስም፤አድራሻና የስልክ ቁጥር የመሳሰሉትን መረጃዎቸን አይጨምርም፡፡ ይልቁንም ለዚህ አገልግሎት ብቻ የሚዉል እርስዎን ለማወቅ የሚያስችል መለያ ቁጥር ጥቅም ላይ እንዲዉል ይደረጋል፡፡በተጨማሪም ስለርስዎ አጠቃላይ የጤና ሁኔታ ለሚቀርቡ አንዳንድ ተጨማሪ ጥያቄዎች መልስ መስጠት ይኖርብዎትአል፡፡

በዚህ ጥናት መሳተፍ የሚያስከትላቸዉ ቸግሮች ምንድን ናቸዉ?

ናሙና በሚሰበሰብበት ወቅት ምንም አይነት የከፋ ችግር አያጋጥምዎትም። ሆኖም ግን ናሙናዉን ለመሰብሰብ ልምድ ያለዉ ባለሙያ ስለሚመደብና አስፈላጊዉ የጥንቃቄ እርምጃ ስለሚወሰድ የህመም ስሜት አይኖርም።

የህክምና መረጃ በሚስጥር ተጠብቆ መቆየት የሚችለዉ እንዴት ነዉ?

ስለራስዎ የሰጡት ማንኛዉም መረጃና ከተወሰደዉ ናሙና ላይ የተገኘዉ የላቦራቶሪ ዉጤት የሚዉለዉ ለጥናቱ አላማ ብቻ ነዉ። ይህን ማህደር ሊያገኙ የሚችሉት የተወሰኑ የጥናቱ ተባባሪ ሰዎች ብቻ ናቸው።ከዚያም በላይ ስለ እርስዎ ያለውን ማንኛውንም መረጃ የተለየ የይለፍ ቃል ባለው የኮምፒውተር የመረጃ ማህደር ውስጥ እንዲቀመጥ ይደረጋል ።

በዚህ ጥናት መሳተፍ የሚያስገኛቸው ጥቅሞች ምንድን ናቸው ?

ይህ ጥናት የማስተርስ ዲግሪ መመረቂያ እንደመሆኑ መጠን በዚህ ጥናት በመሳፈልዎ በገንዘብ የሚያገኙት ጥቅም ባይኖርም ከጥናቱ በሚገኘው ውጤት ግን ተጠቃሚ ነዎት። የእርሶዎ ተሳትፎ የእርስዎንና የወገንዎትን የጉሮሮ ካንሰር ለማወቅና ለማከታተል ከፍተኛ ጥቅም ይኖረዋል።

በዚህ ጥናት ተሳታፊ የመሆንዎ መብቶች ምንድን ናቸው ?

በዚህ ጥናት መሳተፍ ሙሉ በሙሉ በእርስዎ ፈቃደኝነት የተመሰረተ በመሆኑ በማንኛውም ሰዓትና ቦታ የማቋረጥ ሙሉ መብት የተጠበቀ ከመሆኑም በላይ እራስዎን ከጥናቱ በማግለልዎ ምክንያት የሚቀርብዎት ምንም አይነት የሆስፒታል አገልግሎት አይኖርም ።ከዚህም በተጨማሪ ጥናቱን በተመለከተ ማንኛውንም አይነት ጥያቄ የመጠየቅና ገለጻ የማግኘት መብት አለብዎት።የላብራቶሪ ምርመራ ውጤቱንም በነጻ ማግኘት ይችላሉ። ነገር ግን እርስዎ በሚሰጡን መረጃ የችግሩን ስፋት ለመከላከል እና ለመቆጣጠር ጠቃሚ ስለሆነ ለሚቀርብልዎት ጥያቄ ቀጥተኛ መልስ ይሰጡን ዘንድ በታላቅ አክብሮት እንጠይቃለን።

ጥያቄ ካለኝ ወይም ችግር ቢያጋጥመኝ ምን ማድረግ ይገባል?

ይህንን ጥናት በተመለከተ ወይም ከዚህ ጥናት ጋር በተዛመደ መልኩ ስለሚያጋጥሙ ድንገተኛ አደጋዎች ወይም ጥያቄ ካለዎት በሚመለከተው አድራሻ ይጠቀሙ።

ሞባይል፡ +251-941314614 ኢሜል፡ yismueshetu2021@gmail.com

Annex III. Informed consent

Card No…..……

I had been informed that the objective of this study is to Compare cytochemistry and flow cytometry for the diagnosis of acute leukemia. The results of this study have an importance to treat me and o

their patients, and to be used as an input for the future development of strategies H. pylori in Esophageal Cancer and Non-Esophageal cancer. I had been also informed about the confidentiality of this study. The principal investigator requested me to participate in the study that would require my willingness to provide the required data that include blood and stool sample, and filling questionnaire. Therefore, with full understanding of the importance of the study, I agreed voluntarily to provide the requested samples and my benefit will be only from the free laboratory investigation results.

I_____ hereby give my consent for providing the requested information and specimens as the doctors find best for me.

Signature: _____ Date_____

Annex IV. Informed consent (Amharic version)

የሚስጥር ቁጥር ----------

የተሳታፊዉ ስም ----------

እኔ ስሜ ከላይ የተጠቀሰው ተሳታፊ “Magnitude of *Helicobacter Pylori* infection among patients with esophageal cancer and apparently healthy controls at Tikur Anbesa Specialized Hospital visiting oncology Clinic Addis Ababa Ethiopia.” **ጥናት ላይ በቂ ገለጻ ተደርጎልኛል፡፡ ለጥናቱም ደምና የሰገራ ናሙና እንደሚያስፈልግ ተገልጾልኛል፡፡የጥናቱንም አላማዎችም ተረድቻለሁ፡፡**

በቃለ መጠይቁ ላይ የገለጽኳቸው መረጃዎች በሙሉ በሚስጥር የተጠበቁ እንደሚሆኑ ተነግሮኛል ፡፡በጥናቱ ላይ ያለመሳተፍና ማንኛውንም መረጃ ያለመስጠት እንዲሁም በማንኛውም ጊዜ ከጥናቱ ራሴን የማግለል መብቴ የተጠበቀ እንደሆነ ተገልጾልኛል፡፡

ስለዚህ ለዚህ ጥናት መረጃና የስምምነት ቃሌን የሰጠሁት በአጠቃላይ ሁኔታውን በመረዳትና በፍጹም ፍቃደኝነት ነው፡ በተጨማሪም ጥያቄ ለመጠየቅ ተፈቅዶልኝ ለማወቅ የፈለኩትን ያህል ማብራሪያ አግኝቻለሁ ፡፡የዚህ ጥናት ተሳታፊ በመሆኔ የማገኘው ጥቅም የሁሉንም ምርመራ ውጤት በነጻ ማግኘት እንደሆነ ተረድቻለሁ፡፡

በአጠቃላይ እኔ ከላይ በመተማመኛ ቅፅ የተጠቀሱትን ሁሉ በሚገባና በተረጋጋ መንፈስ አንብቤዋለሁኝ፡፡ ስለዚህ በዚህ ጥናት ለመሳተፍ ፈቃደኛ መሆኔን በፊርማዬ አረጋግጣለሁ፡፡

ፊርማ---------- ቀን ----/---/----------

Annex V. Consent form for parents

I have read the information above, or it has been read to me. I have been given the opportunity to ask questions and my questions have been answered to my satisfaction. I voluntarily consent that my child participates in this study provided he/she gives assent.

To give his/her stool

To give his/her Blood

To collect her/his blood and be a participant in this study and understand that I have the right to withdraw my child from the study at any time.

Name of participant, date and signature or thumb impression of participant

_____ ____ /____ /____ (dd/mm/yy) _____

Name of researcher, date and signature of researcher

_____ _____/____/_____ (dd/mm/yy) _____

Annex VI. Questionaries'

1. Card number _____ Age: _____ Gender; Male ____, Female ____
   Body weight _____ Kg?
2. Address: _____ Rural _____Urban _____ Hospital _____ Ward _____
3. Level of Educational? Uneducated _____Elementary _____high school _____ Diploma_____ Degree_____ above_____
4. Type of malignancy EAC_____ ESCC_____ stage of malignancy_____
5. Have you taken any antibiotic? YES_____NO_____, If yes, type of antibiotic _____and for how many Day _____Week, _____ Month_____you use?
6. Do you have a previous history of esophageal cancer? Yes ___NO ___ If "yes" have you been treated? _____
7. Do you have Previous Exposure or infection of H. pylori? yes _____NO____ IF Yes before how many days_____ or week _____and are you treated _____?
8. Do you Drink alcohol? YES_____NO _____ If yes, how much you consume? low _____high ____, what type _____? Frequency? Daily____ weekly_____ Monthly _____ Holiday_____
9. If the answer for question NO 8 is YES when you start drinking alcohol? Current _____ before a year_____ any other time _____
10. Do you use any tobacco? YES ____NO_____, If yes what type? Cigarate _____ any other_____ How much a day in NO____ One pack_____ More ____
11. Do you take Hot Beverages commonly? YES _____NO _____if yes how much per Day___ OR Week _____Do you eat hot food? YES_____NO _____ if yes what type? _____ degree of hotness, Much warmer _____Medium _____ low _____
12. Do you have any esophageal related disease? YES _____NO_____, If yes, what type? Gastric cancer _____, Gastritis_____ colon cancer_____ other _____
13. Do you eat fruits and vegetables? YES_____NO_____ If yes , Low _____, medium ____, high_____
14. Do you have family history of esophageal cancer? YES_____NO_____

15. What type of Job you work? _____your work place_____
16. What is your economy level or income? Lower _____middle _____ higher ____
17. Do you practice a physical exercise? YES ____ NO ____
18. Do you have swallowing problem? YES ___NO____, If yes, what type of food, Solid_____, Semisolid_____, Liquid_____ Saliva _____
19. DO you have a history of radiotherapy? YES ____ NO_____
20. Do you eat read meat? YES ____ NO ___, if yes, is it frequent? YES ___ NO ____

Annex VII. Questionaries (Amharic Version)

አዲስ አበባ ዩኒቨርስቲ ጥቁር አንበሳ ስፔሻላይዝድ ሆስፒታል

ለማስተርስ መመረቂያ ጽሁፍ የሚሆን ቃለ መጠይቅ

1. ካርድ ቁጥር _____ እድሜ _____ ጾታ _____ ክብደት _____
2. የመኖርያ ቦታ _____ የሚታከሙበት ሆስፒታል _____
3. የሚታከሙበት ክፍል _____
4. የትምህርት ደረጃዎትን ይግለጹ? ያልተማረ ☐ የመጀመርያ ደረጃ ☐
   ሁለተኛ ደረጃ ☐ ዲፕሎማ ☐ ዲግሪ ☐ ዶክትሪት ☐
5. የታመሙትን የካንሰር አይነት ይግለጹ _____
6. ከዚህ በፊት መድሀኒት ወስደው ነበር? አዎ ☐ አይደለም ☐
   አዎ ከሆነ መልስዎት የምን አይነት መድሀኒት ወሰዱ_____ ለምን ያክል ጊዜ? _____
7. ከዚህ በፊት የጉሮሮ ካንሰር ነበረብዎት ? አዎ ☐ አይደለም ☐
   ከነበረብዎት ታክመው ነበር?_____ ምን አይነት የጉሮሮ ካንሰር ነው?_____
8. ከዚህ በፊት በሄሊኮባክተር ፓይሎሪ ባክቴሪያ ተይዘ☐ነበር? አዎ☐ አይደለም
   ከተያዙ ከስንት ቀን በፊት _____መድሀኒትስ ወስደው ነበር_____ ለምን ያክል ጊዜ _____
9. አልኮል ይጠጣሉ? አዎ ☐ አይደለም ☐
   ከጠጡ ምን አይነት _____
10. ሲጋራ እና ሌሎች አደንዛዥ እጽ ይጠቀማሉ? አዎ ☐ አይደለም ☐
11. ትኩስ ነገር ወይም ሞቅ ያለ መጠጥ ያዘወትራሉ ወይም ይጠቀማሉ? አዎ ☐
    አይደለም ☐
12. ትኩስ ምግብስ አዘውትረው ይመገባሉ ? አዎ ☐ አይደለም ☐
    አዎ ካሉ የምግቡን አይነት ይጥቀሱ_____
13. ሌሎች ከጉሮሮ ካንሰር ጋር ተያያዥነት ያላቸው በሽታዎች አለብዎት? ☐
    አይደለም ☐
    ካለብዎት ምን አይነት በሽታ_____
14. አትክልት መመገብ ያዘወትራሉ? አዎ ☐ አይደለም ☐
    አዎ ካሉ በምን ያህል ጊዜ _____

15. ከቤተሰብዎ መካከል የጉሮሮ ካንሰር ያለበት ሰው አለ?አዎ ☐ አይደለም ☐

16. ምን አይነት ስራ ነው የሚሰሩት _____

17. የገቢ መጠንዎ ዝቅተኛ ☐ መካከለኛ ☐ ከፍተኛ ☐

18. ያካል ብቃት ንቅስቃሲ ደርጋሉ ? አዎ ☐ አይደለም ☐

19. ጨረር ህክምና ታክመው ነበረ; አዎ ☐ አይደለም ☐

20. ቀይ ስጋ ያዘወትራሉ? አዎ ☐ አይደለም ☐

Annex VIII: Questionaries for Apparently healthy individuals

1. Card number _____ Age: _____ Sex _____Body weight _____ Kg?
2. Address: _____Hospital _____Serial number: _____Ward _____
3. Level of Educational? Uneducated _____Elementary _____high school _____ Diploma_____ Degree_____ Masters____ Phd ___
4. Have you taken any antibiotic? YES_____NO_____, If yes, type of antibiotic _____and for how many Day _____Week, _____ Month_____you use?
5. Do you have a previous history of esophageal cancer? Yes ___NO ___ If "yes" have you been treated? _____
6. Do you have Previous Exposure or infection of H. pylori? yes _____NO____ IF Yes, before how many days_____ or week _____and are you treated _____?
7. Do you use alcohol? YES_____NO _____ If yes, what type _____?
8. Do you use any tobacco? YES ____NO_____, If yes what type? Cigarate _____ any other_____
9. Do you take Hot drink commonly? YES _____NO _____
10. Do you eat hot food? YES_____NO _____ if yes what type? _____
11. Do you have any esophageal related disease? YES _____NO_____, If yes, what type? Gastric cancer _____, Gastritis_____ colon cancer_____ other _____
12. Do you eat fruits and vegetables? YES_____NO_____ if yes for how many times per month_____ or week _____, if NO, what food you commonly eat? _____
13. Do you have family history of esophageal cancer? YES_____NO_____
14. What type of Job you work? _____
15. What is your economy level or income? Lower _____middle _____ higher ____
16. Do you practice a physical exercise YES ☐ NO? ☐

17. Do you practice a physical exercise? YES ____ NO ____
18. Do you have swallowing problem? YES ___NO____, If yes, what type of food, Solid_____, Semisolid_____, Liquid_____ any other _____
19. DO you have a history of radiotherapy? YES ____ NO_____
20. Do you eat read meat? YES ____ NO ___, if yes is it frequent? YES ___ NO ____

Annex X. Data collection Format

Study participant										
cases						**control**				
No	Cancer type	Age	Sex	**Result**		No	Age	Sex	**Result**	
				H.p stool antigen	H.p serum antibody				H.p stool antigen	H.p serum antibody
01						01				
02						02				
03						03				
etc.						etc.				

Annex XI. Laboratory procedure

I. H. pylori stool antigen test

1. Unpackage the aluminum bag of the test device by tearing
2. Check sample collection part
3. Shake the sampling bottle thoroughly
4. Be sure that the sampling bottle is at the vertical direction in the manner that the top part is correctly positioned then break of the plastic cover carefully
5. Put 3-4 drops from sample solution in to dropping hole as shown in the picture.
6. Read out the test result after 5-10 minutes, do not take the result in to consideration after 15 minutes.

Interpretation of the test

The visible line in the T space confirms positive result, unless this visible line is seen in the T space the test is negative. However, there is a color line every time in the control space.

II. Serum H. pylori antibody test

1.Serum sample is first equilibrated to room temperature before testing

2. Remove the device from the pack and label the device before performing the assay

3. Add 1 drop of fresh serum to sample well marked as **S** allow about 30 second for specimen to be absorbed totally

4. Discard 3 drop of wash buffer and add 3 drop of wash buffer in to sample well.

5**.** Strong positive result observed 2-3 minute and weak positive may take up to 7 minutes.

Interpretation of the test

The visible line in the T space confirms positive result, unless this visible line is seen in the T space the test is negative. However, there is a color line every time in the control space

Annex XII. Declaration

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

M.Sc. candidate: Yismu Eshetu (BSc.)

Signature: _____

Date of submission: _____

This Thesis has been submitted with our approval as advisors.

Advisor:

Mr Kassu Desta (MSc, PhD candidate)

Signature: _____

Date: _____

Place: Addis Ababa, Ethiopia.

Advisor: Dessie Abera (MSc)

Signature: _____

Date: _____

Place: Addis Ababa, Ethiopia.

Professor Aster Tsegaye (MSc, PhD)

Signature: _____

Date: _____

Place: Addis Ababa, Ethiopia.