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***Helicobacter pylori* infection among dyspeptic and non-dyspeptic HIV patients
at Yeka Health center, Addis Ababa, Ethiopia.**

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This is to certify that the thesis prepared by GetachewSeid , entitled: Magnitude of *Helicobacter pylori* infection among dyspeptic and non-dyspeptic *HIV* patients at Yeka health center, Addis Ababa, Ethiopia and submitted in partial fulfillment of the requirements for the degree of Master of Science in Clinical Laboratory Sciences (Diagnostic and Public Health Microbiology Specialty) complies with the regulations of the University and meets the accepted standards with respect to originality and quality.

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List of Abbreviations

AARL:	Addis Ababa Regional Laboratory
AAU:	Addis Ababa University
AIDS:	Acquired Immune Deficiency Syndrome
ART:	Anti-retroviral Therapy
BD:	Benson Dickinson
BMI:	Body Mass Index
CBC:	Complete Blood Count
CDC:	Center for Disease Control
CD3:	Cluster of Differentiation 3
CD4:	Cluster of Differentiation 4
CD8:	Cluster of Differentiation 8
CI:	Confidence Interval
95% CI –	95% confidence interval
CLSI :	Clinical Laboratory Standard Institution
DRERC:	Department Research and Ethical Review Committee
EDTA:	Ethylene Diamine Tetra Acetic Acid
ELISA:	Enzyme Linked Immune Sorbent Assay
GI:	Gastro Intestinal
HAART:	Highly Active Anti retro Viral Therapy
HIV:	Human Immune Deficiency Virus
<i>H. pylori</i> :	<i>Helicobacter pylori</i>
HpSA:	<i>Helicobacter pylori</i> Stool Antigen Test
ICSH:	International Committee for Standardization in Hematology
MALT:	Mucosal Associated Lymphoid tissue
NUD:	Non Ulcer Dyspepsia
OR:	Odds Ratio
PI:	Principal Investigator
PU:	Peptic Ulcer
RNA:	Ribo Nucleic Acid

SOPs: Standard Operational Procedures

SPSS: Statistical Package for Social Sciences

WHO: World Health Organization

YHC: Yeka health center

Operational definitions

Dyspepsia: abdominal pain or discomfort bloating, nausea, vomiting and early satiety for > 1 months

Non -dyspeptic: patient who does not have any symptom of bloating,nausea ,vomiting and early satiety for greater than one month.

Case: are HIV patients who have dyspepsia.

Control: are HIV patients who are non- dyspeptic.

Current CD4 T cell count: is CD4 count that is done during the study.

Abstract

Background: *Helicobacter pylori* is a potent producer of urease. HIV infected patients experience many form of opportunistic infections including gastrointestinal symptoms. The exact role of *H. pylori* infection among HIV infected patients in causing gastrointestinal symptoms such as dyspepsia remains unclear. There are limited data regarding *H. pylori* infection in HIV-positive patients in relation to CD4 count in our region and country.

Objectives: To assess the prevalence of *Helicobacter pylori* infection using stool antigen among dyspeptic and non -dyspeptic HIV patients at Yeka health center, Addis Ababa, Ethiopia.

Methods: A case control study enrolling 370 study participants was conducted from January-June 2017. Socio-demographic and clinical data were collected and 3 milliliter blood was drawn aseptically for CD4+T cell count and complete blood count. Stool samples were collected for *H. pylori* stool antigen test. All necessary data were recorded on questionnaires and data sheet. Data entry and analysis was done using SPSS version 20. Logistic regression analysis was used to see the relation between dependent and independent variables.

Results: Of the 370 (185 dyspeptic and 185 non-dyspeptic) study participants, *H. pylori* antigen was detected in 117 (31.62%) participants. The prevalence of *H. pylori* infection was slightly higher in dyspepsia patients 60/185 (32.43%) than non-dyspepsia 57/185 (30.81%) individuals [AOR=1.097, 95% CI: 0.689-1.747, p=0.697]. The infection was significantly associated with gender in non dyspepsia patients [AOR=2.612, 95% CI: 1.338-5.099, p=0.015] while in non-dyspepsia patients the association was not significantly [AOR=0.805, 95% CI: 0.393-1.648), p=0.639]. In both patient groups, no significant association was observed in the prevalence of *H. pylori* with age, family size, educational status, marital status, toilet use habit, occupation, alcohol drinking, cigarette smoking and *khat* chewing (p>0.05). In both case and control group *H. pylori* infection was higher in patients with CD4 T cell count greater than 200 cells/ μ l.

Conclusion: The prevalence of *H. pylori* infection was slightly high among symptomatic than non-symptomatic patients. The infection was not significantly associated with socio-demographic and behavioral factors while it was higher in patients with higher CD4 T cell count. The observed prevalence of *H. pylori* stool antigen positivity necessitates the need to design and apply intervention measures that could decrease transmission and thus minimize the clinical consequences of infection.

Key words: Dyspepsia, Non-dyspepsia, *Helicobacter pylori*, Prevalence, Stool Antigen Test

1. Introduction

1.1 Background

Helicobacter pylori is a spiral bacterium with flagella and a potent producer of urease. It is through the production of bicarbonate, by metabolizing the urea in gastric fluid, that the organism is able to survive in the low pH environment of the stomach. There the infection induces a host immune response which results in mucosal damage and a chronic active gastritis. This occurs initially in the non – acid secreting areas of the stomach in the antrum [1].

Helicobacter pylori is a causative organism for chronic gastritis and is associated with peptic-ulcer disease. Infection may be asymptomatic as well. The prevalence of *Helicobacter pylori* varies among continents and countries around the world, and its prevalence varies from 9% to 82% . It is clear that, among the several types of *Helicobacter pylori*, only the carriers of the Cag A gene, that signalizes a pathogenicity island, with a myriads of pathogenic genes such as Bab A, OIp A and many others and also the carriers of the VaC A gene can induce the disease. So, only CagA and VaC A gene carriers could develop disease including ulcers. All the others genes combinations will not develop any disease and are asymptomatic [2].

More than half of all humans are colonized in their stomachs by *Helicobacter pylori*. That carriage is nearly universal among adults in developing countries suggests that in earlier times most humans carried these organisms. However, it is not known whether *H. pylori* have been with all humans for hundreds of years, or for millions of years [3]. In either case, with the changing conditions associated with improved socioeconomic status, *H. pylori* is disappearing in industrialized countries and declining in prevalence in some developing countries as well [4].

HIV-1 infected patients experience many form of opportunistic infections including gastro intestinal symptoms [5].The exact role of *H. pylori* infection among HIV-1infected patients in gastro duodenal lesions might be different from the general population. It remains unclear if upper gastrointestinal symptoms such as dyspepsia are highly active antiretroviral therapy (HAART) related adverse effects as a result of *H. pylori* infection [6]. The co-morbidity of *H.*

pylori infection in HIV patients is not well defined and the role of *H. pylori* in gastro duodenal lesions might be different between the general population and AIDS patients [7].

Previous studies done on prevalence of *H. pylori* in association with HIV infection have suggested that cell mediated immune deficiency does not appear to increase the risk of infection with *H. pylori* [8]. The involvement of gastrointestinal tract may be because it represents the largest reservoir of HIV in the body with abundant morphological changes in the upper-gastrointestinal tract mucosa and medication complications [9]. Studies have shown that the prevalence of *H. pylori* infection in HIV-1 positive patients is remarkably low compared with the general population [10]. The use of the stool antigen test is recommended for targeted high risk populations [11].

Some studies have indicated that the CD4/CD8 ratio in gastric mucosa is different in subjects with and without *H. pylori* infection. Therefore, CD4 lymphocytes, which are depleted in AIDS patients, might be associated with a different presentation of *H.pylori* infection [12]. Researchers investigated the role played by the level of *CD4+* T-cells in the acquisition of *Helicobacter pylori* infection. Other studies depicted a reduction in the frequency of *H.pylori* infection among severely immune suppressed patients, with acquired immunodeficiency syndrome (AIDS) [13].

The overall prevalence of *H. pylori* is suggested to be correlated with socioeconomic conditions. Gender, occupation, low socioeconomic status, educational level, and alcohol consumption are known risk factors for *H. pylori* infection. However; persistent colonization depends on the host immune responses to the bacterium [14]. Infection may also occur in adult life, which would explain the above average rates of infection in some occupational groups. In some studies, gastroenterologists have been shown to be significantly more likely to be infected than aged matched controls [4, 15, 16].

Three modes of transmission have been described. These include: oro-faecal, oro-oral and water borne. The infection is mainly acquired in childhood in both industrialized and developing countries, and persists throughout life unless treated [17].

Helicobacter pylori stool antigen detection by immunochromatographic or immunoenzymatic methods has a sensitivity of 91–98% and a specificity of 94–99% [18], but its accuracy may be decreased by gastrointestinal bleeding or treatment with proton pump inhibitors [19].

1.2. Statement of the problem

Human immunodeficiency virus (HIV) currently infects over 35 million persons and causes 1.6 million deaths each year. The study of *H. pylori* – HIV co-infection represents an important research issue, as these germs are responsible for two of the most significant present-day pandemics [20]. There is 20% prevalence of infection with *H. pylori* among adolescents in United States in comparison to infection rates exceeding 90% in the developing countries [21].

Infection with *H. pylori* has been recognized as a public health problem worldwide. Though half of the world's population is infected with *H. pylori*, only approximately 30 % of the infected people are symptomatic with more than 7% of them remaining asymptomatic [22]. Previous sero – epidemiologic studies indicated that about 50% of adults in the developed countries and nearly 90% of adults in developing countries were sero - positive for *H. pylori*. Recent studies in Ethiopia showed that the overall prevalence of *H. pylori* infection in adult dyspeptic patients, as found by the different diagnostic methods, varied between 69 and 91% [15].

Among various co-infections incriminated in human immunodeficiency virus (HIV)-associated pathology, *Helicobacter pylori* plays an important role, but under-investigated. Many HIV-infected patients have dyspeptic complaints and, though there is a recognized association between *Helicobacter pylori* and peptic ulcer among general population, the etiology of gastrointestinal disorders varies greatly among HIV-positive individuals, ranging from opportunistic infections to medication adverse effects [13].

Although some studies have shown that *H. pylori* infection is less common among HIV-positive individuals with GI symptoms, other investigations suggested a higher prevalence of *H. pylori* infection in HIV-positive patients as a result of immune suppression. Hence, the relationship between *H. pylori* infection and HIV remain controversial [14].

Moreover, there are limited data regarding the burden of *H. pylori* in HIV-positive patients, which is done by *H.pylori* stool antigen test, particularly in our region and country. This study aims to assess the burden of *H.pylori* infection among dyspeptic and non-dyspeptic HIV-patients and its correlation with CD4⁺ cell count and complete blood cell count level. It also examines related risk factors for the prevalence of the infection.

1.3. Significance of the study

- The results obtained in this study give relevant information on the extent of the infection in symptomatic patients and asymptomatic HIV patients that helps for treatment of the patient.
- The study on provides relevant information on the association of the infection with CD4 count and hemoglobin level, helps to identify selection of appropriate antibiotics for empiric treatment.
- The study may provide information on the risk factors for the infection of *Helicobacter pylori* in HIV patients for appropriate action.
- The study may give information on the contribution of *H.pylori* to cause dyspepsia in HIV patients so helps to improve their clinical management.

2. Literature review

2.1 *H.pylori* infection among dyspeptic and non-dyspeptic patients

A cross-sectional study, study by *Aje Joseph et al.* in Nigeria among patients infected with HIV-1 on antiretroviral therapy using *H. pylori* stool antigen. Of 139 diagnosed HIV-1 infected patients who participated in the study, the prevalence rate of *H.pylori* infection was 46.8%, 18 (13.0%) were men and 38 (33.8%) were women[5].

H. pylori causes dyspepsia, and then the prevalence of the infection should be higher in symptomatic patients. Some investigators have reported a higher prevalence of *Helicobacter pylori* in dyspepsia than in controls, others have found no difference in the prevalence between the two groups, or even a higher prevalence in the controls [23]. Stool antigen tests have recently been welcomed with great expectations as they are convenient and require little technical expertise to perform even in less equipped laboratories [24, 25].

A cross sectional study conducted by *Feleke M et al.* at the Hospital of Gondar University, Northwest Ethiopia, on dyspeptic patients found that the prevalence of HIV among the patients was about 20.5% (44/215). The HIV seropositivity was not associated with *H. pylori* seropositivity as only 19.6% (36/184) of the patients who were positive for *H. pylori* were also positive for HIV while 25.8% (8/31) patients who were negative for *H.pylori* were positive for HIV [26].

A cross sectional study conducted by *Tsega E ,et al.* in Ethiopia in non-ulcer dyspepsia and asymptomatic patient found that 136 patients with NUD and 71 controls had six endoscopic biopsies from different sites of the gastric mucosa for histological diagnosis. *H. pylori* was found in 82(65%) patients with NUD and in 38(56%) asymptomatic controls [27].

A comparative cross-sectional study was conducted by *Tebelay Dilnessa,et al.* among dyspepsia and non-dyspepsia adults at Assosa General Hospital in Ethiopia. *H.pylori* was done by stool antigen test. Of a total of 230(115 dyspeptic and 115 non-dyspeptic) study participants, *H. pylori* antigens were detected in 112(48.7%) of them. Further, the prevalence of *H. pylori* infection was

significantly higher in dyspepsia patients 67/115 (58.3%) than non-dyspepsia 45/115 (39.1%) individuals [28].

2.2 *H.pylori* infection and CD4+ T cell count

A prospective cross-sectional study was carried out by Fialho, *et al.* at the Hospital São José, Brazil from May 2001 to April 2003. Two hundred and fifty four participants were included: 113 HIV-infected dyspeptic patients and 141 age-matched controls. *H.pylori* status was evaluated by urease test and histology. The overall prevalence of *H. pylori* infection was significantly lower in HIV-infected patients (37.2%; 42/113) when compared with the controls (75.2%; 106/141); and did not increase with age. A non-significant lower prevalence of *H. pylori* infection was observed in the patients with CD4 T cell count below 200 [29].

In a case-control study by Abdollahi, *et al.* at Imam Hospital in Tehran, Iran, 114 patients already diagnosed as HIV-positive cases were consecutively enrolled. None of the HIV-positive patients and controls had symptoms referable to upper GI tract. Ig A and Ig G anti-*H.pylori* antibody titer was measured by ELISA techniques. IgG antibody titre was positive in 66 HIV-positive patients (57.9%). On the other hand, IgA titer was positive in 3 HIV-positive patients (2.64%). Of 114 HIV-positive patients, 79 (69.3%) were receiving antiretroviral therapy (ART). HIV patients were classified into three groups based on their CD4⁺ cell count accordingly; CD4⁺ < 200, 200 ≤ CD4⁺ < 500, and CD4⁺ ≥ 500. Serum anti-*H.pylori* IgG and IgA seroprevalence were compared between these groups. Although there was an increasing trend of higher IgG and IgA titer by increasing CD4 cell count in HIV-positive patients, it was not reach statistical significance [14].

A cross sectional study was conducted by Ejilude O, *et al.* at Sacred Heart Hospital, Abeokuta; Nigeria. Out of 230 dyspepsia patients examined, 109 (47.4%) were HIV positive and with clinical co-infection with *H. pylori*. A high prevalence rate of 29.4% was recorded in the age group 26- 35 while a low rate of 3.5% was recorded in 66-75 age group. Female participants were more predisposed to the diseases (52.7%) than male individuals showing 47.4%. Socio-economic status of the patients tested show that unemployed are the high risk group to *H. pylori* infection. Low CD4 count of 213 cell/mm³ among 17.4% unemployed and 405 cell/mm³ among

36.7% artisan was observed. The prevalence rate of *H. pylori* in HIV patients was 47.4%, indicating a very high prevalence HIV and *H. pylori* co-infection in apparent dyspepsia patients [30].

Consecutive adult HIV-infected patients presenting to the HIV outpatient clinic, and HIV-negative blood donors were enrolled in a cross-sectional study conducted at the Komfo Anokye Teaching Hospital in the Ashanti Region of Ghana Between November 2011 and November 2012. *H. pylori* was tested by stool antigen test. Among ART-naïve HIV patients, those with *H. pylori* co-infection had higher CD4 T cell counts (312 vs. 189 cells/ μ l), higher CD4/CD8 ratios (0.31 vs. 0.19, $p < 0.0001$) and lower HIV-1 viral loads (4.92 vs. 5.21 log₁₀ copies/ml) compared to those without *H. pylori* co-infection. In the HIV-infected, ART-exposed group, no significant associations between *H. pylori* status and CD4+ T cell count, HIV-1 viral load, or the proportion of patients with undetectable viral load were observed. However, significantly lower CD8+ T cell counts (858/ μ L vs. 990/ μ L), and a trend towards higher CD4/CD8 ratios (0.55 vs. 0.49), as possible indicator of decreased immune activation, were noted among those patients with *H. pylori* co-infection [31].

As revealed by a case control study in Cameroon, the prevalence rate of *H. pylori* infection in patients belonging to four CD4 groups was as follow: Group A (HIV-positive patients with a CD4count below 200 cells.uL) 42.1% (8/19), group B(HIV-positive patients with a CD4count from 200 to 499) 65.4% (17/20), group C(HIV-positive patients with a CD4 count higher or equal to 500) 27.3 % (3/11) and group D (HIV negative control patients) 55.4%(31/56). The prevalence of *H. pylori* infection in HIV-positive patients did not differ between patients with and without peptic ulcer (PU). Compared with HIV-negative controls, HIV-positive patients with a CD4 count less than 200 had a low prevalence rate of *H. pylori* infection, this difference was not significant [32].

A case control study was conducted in Addis Ababa by *Brhanu T, et al.* to determine the sero prevalence of *H. pylori* in HIV positive patients. The mean age of the participants was 33years with the range of 18 - 60 years. Majority (78.3%) were in the age range of 18 - 40 years. Females constituted 64 % of the total study participants. Only 2.8% had monthly income of 1500 to 2499

birr. Among the illiterate participants (n=35), 60% of them were positive for *H. pylori* antibody. Of the total, 79% (89 of 112) were unemployed of whom 52.8% were *H.pylori* positive. The gender specific prevalence of *H. pylori* infection is the same in the two groups (56.8 % in males and 56.6% in females). The overall prevalence of *H. pylori* infection in this study population was 57.08 % (120 / 212). Sixty eight of the 106 (64.2%) HIV positive participants were *H.pylori* positive while fifty two of the 106 (49.1%) HIV negative were positive for *H. pylori*. The HIV positive patients were further sub classified in to two groups according to their CD4+ lymphocyte count (mean=393.57/mm³; median=406/mm³ and range=693/mm³). Among the HIV positive patients there was no significant difference in the prevalence of *H.pylori* infection between CD4+ count less than 200 cells/ μ l and CD4+ count greater than 200 cells/ μ l [33].

2.3 *H.pylori* infection and Hemoglobin level

A cross-sectional study was conducted at the Komfo Anokye Teaching Hospital in the Ashanti Region of Ghana Between November 2011 and November 2012. The study involved consecutive adult HIV-infected patients presenting to the HIV outpatient clinic, and HIV-negative blood donors. *H. pylori* was tested by stool antigen test. Among ART-naïve HIV patients *H. pylori* positive patients had higher mean hemoglobin levels (11.1 vs. 10.4 g/dl,),and lower platelet counts (262.4 vs. 314.9 x1000/ μ l) [31].

A case- control study was carried out by *Abdollahi, et al.* at Imam Hospital in Tehran, Iran. In the 114 patients already diagnosed as HIV-positive cases and controls none of them had symptoms referable to upper GI tract. In HIV-positive patients, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and IgG titre had a significant relationship with *H. pylori* seropositivity. In contrast, other hematological parameters did not reveal a statistically significant difference [14].

2.4 *H.pylori* infection and risk factors

A case control study by Sud A, *et al.* in Chandigarh, India, was including 73 consecutive HIV infected patients in the study. Antibodies (IgG) to *H. pylori* were tested by *ELISA*. Eleven patients presented with gastrointestinal symptoms. Thirty-five of the 73 (47.9%) patients had serological evidence of *H. pylori* infection. Six of them had gastrointestinal symptoms. These

were odynophagia in 5, dyspepsia in 4 and recent diarrhea in 2. Twenty-four patients with *H.pylori* infection had AIDS. There was no difference in the prevalence of *H.pylori* infection between patients with and without AIDS [34].

A cross-sectional study by Joseph, *et al.* in Jos, North-Central, Nigeria studied 139 HIV-1 infected patients. The prevalence rate of *H.pylori* infection was 46.8%, 18 (13.0%) were men and 38 (33.8%) were women. *H. pylori* stool antigen (HpSA) test was used to detect *H. pylori* antigen. With respect to age category, 17-26 year-olds had the highest frequency (15.1%, n=21), followed by 27- 36 year-olds (12.2%, n=17), 37-46 year-olds (11.0%, n=15), and \geq 41 year-olds (8.6%, n=12). Thirty eight (27.3%) were married and 27 (19.4%) were single. Most (25.2%, n=35) were unemployed, 21 (15.1%) worked in the private sector or were traders, and 9(6.5%) were public servants. A history of alcohol consumption was recorded in 27 (19.4%) patients while 7 (5.0%) smoke cigarettes. The use of pit latrine, water system, and bush for sanitary purposes, was reported by 29 (21.0%), 11 (8.0%), and 25 (18.0%) of the patients, respectively. regarding water source, 14 (10.1%), 12 (8.6%), and 39 (28.0%) patients used tap water, borehole, and well for drinking water, respectively. There were no significant differences between *H. pylori* infection in the patients' with respect to age, marital status, occupation, alcohol consumption, smoking habit, housing accommodation, and drinking water source [5].

A case control study in Nakuru, Kenya, found that Sero-positivity for *H. pylori* was found in 98 (71%) of 138 symptomatic and 70(51%) of 138 asymptomatic participants. In the asymptomatic participants, the prevalence of *H. pylori* infection increased with age, from 18% in the 0- to 10-year age group to 48% in the 11- to 20-year age group, peaking (68%) in the 31- to 40-year age group. In the dyspeptic patients, the prevalence of *H. pylori* infection was 60% to 73% in all age groups. Among persons <21 years old, *H. pylori* infection was more prevalent in those with symptoms than those without (17 [71%] of 24 vs.12 [38%] of 31. Similarly, *H. pylori* seropositivity showed a significant association with dyspepsia among persons 21–30 years of age (35 [73%] of 48 vs. 36 [48%] of 74, but not among persons >30 years of age (46 [70%] of 66 vs. 20 [63%] of 32 [35].

A comparative cross-sectional study was conducted by Tebelay Dilnessa, *et al.* among dyspepsia and non-dyspepsia adults at Assosa General Hospital in Ethiopia. No significant

relationship was observed between prevalence of *H. pylori* and family size, educational status, marital status, source of water and type of work . There was no significant association between occurrence of *H. pylori* and family income. Among personal habits: alcohol drinking, coffee drinking, cigarette smoking and *khat* chewing had no significant association with *H. pylori* infection. A higher prevalence of *H. pylori* was obtained among patients who had not have the habit of hand washing after toilet use 43(66.1%) than having hand washing after toilet 69 (41.8%)[28].

3. Objectives

3.1. General objectives

- To assess the prevalence of *Helicobacter pylori* infection by stool antigen test among dyspeptic and non -dyspeptic *HIV* patient at Yeka health center, Addis Ababa, Ethiopia.

3.2. Specific objectives

- To assess overall prevalence of *H. pylori* infection among HIV patients using stool antigen test
- To compare the prevalence of *H.pylori* infection among dyspeptic and non -dyspeptic HIV patients using stool antigen test.
- To assess the various factors including CD4 T cell count and hemoglobin level associated with *H.pylori* infection in HIV patients.

3.3. Hypothesis

- The prevalence of *Helicobacter pylori* infection based on stool antigen detection is not different among dyspeptic and non -dyspeptic HIV patients

4. Materials and methods

4.1. Study area

The study was conducted at *Yeka* health center which is located in the center of Yeka sub city around megenagna, Addis Ababa. Yeka health center is one of the health centers under yeka sub city health office that has been giving routine health services whose catchment area is the community of yeka woreda 5 and other people in the city. It is one of the oldest health centers in the city. It is one of the primer health center which start ART service in the city. This health center has ART clinic, delivery department; laboratory, pharmacy outpatient departments and other community based services. There are over 1350 patients who attend the ART clinic and there number increase through time. The study was carried out from January 1, 2017 to June, 2017.

4.2. Study design and study period

A case control study was conducted to determine the prevalence of *Helicobacter pylori* based on stool antigen detection among dyspeptic and non -dyspeptic HIV patients at *Yeka* public health center from January, 2017 to June, 2017.

4.3. Population

4.3.1. Source Population

Source of population of the study were dyspeptic and non- dyspeptic HIV patients attending ART clinic of *yeka* health center.

4.3.2. Study population

All dyspeptic and non- dyspeptic HIV patients who were attended the ART clinic of yeka health center within the study period and fulfill the inclusion criteria.

4.4. Inclusion and exclusion criteria

Inclusion criteria

- All patients attending the ART clinic and consented to participate in this study were included.

- HIV Patients with symptoms of dyspepsia for cases and those without dyspepsia for control group
- Age of 18 years and above.

Exclusion criteria

- Patients who took antibiotics for *Helicobacter pylori* in the last two weeks for cases and controls during data collection time .
- those who have surgery in the last six month

4.5. Variables of the Study

4.5.1 Dependent variables:

- Prevalence of Helicobacter pylori stool antigen positivity

4.5.2 Independent variables:

- Socio demographic factors;
 - Age
 - Sex
- -Treatment status for HIV,
- Stage of HIV/ WHO staging
- Weight, nutritional status,
- Treatment condition
- CD4 level, hemoglobin level
- Water source
- Duration on ART

4.6 .Sample size determination and Sampling

4.6.1. Sample size determination

The sample size of the study was determined by two population proportion using the following formula:

$$n1 = \frac{[Z\alpha/2\sqrt{pq}(1 + 1/\lambda) + Z\beta\sqrt{p1q1 + p2q2/\lambda}]^2}{\Delta^2}$$

Where $n_2 = n_1 \lambda \bar{p} = (p_1 + \lambda p_2) / (1 + \lambda)$

Where, $p_1 = 0.642$, from previous studies [33] $p_2 = 0.50$ (at the time of the study we cannot get any literature that show its prevalence so we use 50% prevalence of *H.pylori* in non-dyspeptic HIV patient) 95% confidence interval and at 80% power, $n_1 = 168$, $n_2 = 168$ so 168 cases and 168 control groups were selected and including 10 % contingency the total sample size were **370** subjects.

4.6.2. Sampling procedures

Dyspepsia is a complex set of symptoms, rather than an indication of a specific disease. Dyspepsia can be defined as painful, difficult, or disturbed digestion, which may be accompanied by symptoms such as nausea and vomiting, heart burn, bloating, and stomach discomfort [1]. Those patient who fulfill these symptoms were included in the case group and those which did not have these symptoms (non - dyspeptic) will be included in the control group.

Convenience sampling technique was employed to include study participants who meet the inclusion criteria until the achievement of the sample size.

4.7. Data collection procedures

4.7.1. Demographic characteristics and exposure to risk factors

Data collectors were identified, trained and informed to collect the data as per the pre-structured questionnaire. The purpose of the study as well as any related harm and benefit was explained to the study participants accordingly. Demographic data and potential risk factor of *Helicobacter pylori* infection including medical history, presence symptom, life style and per-individual monthly income were recorded.

4.7.2. Specimen collection and transportation

Based on WHO guideline, we have collected 3 ml of blood venous blood [36]. After collection, the blood sample was mixed gently in the EDTA test tube then transported to the Immunohematology laboratory for hematological analysis. The CD4 T cell counts of the patients were taken from the register of the health center.

FACSCount for CD4 T-lymphocyte count

The FACSCount System is designed to use analyses whole blood, collected in liquid EDTA. When whole blood is added to the tubes of a sample reagent pair, the fluorochrome-labeled

antibodies bind specifically to antigens on the surface of lymphocytes. The FACSCount instrument detects two colors and measures relative cell size. The CD3 cells will fluoresce red and the CD4 cells will fluoresce yellow when analyzed on the FACSCount instrument [37].

A known number of reference beads is contained in each reagent tube and functions as a fluorescence and quantitation standard for calculating the absolute counts for the CD4+ and CD3+ T lymphocytes. Fixative solution is added to the stained samples prior to analysis to preserve the integrity of the antibody binding. No lysing is necessary [38].

The FACSCount System provides a report quantifying the CD4+, and CD3+ T lymphocytes as absolute numbers of lymphocytes per μL (mm^3) of blood. The reportable ranges generated by the FACSCount System for each lymphocyte subset are as follows: [39]

CD4 50–2000 cells/ μL

CD3 100–3500 cells/ μL

Hematology analysis by Horiba ABX Micros Es 60 analyzer

Fresh whole blood specimen was used. The ICSH (International Committee for Standardization in Hematology) defines a fresh blood specimen as “One processed within 4 hours after collection”. Well mixed whole blood specimens, collected in EDTA anti-coagulant and run within eight hours after collection, provide the most accurate results for all parameters. The white cell size distribution may shift when specimens are assayed between 5 and 20 Minutes after collection and not more than 8 hours after collection [40].

***Helicobacter pylori* stool antigen test**

Stool samples requested from each participating patient were collected in airtight containers at the time of the encounter, the end of the day, or the following morning. The samples were analyzed as much as possible or stored in a $+4^\circ\text{C}$ fridge for a maximum of 72 hours before analysis. The wondfo one step *Helicobacter pylori* antigen test, is a rapid lateral-flow immunoassay that utilizes a monoclonal *anti-H. Pylori* antibody as the capture and detector antibody. It is produced in China. Approximately 100 μL of stool was transferred into the sample diluents vial and vortexed for 15 seconds. Three drops of the specimen were applied to the test and the result was read after fifteen minutes. The results were reported as positive or negative

based on the manufacturer's cut-off values [41, 42]. The CD4 T lymphocyte count and hematological analysis of the patient was done by collecting sample on the same day.

4.8 Data management and Quality control

Site assessment and pre-test were done prior to data collection. All data quality control tools (pre-analytical, analytical and post-analytical stages) of quality assurance that were incorporated in standard operating procedures (SOPs) of the serology and immunohematology laboratory were strictly followed. Adequate specimen was collected using appropriate equipment and method. The specimen was kept free of contamination. All materials, equipment and procedures were adequately controlled. The stool antigen test kit was tested for its performance. The FACSCount machine and hematology machine were checked their quality control and calibration before the test was done . Three level hematology and FACSCount controls were employed. The data was checked for completeness and representativeness prior to entry. The reliability of the study findings were guaranteed by implementing quality control (QC) measures throughout the whole processes of the laboratory work. The quality of the stool antigen test kit has been checked by its internal and external quality control material.

4.8.1. Pre-analytical phase

First of all we asked the participant verbally and by written consent for their willingness and then we filled all the information on the preformed questioner. Labeling the EDTA test tube with patient's identification number and blood was aseptically drawn by using sterile syringe. Following collection, specimens were transported to the immunohematology laboratory. The stool sample was collected aseptically in clean, labeled and appropriate container.

4.8.2. Analytical phase

All materials, equipment and Procedures were adequately controlled for their expiration date and appropriateness. FACSCount machine and hematology analyzer was tested by control material before the real sample processed. The rapid one step stool antigen test kit was checked for its performance by doing control. Standard operating procedures (SOPs) of the BD FACS count machine and ABX 60 hematology analyzer were strictly followed and the results were checked by the supervisors.

4.8.3. Post-analytical phase

The results were recorded with the patients' identification number. In order to avoid the errors in the results of the test, the report was repeatedly checked and approved by another laboratory personnel who does not work the test before the results will be given to the ART clinic. Appropriate action(s) was taken when a result was positive.

Every laboratory test result was interpreted based on the SOPs of Addis Ababa Regional Laboratory and CLSI guidelines.

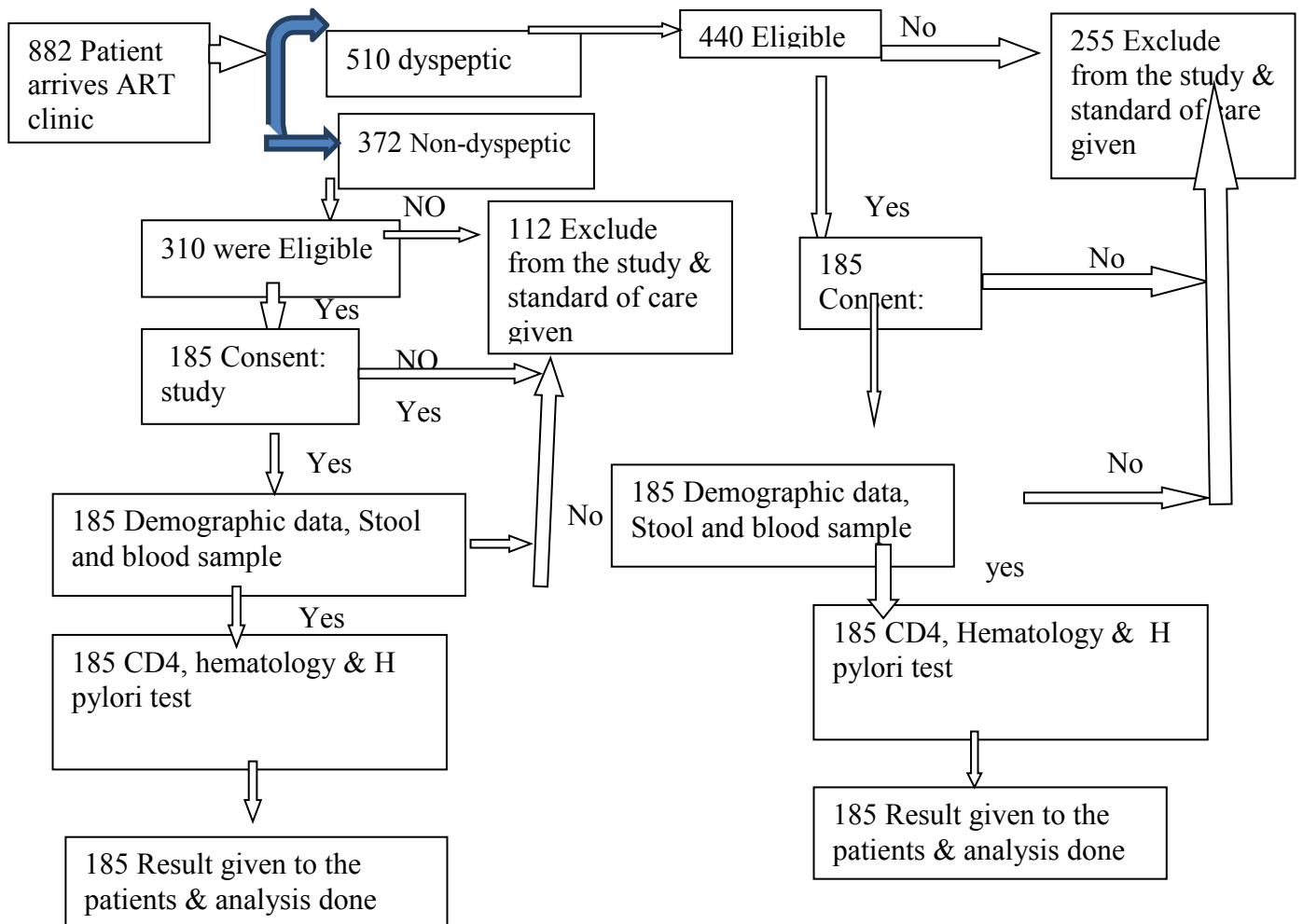


Figure 1. Work flow for the study procedure

4.9. Data Processing and Analysis

Information from the laboratory analysis was cleaned, coded; data entry and analysis were done using SPSS statistical software version 20. Descriptive statistics were employed to describe socio-demographic and clinical characteristics; logistical regression was used to estimate crude and adjusted odds ratio (ORs) with 95% confidence interval (CI) of positive responses to the different variables. Comparison between groups was done with Chi-square and P-value of < 0.05 was considered significant. Tables and graphs were used to describe the results. Percentage, frequency and chi-square test was performed to check the presence of association between dependant and independant variables.

4.10. Ethical consideration

The study was conducted after it was ethically reviewed and approved by the Department of Research and Ethical Review Committee (DRERC) of Department of Medical Laboratory Sciences, College of Health Sciences, Addis Ababa University. Ethical clearance was obtained from Addis Ababa Health Bureau. Then a letter informing the health center was written from the health bureau and permission was obtained from *Yeka* health center. Informed written consent was obtained from participants before data collection. The respondents were informed about the right to refuse to take part in the study as well as to withdraw at any time during the study period. All the information obtained from the study participants was coded to maintain confidentiality. When the participants were found to be positive for *Helicobacter pylori*, they were informed by the health center clinician and receive proper treatment.

4. 11. Dissemination of results

After conducting the research, the results of the study was submitted to Department of Medical Laboratory Sciences (DMLS) Addis Ababa University (AAU). Oral presentation of the thesis was made. Reports will also be submitted to *Yeka* health center, Addis Ababa Health Bureau, presented at annual conferences of professional societies and other concerned bodies. Since it is said that scientific work is incomplete until published, the manuscript will be submitted to peer reviewed journals for publication.

5. Results

5.1 Socio-demographic and clinical characteristics

A total of 370, study participants were enrolled in this study, 185 dyspeptic and 185 non-dyspeptics, of which 126(34.0%) were males and 244 (66.0%) females. The mean (SD) age of the study subjects was 37(10.44) years with the range of 18 - 70 years, (dyspeptic, mean(SD) 38(and non- dyspeptic, mean 34). Majority of the participants (82.4%) were in the age range of 18 - 42 years. In dyspeptic group gender distribution was 35.1% male: 64.9% female (Table 1) and 33% male: 67.0% female in the non-dyspeptic group ($p>0.05$). Females constituted 65.94 % (n=244) of the total study participants from these 32.43 % (n=120) were dyspeptic and 33.5 % (n=124) were non-dyspeptic. Around half of the total participants (50.3%, 186/370) reported that they lived with more than two siblings in the same house; 98 (53.0%) were from the dyspeptic and 88 (47.6%) were from the non-dyspeptic group. Only 2 (1.1%) of the dyspeptic and 9 (4.9%) of the non-dyspeptic study participants had monthly income of greater than 3000 birr (Table 1).

Majority of the study participants attained elementary school education 163(44.05%); 78 (42.1%) of the dyspeptic and 85 (45.9%) of the non-dyspeptic had elementary level education. Only 65(17.56%) of the study groups were college or university graduates; 15.1 % (n=28) were dyspeptic and 20.0 % (n=37) were non-dyspeptic (Table 1)

Married were 67.0 % (n=124) in dyspeptic and 68.6 % (n=127) in the non-dyspeptic group. Of the total study participants, 31.62% (n=117) were unemployed; 61 of the 185 (33%) dyspeptic and 56 of the 185 controls (30.3%) were unemployed (Table 1).

History of alcohol consumption was reported in 57(30.8%) dyspeptic and 43(23.2%) controls. Among the study participants, equal proportion (1.6%, n=3 from each of dyspeptic and controls disclosed cigarettes smoking habit. While 3 (1.6%) in dyspeptic and 7 (3.8%) in the controls had history of *khat* chewing ($p>0.05$)(Table 1).All participants use tap water as water source and 169 (91.4%) in the dyspeptic and 177 (95.7%) non-dyspeptic group use pit latrine (Table 1).

Table 1: Socio-demographic characteristics of *Helicobacter pylori* infection among HIV-1 Patients attending at Yeka health center, Addis Ababa, 2017.

Variable	Dyspeptic n =185(%)	Non-dyspeptic n=185(%)	p- value
Age (Years)			0.0631
18-30	51(27.6)	68(3.7)	
31-50	109(58.9)	105(56.8)	
>50	25(13.5)	12(6.5)	
Gender			0.898
Male	65(35.1)	61(33.0)	
Female	120(64.9)	124(67.0)	
Marital status			0.143
Single	35(18.9)	45(24.3)	
Married	124(67.0)	127(68.6)	
Divorced	11(5.9)	9(4.8)	
Widowed	15(8.1)	4(2.2)	
Occupation			0.177
Unemployed	61(33.0)	56(30.2)	
Government employed	38(20.5)	40(21.6)	
Private employed	84(45.4)	79(42.7)	
Other	2(1.0)	10(5.4)	
Education			0.322
Illiterate	16(8.6)	12(6.5)	
Elementary	78(42.1)	85(45.9)	
High school	63(34.1)	51(27.6)	
College and above	28(15.1)	37(20.0)	
Monthly income			0.272
No	3(1.6)	7(3.7)	
<1000 birr	79(42.7)	82(44.3)	
1000 to 2000 birr	92(49.7)	83(44.8)	
2001 to 3000 birr	9(4.8)	4(2.1)	
>3000 birr	2(1.0)	9(4.8)	
Siblings			0.471
None	8(4.3)	16(8.6)	
One	22(11.9)	31(16.7)	
Two	57(30.8)	50(27.0)	
Greater than two	98(53.0)	88(47.5)	
Alcohol consumption			0.163
Yes	57(30.8)	43(23.2)	
No	128(69.2)	142(76.8)	
Source of drinking water			
Tap	185(100)	185(100)	
Well	0(0)	0(0)	

Smoking habit			0.769
Never	182(98.3)	182(98.3)	
Currently	3(1.7)	3(1.7)	
Past	0(0)	0(0)	
Khat chewing			0.068
Yes	5(2.7)	2(1.1)	
No	180(97.3)	183 (98.9)	
Latrine type			0.613
Pit	169(91.4)	177(95.7)	
Modern (with flash)	17(8.6)	8(4.3)	
Open defecation	0(0)	0(0)	

The common signs of dyspepsia among cases (n=185) was bloating (93, 50.3%) followed by abdominal pain (n=71, 38.4%) as displayed in Figure 2.

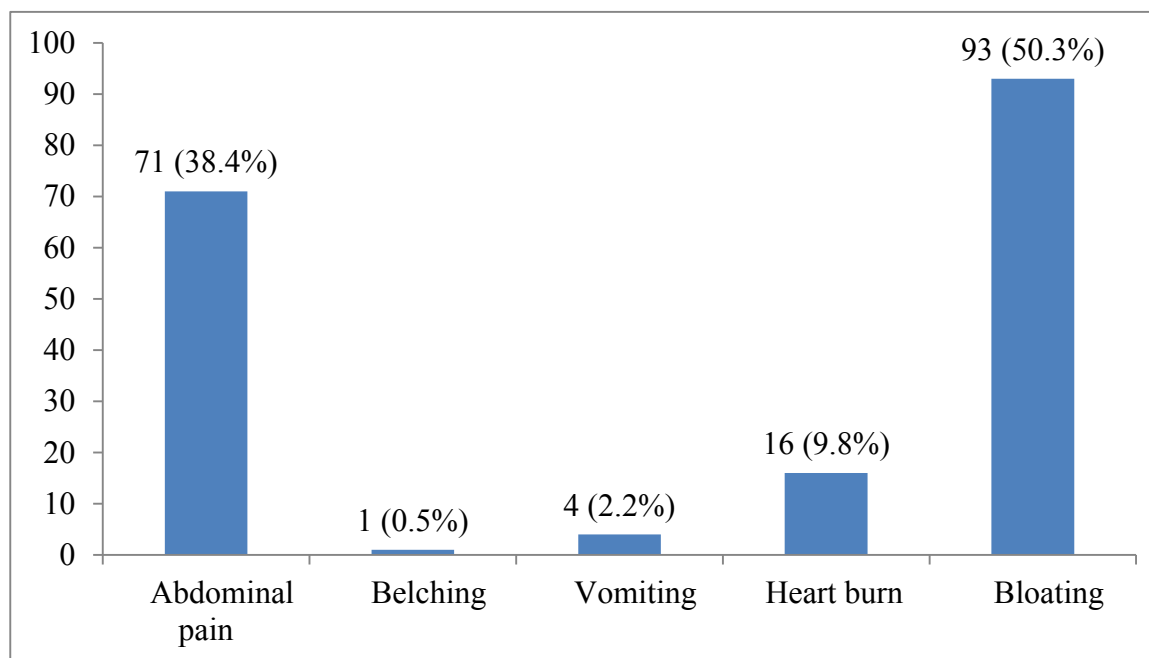


Figure 2. Signs of dyspepsia among cases (n=185) at *Yeka* health center, Addis Ababa, 2017.

5.2 *Helicobacter pylori* infection

The overall prevalence of *H. pylori* infection as determined by stool antigen positivity was 31.6 % (n=117); The gender specific prevalence of *H. pylori* infection was 37.3% (n=47) in males and 28.7% (n=70) in Females .The *H.pylori* prevalence was high at the age group of above 50 years (35.1% n=13). Eight out of 19 (42.1%) widowed study participants were *H.pylori* positive.

Higher prevalence of *H.pylori* (38.4%) was found in government employed study participants. Alcohol consumers had slightly higher *H.pylori* prevalence than non-consumers (34.0% vs.30.7%) (Table 2)

Table 2. Socio demographic characteristics of study participants in relation to *Helicobacter pylori* stool antigen status at Yeka health center 2017.

Variable	N	<i>H. pylori</i> stool antigen status		P value
		<i>H.pylori</i> positive n=117(%)	<i>H.pylori</i> negative n=253 (%)	
Age				0.627
18-30	119	31(26.0)	88(74.0)	
31-50	214	73(34.1)	141(65.9)	
>50	37	13(35.1)	24(64.9)	
Gender				0.175
Male	126	47(37.3)	79(62.7)	
Female	244	70(28.6)	174(71.4)	
Marital status				0.876
Single	80	22(27.5)	58(72.5)	
Married	251	81(32.2)	170(67.8)	
Divorced	20	6(30.0)	14(70.0)	
Widowed	19	8(42.1)	11(57.9)	
Occupation				0.673
Unemployed	117	28(23.9)	89(76.1)	
Government employed	78	30(38.4)	48(61.6)	
Private employed	163	56(34.3)	107(65.7)	
Other	12	3(25.0)	9(75.0)	
Education				0.416
Illiterate	28	7(25.0)	21(75.0)	
Elementary	163	52(31.9)	111(68.1)	
High school	114	32(28.0)	82(72.0)	
College and above	65	26(40.0)	39(60.0)	
Monthly income				0.211
No	10	2(20.0)	8(80.0)	
<1000 birr	161	39(24.2)	122(75.8)	
1000 to 2000 birr	175	67(38.2)	108(61.8)	
2001 to 3000 birr	13	7(53.8)	6(46.2)	
>3000 birr	11	2(18.1)	9(81.9)	
Siblings				0.785
None	24	9(37.5)	15(62.5)	
One	53	15(28.3)	38(71.7)	
Two	107	30((28.0)	77(72.0)	
Greater than two	186	63(33.8)	123(66.2)	
ART naïve				0.937
Yes	331	105(31.1)	226(68.2)	
No	39	12(30.7)	27(69.1)	

Alcohol consumption				0.618
Yes	100	34(34.0)	66(66.0)	
No	270	83(30.7)	187(69.3)	
Source of drinking water				
Tap	370	117(31.6)	253(68.3)	
Well	0	0	0	
Cigarette smoking				0.684
Yes	6	2(33.3)	4(66.6)	
No	364	115(31.5)	249(68.4)	
Khat				0.427
Yes	7	1(14.2)	6(85.7)	
No	363	116(31.9)	247(68.0)	

5.2.1 *Helicobacter pylori* status among dyspeptic HIV patients

The prevalence of *H.pylori* in the dyspeptic study group was 32.4% (n=60) . Among *H. pylori* positives, the majority were in the age group 31-50 years (63.3%, n=38), females (66.7%, n=40), married (68.3%, n=41), private employed (48.3, n=29), and at elementary education level (46.7%, n=28), *Helicobacter pylori* status among dyspeptic HIV patients by socio-demographic characteristics is displayed in Table 3.

Table-3. Demographic characteristics and *Helicobacter pylori* status among dyspeptic HIV patients at Yeka health center 2017.

Variable	Dyspeptic n =185(%)				
	N	<i>H.pylori</i> positive n=60(%)	<i>H.pylori</i> negative n=125(%)	X ²	p- value
Age				23.766	0.941
18-30	51	14(23.3)	37(29.6)		
31-50	109	38(63.3)	71(56.8)		
>50	25	8(13.4)	17(13.6)		
Gender				0.894	0.639
Male	65	20(33.3)	45(36.0)		
Female	120	40(66.7)	80(64.0)		
Marital status				1.290	0.863
Single	35	9(15.0)	26(20.8)		
Married	124	41(68.3)	83(66.4)		
Divorced	11	4(6.7)	7(5.6)		
Widowed	15	6(10.0)	9(7.2)		
Occupation				16.690	0.476
Unemployed	61	17(28.3)	44(35.2)		

Government employed	38	13(21.7)	25(20.0)		
Private employed	84	29(48.3)	55(44.0)		
Other	2	1(1.7)	1(0.8)		
Education				8.721	0.559
Illiterate	16	4(6.7)	12(9.6)		
Elementary	78	28(46.7)	50(40.0)		
High school	63	17(28.3)	46(36.8)		
College and above	28	11(18.3)	17(13.6)		
Monthly income				14.499	0.632
No	3	1(1.6)	2(1.6)		
<1000 birr	79	21(35.0)	58(46.4)		
1000 to 2000 birr	92	34(56.7)	58(46.4)		
2001 to 3000 birr	9	4(6.7)	5(4.0)		
>3000 birr	2	0	2(1.6)		

5.2.2 *Helicobacter pylori* status among non-dyspeptic HIV patients

The prevalence of *H.pylori* among the non-dyspeptic HIV patient was 30.8% (n=57). As shown in the Table 4, among the *H. pylori* positives, the majority were in the age group 31-50 years (61.4%, n=35), females (52.6%, n=30), married (70.2%, n=40), private employed (47.4%, n=27), and at elementary education level (42.1%, n=24).

Table-4 Demographic characteristics and *Helicobacter pylori* status among non-dyspeptic HIV patients at Yeka health center 2017.

Variable	Non-dyspeptic n=185(%)				
	N	<i>H.pylori</i> positive n=57(%)	<i>H.pylori</i> negative n=128(%)	X ²	p-value
Age				46.706	0.216
18-30	68	17(29.8)	51(39.8)		
31-50	105	35(61.4)	70(54.7)		
>50	12	5(8.8)	7(5.5)		
Sex				8.406	0.015
Male	61	27(47.4)	34(26.5)		
Female	124	30(52.6)	94(73.5)		
Marital status				0.684	0.953
Single	45	13(22.8)	32(25.0)		
Married	127	40(70.2)	87(68.0)		
Divorced	9	2(3.5)	7(5.4)		
Widowed	4	2(3.5)	2(1.6)		
Occupation				20.974	0.399

Unemployed	56	11(19.2)	45(35.1)		
Government employed	40	17(29.8)	23(17.9)		
Private employed	79	27(47.4)	52(40.6)		
Other	10	2(3.5)	8(6.2)		
Education				17.001	0.049
Illiterate	12	3(5.3)	9(7.0)		
Elementary	85	24(42.1)	61(47.3)		
High school	51	15(26.3)	36(28.1)		
College and above	37	15(26.3)	22(17.2)		
Monthly income				25.654	0.140
No	7	1(1.7)	6(4.7)		
<1000 birr	82	20(35.0)	62(48.4)		
1000 to 2000 birr	83	31(54.4)	52(40.6)		
2001 to 3000 birr	4	3(5.3)	1(0.8)		
>3000 birr	9	2(3.5)	7(5.5)		

5.3 Associations between *Helicobacter pylori* infection and dyspepsia sign, risk factor, current CD4 T cell count and Hemoglobin level

5.3.1 *H.pylori* infection and dyspepsia

The prevalence of *H. pylori* infection in non -dyspeptic HIV patient group and dyspeptic HIV positive patient group is not statistically different (57/185; 30.8% Vs. 60/185; 32.4%, $P > 0.05$) (Table 5).

Table 5. Associations of *H. pylori* prevalence among dyspeptic and non-dyspeptic HIV patients at Yeka public health center, Addis Ababa, 2017 (n=370)

<i>H pylori</i> status	Dyspepsia		COR(95%CI)	P-value
	Yes n (%)	No n (%)		
Positive	60(16.2)	57(15.4)	.952(0.745-1.217)	0.697
Negative	125(33.8)	128(34.6)	1.044(0.838-1.302)	0.276

COR=Crude odd ratio, CI= Confidence interval

5.3.2 *H.pylori* infection and risk factors

As displayed in Table 6, potential risk factors for *H. pylori* in dyspeptic and non-dyspeptic study participants were analyzed. However, none of them showed significant association. Among the clinical factors, ART duration and WHO staging were investigated for potential association. Both in case and control group, based on the CDC clinical classification of HIV infection, the majority were in stages 1 and 2. Among *H. pylori* infected dyspeptic patients, majority 32 (53.3%) were in stage 2 and had a duration of 1-5 years (51.7%, n=31). Nevertheless, there was no significant association with neither staging or ART duration with *H. pylori* infection (Table 6).

Table-6 The association of risk factors and *Helicobacter pylori* status among dyspeptic and non-dyspeptic HIV patients at Yeka health center, Addis Ababa, 2017.

Variable	N	Dyspeptic (n=185)				Non-dyspeptic n=185(%)				
		<i>H. pylori</i> positive (n=60) n (%)	<i>H. pylori</i> negative (n=125) n (%)	X ²	p-value	N	<i>H. pylori</i> positive n=57(%)	<i>H. pylori</i> negative n=128(%)	X ²	p-value
Siblings				2.037	0.916				10.435	0.107
None	8	4(6.6)	4(3.2)			16	5(8.7)	11(8.6)		
One	22	7(11.6)	15(12.0)			31	8(14.1)	23(18.0)		
Two	57	18(30.0)	39(31.2)			50	12(21.1)	38(29.7)		
Greater than two	98	31(51.6)	67(53.6)			88	32(56.1)	56(43.7)		
Alcohol consumption				0.777	0.378				2.488	0.115
Yes	57	16(26.6)	41(32.8)			43	18(31.6)	25(19.5)		
No	128	44(74.4)	84(67.2)			142	39(68.4)	103(80.5)		
Source of drinking water				-	-				-	-
Tap	185	60(100)	125(100)			185	57(100)	128(100)		
Well	0	0	0			0	0	0		
Cigarette smoking				0.984	0.321				1.85	0.173
Yes	3	1(1.7)	2(1.6)			3	1(1.7)	2(1.5)		
No	182	59(98.3)	123(98.4)			182	56(98.3)	126(98.5)		
Khat chewing				2.50	0.113				2.272	0.132
Yes	5	1(1.6)	4(3.2)			2	0(0)	2(1.5)		
No	180	59(98.4)	121(96.8)			183	57(100)	126(98.5)		
Animal contact				0.216	0.610				0.392	0.822
Yes	12	5(8.3)	7(5.6)			9	4(7.0)	5(3.9)		

No	173	55(91.7)	118(94.4)			176	53(93.0)	123(96.1)		
On ART duration				15.605	0.520				23.682	0.166
Not on ART	9	2(3.3)	7(5.6)			29	10(17.5)	19(14.8)		
<1 year	6	2(3.3)	4(3.2)			7	3(5.3)	4(3.1)		
1-5 year	109	31(51.7)	78(62.4)			93	26(45.7)	67(52.3)		
>5 year	61	25(41.7)	36(28.8)			56	18(31.5)	38(29.6)		
WHO stage				1.070	0.899				3.964	0.265
Stage 1	88	26(43.3)	62(49.6)			102	27(47.4)	75(58.5)		
Stage 2	92	32(53.3)	60(48.0)			78	27(47.3)	51(39.9)		
Stage 3	3	1(1.7)	2(1.6)			4	2(3.5)	2(1.6)		
Stage 4	2	1(1.7)	1(0.8)			1	1(1.7)	0(0)		

In non-dyspeptic study participants alcohol consumption, cigarette smoking, sibling water source, animal contact and *khat* chewing were not risk factors ($P > 0.05$, $OR > 1$) for stool antigen *H. pylori*. From the total 57 *H. pylori* positive non-dyspeptic control group, 18 (31.6%) consume alcohol. Those who live with more than two people in the same house had higher (56.1%, $n=32$) positive prevalence of *H. pylori* than who live with less number (Table 6).

5.3.3 *H. pylori* infection and CD4 T cell count

CD4+ T-cells count had a median level of 364 cells/ μ L among Dyspeptic study participants and 312 cells/ μ L in non-dyspeptic study group. The study participants were further sub classified in to three groups according to their current *CD4 T* lymphocyte count (mean=350.10/ mm^3 ; median=341.50/ mm^3). Among the study participants there was significant difference in the prevalence of *H. pylori* infection between *CD4 T* count less than 200 cells/ μ L, 200 to 500 cells/ μ L and *CD4 T lymphocyte* count greater than 500 cells/ μ L ($n=40$; 40%, $n=56$; 29.62% and $n=21$; 25.92% p – value 0.129 OR 0.001) (Table 7).

In dyspeptic individuals *H. pylori* prevalence increases in parallel with *CD4+ T* cell counts, from 26.6% in patients with less than 200 CD4 T cells/ μ L, to 50.0% in patients with 200 to 500 CD4 T cells/ μ L. The same trend was observed in non-dyspeptic HIV individuals, without reaching statistical Significance (Fig .3). The , mean of CD4 T cell count, among dyspeptic and non-dyspeptic was 370 vs. 329 respectively and its value in *H. pylori* positive and negative was 305 vs. 373 respectively .

Table 7. CD4 T cell count and *H.pylori* status of the study participants at yeka health center 2017

CD4	N=370(%)	<i>H.pylori</i> status		p-value
		<i>H.pylori</i> positive n=117 (%)	<i>H.pylori</i> negative n=253(%)	
<200	100	40(34.2%)	60(23.71%)	
200 to 500	189	56(47.8%)	133(52.56%)	
>500	81	21(17.9%)	60(23.7%)	

46(24.9%) dyspeptic group patient and 54(29.2%) Non-dyspeptic group patients were with CD4+ T-lymphocytes level below 200cells/ μ l. From these 16(8.6%) dyspeptic and 24 (12.97%) non –dyspeptic group patients were *H.pylori* positive. 89(48.1%) dyspeptic group patient and 100(54.0%) Non-dyspeptic group patients were with CD4+ T-lymphocytes level between 200 to 500cells/ μ l. From these 30(16.2%) dyspeptic and 26 (14.0%) non –dyspeptic group patients were *H.pylori* positive. However, our study did not find a statistically significant association between CD4 T cell count and the existence of *Helicobacter pylori* both in dyspeptic and non -dyspeptic study group ($p>0.05$)(Table 8).

Table 8. CD4 T cell count and *H.pylori* status among dyspeptic and non -dyspeptic HIV patients at Yeka health center 2017.

CD4 T cell count	Dyspeptic (N=185)				Non-dyspeptic (N=185)			
	N	<i>H.pylori</i> positive n=60 (%)	<i>H.pylori</i> negative n=125(%)	p=0.320 X ² =14.00	N	<i>H.pylori</i> positive n=57(%)	<i>H.pylori</i> negative n=128	p=0.479 X ² =6.055
<200	46(24.9)	16(8.6)	30(16.21)		54(29.2)	24(12.97)	30(16.21)	
200-500	89(48.1)	30(16.21)	59(31.89)		100(54.0)	26(14.05)	74(40.0)	
>500	50(27.0)	14(7.56)	36(19.45)		31(16.8)	7(3.78)	24(12.97)	

When we see the mean and median CD4 T cell count of dyspeptic study participants it was (mean 370, median 364 cell/dl) while in the control group it was (mean 329, median 312 cell/dl).

The mean of CD4 T cell count, among dyspeptic and non-dyspeptic was 370 vs. 329 respectively and its value in *H.pylori* positive and negative was 305 vs. 373 respectively (Table 9) .

Table 9. CD4 T cell count in Relation with dyspepsia and *H.pylori* status at yeka health center 2017

Variable	CD4 T cell count						
	Mean	St deviation	Minimum	Maximum	p-value	X ²	Std error of mean
<i>H.pylori</i>					0.244	184.479	
Positive	305	205.981	8	865			4.275
Negative	373	206.610	19	995			17.588
Dyspepsia sign					0.703	161.662	
Dyspeptic	370	218.324	18	995			21.408
Non-dyspeptic	329	197.100	8	885			19.144

In dyspeptic individuals *H. pylori* prevalence increases in parallel with CD4+ T cell counts, from 26.6% in patients with less than 200 CD4 T cells/ μ L, to 50.0% in patients with 200 to 500 CD4 T cells/ μ L. The same trend was observed in non-dyspeptic HIV individuals, without reaching statistical Significance(Fig .3).

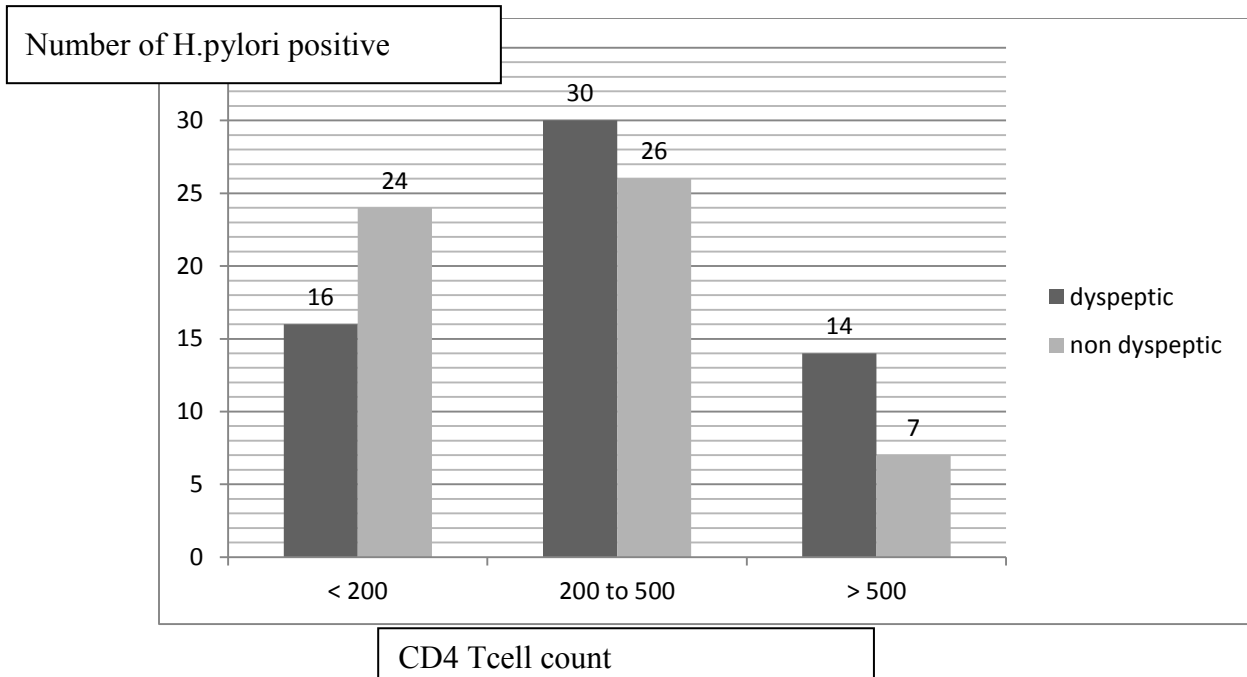


Figure 3. Comparison of *H. pylori* prevalence according to *CD4 T* cell count/ μ L for Dyspeptic HIV positive participants ($p = 0.129,$) and for non-dyspeptic HIV positive participant ($p = 0.244,$);at yeka health center Addis Ababa,Ethiopia from January to June 2017.

5.3.4 *H.pylori* infection and Hemoglobin level

The mean Hemoglobin of the study participant was 13.64 g/dl (median 13.98g/dl and range 9g/dl).The mean Hemoglobin of *H.pylori* positive and negative subjects was 11.1 g/dl and 13.1 gm/dl respectively. There was significant association between *H.pylori* status and Hemoglobin level(p -value < 0.02).The dyspeptic study group had *Hemoglobin* (mean13.76and median 14.00 g/dl)while non -dyspeptic had a Hemoglobin (mean 13.54g/dl ,median14.00 g/dl).

6. Discussion

The aim of this study was to assess the prevalence of *Helicobacter pylori* infection based on stool antigen positivity among dyspeptic and non-dyspeptic HIV positive patients at *Yeka* health center in Addis Ababa. This study systematically investigates the interplay between *H. pylori* and dyspepsia in HIV infected patients at *Yeka* health center. We assessed the prevalence of *H. pylori* co-infection in adult dyspeptic HIV infected individuals and non-dyspeptic HIV infected controls, and its association with risk factors, *CD4 T* cell count and *Hemoglobin* level. Our primary group of study consisted of 185 dyspeptic HIV-infected patients, to which we paired a second group formed by 185 non-dyspeptic HIV-infected subjects .

In our study, *Helicobacter pylori* infection registered a frequency of 30.81% among non-dyspeptic HIV-positive patients, slightly lower than among dyspeptic HIV-positive subjects – 32.43%, but not enough to provide a statistically significant association between the dyspepsia sign and the presence of *Helicobacter pylori* ($p=0.679$, $OR=0.81$). This was similar to the conclusion of Ankouane *et al* [32], but different from others [27,35].

The prevalence of *H. pylori* antigen in stool of dyspeptic HIV positive patients in this study was not significantly different from non- dyspeptic HIV positive patients ($p = 0.679$, 32.4% vs. 30.8%). But it disagrees with previous study, in the general population *H.pylori* prevalence was (65%) patients with NUD and in (56%) asymptomatic controls [27]. The reasons for such lower prevalence might be due to the method difference that the studies were done (previous endoscopy vs. stool antigen test). But it agree with the study in Cameroon which showed the prevalence of *H. pylori* infection in *HIV*-positive subjects did not differ between patients with and without PU [32].

The absence of a statistically significant difference regarding the frequency of *Helicobacter pylori* infection in dyspeptic HIV patients and non-dyspeptic HIV positive patients opens the gate for exploring other causes for dyspepsia in HIV-infected persons, ranging from fungal and viral digestive infections [1] to medication adverse effects [13], as some authors state that more than half of all HIV-positive individuals complain of gastro-intestinal symptoms [34].

Our results showed low prevalence of *H. pylori* infection (31.6%) in *HIV* patients which differs remarkably from that previously reported study for *HIV*-positive adults in *Nigeria* (46.8%) [5]. This difference may be due to geographical and life habit difference of the study subjects. This study was stool antigen based, but we observed similar prevalence against the earlier reported study in *Brazil* 36.7% [29]. Tadege *et al* [14] indicated that the lower prevalence of *H. pylori* infection in *HIV*-positive patients is due to the suppressed immune response. As the symptoms of dyspepsia are more common among *HIV*-positive population, taking medications such as PPIs and more attempts to eradicate *H. pylori* infection by the physicians may result in decreased infection rate of such microorganism [14]. Decreased secretion of gastric acids has been accounted as another explanation; hence, other opportunistic infections such as cytomegalovirus may emerge to compete with *H. pylori*. This in addition to the decreased acid secretion may lead to inappropriate environment for colonization of *H. pylori* [14]. Similarly, studies have reported different prevalent rates using sera (*H.pylori* IgG antibodies) in st Paul's Hospital, Ethiopia (57.08 %) and Chandigarh, India, (47.9%)[34,33,].

In the present study, it is found that the overall prevalence of *H. pylori* infection was 31.6%. This result contradicted with studies in st Paul's hospital, Addis Ababa, Ethiopia which showed high prevalence 57.08 % [33]. This disagreement might be due to use of different methods for the diagnosis of *H.pylori* between the present study and the previous study that was stool antigen test on our study which had higher specificity [24].

This study revealed that high proportion (35.1%) of participants aged > 50 years were infected with *H. pylori* as compared to younger age categories, and this was consistent with earlier reported findings in Nigeria [5], and Iran [14]. Similarly, the study observed high proportions of *H. pylori* infection among women with no significant risk of association, though larger percentage of women had participated compared to men.

In both case and control group we did not found a significant association with age group, marital status ,income, education, sibling, alcohol consumption, cigarettes smoking, *khat* chewing, house accommodations and water source($p>0.05$) and a similar finding was reported from Malaty and Graham, [17]. The absences of association between *H.pylori* status and *khat* chewing in this study might be due to less number of chewers that cause difficulty to compute the association.

Although it has been hypothesized that poor education, including incorrect sanitary practices, might indirectly influence the presence of *Helicobacter pylori* [14], we did not register statistically significant differences regarding the level of education ($p=.416$), siblings($p=0.785$), alcohol consumption($p=0.618$), cigarette smoking and lack of employment ($p=0.673$) between *H.pylori* positive and negative subjects in both dyspeptic and non-dyspeptic study participants. This could be explained by the high rates of unemployment and incomplete education among both subgroups of HIV-infected patients from our geographic area.

In the study no difference in *H. pylori* prevalence in relation to dyspepsia sign, being 32.4% in dyspeptic HIV positive individual and 30.8% in non-dyspeptic HIV-positive individuals which is similar from that previous reports in brazil on dyspeptic HIV patients (37.2%)(26). This study also categorized HIV patients with dyspepsia and without dyspepsia, and found no significant difference in stool antigen *H. pylori* distribution between two groups. In these study participants

prevalence of *H.pylori* stool Antigen increases in increase of CD4 T cell count. In both dyspeptic and non-dyspeptic HIV positive individuals, high *H. pylori* prevalence found in CD4+ T cell counts range from 200 to 500 cell count/ μ l, although statistical significance was not attained. Hypochlorhydria has been described in HIV-positive patients. Previous studies have shown that HIV-positive patients with overt AIDS have significantly increased serum levels of gastrin and pepsinogen II compared with HIV-positive patients without overt AIDS. Hypochlorhydria may provide a less suitable environment for *H. pylori* and predispose to overgrowth of other bacteria. Inhibition of *H. pylori* by competition with other opportunistic pathogens such as Cytomegalovirus via unknown mechanisms has been also suggested [33].

In the present study in both case and control group, an increase in stool antigen prevalence of *H. pylori* is observed by increasing the CD4 T cell count which did not reach statistical significance. The study agreed with some investigations [31, 33]. This could be explained due to the excessive administration of antibiotics in HIV-positive patients to control the infectious complications. The underlying mechanisms responsible for this association between immune competence and *H. pylori* prevalence are still unclear, although several hypotheses have been offered [14]. The most popular is that more frequent bacterial infections in HIV patients, especially those with advanced disease stages, lead to antibiotic treatment courses, probably resulting in unintended *H. pylori* eradication [14].

Hemoglobin parameter was also compared between *H. pylori* stool antigen positive and negative cases in HIV-positive group; it shows a significant difference in the parameter level was observed between *H. pylori* stool antigen negative and positive HIV patients. *Hemoglobin* level did not show significant difference between the case and control group. There was no single study in the literature to be compared with this finding.

7. Strength and Limitation of the study

7.1 Strength of the study

The study was tried to assess the various risk factors including clinical factors.

7.2 Limitation of the study

The great limitation of this study was that, it was done in single institution. This study considered as a case control study for the assessment of contributing factors for the infection. Therefore, it is difficult to establish temporality of the association between *H. pylori* and its risk factors. There was limited literature on prevalence of *H.pylori* on HIV patients done by stool antigen test in our country that shorten our discussion.

No single study that was done on dyspeptic and non- dyspeptic *HIV* patients on the same paper to compare our study. We used *H.pylori* stool antigen test other studies have performed endoscopic biopsy or urease breath test to confirm the diagnosis of this infection.

8. Conclusion and recommendation

8.1 Conclusion

The overall stool antigen prevalence of *H. pylori* among *HIV* patients at yeka Health center was 31.6%. There was slight difference in the detection of *H. pylori* stool antigen in dyspeptic patients and in the non-dyspeptics i.e. 32.4% versus 30.8%. However, a statistically significant association was not found between prevalence of *H. pylori* infection and dyspepsia symptom, which is similar to previous studies in other country. This suggests the need to investigate alternative etiologies for dyspepsia in *HIV*-positive patients, besides *Helicobacter pylori* infection. In our study we did not get any association between *H. pylori* and its risk factors which needs further study.

In Both dyspeptic and non-dyspeptic study participants, patients with severely impaired immune status, reflected by CD4+ T-lymphocyte levels below 200 cells/ μ L, are less probable to be infected with *Helicobacter pylori*. So those patients who have CD4 T cell count greater than 200 cells/ μ L must not ignore for test of *H. pylori*. The mean CD4 T cell count in dyspeptic and non-

dyspeptic *HIV* patient was 370 vs.329 respectively. This indicates slight difference in CD4 T cell count among dyspeptic and non- dyspeptic HIV patients.

There was significant association between *H.pylori* status and Hemoglobin level (p-value < 0.02).The mean Hemoglobin of the study participant was 13.64 g/dl (median 13.98g/dl and range 9g/dl).The mean Hemoglobin of *H.pylori* positive and negative subjects was 11.1 g/dl and 13.1 gm/dl respectively. The mean and median hemoglobin of the dyspeptic and non-dyspeptic study participants were (13.76 g/dl:14.00 g/dl vs.13.54 g/dl:14.00 g/dl).

8.2 Recommendations

_ This study found slight difference of *H. pylori stool antigen* in dyspeptic patients and non dyspeptic HIV patients. Therefore, both patients with and without sign of dyspepsia should be tested for *H.pylori*.

-This study found that the *H.pylori* positivity increases as the CD4 count increases. There for patients with higher CD4 count should also tested for *H.pylori*.

_ Due to undersized number of participants in some of the studied variables, statistically Significant associations were not found. As a result, further investigation on these variables is recommended.

-public health awareness on HIV spread should be encouraged while risk factors to *H. pylori* infection must be emphasized with adequate HIV management.

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10. Annexes

Annex I: English Versions of Participant Information Sheet

My name is Getachew Seid. I am a laboratory technologist postgraduate student at Addis Ababa University. Now I am carrying out a study entitled *Helicobacter pylori* infection among dyspeptic and non –dyspeptic *HIV* patients at Yeka health center, Addis Ababa Ethiopia.

You are invited to participate in this study. Please read the following statements and ask any unclear points before you agree to participate. If you agree to be included in this study, I will like to ask you to sign on a document to show your agreement; participate accordingly, and give clinical specimen.

Introduction

The topic of this study is “*Helicobacter pylori* infection among dyspeptic and non- dyspeptic *HIV* patient at Yeka health center, Addis Ababa Ethiopia”.*Helicobacter pylori* is an organismthat

infects the stomach and is known to cause peptic ulcer disease and other diseases .since *Helicobacter pylori* infection is one of the major health problems in our country, the result of the study can be helpful in planning and intervention to solve the problem and for patient treatment. Participation in this study is exclusively voluntarily. If you are not interested to participate or if you once decide to participate and withdraw yourself at any time, there will be no consequences and you will get all the services provided in the health center without any problem. If you decide to participate, you have to sign on the assent/ permission template form and you may obtain a copy of this information sheet.

Expected from participants

As a participant of this study, you are expected to give stool and blood sample. The stool sample tested for *Helicobacter pylori* infection and the blood for *CD4* and hematological analysis. Being asked to give sample does not necessarily mean that you have the disease. When you are found to be positive for the bacteria, you will be informed by the health worker and receive proper treatment. You need to know that your results might be discussed with other appropriate individual out of this health center. But your name, address will not be disclosed rather an identification code will be used in such conditions.

Time required for participate

You will spend 10-20 minute until the specimen is collected and permission form is signed.

Risks of participant

Specimen collection will have no effect as usual and you will not get any risk as the sample will be collected by well trained professionals. As you know, you may feel minor temporary pain during sample collection.

Confidentiality

The information in your records is strictly confidential. All information that you give and the results from your specimen will be used for this study only. Only limited numbers of professional that know you previously will have access the information. The information will be encoded in a computer and will save with password protection.

Benefits of participation

By participating, you will get no financial benefits. Even though there is no direct benefit due to participation in this study, the findings of the study is useful for better management of you and other patients on the infection. You will also obtain all the results of the analysis for free and communicated to your physician for the appropriate management.

Rights of participants

Your participation is completely voluntary, and you can refuse to participate or withdraw from the study at any time. Refusal to participate will not result in loss of medical care provided or any other benefits. You can get your results of the analysis.

Communication

In case if you have any questions, unclear ideas and doubt about the project, contact addresses are:

Investigator: Getachew Seid (BSc); AAU +251912131707

Email- gechgsa@gmail.com

For additional information, please contact Addis Ababa University, College of Health Sciences, Department of Medical Laboratory Sciences at: Telephone +251112755170

Your signature below indicates that you have read /or listened, and understand the information provided for you about the study. Before you sign, please understand purpose of the study, procedure, risks and benefits of participation, right to refuse or withdraw, confidentiality and privacy, and who to contact if you have question. I have read /or listened to the description of the study and I understand what procedures are and what will happen to me in the study.

Agree to participate? Yes----- No-----

Annex II: Amharic Versions of Participant Information Sheet

እኔ ጌታቸው ሰይድ እባላለሁ። በአዲስ አበባ ዩኒቨርሲቲ፣ ጤና ሳይንስ ኮሌጅ፣ የህክምና ላቦራቶሪ ሳይንስ ትምህርት ክፍል የሁለተኛ ዲግሪ ተማሪ ስሆን የምርምር ስራየን በመስራት ላይ እገኛለሁ። በመሆኑም እርስዎም በዚህ ጥናት ላይ እንዲሳተፉት ጋብዘዋል።

በጥናት ለመሳተፍ ፈቃደኛ ሆነው ከተሰማሙ መስማማትዎን የሚያሳይ ዶክመንት ላይ እንዲፈረም እጠይቃለሁ።

መግቢያ

የጥናቱ ርዕስ “ሄሊኮባክተር ፓይለሪ የተባለው ደቂቅ ህዋስ የጨንጉራ ህመም ምልክት ባለባቸው እና በሌለባቸው የኤች አይ ቪ ህመምተኞች ያለው ስርጭት በየካ ጤና ጣቢያ ታካሚዎች፣ አዲስ አበባ፣ ኢትዮጵያ” በሚል ርዕስ እያጠናሁ እገኛለሁ። ይህ ጥናትም በተሳታፊ ሙሉ ፈቃደኝነት ላይ ተመስርቶ ሄሊኮባክተር ፓይለሪ የተባለው ደቂቅ ህዋስ የጨንጉራ ህመም ምልክት ባለባቸው እና በሌለባቸው የኤች አይ ቪ ህመምተኞች ያለው ስርጭት ለማወቅ እና ታካሚዎች ተገቢውን ህክምና እንዳገኙ ያስችላል።

ከጥናቱ ተሳታፊ የሚጠበቁ

በዚህ ጥናት ለመሳተፍ የሚሰማሙ ከሆነናሙና (ደም እና ሰገራ) እንዲወሰድ እና ለጥናቱ እንዲወልዱ መስማማት ይጠበቅቦታል። የጤና ባለሙያ ከእርስዎና ሙናውን ይሰበስባል። ከተወሰደውም ሙና ላይ የሚገኙ መረጃዎች ከዚህ ጤና ጣቢያ ወጭ ለሚገኙ ለሥራው አግባብነት ላላቸው ሰዎች ቢነገር የማይቃወሙ መሆኑን መስማማት ይጠበቅቦታል። ይሁን እንጂ ይህ ዓይነቱ መረጃ የእርስዎን ማንነት የሚገልጹ ማስረጃዎን ማለትም ስም፣ አድራሻና የመሳሰሉት መረጃዎች አይጨምርም።

: ይልቁንም ለዚህ ጥናት አገልግሎት ብቻ የሚወል መለያ ቁጥር ጥቅም ላይ እንዲውል ይደረጋል።
ናሙና ሰጠው ማለት በሽታው ይገኝ ብዎታል ማለት አይደለም።

በእርሰዎና ሙናው ስጥ የበሽታ አምጭ ተህዋስ ቢገኝ ከጤና ባለሙያ ወይም አስፈላጊውን ህክምና ያገኛሉ።

ተሳታፊው የሚያጠፋው ጊዜ

የተዘጋጀውን የስምምነት ቅጽ ለመፈረምና ሙና ለመስጠት 10-20 ደቂቃ ያስፈልጋል።

በጥናቱ በመሳተፍ የሚሰከትላቸው ችግሮች

ናሙና በሚሰበሰብበት ወቅት ምንም አይነት ችግር አያስከትል በዎትም። ሆኖም ሙናው በሚወሰድበት ጊዜ ትንሽ የህመም ስሜት ሊኖር ይችላል።

የመረጃው ሚስጥራዊነት

ማንኛውም የሰጡት መረጃ እና ከተወሰደው ሙና ላይ የተገኘው የላቦራቶሪ ውጤት የሚወለወው ለጥናቱ አላማ ብቻ ነው። ይህን ማህደር ሊያገኙ የሚችሉ የተወሰኑ የጥናቱ ተባባሪ ሠራተኞች ብቻ ናቸው። ከዚህም በላይ ስለ እርስዎ ያለውን ማንኛውንም መረጃ የይለፍ-ቃል ባለው የኮፒወተር የመረጃ ማህደር ውስጥ እንዲቀመጥ ይደረጋል።

በጥናቱ በመሳተፍ የሚያስከትላቸው ጥቅሞች

ይህ ጥናት የማስተርስ ዲግሪ መመሪያ እንደመሆኑ መጠን በመሳተፍ የሚያገኙት የገንዘብ ጥቅም ጥቅም የለም። ለወደፊት በተመሳሳይ ሁኔታ ውስጥ ላሉ በሽተኞች በመረጃ ላይ የተመሰረተ ህክምና ለመስጠት ያግዛል። ከፈለጉ የላቦራቶሪ ውጤትዎን በነፃ ያገኛሉ እንዲሁም ስለ አስፈላጊው ህክምና ከህኪም ጋር ይነጋገራሉ።

የጥናቱ ተሳታፊዎች መብት

ትብብር ያለው ለመሆኑ በፍቃድ እንዲሁም የተመሠረተና ተሳትፎ ያለውን መተውና በማንኛውም ሰዓት ጥናቱን ማቆም ይችላሉ። በጥናቱ ውስጥ ያለውን ተሳትፎ በማንኛውም ጊዜ የማቆረጥ ሙሉ መብትዎ የተጠበቀ ከመሆኑም በላይ ራሶን ከጥናቱ በማግለል ያለ ምክንያት የሚቀርብዎት ምንም ዓይነት የጤና ጣቢያው አገልግሎት አይኖርም። ከዚህም በተጨማሪ ጥናቱን በተመለከተ ማንኛውንም ዓይነት ጥያቄ የመጠየቅና ገለፃ የማግኘት መብት አለዎት። የላቦራቶሪ ምርመራ ውጤቱንም በነፃ ማግኘት ይቻላል።

ግንኙነትና ጥያቄ

ይህን ጥናት በተመለከተ ወይም ከዚህ ጋራ በተዛመደ መልኩ ስለሚያጋጥሙ ድንገተኛ ችግር ወይም ጥያቄ ካሎት በሚከተለው አድራሻ ይጠቀሙ።

ተመራማሪ፣ ጌታቸው ሰይድ (ቢ.ኤስ.ሲ.) ሞባይል +251912131707
ኢ-ሜይል፣ gechgsa@gmail.com

ለተጨማሪ መረጃ አዲስ አበባ ዩኒቨርሲቲ፣ የሕክምና ላቦራቶሪ ሳይንስ ትምህርት ክፍል ይጠይቁ;
ስልክ-+251112755170
ከዚህ በታች የሚገኘው ፊርማ ለእርስዎ የተሰጠውን መረጃ ማንበብ ያስጠይቃል።
ከመፈረም በፊት እባክዎትን የጥናቱን ዓላማ፣ የተሳትፎ ጉዳትና ጥቅሙ፣ የመተው፣ የማቆረጥ፣ መብትና ነፃነት እንዳለዎት ይረዱ።

ተስማምተዋል? የጥናቱን መግለጫ አንብብ ያለሁ/ሰምቻለሁ እናምተረድቻለሁ።
 መመሪያውን እንደሆነና በእኔምን ሊከሰት እንደሚችልተረድቻለሁ። በጥናቱ ላይ ለመሳተፍ፤
 እስማሙ ----- አልስማም -----

Annex III: English Versions of Consent form

This page contains an agreement signature to participate in the study entitled with “*Helicobacter pylori* infection among dyspeptic and non - dyspeptic HIV patients at Yeka health center, Addis Ababa Ethiopia”. So please read the following points and sign your signature at the end in the space provided.

1. I understand the objective of the study “Magnitude of *Helicobacter pylori* infection among dyspeptic and non- dyspeptic HIV patients at Yeka health center, Addis Ababa Ethiopia”
2. I know that the information/ specimen that I will give used for this study only.
3. I understand that, all the information given for the study and the results are confidential.
4. I understand that I will not get any money for my participation.
5. I understand that I have a right to stop from participation any time in the study.
6. I understand all the information which is explained by specimen collector/laboratory technician/technologist.

Signature of the participant: _____

Address of the participant: _____

Date: _____

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

Getachew Seid

Department of Medical Laboratory Sciences, College of Health Sciences

Addis Ababa University

Mobile +251912131707

Email- gechgsa@gmail.com

For additional information, please contact Addis Ababa University, College of Health Sciences, Department of Medical Laboratory Sciences at: Telephone +251112755170

Annex IV: Amharic Versions of Consent form

የተሳታፊ ስምምነት ቅጽ

ይህ ገጽ “*Helicobacter pylori* infection among dyspeptic and non-dyspeptic HIV patients at Yeka health center, Addis Ababa, Ethiopia” ማለትም “ሄሊኮባክተር ፓይሎሪ የተባለው ደቂቅ ህዋስ የጨንጉራ ህመም ምልክት ባለባቸው እና በሌለባቸው የኤች አይ ቪ ህመምተኞች ያለው ስርጭት በየካ ጤና ጣቢያ ታካሚዎች፣ አዲስ አበባ፣ ኢትዮጵያ” በሚል ስም የተሰጠው ስምምነት ቅጽ ነው። በመሆኑም እባክዎን ከዚህ በታች የተዘረዘሩትን ጥቦች ይረዱና፤

ለመሳተፍ ፈቃደኛ ሆነው ከተስማሙ መስማማት ያለውን ማረጋገጫ ይሰጡ እንዲፈረም እጠይቃለሁ።

1. እኔ “ሄሊኮባክተር ፓይሎሪ የተባለው ደቂቅ ህዋስ የጨንጉራ ህመም ምልክት ባለባቸው እና በሌለባቸው የኤች አይ ቪ ህመምተኞች ያለው ስርጭት በየካ ጤና ጣቢያ ታካሚዎች፣ አዲስ አበባ፣ ኢትዮጵያ” በሚል የሚለው ጥናት አላማ በደንብ ተገንዝቤ አለሁ።
2. ከእኔ የሚወሰደውና ለጥናቱ አላማ ብቻ እንደሚወልድ ረድቻለሁ።
3. ሁሉም መረጃዎች እና የጥናት ውጤቱ ምስጢራዊ መሆኑን ተገንዝቤ አለሁ።
4. በጥናቱ ላይ በመሳተፍ ምንም የገንዘብ ክፍያ እንደማላገኝ ተረድቻለሁ።
5. በጥናቱ ያለ መሳተፍ እንዲሁም በማንኛውም ሁኔታ ለጥናቱ ስሜን ለማግኘት እንዳለኝ አወቁ አለሁ።
6. ሁሉም መረጃዎች በአስተባባሪ ወይ/ዎች ተገልጾ ለደንብ ተረድቻለሁ።

የተሳታፊ ፊርማ: -----

የተሳታፊ አድራሻ:-----

ቀን:-----

ይህንጥናት በተመለከተ ጥያቄ ቢኖርዎት ወይም ከዚህ ጋራ በተዛመደ መልኩ ስለሚያጋጥመዎት ደንበኞች ችግር በሚከተለው አድራሻ ይጠቀሙ::

ጌታቸው ሰይድ ሞባይል+251912131707

የሕክምና ላብራቶሪ ሳይንስ ትምህርት ክፍል፣ የጤና ሳይንስ ኮሌጅ፣ አዲስ አበባ ዩኒቨርሲቲ

ኢ-ሜይል፣ gechgsa@gmail.com

ለተጨማሪ መረጃ፣ አዲስ አበባ ዩኒቨርሲቲ ፣ የሕክምና ላብራቶሪ ሳይንስ ት/ክፍል ይጠይቁ:: ስልክ+251112755170

Annex V: Collection and running of blood for hematological analysis by ABX MICROS Es 60 hematology analyzer Horiba

Sample collection

Fresh Whole Blood specimens’ are recommended! The ICSH (International Committee for Standardization in Hematology) defines a Fresh blood specimen as “One processed within 4 hours after collection”. Well mixed Whole Blood specimens, collected in EDTA anti-coagulant and run within eight hours after collection, provide the most accurate results for all parameters. The White cell size distribution may shift when specimens are assayed between 5 and 20 Minutes after collection and not more than 8 hours after collection[40].

Test procedure

Run the Quality Control blood, all (3) levels and verify that the results are within their specified Limits. Run the patient blood samples. Follow the steps listed:

- 1 - Mix the blood sample gently and thoroughly.
- 2 - Rotate the Tube Holder to the position desired for the sample.
- 3 - Press the “ID” key and enter the patient Identification if (US Mode) is selected in setup. Press the “ID” key and enter the Run # if (Standard Mode) is selected in set-up.
- 4 - Mix and place the sample into the tube holder, Close the tube holder door. The analysis will begin if the system is set for “Auto - Start” in the set-up menu. Press the “START” key on the front panel if the system is set for “Manual -Start” in the set-up menu.

5. The machine analyzes the sample and displays the result within a minute. At the end of the cycle, the result prints out, the LED on the front panel turns from “Red” to “Green”, and the ABX MICROS Es 60 is ready for the next analysis.

6. If you want you can print out the result

If any results are out of their acceptable ranges, Controls and Normal Patient samples, perform the following steps:

1 - Rerun the Control or patient sample.

2 - Clean the system, Service functions, and (Concentrated Cleaning) .Re-run the sample.

3 - Open a New vial of Control material.

4 - Call your local ABX Technical Support Representative before re-Calibrating the system.

Result interpretation

Normal ranges of hemoglobin male:13.5-16.5 g/dl and females 12.0-15.0g/dl

Annex VI: Collection and running of blood for CD4 T lymphocyte count by FACSCount machine

Test principle

FACSCount is a complete system incorporating instrument, reagents, controls, and software. It employs a direct two-color immune fluorescence method for enumerating absolute lymphocyte counts (cells/ μ L whole blood) of the following mature human lymphocyte subsets: T lymphocytes (CD3+), T-helper/inducer lymphocytes (CD3+CD4+), and T-suppressor/cytotoxic Lymphocytes (CD3+CD8+). In addition, the helper/suppressor T-lymphocyte (CD4+/CD8+) ratio is provided. These data can provide important information for staging and monitoring patients infected with HIV [37].

The FACSCount System is designed to use analyses whole blood, collected in liquid EDTA. When whole blood is added to the tubes of a sample reagent pair, the fluorochrome-labeled antibodies bind specifically to antigens on the surface of lymphocytes. The FACSCount instrument detects two colors and measures relative cell size. The CD3 cells will fluoresce red and the CD4 and CD8 cells will fluoresce yellow when analyzed on the FACSCount instrument. A known number of reference beads is contained in each reagent tube and functions as a fluorescence and quantitation standard for calculating the absolute counts for the CD4+, and CD3+ T lymphocytes. Fixative solution is added to the stained samples prior to analysis to preserve the integrity of the antibody binding. No lysing is necessary [39].

Test kit

The FACSCount Reagent Kit consists of 50 paired reagent sets containing a mixture of monoclonal antibody reagents conjugated to two fluorochromes and a known number of fluorochrome-integrated polystyrene beads. The first tube in each pair contains CD4 and CD3 antibodies. The kit also contains two vials of formaldehyde fixative (fixative solution), sufficient to prepare 50 tube pairs. The FACSCount Control Kit consists of paired control bead sets containing fluorochrome-integrated (2- μm) polystyrene beads at four levels (zero, low [50 beads/ μL], medium [250 beads/ μL] and high [1000 beads/ μL]). The control kit allows 25 control runs [39].

Analysis

The FACSCount System software provides automated analysis, requiring no operator intervention. The FACSCount System provides a report quantifying the CD4+, CD8+, and CD3+ T lymphocytes as absolute numbers of lymphocytes per μL (mm^3) of blood. The absolute CD3+ lymphocyte count is reported as the average of the total CD3+ values from the same blood sample stained with the two reagent tubes.

The reportable ranges generated by the FACSCount System for each lymphocyte subset are as follows: [39]

CD4 50–2000 cells/ μL

CD3 100–3500 cells MI

The Procedure

FACSCount sample preparation consists of three easy steps with minimal hands-on time. This reduces the risk of exposure to biohazards and improves laboratory efficiency.

1. Pipette 50 μL whole blood into each tube, vortex and incubate.
 - Add 50 μL blood to each of the two tubes.
 - Cap, vortex, and incubate for 60 minutes.
 - Add 50 μL fixatives to each tube.
 - Cap and vortex.
2. Pipette 50 μL of fixative solution into each tube and vortex.
 - Add 50 μL fixatives to each tube.
 - Cap and vortex.
3. Run on the FACSCount instrument.

-Run the tubes on the FACSCount [43].

Annex VII. Stool antigen test using one step *Helicobacter pylori* feces test

Specimen handling

One step *Helicobacter pylori* feces test is a sample test that utilizes a combination of *Helicobacter pylori* antibody is coated particles and anti-mouse IgG to qualitatively and selectively detect *H. pylori* antigens in feces in just minutes. Collect feces from specimen caught in clean cup. Contamination from toilet water and urine should be avoided. The stool specimen can be stored up to 3 days at 2-8⁰c before testing [41, 42].

Kit components

-25 individual sealed pouches, each containing:

=test strip

=desiccant pouch

-25 specimen collection tube .each 1.5 ml buffer solution

Test procedure

Bring the device and the extracted feces to room temperature (10^oc - 30 ^oc), if it is refrigerated .then mix the sample solution well gently by gently shaking the collection tube for a few times.

1. Remove the test device from the foil pouch by tearing at the notch and place it on a level surface.

2. Holding a sample collector upright, squeeze 3 drops (about 80 μ l) of sample solution to the sample pad below the mark line.

3. Wait for 15 minutes and read the result, don't read the result after 30 minutes.

Interpretation of the result

Positive- rose pink bands are visible both in the control region and the test region .a positive result indicates the presence of *Helicobacter pylori* antigen.

Negative -rose pink bands are visible both in the control region. No color band appears in the test region .a negative result indicates the presence of *Helicobacter pylori* antigen is lower than the detection of the test.

Invalid – no visible band at all or there is visible band only in the test region but not in the control region.

Quality control

If there is control line band appears in the control band, it confirms sufficient specimen volume, adequate membrane wicking and correct procedural technique.

Storage and stability

1. Store at 4 °c – 30 °c in the sealed pouch within the expiration date.
2. Keep away from sun light, moisture and heat.
3. Do not freeze.

Annex VIII. Questionnaire

Addis Ababa University Collage of Health Sciences, School of Allied Health Science
Department of Medical Laboratory Science. Questionnaire for the demographic characteristics
and assessment of risk factors of *Helicobacter pylori* infection among dyspeptic and non-
dyspeptic HIV patients at Yeka health center, Addis Ababa Ethiopia.

Patient Identification

Patient name _____ Participant code _____
Participants residence address _____ Telephone _____ signature _____
Data collector Name _____ date _____ signature _____

I. Socio- Demographic Characteristics of the Study participants.

1. Age _____
2. Sex 1) Male 2) Female
3. Height _____ ,weight _____ BMI _____
4. Monthly income (in Ethiopian birr) _____
5. Occupation _____
6. Marital status _____
7. Education level _____

II. Medical history of the study participant

8. Which of the following symptom does the patient have as he come to the ART clinic
- A. Dyspepsia B. Non dyspepsia
9. Have you ever been investigated for *Helicobacter pylori* ?
- A. yes B no C.don't know
10. If yes when you tested?
- A. within the last two weeks B. Before 1 month C. before 2 months D. don't know
11. If yes what is your result
- A. positive for *Helicobacter pylori* B. Negative for *Helicobacter pylori* C .don't know
12. What sample do you gave for the test?
- A. Stool B. Blood C .I don't remember
13. If you are positive have you ever treated *Helicobacterpylori*?
- A. Yes B.No C. I don't remember
14. Are you on ART?
- A. Yes B. No
15. If yes how long you are on ARV treatment _____
16. WHO stage of AIDS of the patient
- A. Stage 1 B. Stage 2 C. Stage 3 D. Stages 4

III. Associated symptoms in current illness

Symptom	Yes	No
Diarrhea		
Vomiting		
Loss of appetite		
Any abdominal pain		

IV. Nutritional and live condition

1. What do you eat most of the time _____
2. How much times do you eat per day _____
3. Do you drink alcohol? A. yes B. No
4. If yes how much litter alcohol per day _____
5. What type of alcohol do you drink?

A. beer B. draft C. local alcohol D. other

6 .source of water that you drink_____

7. Currently Do you smoke cigarettes ? A yes B. no

8. currently do you chow chat? A yes B. no

V. Laboratory result

1. Laboratory result of H. pylori one step stool antigen test?

A. negative B. positive

2. current CD4 T-lymphocyte result_____

3. Hemoglobin value_____

VI. Comments_____

Name of principal investigator _____Signature

_____Date _____

IX ቃለ-መጠይቅ:

በአድስ አበባ ዩኒቨርሲቲ የአላይድ ጤና ሳይንስ ትምህርት ቤት የሜድካል ባቦራቶሪ ሳይንስ ትምህርት ክፍል። በየካ ጤና ጣቢያ ለሚሰራውማለትም ሄሊኮባክተር ፓይለሪ የተባለው ደቂቅ ህዋስ የጨንጉራ ህመም ምልክት ባለባቸው እና በሌለባቸው የኤች አይ ቪ ህመምተኞች ያለው ስርጭት በየካ ጤና ጣቢያ ታካሚዎች፤ አዲስ አበባ፤ ኢትዮጵያ የተሳታፊዎች የግል ሁኔታ ቃለመጠይቅ።

ታካሚዎች መለያ

የታካሚው ስም-----ኮድ-----

የመኖሪያ አድራሻ-----ስልክ-----ፊርማ-----

መጠይቁን የሞላው ሰው ስም-----ቀን -----ፊርማ-----

I. የታካሚው የግል ሁኔታ

- 1.እድሜ-----
- 2.ጾታ ሀ.ወንድ ለ.ሴት
- 3.ቁመት----- ክብደት-----
- 4.ወርሀዊ ገቢ----- ቁመት-----
- 5.የስራ ሁኔታ-----
- 6.የጋብቻ ሁኔታ-----
- 7.የትምህርት ደረጃ-----

II. የተሳታፊው የህክምና ሁኔታ

8. ታካሚው የጨጋራ ህመም(Dyspeptia) ምልክት አለበት የሚያሳየው የህመም ምልክት ሆኖ

ለ. የለበትም

9. ከዚህ በፊት የሄልኮባክተር ፓይለሪ ምርመራ አድርገው ያውቃሉ

ሀ. አውቃለሁ ለ. አላውቅም ሐ. አላስታውስም

10. አዎ ከሆነ መልስዎ መቸ ነው የተመረመሩት

11. አዎ ከሆነ መልሰዎውጤትዎ ምን ነበር

ሀ. ፖዘቲፍ ለ. ነጋቲፍ ሐ. አላስታውስም

12. ፖዘቲቭ ከነበሩ ህክምና አግኝተው ነበር

ሀ. አዎ ለ. አላገኘሁም ሐ. አላስታውስም

13. ምን አይነት ናሙና ነበር የሰጡት

ሀ. ሰገራ ለ. ደም ሐ. አላስታውስም

14. የጸረ ኤች አይ ቪ መድሀኒት እየወሰዱ ነው

ሀ. አዎ ለ. አይደለም

15. መልስዎ አዎ ከሆነ ለስንት ጊዜ የጸረ ኤች አይ ቪ መድሀኒት እየወሰዱ ነው

16. የአለም ጤና ድርጅት በሽተኛው ያለበት ደረጃ

ሀ. ደረጃ 1 ለ. ደረጃ 2 3. ደረጃ 3 ሐ. ደረጃ 4

IV. በአሁኑ ወቅት ያሉ ተጋዳኝ የህመም ምልክቶች

የህመም ምልክት አለ የለም
ተቅማጥ ትውከት
የምግብ ፍላጎት መቀነስ
የሆድ ህመም

V. የኑሮ ሁኔታ

1. ብዙ ጊዜ የሚመጡት የምግብ አይነት -----

2. በቀን ምን ያህል ጊዜ ይመጣሉ-----

3. የአልኮል መጠጥ ይጠጣሉ ሀ. አዎ ለ. አልጠጣም

4. አዎ ከሆነ በቀን ምን ያህል ሊትር ይጠጣሉ-----

5.አዎ ከሆነ ምን አይነት መጠጥ ነው የሚጠጡት

ሀ.ቢራ ለ.ድራፍት ሐ. የሀገር ባህል መጠጥ መ.ሌሎች

6.በ አሁኑ ሰአት ጫት ይቅማሉ;ሀ.አዎ ለ.አልቅምም

7. በ አሁኑ ሰአት ሲጋራ ያጨሳሉ ሀ.አዎ ለ.አላጨስም

V.የላቦራቶሪ ውጤት

1. የሄሊኮባክተር ፓየሎሪ ውጤት

ሀ.ፖዘቲቭ ለ.ነጋቲቭ

2.የሲድ ፎር ውጤት-----

3.የሄሞግሎቢን ውጤት-----

VI. አስተያየት-----

የተመራማሪው ስም -----ፊርማ-----ቀን-----

Annex VI: Declaration

Title of project: *Helicobacter pylori* infection among dyspeptic and non - dyspeptic HIV patients at Yeka health center, Addis Ababa Ethiopia.

I, the undersigned, declare that this MSc research project is my original work. It has not been presented for a degree in any other University. False statements could be cause for invalidating this research project and may lead to other administrative or legal action.

Principal investigator:

Name: **Getachew Seid(BSc)**

Address: Department of Medical Laboratory Sciences, AAU

Signature: _____ Date: _____

Advisor (s):

Name: **Kassu Desta (MSc, PhD fellowship)** Signature: _____ Date: _____

Aster Tsegaye (MSc,PhD) Signature: _____ Date: _____

Address: Department of Medical Laboratory Sciences, AAU