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COLLEGE OF HEALTH SCIENCES

SCHOOL OF ALLIED HEALTH SCIENCES

DEPARTMENT OF MEDICAL LABORATORY SCIENCES

H.PYLORI INFECTION AND ITS ASSOCIATION WITH CD4 T CELL COUNT AMONG HIV INFECTED INDIVIDUALS WHO ATTENDED THE ART SERVICE IN KOTEBE HEALTH CENTER, YEKA SUBCITY, ADDIS ABABA, ETHIOPIA.

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This is to certify that the thesis prepared by Eden Ababu entitled:

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LIST OF ACRONYMS

AIDS: Acquired immune deficiency syndrome

ART: Anti-retroviral treatment

BD: Becton Dickinson

CBC: Complete Blood Count

CMV: Cytomegalovirus virus

DNA: Deoxyribonucleic Acid

EDTA: Eylene Diamine Tetra Acetate

ELISA: Enzyme-Linked Immunosorbent Assay

FACS: Fluorescent Activated Cell Sorter

GI: Gastrointestinal

HAART: Highly Active Anti-retroviral Treatment

HCT: Hematocrit

HIV: Human Imunodeficinet Virus

Hg: Hemoglobin

HPSA: *H.pylori* Stool Antigen Test

H.pylori: *Helicobacter pylori*

ID: Identification

MALT: Mucosa-associated lymphoid-tissue

ROS: Reactive Oxygen Species

SOP: Standard Operating Procedure

ABSTRACT

Background: *H.pylori* is a gram-negative bacterium found on the luminal surface of the gastric epithelium. At least 50% of the world's human population has *H.pylori* infection. Studies have shown an increased prevalence of this bacteria in people infected with HIV relied on the CD4 count level.

Aim: To determine the burden *H.pylori* and its association with CD4 count among HIV infected individuals who attend the ART service in Kotebe Health Center, Yeka Sub City, Addis Ababa, Ethiopia.

Methods:A cross sectional Study was conducted from October to November 2016 on 388HIV infected individuals at Kotebe Heath Center, Yeka Sub City Addis Ababa. Convenient sampling technique was employed to include study participants who met the inclusion criteria. CD4 count and hemoglobin level with demographic and other variables such as habit of drinking alcohol, habit of eating chat, habit of personal hygienic practice, and also direct stool microscopy examination was included in the study.

Results: The prevalence of *H.pylori* infection among study subject was 54.8%, *H.pylori* infection among female participant was 51.8% (133/256) and among male participant was 60.6% (87/143) with $X^2 = 2.745a$ and P-Value 0.098. The prevalence of *H.pylori* infection in patient with CD4 count level less than 500 was 54.4% (131/141) and with CD4 count level greater than 500 was 55.1% (81/147).The prevalence of *H.pylori* was 67.1% (61/91) among HIV clients with people in the house hold greater than four. The prevalence of *H.pylori* infection among HIV clients those use tanker water, wheel water, pipe water was 50% (2/4), 25% (4/16) and 55.9% (226/401) respectively ($X^2= 6.114^a$ P. value=0.047).

Conclusion: There was no significant association between the prevalence of *H.pylori* and level of CD4 cell count. But there was significant association between number people in the house hold and type of water used for drinking. And there was no significant association between with socio demographic data like age, sex, marital status, educational level, stage of ART taking, habit for drinking of alcohol, smoking, eating of chat, and habit of hand washing, and pervious history of GIT. The health facility and other responsible body must be conscious in creating awareness on the possible transmission of the disease.

Key words: *H.pylori* stool antigen test, CD4 cells, HIV, ART.

1. INTRODUCTION

1.1 BACK GROUND

H.pylori is a gram-negative, microaerophilc, motile bacterium found on the luminal surface of the gastric epithelium, were first isolated by Warren and Marshall in 1983(1).The infection is usually contracted in the first few years of life and tends to persist indefinitely unless treated. The higher prevalence in older age groups is thought to reflect a cohort effect related to poorer living conditions of children in previous decades. At least 50% of the world's human population has *H.pylori* infection and varies markedly around the world. The organism can survive in the acidic environment of the stomach partly owing to its remarkably high urease activity (2).

Infection with *H.pylori* is a cofactor in the development of three important upper gastrointestinal diseases: duodenal or gastric ulcers (reported to develop in 1 to 10% of infected patients), gastric cancer (in 0.1 to 3%), and gastric mucosa-associated lymphoid-tissue (MALT) lymphoma (in <0.01%). The risk of these disease outcomes in infected patients varies widely among populations. The great majority of patients with *H.pylori* infection will not have any clinically significant complications .Although its exact route of transmission and specific prevention methods are unknown, some research indicates that *H.pylori* is transmitted by the faecal–oral route or by the oral–oral route through vomitus or saliva (3).

The overall burden of *H.pylori* is suggested to be correlated with socioeconomic conditions; gender, occupation, 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 (4).

Among the several types of *H.pylori* genes, the carriers of the Cag A gene that signalizes a pathogenicity island with myriads of pathogenic genes such as Bab A, OIpA and also the carriers of the VaC A gene can induce the disease (5, 6). Although *H. pylori* avoid many innate immune receptors, specific virulence factors (including those encoded on the cag pathogenicity island) stimulate innate immunity to increase gastric inflammation and increase disease risk and also acquired T helper 1 response up regulates local immune effectors (7,8). *H.pylori* also known by

its formation of reactive oxygen species (ROS) in the stomach results in oxidative stress(9) and this oxidative stress plays an important role in proliferation, apoptosis, and inflammation in gastric epithelial cells infected with *H.pylori* (10).

The immune deficiencies caused by HIV allow for many different GI opportunistic infections, usually affecting the esophagus or intestine. Bacterial, viral, fungal, and protozoan pathogens have all been reported to cause gastritis, with the likelihood of different infections dependent on an individual patient's degree of immunosuppression (11). A number of studies have addressed *H.pylori* infection is more or less frequent in people infected with human immunodeficiency virus (HIV). Some studies demonstrating no difference in prevalence compared to HIV negative population, while others show a lower or a higher prevalence (12).

CD4 cells are white blood cells that play an important role in the immune system. A higher number indicates a stronger immune system. *H.pylori*, which is present in 90% of immunocompetent patients with chronic active gastritis, continues to be extremely common in those whose CD4 cell counts are greater than 300/μL in HIV infected individuals (13). It is well known that CD4 cells play a role in inducing gastritis and that this gastritis may be a mechanism by which *H.pylori* colonization is enhanced (14). Some studies have showed HIV-infected patients with *H.pylori* have higher mean CD4 count than HIV-infected patients without *H. pylori* and low CD4 counts are apparently associated with low-grade *H.pylori* infection (15).

Serologic tests are available for identification of *H.pylori* and they are relatively cost-effective tests which are often used for screening or documentation of infection in patients whose other tests yielded borderline results. However, these tests are not suitable to diagnose active infection or follow-up of eradication because of its low accuracy (16).Urea breath tests are considered to be the gold standard of diagnosis, with sensitivity and also specificity from 90% to 100%.The stool antigen tests, have been tested in several laboratories of the world. The results have been satisfactory and in some researches it has been considered to be the preferable strategy for diagnosis of *H.pylori* in primary care (17, 18). In this paper stool antigen tests was applied to identify *H.pylori* infection.

1.2 STATEMENT OF THE PROBLEM

The prevalence of *H.pylori* infection varies among continents and countries around the world, and its prevalence varies from 9% to 82% (19). Overall prevalence is high in developing countries and lower in developed countries. The prevalence of *H.pylori* infection in developed countries is 30-40% and 80-90% in developing countries (20).

The gastrointestinal tract is the largest immunological site of the body and HIV infection profoundly impacts on gut function (21). HIV sero-positive patients frequently experience upper gastrointestinal-tract symptoms that cause considerable morbidity and are due to multiple etiologies. HIV infection predisposes to a multitude of opportunistic infections, many of them resulting in gastrointestinal symptoms (22). *H.pylori* is a causative organism for chronic gastritis and is associated with peptic-ulcer disease. Infection may be asymptomatic as well (23).

It is well-known that the acute and chronic gastritis are asymptomatic, in general population, the HIV patients could be more susceptible for diseases, and Gastrointestinal (GI) discomfort is a common complaint among patients infected with HIV. GI symptoms can be caused by a myriad of factors, including, but not limited to, co-infections, antiretroviral therapy, medications for opportunistic infections, and nutritional status (24).

Some studies show the role of *H.pylori* infection in gastro duodenal lesions might be different between the general population and acquired immunodeficiency syndrome (AIDS) patients and it remains unclear up to now. Several epidemiologic studies have examined the relationship between *H.pylori* infection and HIV. Some researchers have hypothesized that *H.pylori* infection may be more common among HIV-infected patients as a result of immune suppression. An increased incidence of *H.pylori* infection would contribute to the prevalence of GI complaints in this population (25).

In contrast to the established role of *H.pylori* in gastritis and duodenal ulcers in general, conflicting results have been reported in patients with Human Immunodeficiency Virus (HIV) infection. The prevalence of *H. pylori* in patients infected with human immunodeficiency virus (HIV) has been reported to be remarkably lower; reason for these lower rates is unclear (26).

There is great controversy about the prevalence of patients co-infected with HIV and *H.pylori*, especially after the advent of the use of HAART to control HIV infection. Studies have shown an increased prevalence of this bacteria in people infected with HIV relied on the CD4 count: increase in HIV/AIDS patients more than 200 cells/mm3, and is decreased in HIV/AIDS patients with less than 200 cells/mm3 (27).

Therefore, the CD4 count in gastric mucosa is different in participants with and without *H. pylori* infection. CD4 lymphocytes, which are depleted in AIDS patients, might be associated with a different presentation of *H.pylori* infection. Also, the frequent use of antibiotics by AIDS patients could lead to *H. pylori* eradication from gastric mucosa, explaining the lower prevalence described in this population (28).

Hence, it had been given little emphasis concerning the prevalence *of H.pylori* infection among HIV infected individuals in the context of Ethiopia region. And many researchers had reported that there is a significance association between *H.pylori* infection and level of CD4 count among HIV infected individuals, and yet little emphasis given to the burden *H.pylori* and its association with CD4 count among HIV infected individuals, in Ethiopia region, so this study will address the issue and may fill the gap.

1.3 SIGNIFICANCE OF THE STUDY

H.pylori is one of the causative agent for the gastro-intestinal tract infection among HIV infected individuals. This study helps to know the prevalence of *H.pylori* in this study population and also helps to determine if there is a significance association between CD4 count profile and *H.pylori* infection. Additionally, this study determines the association between *H.pylori* infection with different variables.

In conclusion, this paper aims to determine the burden *H.pylori* infection and its association with CD4 count among HIV infected individuals in this study participants. As a result, this paper helps to create awareness about the burden of *H.pylori* among study participants and may help to take any kind of preventive or treatment measurement by the health center and also by any other responsible personnel. And about lastly the study serves as base line data for the upcoming researchers in these study participants.

2. LITERATURE REVIEW

I. Identification of *H.pylori* infection using Stool antigen test

Retrospective, analytical case-upon two groups of patients with gastro-intestinal symptoms tested for *H.pylori* stool antigen at the Infectious Diseases Laboratory, Clinical County Hospital Mureş, Romania. In group A 44 HIV-positive patients, group B: 58 HIV-negative participants were included. They first compared groups A and B regarding the frequency of *H.pylori* infection. Group A was divided into two sub-groups, according to the status of *H.pylori* infection: group A1: 5 *H.pylori*-positive subjects, group A2: 39 *H.pylori*-negative ones. The frequency of *H.pylori* infection was 11.36% among HIV-positive patients and 13.79% in HIV-negative ones, without statistically significant difference. They found no statistically significant differences between subgroups A1 and A2 regarding CD4+ Tlymphocytes level, HIV-RNA plasma viral load, antibiotic/antiretroviral therapy (29).

A prospective cross sectional study was done in the immunology section of microbiology, University College of Medical Sciences (UCMS) and Guru Teg Bahadur (GTB) Hospital, Delhi India. Stool samples from 50 HIV reactive participants (cases) 16 years-65 years age group presenting with diarrhea were screened for the presence of *H.pylori* antigen along with equal number of HIV nonreactive (control) participants presenting with diarrhea by ELISA. *H.pylori* antigen was detected in 10% of cases while none of the controls was found to be positive for the presence of *H.pylori* antigen ($p<0.05$). No significant correlation was found between risk of acquisition of infection and age or sex (30).

Study conducted in Nigeria, to determine the prevalence of *H.pylori* infection among patients infected with HIV-1 on antiretroviral therapy using *H.pylori* stool antigen. 139 patients infected with HIV-1 were recruited, stool samples were collected and the *H.pylori* stool antigen (HpSA) test was used to detect *H.pylori* antigen.46.8% of the respondents were positive for *H.pylori* and 53.2% were negative, 18 (13%) were men and 47 (33.8%) were women.. They also observed that the prevalence of *H.pylori* was low in these patients compared with the general population (31).

Study conducted at a university hospital in Ghana on HIV-patients (n = 1,095) and HIV-negative individuals (n = 107),*H. pylori* status was determined using stool antigen testing. The prevalence

of *H.pylori* infection was significantly lower in HIV-positive compared to HIV-negative individuals (51.5% vs. 88%, p<0.0001). In HIV patients, *H.pylori* prevalence decreased in parallel with CD4+ T cell counts. In ART-naïve HIV-infected individuals, but not in those taking ART, *H.pylori* infection was associated with higher CD4 cell counts (312 vs. 189 cells/μL, p<0.0001) and lower HIV-1 viral loads (4.92 vs. 5.21 log10 copies/mL, p = 0.006). Having no access to tap water and higher CD4+ T cell counts were identified as risk factors for *H.pylori* infection (32).

II. Identification of *H.pylori* using PCR

Study conducted in sexual infection control center, Imam Khomeini Hospital, Tehran, Iran, the prevalence *H.pylori* infection was determined using PCR from stool of 43 HIV infected individuals. *H.pylori* infection was found in 30 patients (69.76%) and 35 of the patients (81.39%) had CD4+ count below 200. There was no significant relationship between CD4+ count and presence of *H.pylori* (P value > 0.05) (33).

III. Identification of *H.pylori* using serologic tests

A case-control study in a Greek hospital: to evaluate the prevalence and morbidity of *H.pylori* in HIV-infected patients. HIV-seropositive patients were infected by *H. pylori* less often than HIV-seronegative controls [12/58 (20.7%) versus 38/58 (65.5%), p< 0.001]. The mean CD4 count was lower for *H.pylori*-negative than *H.pylori*-positive HIV-infected patients (p < 0.007). Also, among HIV patients, prior use of antibiotics or proton pump inhibitors was more common in those without *H. pylori* infection, however, this difference was not statistically significant (p = 0.06) (34).

Study conducted in Kenya, Prevalence of *H.pylori* in HIV seropositive patients was 73.1% [95% CI 59.9-83.8] and in HIV seronegative patients was 84.6% [95% CI 72.9-92.6]. The prevalence of *H.pylori* in HIV seropositive patients was lower than in HIV seronegative patients but the difference was not statistically significant (p=0.230). The prevalence *of H.pylori* was stratified according to CD4+ counts in HIV seropositive patients. The prevalence of *H.pylori* decreased with decreasing CD4 cell counts, being significantly lower in those with CD4 cell counts <50/mm3 (57.1%) as compared to those with a count > 50/mm3 (86.6%) (35).

A comparative cross - sectional study was conducted in St.Paul's General Specialized Hospital, Addis Ababa, Ethiopia, on 106 HIV positive participants, 68 (64.2%) were positive for anti-

*H.pylori*IgG antibodies; and of the 106 HIV negative controls, 52 (49.1%) were positive for anti-*H.pylori*IgG antibodies (p=0.037). There was no significant difference of *H.pylori* seroprevalence between relatively higher and lower CD4 cell counts in the HIV positive cases (p>0.05). There was no significant difference in the prevalence of *H.pylori* between different CD4+ cell counts in the HIV positive study group (36).

IV. Identification of *H.pylori* using different histology and pathologic tests

A retrospective study was conducted in New York, U.S.A, upper endoscopic and pathologic findings of the stomach and duodenum were recorded. A total of 106 patients were included in the study *H.pylori* was present in only 15 of 106 (14%) specimens. Active *H.pylori* infection was confirmed by pathology. The major indications for upper endoscopy were abdominal pain, reflux, nausea, and vomiting which accounted for 70% of cases. Forty-seven percent of the *H.pylori* positive patients presented with dyspepsia. The Increased frequency of *H.pylori* infection are common in HIV+ patients and that the prevalence of *H. pylori* studies is higher in patients with CD4+ cell counts greater than 200/μL (37).

Another study conducted in New York, U.S.A, on Seventy-two patients (48 HIV-positive and 24 HIV-negative) with GI symptoms were evaluated with upper endoscopy and astral gastric biopsy. The prevalence of *H. pylori* in HIV-positive patients with CD4 count greater than 200 is 69% (11/16), the prevalence of *H.pylori* in HIV-positive patients with CD4 count less than 200 is13% (4/32) and it is significantly lower ($p < 0.001$) than that found in HIV-negative patients. The number of peptic ulcers in the HIV-positive group with CD4 < 200 was significantly less ($p = 0.035$) than that of the HIV-negative patients. These results suggest a role of CD4 cell and immune function in sustaining *H.pylori* infection and *H.pylori*-related peptic ulcer disease. Lower *H.pylori* infection and peptic ulcer disease prevalence in patients with AIDS and suppressed CD4 counts (38).

Study conducted in Italy, Sixty-seven consecutive patients infected with the human immunodeficiency virus (HIV-1), the infection was studied by performing both histological examination of gastric biopsies and serological testing for anti-*H.pylori* IgG antibodies. The *H. pylori* prevalence rate was 55% in histology; Positive histological testing appeared to be directly related to the peripheral CD4 lymphocyte count (minimum rates of 43% were detected in patients

with CD4 count 100/liter and maximum rates of 78% in patients with CD4 count 200/liter, respectively; *P*- 0.05) and inversely related to the frequency of antibiotic treatments performed over the six months prior to endoscopy. Low CD41 counts were also apparently associated with low-grade *H. pylori* infection (39).

Study conducted in Germany: A significantly lower proportion of *H.pylori* infected individuals was observed among those HIV patients who had AIDS-defining diseases. Furthermore, a substantial but insignificant decrease of *H.pylori* infection prevalence was noted in HIV patients with an extensive decline of CD4 cell count (< 100/microl). HIV patients who had received antimicrobial or H2-antagonizing drugs within 12 months prior to the study commencement also were found to have a remarkably decreased frequency of *H.pylori* infections independently of their CD4 cell count. No association between *H.pylori* infection prevalence and patient's age, sex, risk group and the type of their antiretroviral treatment was found (40).

Study conducted in China: total of 151 patients (122 HIV-positive and 29 HIV-negative) with gastrointestinal symptoms were examined by upper endoscopy and biopsy. The prevalence of *H.pylori* was less common in HIV-positive patients (22.1%) than in HIV-negative controls (44.8%; *P*< 0.05), and the prevalence of *H.pylori* displayed a direct correlation with CD4 count stratification in HIV-positive patients. In comparison with HIV-negative group, HIV-positive patients had a lower incidence of peptic ulcer (20.7% *vs* 4.1%; *P*< 0.01), but a higher prevalence of chronic atrophy gastritis (6.9% *vs* 24.6%; *P*< 0.05)(41).

Study conducted in Taiwan, One hundred and fifty-six patients (52 HIV-positive, 104 HIV-negative) with gastrointestinal symptoms were evaluated with upper gastrointestinal endoscopy and biopsy. AIDS patients had a lower prevalence of *H.pylori* infection (P < 0.0001) but a higher prevalence of CMV infection (P < 0.0001). The low prevalence of *H.pylori* infection and peptic ulcer in AIDS patients suggests a different role of *H.pylori* infection in peptic ulcer or even a different mechanism of peptic ulcerogenesis in HIV-positive participants (42).

Prospective cross-sectional study was carried out at the Gastroenterology Division of the Hospital Fern´andez in Argentina, on 209 individuals (102 HIV-positive patients and 107 non-HIV infected individuals) who underwent upper Gl endoscopy. The prevalence of *H. pylori* infection was 41.1% in HIV-infected patients and 49.5% in non-HIV patients (P = 0.22, chi2 = 1.47, NS). In HIV-

positive patients infected with *H.pylori* the mean CD4 count was higher than in HIV-positive patients without *H.pylori* (364 and 228 cells/mm3, respectively; P = 0.0001)(43).

Study conducted in Northeastern Brazil, 113 HIV-positive and 141 age- HIV-negative patients, who underwent upper gastrointestinal endoscopy for dyspeptic symptoms. *H.pylori* status was evaluated by urease test and histology. The prevalence of *H.pylori* infection was significantly lower ($p < 0.001$) in HIV-infected (37.2%) than in uninfected (75.2%) patients. There were no significant differences between *H.pylori* status and gender, age, HIV viral load, antiretroviral therapy. A lower prevalence of *H.pylori* was observed among patients with T CD4 cell count below 200/mm^3; however, it was not significant. *H.pylori* infection was significantly associated with chronic active gastritis in the antrum in both groups, but it was not associated with corpus chronic active gastritis in the HIV-infected patients (44).

3. OBJECTIVES

3.1 GENERAL OBJECTIVES

- To determine *H.pylori* infection and its association with CD4 count among HIV infected individuals who attend the ART service in Kotebe Health Center, Yeka Sub City, Addis Ababa, Ethiopia. From October – November 2016

3.2 SPECIFIC OBJECTIVES

- To determine *H.pylori* infection among HIV infected individuals who attend the ART service in Kotebe Health center, Yeka Sub City, Addis Ababa, Ethiopia.
- To assess the association of *H.pylori* infection with CD4 profiles among study participants in Kotebe Health center, Yeka Sub City, Addis Ababa, Ethiopia.
- To assess the association of *H.pylori* infection with different variables among study participants in Kotebe Health center, Yeka Sub City, Addis Ababa, Ethiopia.

4. METHODS AND MATERIALS

4.1. STUDY DESIGN AND PERIOD

- A cross sectional Study was conducted from October to November 2016.

4.2. STUDY AREA

The study was conducted in Kotebe Heath Center, Yeka Sub City Addis Ababa. This health center sited around Kotebe Zero Hulet, and generally gives service for a total of approximately 2000 ART clients about more than 50 clients per day (45).

Kotebe health center laboratory is providing services in the health center comprised all disciplines, and participating in quality assurance and laboratory accreditation program (star level 3), other than cultures, molecular techniques and electrolytes analysis.

4.3. SOURCE AND STUDY POPULATION

4.3.1. SOURCE OF POPULATION

- All HIV clients who were attending the ART service in Kotebe heath center during the study period.

4.3.2. STUDY POPULATION

- All HIV clients who were attending the ART service and also startedCD4 count follow-up in Kotebe heath center and willing to involve in this study was the study population.

4.4 INCLUSION AND EXCLUSION CRITERIA

4.4.1 INCLUSION CRITERIA

- All HIV clients those ages were above 18 years old, also attending the ART service and had started CD4 count follow up in the study period and who were willing to participate in the study.

4.4.2 EXCLUSION CRITERIA

- Who were already started treatment for *H.pylori*.

4.5 STUDY VARIABLES

4.5.1 DEPENDENT VARIABLES

- *H.pylori* infection

4.5.2 INDEPENDENT VARIABLE

- Socio-demographic (age, sex/gender, marital status, educational level, occupation and number of people in the house hold), some behavioral variables (smoking habit, drinking habit, and chat chewing habit, and habit of drinking coffee), hygienic application (water use for drinking, habits of hand washing before and after meal and after toilet used), level of CD4 count, hemoglobin level, stage antiretroviral drug and stool microscopy examination.

4.6 SAMPLE SIZE AND SAMPLING TECHNIQUES

4.6.1 SAMPLE SIZE DETERMINATION

- The sample size was calculated using study conducted in St.Paul's General Specialized Hospital, Addis Ababa, Ethiopia, on 106 HIV positive participants, the prevalence of *H.pylori* infection was 68/106 (64.2%). Therefore, by taking the prevalence 64.2% and using single population proportion:

$n = z(\alpha/2)^2 p(1-p)/d^2$

n =sample size P= 0.642

$z\ (\alpha/2)^2$ =z score at 95%

(Confidence interval which is 1.96)

Level of significance=0.05

Marginal Error=5%=0.05

$$n = \frac{(1.96)^2\ 0.642(1-0.642)}{0.05^2}$$

10% non-response rate = $\frac{10 * 353}{100} = 35.3$

sample size = 353+35.3= 388

4.*6.2 SAMPLING TECHNIQUES*

Convenient sampling technique was employed to include study participants who met the inclusion criteria from October to November 2016.

4.7 DATA COLLECTION AND PROCESSING

Demographic characteristics and exposure variables

Socio-demographic variables data like (age, sex/gender, marital status, educational level, occupation, number of people in the house hold and other relevant data such as history of gastrointestinal illness, water use for drinking, habits of hand washing before and after meal and after toilet used, habits of smoking, alcohol drinking, chat chewing, drinking coffee) were obtained using a predesigned questionnaires through interview by data collectors.

Sample collection and processing

Well trained laboratory technologist/technician collected stool specimens in order to ensure that appropriate stool specimen was obtained. Blood Sample for CD4 count and hemoglobin was collected when clients came during their follow up and the result was taken from the request paper. For CD4 count test BD FACS count and for hemoglobin test CBC SYSMEX machines were used. Stool specimen collected when clients came for result in the next 2-4 day after blood sample collection and *H.pylori* stool antigen test and direct stool microscopy examination was performed.

Stool sample collection and transportation

Stool specimen collection and handling: stool sample was collected in a clean and dry stool cup. During the study total of 388 fresh stool samples were collected strictly following SOP with sterile stool cup. Proper stool specimen was taken from each individual to reduce the occurrence chance of false negative and excess stool sample might lead to an invalid test result.

Transportation of specimens: following collection from each individual, the specimen was transported by placing each a separate sterile stool cup to the laboratory within 30 minutes (46).

Stool sample processing for *H.pylori* test: following fecal sample collection, the *H.pylori* test strip was removed from the pouch and place on a clean, flat surface and the stool specimen and test components were brought to room temperature; the plastic dropper was filled with stool specimen. Holding the dropper vertically one drop of specimen was dispensed in to the sample well of the test cassette, the specimen migrate by capillary action across the cassette. *H.pylori* antigens if present in the specimen was bind to the anti-*H.pylori* conjugates. The immune complex was then captured on the membrane by protected antibody, forming a boundary colored T-band, indicating an *H.pylori* positive test result. Absence T-band suggests that the concentration of

H.pylori antigens in the specimen was below the detectable level, indicating an *H.pylori* negative test result (47).

Stool sample processing wet mount identification technique for direct microscopy: to prepare a wet mount, we obtained a microscope slide and added a drop or two of saline to the slide. Took a small amount of the specimen and place it on a microscope slide. Using a rod or stick, and was emulsified the stool specimen in the saline and was mixed the stool specimen with saline on slide and covered it with cover slip. Then we examined the preparation microscopically using the 10x objective with the condenser iris closed sufficiently to give good contrast. We used 40X objective to examine small cysts and eggs (48).

Stool sample processing Formol ether concentration technique for direct microscopy: using a rod or stick, we emulsified an estimated 1 g (pea-size) of faeces in about 4 ml of 10% formol water contained in a screw-cap bottle or tube. Added a further 3–4 ml of 10% v/v formol water, cap the bottle, and mixed well by shaking. Transferred the suspension to a conical (centrifuge) tube and added 3–4 ml of diethyl ether or ethyl acetate and mixed for 1 minute and it was centrifuge immediately at 750–1 000 g (approx. 3000 rpm) for 1 minute. Using a stick or the stem of a plastic bulb pipette, was loosen the layer of faecal debris from the side of the tube and inverted the tube to discard the ether, faecal debris, and formol water. The sediment was remained. We returned the tube to its upright position and allow the fluid from the side of the tube to drain to the bottom. Tapped the bottom of the tube to resuspend and mix the sediment. We transferred the sediment to a slide, and covered with a cover glass. We examined the preparation microscopically using the 10X objective with the condenser iris closed sufficiently to give good contrast. The 40X objective to examine small cysts and eggs (48).

4.8 QUALITY ASSURANCE AND QUALITY CONTROL

SOP was strictly followed and internal controls materials were included from the test kits in order to perform based on manufacture instructions. The questionnaires prepared was checked by advisors and pretested before the details work was done. Data collectors were trained prior to data collection. In addition, there was a daily follow up by the principal investigator and supervisors.

4.8.1 PRE ANALYTICAL PHASE

Stool sample was collected from the study participants and properly labeled with their identification name or ID or card number. The stool sample was collected by the trained laboratory personnel to collect good quality sample and analyzed it and produced reliable and valid data.

4.8.2 ANALYTICAL PHASE

The test was done by trained laboratory personnel according to SOP of each test methods. The reagent, kits and the method was assessed with known positive and negative control materials, well trained and experienced laboratory professionals were participated in the laboratory analysis procedure.

4.8.3 POST ANALYTICAL PHASE

The results were recommended with patient's identification in order to avoid the error in the result of the test. And results were repeatedly checked before reporting to the ordering physician/health officer or nurse if they needed to order treatment or support for patients.

4.9. DATA MANAGEMENT

Data quality was ensured through use of standardized data collection materials, pretesting of the questionnaires, proper training was given before the start of data collection by the principal investigator. The confidentiality of this data was kept secret and the result was disclosed by code. Cross checking of the results was done for each individual. The health center had the right to get the copy of the findings.

4.10. DATA ANALYSIS AND INTERPRETATION

Data was entered and analyzed using Excel, SPSS (version 20) and the results were expressed using frequency and percentage. Chi square was done to identify variable associated with *H.pylori* infection. Figures and tables were used for data presentation. Association with the prevalence *H.pylori* infection with different variables was done by using X^2 test. In all case 95% confidence interval was used and P-value less than 0.05 were considered statistically significant. CD4 count level was grouped as of those immunecompetent CD4 count greater than 500 cells/$mm^3$and

immunecompromisedCD4 count, less or equal to 500 cells/mm^3 (49). Anemia was defined as hemoglobin level is less than 12g/dl for female and less than 13 g/dl for male (50).

4.11. ETHICAL CONSIDERATIONS

This research project first approved by Addis Ababa University, College of Health Sciences, Department of Medical Laboratory, Departmental Research and Ethics Review Committee and ethically approved by Addis Ababa Health Office, Public Research and Emergency Management Core Process Ethical Committee. Further more privacy and confidentiality was assured for all study participants. The right of any individual not to participate or need to withdraw from the study at any time was fully respected. Data collection from each study subject was started after study participants had been given informed consent. Permission for data collection was also obtained from Kotebe Health Center administration. Results were communicated with physician/health officer/nurses for proper management of the study participants

4.12. DISSEMINATION OF RESULTS

The result of this study was presented to department of medical laboratory sciences and to the scientific community through scientific presentation and finally will be send to duplication on peer reviewed scientific journals. The result of this study finding was communicated to the attending health personnel in the health center.

5. RESULTS

5.1 STUDY PARTICIPANTS

In this study, total of 388 individuals were involved to evaluate *H.pylori* infection and its association with level of CD4 count. Participants with ages greater than 18years were included in the study. The total age range of this variable was 52 and the mean (± SD) age of 37.5±9.06 years. From this 132 (34%) were males and 256 (66%) were females (Table 1).

From the study participants (54.9%) were married from these 49.4% (126/256) were females and 65.9% (87/132) were males, and 18.3% (79/388) study participants were divorced. From the study participants only 4.4% (17/388) were graduated from university, and only 0.5 % (2/388) females were university completed. And most of HIV client those participated in the study (60.1%; 233/388) had their private work. Some of the study participant (23.5%; 91/388) were live with family number greater than or equal to four. Majority of the study participant 95.1% (368/388) are at stage one of ART drug taking from this 94.9% (241/256) were females and 95.5% (126/132) were males (Table 1) (Table 2).

Among all HIV clients in the study participant few males had habit for consumption of alcohol, smoking, chewing of chat. Some of the study participants 33.2% (129/388) had habit for consumption of coffee. Most of them (60.1%; 233/388) had history of gastro intestine, from this 63.5% (162/256) were females and 53.8% (71/132) were males. All of study participant had habit for washing hand before and after meal, and after toilet usage. Majority of them 94.8% (368/388) use pipe water as source for drinking purpose (Table 3) (Table 4).

Two hundred forty one (62.1%; 241/388) of study participants were those their CD4 count was less than 500 from this 56.1 % (143/256) were females and 73.5% (97/132) were males. And 51 (20%; 51/256) females and 17 (12.9%; 17/132) males were anemic, (Hg <12g/dl for female and Hg value < 13g/dl for male). From the study individuals 16.8% (65/388) were positive for intestinal parasite (only Entamoeba histolytica and Giardia lamblia was observed using both direct stool microscopic and formol ether concentration technique) (Table 3) (Table 4).

Table 1:- Socio-demographic characteristics in frequency and percentage of study participants in Kotebe Health Center from October – November 2016. (n=388)

Characteristics	Frequency	Parentage (%)
Sex		
Male	132	34
Female	256	66
Age in group		
19-25	25	6.4
26-35	142	36.6
36-45	155	39.9
46-55	47	12.1
≥56	19	4.5
Marital status		
Married	213	54.9
Single	37	9.5
Divorced	71	18.3
Widowed	67	17.3
Level of education		
Illiterate	82	21.1
primary	165	42.5
Secondary	124	32.0
University comp	17	4.4
Working status		
Government	50	12.9
Non- government	18	4.6
Private	233	60.1
House wife	80	20.6
House made	7	1.8
Number of people in the household		
One	40	10.3
Two	91	23.2
Three	115	29.6
Four	51	13.1
Greater than four	91	23.5
Stage of taking ART drug		
First	367	95.1
Second	8	1.5
Third	2	0.5
Never started taking ART drug	11	2.8

Table 2:- Socio-demographic characteristics of female and male population among the study participants in Kotebe Health Center October – November 2016.

	Female			Male		
Characteristics	Frequency	Percentage (%)(n=256)	Percentae (%)(n=388)	Frequency	Percentage (%)(n=132)	Percentage (%)(n=388)
Age in group						
19-25	15	5.9	3.9	10	7.6	2.6
26-35	101	39.5	26	41	31.1	10.6
36-45	101	39.5	26	54	40.9	14.0
46-55	28	10.9	7.2	19	14.4	4.9
≥56	11	4.3	2.8	8	6.1	2.1
Marital status						
Married	126	49.4	32.6	87	65.9	22.5
Single	16	6.3	4.1	21	15.9	5.4
Divorced	56	22.0	14.5	15	11.4	3.9
Widowed	57	22.4	14.7	9	6.8	2.3
Level of - education						
Illiterate	75	29.4	19.4	7	5.3	1.8
Primary	100	39.2	25.8	64	48.5	16.5
Secondary	78	30.6	20.2	46	34.8	11.9
University-completed	2	0.8	0.5	15	11.4	3.9
Working status						
Government	26	10.2	6.7	24	18.2	6.2
Non-Government	13	5.1	3.4	5	3.8	1.3
private	138	54.1	35.7	94	71.2	24.3
House wife	73	28.6	18.9	7	5.3	1.8
House made	5	2.8	1.3	2	1.5	0.5
Number of people in the household						
One	22	8.7	5.1	18	13.6	4.7
Two	61	24.0	15.8	28	21.2	7.3
Three	85	33.5	22.0	30	22.7	7.8
Four	33	13.0	8.5	24	18.2	6.2
Greater than four	53	20.9	13.7	32	24.2	8.3
Stage of taking ART drug						
First	241	94.9	62.7	126	95.5	32.6
Second	4	1.6	1.0	2	1.5	0.5
Third	1	0.4	0.3	1	0.8	0.3
Never started taking ART drug	8	3.1	2.1	3	2.3	0.8

Table 3:- Behavioral characteristics, hygienic practice, CD4 count level and some variables of the study participants in Kotebe Health Center; From October – November 2016. (n=388)

Characteristics	Frequency	Parentage (%)
Habit for consumption of alcohol		
No	378	97.4
Yes	10	2.6
Habit for smoking of cigarette		
No	376	96.9
Yes	12	3.1
Habit for chewing of chat		
No	387	99.7
Yes	1	0.3
Habit for consumption of coffee		
No	259	66.8
Yes	129	33.2
Experience of Gastro Intestine		
No	155	39.9
Yes	233	60.1
Water source for drinking		
Tanker	4	1.0
Wheel	16	4.1
pipe	368	94.8
CD4 count result		
≤500	241	62.1
>500	147	37.9
Hemoglobin result		
Normal	320	82.5
Anemic	68	17.5
Stool examination result		
Negative	323	83.2
positive	65	16.8

Table 4:- Behavioral characteristics, hygienic practice, CD4 count level and some variables of females and males among study participants conducted in Kotebe Health Center from October – November 2016. (n=388)

	Female			Male		
Characteristics	Frequency	Parentage (%) (n=256)	Parentage (%) (n=388)	Frequency	Parentage (%) (n=132)	Parentage (%) (n=388)
Habit for consumption of alcohol						
No	256	100	66	122	92.4	31.5
Yes	0	0	0	10	7.6	2.5
Habit for smoking of cigarette						
No	256	100	66	120	90.9	31.0
Yes	0	0	0	12	9.1	3.0
Habit for chewing of chat						
No						
Yes	256	100	66	131	99.2	33.9
	0	0	0	1	0.8	0.3
Habit for consumption of coffee						
No	155	60.8	40.1	103	78	26.6
Yes	100	39.2	25.8	29	22	7.4
Experience of Gastro Intestine						
No	93	36.5	24.0	61	46.2	15.7
Yes	162	63.5	41.9	71	53.8	18.3
CD4 count result						
<500	143	56.1	37.1	97	73.5	25.1
>500	112	43.9	28.9	35	26.5	9.0
Hemoglobin result						
Normal	204	80	52.7	115	87.1	29.7
Anemic	51	20	13.3	17	12.9	4.4
Stool examination result						
Negative	215	84.3	55.6	107	81.1	27.6
positive	40	15.7	10.3	25	18.9	6.5

5.2 HELICOBACTER INFECTION

The overall prevalence of *H.pylori* infection among the study participants was 54.8%. *H.pylori* infection among female participant was 51.8% (133/256) and among male participant was 60.6% (80/132) with $X^2 = 2.745^a$ and P-Value 0.098. HIV clients within age group, participants whose age were 46-55 (32/47) had the highest prevalence of *H.pylori* infection ($X^2= 4.836^a$ and P-Value= 0.305). And within educational level, study participants those completed university had the highest prevalence of *H.pylori* infection 65.2% (12/17) (Table 5).

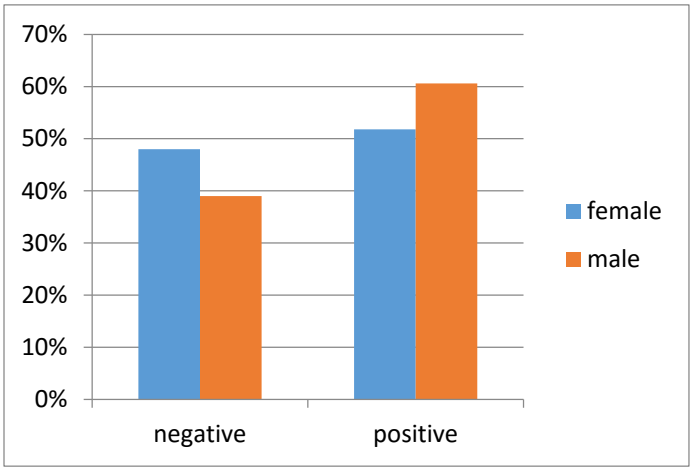

Figure 1:*H.pylori* infection in percentage within sex among male and female population from the study participant in a Kotebe Health Center from October – November 2016. (n=388)

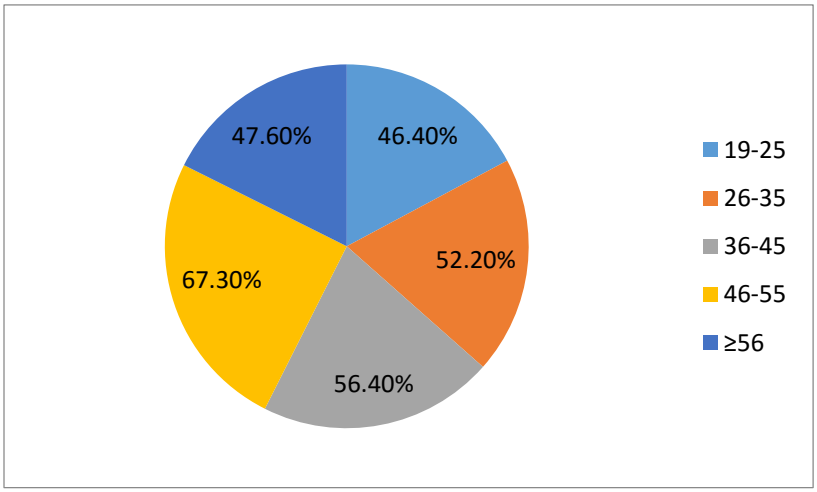

Figure 2: *H.pylori* infection in percentage within age group of the study participant in Kotebe Health Center from October – November 2016. (n=388)

There were no difference between prevalence of *H.pylori* infection and some socio-demographic characteristics like marital status, occupational status of the study subject. But there was increment of *H.pylori* infection as the number of people in the house hold increased (X^2=12.369[a]; P.value= 0.015). The prevalence of *H.pylori* was 67.1% (61/91) among HIV clients with people in the house hold greater than four. Most of the study participants were at stage one for taking ART drug and the prevalence of *H.pylori* infection was 55.4% (204/368) (Table 5).

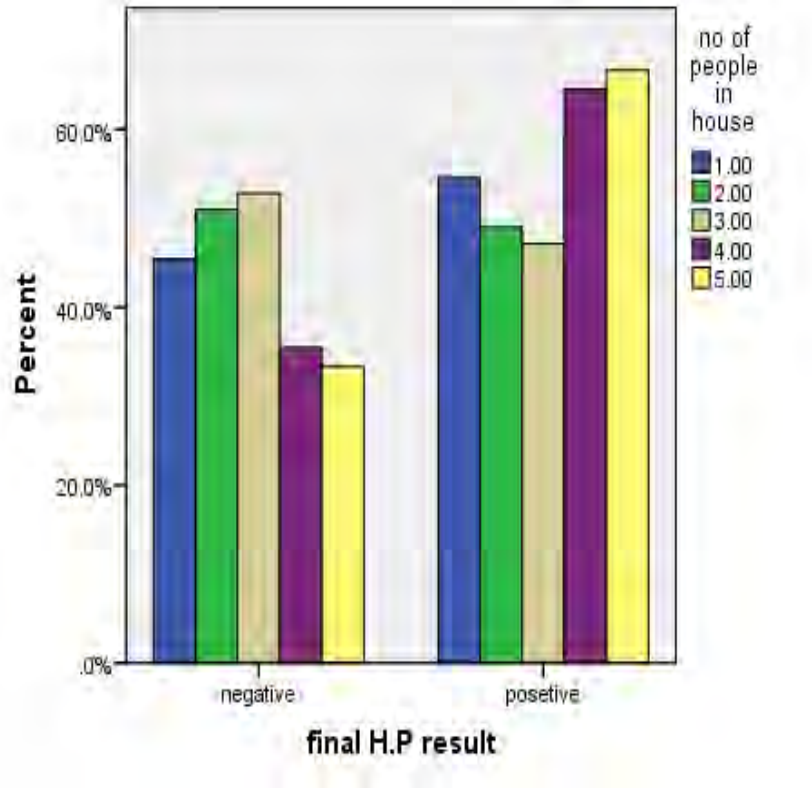

Figure 3: *H.pylori* infection in percentage within number of people living in the house hold among the study participant in a Kotebe Health Center from October – November 2016. (n=388)

Table 5:- *H.pylori* infection and socio-demographic characteristics of the study participant in Kotebe Health Center from October – November 2016. (n=388)

Characteristics	*H.pylori* Stool AntigenTest		X^2	P-Value
	negative	positive		
Sex				
Female	123	133	2.745[a]	0.098
Male	52	80		
Age in group				
19-25	12	13		
26-35	70	72	4.836[a]	0.305
36-45	69	86		
46-55	15	32		
≥56	10	9		
Marital status				
Married	96	117		
Single	16	21	1.237[a]	0.744
Divorced	36	35		
Widowed	28	39		
Level of education				
Illiterate	41	41		
Primary	74	91	2.476[a]	0.48
Secondary	56	68		
University comp	5	12		
Working status				
Government	20	30		
Non-Government	8	10	0.976[a]	0.913
Private	106	127		
House wife	39	41		
House made	3	4		
Number of people in the household				
One	19	21		
Two	48	43	12.369[a]	0.015
Three	61	54		
Four	18	33		
Greater than four	30	61		
Stage of taking ART drug				
First	165	204		
Second	2	4	4.990[a]	0.288
Third	1	1		
Never started taking ART drug	8	3		

There was no significant association between prevalence of *H.pylori* infection and CD4 count level less than or equals to 500 and with CD4 count level greater than 500 the prevalence was 54.4% (131/241) and 55.1% (81/147) respectively (X^2=0.020[a]; p=0.886). Majority of the study participants had no habit of smoking, habit for drinking of alcohol, habit for chewing of chat. But some of them had habit for dinking of coffee. The prevalence of *H.pylori* from HIV clients those had habit for drinking of coffee was 53.5% (69/129). And 60.4% of *H.pylori* infected participant had history of GIT (Table 6).

All of study participants had habit for hand washing before and after meal, and after use of toilet. But some of the study participants use tanker and wheel water as a source for dirking purpose. The prevalence of *H.pylori* infection among HIV clients those use tanker water, wheel water, pipe water was 50% (2/4), 25% (4/16) and 55.9% (206/368) respectively (X^2= 6.114[a] P value=0.047). The prevalence of *H.pylori* infection among anemic HIV client was 44.1% (30/68) and intestinal parasites were identified in 15.5% (33/213) of *H.pylori* infected study participant (Table 6).

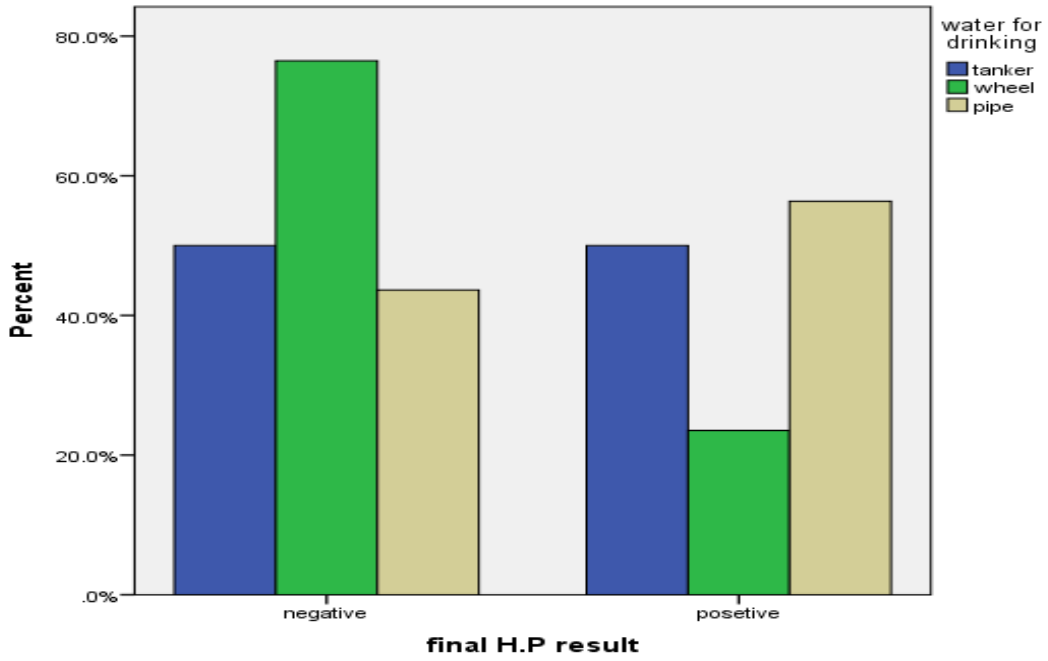

Figure 4: *H.pylori* infection in percentage within source of water for dirking among the study participant in Kotebe Health Center from October – November 2016. (n=388)

Table 6:- *H.pylori* infection and behavioral characteristics, hygienic practice, CD4 count level and with some variables of the study participants in Kotebe Health Center from October – November 2016. (n=388)

Characteristics	*H.pylori* result		X^2	P-value
	negative	positive		
Habit for consumption of alcohol No Yes	171 5	207 5	0.00	1.0
Habit for smoking of cigarette No Yes	171 5	205 7	0.00	1.0
Habit for chewing of chat No Yes	175 1	212 0	0.01	0.926
Habit for consumption of coffee No Yes	116 60	143 69	0.0.45	0.831
History of Gastro Intestine No Yes	71 105	84 128	0.02	0.968
Water source for drinking Tanker Wheel pipe	2 12 162	2 4 206	6.114	0.047
CD4 count result ≤500 >500	110 66	131 81	0.020[a]	0.886
Hemoglobin result Normal Anemic	138 38	182 30	3.683[a]	0.886
Stool examination result Negative positive	144 32	179 33	0.472[a]	0.492

6. DISCUSSION

In this study it is found that the overall prevalence of *H.pylori* was 54.8%, and 40% & 60% was for female and male participants respectively. There was no significant difference in the prevalence of *H.pylori* with level of CD4 count. This finding is different from study conducted in Romania by Nina S et.al 2014, the prevalence of *H.pylori* infection was 11.36%. But they found no statistically significant differences between *H.pylori* infection regarding to CD4+ T lymphocytes level. And this difference might be due to difference in the study method they used (29).

The prevalence of *H.pylori* infection was10% in study conducted in India by Kashyap B et.al, 2016 and study conducted in Nigeria by Joseph AA et.al 2016, the prevalence was 46.8%. There was slight difference in the prevalence of *H.pylori* infection in this study and study conducted at university hospital in Ghana by Stephen SF et.al, 2015, the prevalence was 51.5%, and had reported that *H.pylori* prevalence decreased in parallel with CD4+ T cell counts. And this difference in prevalence might be due to difference in the sample size and socio-demographic data they used (30, 31, 32).

In the study conducted in Imam Khomeini Hospital in Iran by Hossein SK et.al, 2011,*H.pylori* infection was found in 30 patients (69.76%) and there was no significant relationship between CD4+ count and presence of *H.pylori* (P value > 0.05) this finding was the same in this study but not there was difference in the prevalence of *H.pylori* infection with result in this study. This difference might be the sample size and laboratory method, they used PCR, but in this paper only *H.pylori* stool antigen test was applied (33).

The prevalence in *H. pylori* infection was reported to be20.7% in study conducted in a Greek hospital by George Z et.al, 2001. The mean CD4 count was lower for *H. pylori*-negative than *H. pylori*-positive HIV-infected patients, the prevalence was less than the prevalence *H.pylori* infection in this study (54.8%) and there was no significance association in the prevalence of *H.pylori* with CD4 count level in this study. The difference might be because of case control method they used and the laboratory method used (34).

In the study conducted in Kenya by Alimohamed F et.al, 2002, Prevalence of *H.pylori* in HIV seropositive patients was 73.1%, the prevalence of *H. pylori* decreased with decreasing CD4 cell

counts. But in this study there was no significance association between *H.pylori* prevalence and CD4 count level. In the study conducted in St.Paul's General Specialized Hospital, Addis Ababa, Ethiopia by Brhanu T, the prevalence of *H.pylori* was 64.2%. There was no significant difference of *H.pylori* seroprevalence between relatively high and low CD4 cell counts. There was difference in the prevalence of *H.pylori* with result in this paper that might be because of the serological anti-body test they used in the laboratory method (35, 36).

There was difference in prevalence of *H.pylori* infection from study conducted in U.S.A by Z. Feinberg MD et.al, 2011 and by Cacciarelli AG et.al, 1996, which reported as the prevalence of *H.pylori* decrease as CD4 count decrease. The difference might be because of case control method they used and the laboratory method used, they evaluated with upper endoscopy and astral gastric biopsy. In this study only *H.pylori* stool antigen was used (37, 38).

The prevalence of *H.pylori* infection in this study was almost the same as the prevalence of *H.pylori* which is conducted in Italy by Fabris P et.al, 1997, which was 54.8% but they found that the prevalence of *H.pylori* decreases as CD4 count decreases. In the study conducted in Germany Lichterfeld M et.al, 2001, a substantial but insignificant decrease of *H.pylori* infection prevalence was noted in HIV patients with an extensive decline of CD4 cell count. No association between *H.pylori* infection prevalence and patient's age, sex, risk group and type of their antiretroviral treatment. Their finding was different from result in this paper that there was no decreasing or increasing in the prevalence of *H.pylori* with level of CD4 count, and there was a significant association between number of people in the house hold in this study. The difference might be because of case control method they used and the laboratory method (39, 40).

In the study conducted in China by Lv FJ et.al, 2007; the prevalence of *H.pylori* was 22.1% and low prevalence of *H.pylori* has been reported in Taiwan by Chiu HM, 2004. But in this paper the prevalence was 54.8% and this difference may be because of the sample size and the laboratory method they used, study participants were examined by upper endoscopy and biopsy. But in this study only *H.pylori* stool antigen test was used (41, 42).

The prevalence of *H. pylori* was 42 (41.1%), in the study conducted in Argentina by Martin Olmos et.al, 2004,. And they found that HIV-infected patients with *H.pylori* had a higher mean CD4 count than HIV-infected individuals without *H.pylori*. But in this study there were no difference between

H.pylori prevalence and CD4 count level. And this difference might be due to difference in the sample size, socio-demographic data and laboratory method (43).

Lower prevalence of *H.pylori* was observed as CD4 count level lower in the study conducted in Northeastern Brazil by Andréa BC, et.al, 2011, and the prevalence of *H.pylori* infection was 37.2%. There were no significant differences between *H.pylori* status and gender, age, HIV viral load, antiretroviral therapy; *H.pylori* infection was significantly associated with chronic active gastritis. But in this study there were no significant association in the prevalence of *H.pylori* and CD4 count level but there is a significant difference in the prevalence of *H.pylori* infected people living in the house hold. The difference may be due to sample size, socio- demographic variables and laboratory methods, the former use urease test and histology, but in this study only *H.pylori* stool antigen test was used (44).

7. STRENGTH AND LIMITATION OF THE STUDY

7.1 LIMITATION OF THE STUDY

- There might be some bias because of the convenient sampling technique that is used in this study.
- Another limitation was the determination of *H.pylori* was done only with one method (*H.pylori* stool antigen test), even if HPSA kit employed in this study had a sensitivity 98.8%, specificity 100%, and accuracy 98.9%. (User leaflet of kit)

7.2 STRENGTH OF THE STUDY

- This study attempts to indicate the burden of *H.pylori* infection and its association with CD4 cell count and with different variables among HIV client in this study situation.
- The determination of *H.pylori* infection in this study was using *H.pylori* stool antigen test kits which are more sensitive and more specific for current identification of *H.pylori* infection compared to *H.pylori* anti-body test kits that might indicate past or current infection.

8. CONCLUSION AND RECOMMENDATION

8.1 CONCLUSION

- This study showed remarkable prevalence of *H.pylori* infection among HIV clients in the study area and there were no significant association between prevalence of *H.pylori* infection and level of CD4 count. There was significant association between *H.pylori* infection and number of people living in the house holdand also with type of water used for drinking. There were statistically no significant association with *H.pylori* infection and other socio-demographic characteristics and some variables like smoking habit, alcohol drinking habit, habit for drinking coffee, chat chewing habit, habit for hand washing and previous history of GIT. Also Hg value (anemia) and intestinal parasite showed statistically no significant association with *H.pylori* infection in this study.

8.2 RECOMMENDATION

- The health facility and other responsible body must be conscious in creating awareness on the possible transmission of the disease including people in the house hold and water used for drinking. Even if majority of the study participants used tap water for drinking purpose, it is advised that it needs to be treated.
- Further studies are required in this health facility and in this study area using different diagnostic method to explain the actual role of *H.pylori* in causing infection and its impact in the CD4 count and other immune system.
- We recommend that large scale study could be conducted in order to further explore the interaction of *H.pylori* infection and its association with CD4 count level among HIV clients.

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ANNEXES

ANNEX I. PARTICIPANT INFORMATION SHEET ENGLISH VERSION

Participant information sheet

Department of medical laboratory school, college of health science Addis Ababa University, Addis Ababa, Ethiopia.

Title –*H.pylori* infection and its association with CD4 count among HIV infected individuals who attend the ART service in Kotebe Health Center, Yeka Sub City, Addis Ababa.

Introduction – This information sheet and consent form is prepared by the principal investigator to clarify the study that you are asked to take part. If there is any that is unclear before you decide to participate or not you can ask freely.

Purpose- the main purpose of this study is on the determine the burden *H.pylori* and its association with CD4 count among HIV infected individuals who attend the ART service in Kotebe Health Center, Yeka Sub City, Addis Ababa. This finding will help us to determine the burden *H.pylori* and its association with CD4 count among HIV infected individuals who attend the ART service in Kotebe Health Center, Yeka Sub City, Addis Ababa

The aim of the study- the objective of the study determine the burden *H.pylori* and its association with CD4 count among HIV infected individuals who attend the ART service in Kotebe Health Center, Yeka Sub City, Addis Ababa.

Procedure for sample collection –

Stool sample will be collected in a clean dry clean stool cup. A small amount of fresh stool samples will be collected strictly following SOP with sterile stool cup. Proper stool specimen will be taken from each individual to reduce the occurrence chance of false negative and excess stool sample may lead to an invalid test result. Following collection from each individual, the specimen is transported by placing each a separate sterile stool cup to the laboratory within 30 minutes. The sample will be used for research purpose after the customer willing to participate the study will be

confirmed in their signature and also the result of CD4 and Hg value will be taken from your laboratory request paper. The consent agreement will be made by the physician/health officer/nurse at ART clinic.

Benefit of the study participant- Study participant will not have any financial incentive and also other documents from participants on their study. However their results will be given by the physician/health officer/nurse and will be treated based on *H.pylori* stool antigen test result.

Risk and complication-There will not be risk to the study subject, except little pain when drawing of blood sample, in the participating in the study.

Confidentiality- In order to maintain the confidentiality of participant's information, the name will not be given and the sample will be coded. Participants will not be prohibited from to stop or withdraw at any time of the study. Only interested participant will be retrieve their own laboratory result using their code number. The physician/health officer/nurse is responsible for the interpretation of the results and providing them treatment.

Investigator contact address

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ANNEX II. PARTICIPANT INFORMATION SHEET AMHARIC VERSION

<u>በጥናቱ ላይ ተሳታፊዎች ለሚሆኑ የመረጃ ቅፅ</u>

አዲስ አበባ ዩኒቨርስቲ የጤና ሳያንስ ኮሌጅ ሜዲካል ላቦራቶሪ ክፍል አዲስ አበባ ኢትዮጵያ

<u>አርስት፡-</u>በአዲስ አበባ ከተማ በየካ ክፍለ ከተማ ኮተቤ ጤና ጣቢያ ውስጥ የሚገኙ የHIV ታካሚዎች ሆነው የART ክሊኒክ ተገልጋዮች ላይ የ*H.pylori* ኢንፌክሽን *H.pylori* ከCD4 ጋር ያለውን ግንኙነት ለማወቅ የሚረዳ ጥናት

<u>አጠቃላ ይመረጃ፡-</u>ይህ የመረጃ ቅፅ እና የስምምነት ውል ቅፅ የተዘጋጀው በዳታ ኮሌክተር አማካኝነት ሲሆን የዚህጥናት ተሳታፊዎች በመሆናችሁ ስለጣናቱ ግልፅ የሆነ መረጃ እንዲኖራችሁ ስለተፈለገ ነው። ማንኛውም ያልገባችሁ ወይም ግልፅ ያልሆነ ነገር ካለ ከመስማማታችሁ በፊት መጠየቅ ትችላላችሁ።

<u>ስለጥናቱመረጃ፡-</u>ዋናው የጥናቱ ትኩረት የሆነው የ*H.pylori* ኢንፌክሽን የጨጓራ ባክቴሪያ በኮተቤ ጤና ጣቢያ የሚገኙ የHIV ታካሚዎች ላይ በምን ያህሉ ላይ እንደሚገኝና *H.pylori* (የጨጓራባክቴሪያ) ከCD4 መጠን ጋር ያለውን ግንኙነት ለማወቅ የሚረዳ ሲሆን፤ይህም ጥናት የጨጓ ራባክቴሪያ (*H.pylori*) በኮተቤ ጤና ጣቢያ በሚገኙት የHIV ታካሚዎች በምን ያህል መጠን እንደሚገኝ ለማወቅ ይረዳል።

<u>የጥናቱ አላማ፡-</u>በአዲስ አበባ ከተማ በየካ ክፍለ ከተማ ኮተቤ ጤና ጣቢያ ውስጥ የሚገኙ የHIV ታካሚዎች ሆነው የART ክሊኒክ ተገልጋዮች ላይ የ*H.pylori* ኢንፌክሽን እና *H.pylori* ከCD4 ጋር ያለውን ግንኙነት ለማወቅ የሚረዳ ጥናት

<u>ጥናቱ ለተሳታፊውያለውጥቅምናጉዳት</u>፡-በጥናቱ ላይ ተሳታፊ የሚሆነው ሰው ማንኛውንም አይነት የገንዘብም ሆነ በጥናቱላይ የሚካተቱ መረጃዎችና ቅፆችን የማያገኙ ቢሆንም ግን ለናሙና የሰጡትን ውጤታቸውን በሐኪም፣ በነርስ ወይም በጤና መኮንን አማካኝነት ውጤቱን ማወቅ እንደሚችሉ እና የ*H.pylori* የጨጓራ ባክቴሪያ ካለቦት መድኃኒት ይታዘዝሎታል።

<u>በጥናቱ ተሳትፊዎች ላይ ያለው ጉዳት እና ተዛማጅ ችግር</u>፡- በጥናቱ ተሳታፊ በመሆንዎ ምንም አይነት ችግር አይገጥምዎትም።

<u>ሚስጥ ርአጠባበቅ፡-</u>በዚህ ጥናት ተሳታፊ በመሆናችሁ ምክንያት ማናኛውንም አይነት እናንተን የሚገልፅ መረጃ እንደማይኖር እና ናሙና በምንሰጥበትም ወቅት ናሙናውን መስጫ ዕቃው በመለያ ቁጥር ይለያል፡፡ ተሳታፊዎች በጥናቱ ጊዜ ማንኛውም ሰዓት ተሳትፎአቸውን ማቋረጥ ይችላሉ፡፡ ጥናቱ ውስጥ ተሳትፊዎች ለሚሆኑ ግን የላቦራቶሪ ውጤታቸውን በመለያ ቁጥራቸው አማካኝነት በሐኪም፣ በነርስ ወይም በጤና መኮንን አማካኝነት መውሰድ ይችላሉ፡፡ ሐኪም፣ ነርስ ወይንም ጤና መኮንኖች ውጤቱን አይተው መድኃኒት የማዘዝ ኃላፊነት ይኖርባቸዋል፡፡

ማንኛውንም ጥያቄ ለመጠየቅ ለምትፈልጉ የሚከተለውን አድራሻ መጠቀም ትችላላችሁ፡፡

ስም- <u>ኤደንአባቡ</u>

ስልክቁጥር- 0911808107

ኢሜል- haniababu710@gmail.com

ANNEX III. ENGLISH VERSION OF INFORMED CONSENT

I have been informed about the objective of the study entitled "*H.pylori*infection and its association with CD4 count among HIV infected individuals who attend the ART service in Kotebe Health Center, Yeka Sub City, Addis Ababa" and I am also informed that all information contained within the questionnaires to be kept confidential. And also I have been well informed of my right to refuse at any time and refuse to cooperate and drop out of the study. None of my action will have any bearing at all on my overall health care. Therefore, with full understanding of the situation, I agree to give the entire necessary information and stool sample for laboratory analysis. I will have the opportunity to ask questions about the project and to receive clarification to my situation in language I understand. I am informed that the result for stool sample will be given to the health facility and I may ask the information at any time as I want.

I_____ hereby give my consent for giving of the requested information and specimen for this study.

Participant code_____

Signature _____

Date _____

ANNEX IV. AMHARIC VERSION INFORMED CONSENT

<u>በአማርኛ የተዘጋጀ የስምምነት ውል</u>

በአዲስ አበባ ከተማ በየካ ክፍለ ከተማ ኮተቤ ጤና ጣቢያ ውስጥ የሚገኙ የHIV ታካሚዎች የሆኑት የART ክሊኒክ ተገልጋዮች ላይ የ*H.pylori* ኢንፌክሽን ለማወቅ እና *H.pylori* የጨጓራ ባክቴሪያ ከCD4 መጠን ጋር ያለውን ግንኙነት ለማወቅ የሚረዳ ጥናት ላይ በቂ የሆነ ግንዛቤ አግኝቻለሁ፡፡ እንደገናም ደግሞ ማንኛውም በመረጃ ቅፅ ላይ ያለውን መረጃዎች በሙሉ በሚስጥር እንደሚያዝ ተነግሮኛል፡፡ ከዚህም በላይ በጥናቱ በማንኛውም ሰዓት ላይ ተሳትፎዬን ማቋረጥ እንደምችል ተነግሮኛል፡፡ ይህንንም በማድረጌ በሌላው የህክምና አገልግሎት ላይ ምንም አይነት ችግር እንደማይገጥመኝ ተነግሮኛል፡፡ የምፈልገውንም ጥያቄ መጠየቅ እንደምችል እና በሚገባኝ ቋንቋ ገለፃም እንደሚደረግልኝ ተነግሮኛል፡፡ ለላቦራቶሪ ምርመራ የሰጠሁትን የሰገራ ውጤትም በጤና ጣቢያ ባለሞያ አማካኝነት በማንኛውም ጊዜ መውሰድ እንደምችል ተነገሮኛል፡፡ ስለዚህም ይህንን ሁሉ ግንዛቤ ውስጥ በመክተት መረጃ በመስጠትና የሚጠበቅብኝን የሰገራ ናሙና ለመስጠት ፍቃደኛነኝ፡፡

እኔ_____ የተባልኩት በጥናቱ ውስጥ የሚያስፈልገውን ማንኛውንም አይነት መረጃ እና የሰገራ ናሙና ለመስጠት ፍቃደኛ መሆኔን እገልፃለሁ፡፡

የተሳታፊው የመለያ ቁጥር _____

ፊርማ _____

ቀን _____

ANNEX V. ENGLISH VERSION OF PREPARED QUESTIONNAIRES

Prepared Questionnaires to be asked for the study participant

To determine of *H.pylori* infection and its association with CD4 cunt level among HIV clients visiting a selected health center in Kotebe Health center, Yeka sub City, A.A 2016.

Facility name _____

Participant code_____

Participant address_____ Sub city_____ Telephone No_____

Data collector name _____ Date_____ Signature_____

I. Socio demographic information (tick) for the study participant		
1	Sex(gender)	1. Female 2. Male
2	Age (in years)	
3	Marital status (circle one)	1. Single 3. Divorced 2. Married 4. Widowed
4	What is your level of education (circle one)	1. Illiterate 3. Secondary school 2. Primary school 4. University completed
5	What is your occupational status (circle one)	1. Government 3. private 2. Non government 4. Hose wife 5. House made
6	Number of people in the house hold(circle one)	1. Two 3. Four 2. Three 4. > Four
7	Stage of ART(circle one)	1. First 2. Second 3. Third 4. Forth 5. I didn't start taking ART drug
II. Associated risk assessment for *H.pylori* infection		
8	Do you have experience for habit of the consumption of alcohol?	1. Yes 2. No
9	Do you have experience for habit of smoking?	1. Yes 2. No

10	Do you have experience for habit of chewing chat?	1. Yes 2. No
11	Do you have experience for habit of consumption of tea and coffee?	1. Yes 2. No
12	Did you have possible history of Gastrointestinal?	1. Yes 2. No
	III. Some hygienic application	
13	Washing of hand before and after meal?	1. Yes 2. No
14	Washing of hand after toilet?	1. Yes 2. No
15	Water for drinking	1. Tanker water 2. Wheel water 3. Pipe water
	Final *H.pylori* test result	1. Positive 2. Negative
	Current CD4 count	
	Hg in gm/ dl	
	Stool microscopy result	

ANNEX VI. AMHARIC VERSION OF PREPARED QUESTIONNAIRES

<u>በአማርኛየተዘጋጀመጠይቅ</u>

በአዲስ አበባ ከተማ በየካ ክፍለ ከተማ በኮተቤ ጤና ጣቢያ ውስጥ የሚገለገሉት ART ተጠቅሚዎች የሆኑት HIV ታካሚዎች ላይ የ*H.pylori* የጨጓራ ባክቴሪያ ኢንፌክሽን እና *H.pylori* ከCD4 መጠን ጋር ያለውን ተዛማጅነት ለማወቅ የሚረዳ ጥናት

የጤና ጣቢያ ውስም _____

በጥናቱ ላይ ሚሳተፈው መለያ ቁጥር _____

የተሳታፊው አድራሻ _____ ክ/ከተማ _____ የስልክ ቁጥር _____

መረጃውን የሚሰበስበው ሰው ስም_____ ቀን _____ ፊርማ _____

ተ.ቁ	1. የተሳታፊው የግል መረጃ (ከቀረቡት ምርጫዎች አንዱን ያክብቡ)	
1	ጸታ	1. ሴት 2. ወንድ
2	ዕድሜ (በዓመት)	
3	የጋብቻሁኔታ	1.ያገባ/ች 3.የተፋታ/ች 2.ያላገባ/ች 4.የሞተችበት/ባት
4	የትምህርደረጃ	1.ያልተማረ/ች 3.ሁለተኛደረጃ 2.አንደኛደረጃ 4.ዩኒቨርስቲየጨረሰ/ች
5	የስራ ሁኔታ	1.የመንግስት 3.የግልስራ 2.መንግስታዊያልሆነ 4.የቤትእመቤት 5.የቤትሰራተኛ
6	በቤት ውስጥ ያሉ ሰዎች ብዛት	1.ሁለት 3.አራት 2.ሶስት 4.ከአራትበላይ
7	የART መድኃኒት የሚወስድበት የክትትል ደረጃ	1.1ኛ 2.2ተኛ 3.3ተኛ 4.4ተኛ 5. የART መድኃኒት መውሰድ አልጀመርኩም

H.pylori የጨጓራ ባክቴሪያ ሊያስከትሉ የሚችሉ ተያያዥነት ያላቸው ነገሮች			
8	የአልኮል ሱስ አለቦት ወይ	1.አዎ	2.አይ
9	የሲጋራ ሱስ አለቦት ወይ	1.አዎ	2.አይ
10	የጫት ሱስ አለቦት ወይ	1.አዎ	2.አይ
11	የቡና ሱስ አለቦት ወይ	1.አዎ	2.አይ
12	ከዚህ በፊት የጨጓራ ህመም ታመው ያውቃሉ ወይ	1.አዎ	2.አይ
የግልንፅህናንአጠባበቅልምድ			
13	ከምግብ በፊትና በኃላ እጆትን በሳሙናና በውሃ ይታጠባሉ ወይ	1.አዎ	2.አይ
14	ከሽንት ቤት በኃላ እጆትን በሳሙናና በውሃ ይታጠባሉ ወይ	1.አዎ	2.አይ
15	ለመጠጥ የሚጠቀሙት ውሃ	1.የታንከርውሃ 2.የጉድጓድውሃ 3.የቧንቧውሃ	
	የ*H.pylori* ውጤት	1.አለበት(ፖዝቲቭ) 2.የለበትም(ኔጌቲቭ)	
	የአሁኑየCD4 ውጤት		
	የHg ውጤት በ g/dl		
	የማይክሮስኮፕ የሰገራምርመራ ውጤት		

ስለትብብሮ እና መሰማናለን

ANNEX VII. LABORATORY SOP FOR *H.PYLORI* STOOL ANTIGEN TEST

1. Purpose

For proper preparation, detection, identification of *H.pylori*. ***Helicobacter pylori*** (***H. pylori***), previously named ***Campylobacter pyloridis***, is a Gram-negative, microaerophilicbacterium found in the stomach

2. Abbreviations

H. pylori = helicobacter pylori

IgM : Immuno globulin M.

IgG : Immuno globulin G.

3.Material

Reagents
3.1Device
3.2 Desiccant pouch
3.3 Sample dropper
3.4 Specimen collection tubes, each with 1.5ml buffer solution

Supplies
3.5 Timer
3.6 Disposable gloves
3.7 Clean stool containers
3.8 Control materials

4. Sample

Sample type	Amount required	Transport and Storage	Stability
Human stool	*Upto cover the threaded portion of the sampling stick*	4^0c-30^0c	In case of delay in testing, sample may be stored at 2-8 for maximum up to three days

5. Safety Precautions

5.1 This package insert must be read completely before performing the test. Failure to follow the insert gives inaccurate test results

5.2 Do not open the sealed pouch, unless ready to conduct the assay.

5.3 Do not use expired devices.

5.4 Reagents must be kept to room temperature (15°C-30°C) before use.

5.5. Do not use the components in any other type of test kit as a substitute for the components in this kit.

5.6. Do not use specimen more than one day uless its kept at 2-8^0c

5.7. Wear protective clothing and disposable gloves while handling the kit reagents and clinical specimens. Wash hands thoroughly after performing the test.

5.8. Users of this test should follow the US CDC Universal Precautions for prevention contaminated to stool sample.

5.9. Do not smoke, drink, or eat in areas where specimens or kit reagents are being handled.

5.10. Dispose of all specimens and materials used to perform the test as bio hazardous waste.

5.11. Handle the Negative and Positive Control in the same manner as patient specimens.

5.12. The testing results should be read within 15 minutes after a specimen is applied to the sample well or sample pad of the device. Read result after 15 minutes may give erroneous results.

5.13. Do not perform the test in a room with strong air flow, ie. an electric fan or strong air-conditioning.

6. Quality Control

Using individual OnSite *H. Pylori* AgRapid Test strip as described in the Assay Procedure below, run 1 Positive Control and 1 Negative Control (provided upon request) under the

Negative Control

Only the C band shows color development. The T band shows no color development.

Positive Control

Both C and T bands show color development. The appearance of any burgundy color in the T band, regardless of intensity, must be considered as presence of the band.

7. Procedure

Step	Action

7.1	Collect stool sample by using the sample collection tube provided. First, unscrew the cap of the sample collection tube; take out of the sampling stick.
7.2	Inset the sampling stick into stool sample at 6 different sites. Remove excess from the stick by gently wiping the absorbent tissue.
7.3	Put the sampling stick to the sample collection tube and screw tightly, mix well.
7.4	Remove the test device from the foil pouch by tearing at the notch and place it at level place.
7.5	Holding the sample collector upright, carefully break off the tip of the collector at the break point.
7.6	Squeeze 3 drops (about 80ul) of sample solution to the sample well, as in the illustration.
7.7	Wait for 15 minute and read results, do not read results after 30 minute. *Don't read result after 15 minutes. To avoid confusion, discard the test device after result is reported*

8. Result Interpretation

8.1 NEGATIVE RESULT:

If only the C band is developed, the test indicates that no detectable antigens to *H. Pylori* are present in the specimen. The result is negative.

8.2 POSITIVE RESULT:

If both C and T bands are developed, the test indicates for the presence of antigens to *H. Pylori* in the specimen. The result is positive.Samples with positive results should be confirmed with alternative testing method(s) and clinical findings before a positive determination is made.

8.3 INVALID:

If no C band is developed, the assay is invalid regardless of color development on the T band as indicated below. Repeat the assay with a new device. Error and/or that the test reagent has deteriorated. The test should be repeated using a new strip.

9. Principle

The *H.pylori* antibody is immobilized in the test line region of the device and the *H.pylori* antigen is in human fecal sample. When an adequate volume of test specimen is applied into the sample pad of the strip, the specimen migrates by capillary action across the strip, mixes with the antibody-dye conjugate and floes cross the pre-coated membrane. When the antigens to *H.pylori* levels are at or above the target cutoff (the detection limit of the test) *H.pylori* antigen in the specimen binds to the antibody- dye conjugate and are captured by antibody immobilized in the test region (T) of the device. This procedure a colored Test band and indicates a positive result. When the *H.pylori* antigens levels of specimen are zero or below the target cutoff, there is not a visible colored band in the test region (T) of the device. This indicates negative result. The test contains an internal control (C band) which should exhibit colored band in the control region (C). Otherwise, the test result is invalid and the specimen must be retested with another device.

10. Clinical Utility

10.1 Gastrointestinal diseases included non-ulcer dyspepsia, duodenal and gastric ulcer and active, chronic gastritis.

10.2. The prevalence of *H.pylori*infection could exceed 90% in patients with signs and Symptoms of gastrointestinal diseases. Recent studies indicate an association of *H.Pylori* infection with stomach cancer.

10.3. *H.Pylori* colonizing in the gastrointestinal system elicits specific antibody responses which aids in the diagnosis of *H. Pylori*infection and in monitoring the prognosis of the treatment of

10.4. *H. Pylori* related diseases. Antibiotics in combination with bismuth compounds

have been shown to be effective in treating active *H. Pylori*infection

10.5. *H. pylori* is associated with clinical improvement in patients with gsstro intestinal diseases providing a further evidence.

10.6. The OnSite H. Pylori Ag Rapid Test is a latest generation of chromatographic immunoassay which utilizes a combination of *H.pylori* antibodies coated particles and anti-mouse IgG to qualitatively and selectively detect to *H.pylori* anti-gens in human feces.

ANNEX VIII. LABORATORY SOP FOR DIRECT SMEAR MICROSCOPIC EXAMINATION

Purpose

This procedure provides instruction on how to perform direct smear microscopic examination for parasite identification.

Equipment Requirements:

- Glass slides
- Cover slip (20 x 20 mm)
- Wooden applicator
- Grease pencil.
- Microscope.

Reagents & Stain Requirements:

- Normal Saline (0.9% Sodium chloride solution)
- Diethyl ether or ethyl acetate
- Formol water

How to prepare 0.9% Sodium chloride solution –Normal Saline

Sodium chloride NaCl …… 9 g

Distilled water……1000 ml

How to prepare, Formol water

Formol water, 10% v/v, Prepared by mixing 50 ml of strong formaldehyde solution with 450 ml of distilled or filtered rain water.

Specimen:

Fresh stool sample in small amount is required. Stool sample is transported to the test site within 30min.

Procedure

Always first examine the stool sample macroscopically (with your eyes). Note the colour, consistency and look for mucus, blood stains and worms.
For wet mount • Label a glass slide with the patient name and/or lab number. • Put one drop of Normal Saline (sediment from frmol-ether concentrations procedure) in the middle of the left half of the slide. • You can examine the slide using Normal Saline only but you would not been able to see cysts well. Therefore it is advisable to examine each stool sample with Lugol's iodine and Normal Saline.
Take a small piece of stool with the wooden applicator. (About pea- size). If the stool is formed take the piece from inside and the surface of the sample. If the stool is liquid take a drop. *N.B. If the specimen is very liquid place one or two drops of stool directly onto the slide and cover it with the cover glass, do not add the saline as this would further dilute the specimen.*
Mix the sample first with the drop of Normal Saline on the left half of the slide. If the stool contains mucus or bloodstained parts, prepare a second slide with a drop of Normal Saline and take the piece from the mucus or blood-stained part. *N.B. The Iodine preparation is useful to identify cysts but kills all living organisms in the specimen. 0.5 % Eosin solution helps in the search for trophozoites of protozoa.*

Place a cover slip over each drop.

Put the cover slip slowly letting it move down from the side to avoid air bubbles.

Examine the entire cover slip systematically.

For the saline preparation use the 10x objective and the 40x objective.

N.B. When searching for Cryposporidium and Cyclospora cayetanensis it is better to prepare a modified Ziehl Neelsen stained smear.

Formol Ether stool concentration technique

- Using a rod or stick emulsify an estimated 1 g (pea-size) of faeces in about 4 ml of 10% formol water in a screw-cap bottle or tube.
- Adde a further 3–4 ml of 10% v/v formol water, cap the bottle, and mix well by shaking. Transfere the suspension to a conical (centrifuge) tube
- And add 3–4 ml of diethyl ether or ethyl acetate and mix for 1 minute and centrifuge immediately at 750–1 000 g (approx. 3000 rpm) for 1 minute.
- Using a stick or the stem of a plastic bulb pipette, loose the layer of faecal debris from the side of the tube and inverte the tube to discard the ether, faecal debris, and formol water. The sediment will remain.
- Return the tube to its upright position and allow the fluid from the side of the tube to drain to the bottom.
- Tap the bottom of the tube to re suspend, and mix the sediment.
- Transfer the sediment to a slide and cover it with a cover glass. Examine the preparation microscopically using the 10X objective with the condenser iris closed sufficiently to give good contrast. The 40X objective to examine small cysts and eggs.

Reporting and Interpretation of Results:

Macroscopic examination:

- Always report the colour of the stool sample: The colour of the stool changes with dietary intake. Normal stool in adults is brown or light brown. In infants it is yellow or curd-like.
- Always report the consistency of the stool sample: Formed, semi-formed, soft or watery.
- Always report the visible presence of blood, mucus or parasites. Look for adult worms of Ascaris lumbricoides or Trichuris trichuria, and segments of Taenia species.

Microscopic examination:

- Report the name of the parasite found and the quantity (few, some, many).
- Also report the quantity of white blood cells and red blood cells if found.

Internal Quality Control Procedures and Sources of Error:

- Follow proper collection procedures to ensure accurate diagnosis, e.g. Amobic trophozoites begin to degenerate within 1-2 hours after collection.
- Cystes, flagelates and eggs also undergo changes especially if the stool is left at high temperatures.
- Properly label the specimen with patient name and lab number to avoid confusion.
- Only accept fresh specimens and refuse specimens contaminated with dirt or urine.
- If you cannot examine specimens immediately, leave them in a cool place and not exposed to sun.
- Always examine watery and blood-stained specimens first.
- Store Lugol's iodine in brown bottles. Prepare Lugol's iodine fresh every two weeks.
- Never use tincture iodine as it contains alcohol and it would destroy amoeba trophozoites
- When preparing the smears, select portions of the stool that are coated with blood or mucus.
- Keep prepared slides in a wet chamber to prevent them from drying up.
- Do not touch the stool or the smear with your bare fingers. Stool may contain infectious material, health hazard!
- Refer to pictures and charts if you are in doubt about structures that resemble eggs, cysts or trophozoites.

- If in doubt preserve the stool in 10% formalin for examination by a visiting export or for referral of the specimen.

Principle of the Test Method:

Many parasites cause disease in man. Some of these parasites are excreted in stool; they are called intestinal parasites. Intestinal parasites can be identified by examination of fresh stool samples. In stool samples we can find worms (eg. Ascaris lumbricoides) and segments of worms (e.g. Taenia species) are visible to the eye. By microscopic examination of fresh stool samples, we can find eggs (e.g. Hookworm) and larvae of worms (e.g. Strongyloides stercoralis). We also find protozoa trophozoites (e.g. Amoeba) and cysts (e.g. Cyclospora cayetanensis). In heavy and moderate infection, a direct smear examination with normal saline and/or iodine to stain cysts, is usually sufficient. For light infections, a concentration of the stool sample might be required to find helminth (worm) eggs and protozoa by microscopic examination.

Clinical Significance of the Test:

Many pathogenic parasites are excreted in stool. Often, when a person is infected with intestinal parasites, other symptom such as anaemia, eosinophilia, diarrhoea and malabsorption are also present. However diagnosis by physical examination is not sufficient to identify intestinal parasitic infection. Stool examination is essential to identify parasites that cause the disease.

DECLARATION

I, the under signed, declare that this MSc thesis is my original work and it has not been presented for a degree in any other University. All source of materials used for this thesis and institution who gave support have been duly acknowledged.

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