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Association of *Helicobacter pylori* infection and hyperemesis gravidarum women: A case control study in selected Hospital and two health centers in Kirkos Sub-city, Addis Ababa, Ethiopia

Investigator: Yilikal Assefa

Advisor: Kassu Desta (BSc, Msc,PhD fellow,Assistant Professor)

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As thesis advisor, I hereby certify that I have read and evaluated this thesis prepared under my guidance, by Yilikal Assefa; entitled: 'case control study on the association of *Helicobacter pylori* infection and Hyperemesis gravidarum in pregnant women attending a selected Hospital and health Centres, Kirkos Sub city, Addis Ababa,Ethiopia'.

Yilikal Assefa _____ _____

Principal investigator Signature Date

I recommend it to be submitted as fulfilling the thesis requirement.

Kassu Desta (MSc, PhD fellow) _____ _____

Advisor Signature Date

As members of the Board of Examiners of the M.Sc. Thesis Open Defense Examination, we certify that we have read and evaluated the thesis prepared by Yilikal Assefa and examined the candidate. We recommended that the thesis be accepted as fulfilling the Thesis requirement for the degree of Master of Science in Clinical Laboratory Sciences (Diagnostic and Public Health Microbiology specialty).

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Chairperson Signature Date

_____ _____ _____

Internal Examiner Signature Date

_____ _____ _____

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Abbreviation

Ab	Antibody
Ag	Antigen
ANC	Antenatal care
ELISA	Enzyme linked immunoserbent assay
EQA	External Quality Assurance
FGR	Fetal growth restriction
GI	Gastrointestinal
Hb	Hemoglobin
HCl	Hydrochloric acid
HG	Hyperemesis gravidarum
HGM	Hyperemesis gravidarum mother
HP	Helicobacter pylori
HPSA	Helicobacter pylori Stool Antigen test
IDA	Iron deficiency anemia
IgE	Immunoglobulin E
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IQC	Internal Quality Control
DMLS	Department of medical laboratory Sciences
SOP	Standard operational procedures

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Operational definitions

- **Anemia:** Is a decrease in the RBC count, Hgb and/or HCT values.
- **Helicobacter pylori stool antigen test (HPSA):** Is a lateral flow chromatographic immunoassay for the qualitative detection of *H.pylori* antigen in human faecal specimen.
- **Hemoglobin (Hb) determination:** Is the measurement of concentration of hemoglobin in red cells (whole blood)
- **Hyperemesis Gravidarum (HG)**: Characterized by severe and prolonged vomiting that often results in dehydration in pregnant women.
- **Pregnancy (or gestation)**: Is the development of one or more offspring, known as an embryo or fetus, in the uterus of a woman.
- **Prevalence:** Is a measurement of all individuals affected by the disease at a particular time.
- **Trimester**: One of the three-month periods into which a pregnancy is divided.
- **Cases**: Patients who have a disease or outcome of interest (pregnant women with hyperemesis gravidarum).
- **Controls:** Patients who do not have the disease or outcome (pregnant women without hyperemesis gravidarum)

Abstract

Background: Nausea and vomiting is a common and unpleasant problem during pregnancy with a frequency of 75% to 80% of pregnancies. In some women nausea and vomiting is very severe and does not respond to simple diet manipulation and antiemetic agents. Hyperemesis gravidarum (HG) is one of the many problems during pregnancy; its etiology has not been clearly understood. Inflammatory factors like *Helicobacter pylori* infection have been considered as a risk factor in some studies. However, this information is very limited in the Ethiopian context and this research addressed the information gap.

Objective: The objective of this study was to assess the possible association of *Helicobacter pylori* infection and Hyperemesis gravidarum women visiting a selected hospital and health centres in Kirkos Sub city, Addis Ababa, Ethiopia.

Methods: A case control study was conducted in Kirkos sub city in one hospital and two health centers (Gandhi Memorial Hospital, Kirkos health center and Kazanchis health centers) Addis Ababa, Ethiopia among 50 hyperemesis gravidarum women (cases) and 100 non- hyperemesis gravidarum women (controls). A structured questionnaire was used to collect sociodemographic data of the study participants. Venous blood samples were collected and analyzed for determination of hemoglobin concentration and stool samples were processed for the presence of *H.pylori* infection using stool antigens test kit and the presence of intestinal parasites by direct stool examination (wet smear) and formol- ether concentration techniques. Urine analysis was done for ketone bodies. Data was summarized in frequencies (%) and mean $\pm$ SD as appropriate. Chi-square tests, Student "t" test and logistic regression were used in the analysis as needed. In all cases P-value <0.05 was considered as statistically significant.

Results: The overall rate of *H.pylori* infection among study subjects of pregnant women was 24.7 % (37/150). The prevalence of *H.pylori* infection was higher in pregnant women with hyperemesis gravidarum than pregnant women without hyperemesis gravidurum, 56% (28/50) and 9% (9 /100) respectively (X^2= 39.626 P value = 0.000).In this study there was a statistical association between *H.pylori* infection and low hemoglobin value, (OR=4.121, 95% CI=1.233–

13.771, p=0.024). There was no statistically significance difference between *H.pylori* positive women and sociodemographic characteristics (pvalue > 0.05).

Conclusion: Our study suggested that there was a strong association between *H. pylori* infection and HG. *Helicobcater pylori* should therefore, be considered as one of the risk factors for HG. *H.pylori* infection was associated with low hemoglobin value. *H.pylori* infected HG pregnant women showed higher rates of anemia than pregnant women without HG. Some expected *H.pylori* associated risk factors like presence of intestinal parasites; smoking habit; khat chewing and habit of drinking alcohol do not have significant association with *H.pylori* infection in this study.Further studies are required in the community using different diagnostic methods to explore the actual role of *H.pylori* and warranted to consider weather screening *H.pylori* infection during pregnancy could benefit the mother and the fetus in the near future and investigation of *H.pylori* infection used as a potential factor that might play a role in the managements of occurrence of anemia and hyperemesis gravidarum in pregnant women would be further explored.

Key words: Anemia**,** *H.pylori,* pregnancy, Hyperemesis gravidarum, *H. pylori* stool antigen test

1. Introduction

1.1. Background

Nausea and vomiting is a common and unpleasant problem during pregnancy with a frequency of 75% to 80% of pregnancies. It usually starts between the first and second missed menstrual period and can continue up to 14-16 weeks of pregnancy. In some women nausea and vomiting is very severe and does not respond to simple diet manipulation and antiemetic agents. This culminates in dehydration, electrolytic imbalance and starvation ketosis and is called hyper emesis gravidarum (HG). The frequency of HG is about one in 200 to 1.5% of pregnancies (1) and although its definition has not yet been standardized, the accepted clinical pattern includes persistent vomiting, dehydration, ketosis, electrolyte imbalance and weight loss (more than 5% of body weight).The exact etiology of HG is not clearly defined, but it can be considered as a multi factorial problem, which is under investigation(2). A relationship between *Helicobacter pylori* (HP) infection and HG has recently been reported. Chronic infection with HP has been reported to have a role in producing HG. In one of the studies, 61.8% of pregnant women with HG had positive HP genome, while 27.6% of pregnant women without HG had this genome. The researchers concluded that chronic infection with HP should be considered as an important factor in the pathogenesis of HG, even if it is not the sole factor(3).

Hyperemesis gravidarum (HG), the most severe form of pregnancy-associated nausea and vomiting, is accompanied by weight loss, dehydration, acidosis from starvation, alkalosis from loss of hydrochloric acid in vomitus, hypokalemia, and transient hepatic dysfunction.Estimates of the incidence of HG very widely depending on diagnostic criteria and different study populations. Some sources state a proportion of 0.5 to 2% of all pregnancies, whereas others provide an incidence of 0.3 to 1.5% of all live births (4).

Many hypotheses have been created to explain the etiology and pathophysiology of HG, including psychological factors, gastrointestinal tract dysfunction, endocrinological changes, infections, and immunological, metabolic, and anatomical factors. However, there is still no single theory sufficient to provide an adequate explanation for all the properties of HG. Numerous gastrointestinal and extra digestive pathologies are supposed to be associated with *H.pylori* infection. More recently, some studies performed on patients with HG indicated

causative relationship between H. pylori infection and HG (5). However, there is limited research that investigates the relationship between HG and the H. pylori stool antigen positivity (6, 7).

H. pylori, as a gram-negative flagellated spiral bacterium, colonizes the stomach and creates the basis of pathogenesis of gastric pathologies, including chronic gastritis, duodenal and gastric ulcers, gastric adenocarcinoma, and mucosaassociated lymphoid tissue lymphoma. *H. pylori* infect the stomachs of 50 % of the world population, and it is more prevalent in developing countries. The prevalence of *H. pylori* infection in pregnant women varies according to socioeconomic conditions, geographic area, and even the method used to test *H. pylori* infection (8, 9).

Several studies have shown a possible involvement of *H. pylori* infection in individuals with HG; however, other studies did not agree. On the other hand, the role of some factors such as different populations, geographic areas, ethnicity, and low socioeconomic status is unclear in HG(10).

Among the tests available for the diagnosis of *H. pylori* infection, the invasive ones (endoscopy with biopsy for histology, culture, and rapid urease test) are not suitable for pregnant women. Serological tests do not discriminate between current and past infections. The noninvasive "gold standard" C urea breath test is not available for this study for logistic reasons. The stool antigen test offers a simple, yet robust alternative (11) which has been recommended by the European *Helicobacter* Study Group as one of two noninvasive tests (the other being the urea breath test) (12).

1.2. Statement of the problem

Excessive nausea and vomiting that starts between four to 10 weeks gestation and resolve before 20 weeks, requiring intervention, is known as hyperemesis gravidarum. It affects 0.3-3% of all pregnant women, is associated with dehydration, electrolyte imbalance and weight loss of up to 10 % of prepregnant weight and should not be confused with the common symptoms of nausea and vomiting of pregnancy that are self-limiting (13).

Hyperemesis gravidarum causes uncontrollable vomiting, severe dehydration and muscle wasting in pregnancy and usually requires weeks or months of intravenous fluid therapy. If hyperemesis gravidarum is left untreated the mother's condition worsens. Wernicke's encephalopathy is a complication associated with a lack of vitamin B1 (thiamine). Hepatic and renal involvement leads to coma and death. Termination of pregnancy may reverse the condition

and has a place in preventing maternal mortality. Hyperemesis gravidarum persisting into the third trimester should be further investigated (14).

The biggest danger with hyperemesis gravidarum is that the woman will become dehydrated and no longer be able to provide the fetus with essential nutrients for growth. Prolonged hospitalization or home care with this disorder may result in social isolation. Hyperemesis gravidarum is a high-risk problem because it increases chances for pregnancy loss, intra-uterine growth retardation, maternal activity restriction, fatigue and depression (15).

Classified as a class one carcinogen, *Helicobacter pylori* microaerophilic, flagellated organism that has chronically infected more than 50% of the world's population. Significant evidence exists that links the bacterium to the pathogenesis and development of certain diseases such as gastric ulcers, chronic gastritis and stomach cancers, although most of the people harboring this organism are asymptomatic (16).

The prevalence of infection caused by this organism increases with advancing age and is reported to be higher in developing countries and among low socioeconomic populations, probably owing to conditions that favor the infection such as poor hygiene, crowded living conditions, and inadequate or no sanitation. The prevalence of this infection in human varies with geographical location and socio-demographic characteristics of the population; however it does not parallel the incidence of morbidity caused by the infection(17).In industrialized countries there is generally a low prevalence of *H. pylori* infection and yet a relatively high prevalence of gastric cancer. On the other hand, some countries with high *Helicobacter* prevalence rates have low gastric cancer prevalence (18).

It is, however, confirmed that this organism causes of 90% of all duodenal ulcers, 75% of all gastric ulcers and two forms of stomach cancer; adenocarcinoma and mucosa-associated lymphoid tissue (MALT) lymphoma. The evidence of its association with gastric cancer, led to its classification as a class 1 carcinogen by the International Agency for Research on Cancer and the World Health Organization. *H. pylori* are the first bacterium, and the second infectious organism after hepatitis B virus to be classified a carcinogen. A majority of *H. pylori*-infected individuals (80–90%) have clinically asymptomatic gastritis, 10–15% develop peptic ulcer, and 1–2% gastric malignancies (19).

H. pylori infection may have a role in the pathogenesis of various pregnancy-related disorders through different mechanisms: depletion of micronutrients (iron and vitamin B_{12}) in maternal anemia and fetal neural tube defects; local or systemic induction of pro-inflammatory cytokines release and oxidative stress in gastrointestinal disorders and; cross-reaction between specific anti-*H.pylori* antibodies and antigens localized in placental tissue and endothelial cells (pre-eclampsia, fetal growth restriction, miscarriage). Since *H. pylori* infection is most likely acquired before pregnancy, it is widely believed that hormonal and immunological changes occurring during pregnancy could activate latent *H. pylori* with a negative impact not only on maternal health (nutritional deficiency, organ injury, death), but also on the fetus (insufficient growth, malformation, death) and sometime consequences can be observed later in life(20).

The severity of this disease may affect the physical and psychological/emotional health of pregnant women, as well as family, social and occupational functioning and the states of maternal role. Some women also considered termination of otherwise wanted pregnancies because of severe and prolonged nausea and vomiting. In Ethiopia, there is no case control study with emphasis on HG to provide additional guidelines for the diagnosis, treatment as well as for protection against and relief from hyperemesis gravidarum.

1.3. Significance of the study

So far, to our knowledge, there is no study that investigates the association of HP with hyperemesis gravidarum and the infection rate in pregnant women in Addis Ababa particularly and in Ethiopia in general. However generating valuable information about the cause of hyperemesis gravidarum particularly in relation to *Helicobacter pylori* is highly required.

The study also helps to explain the association between *H.pylori* infection, intestinal parasite and anemia among the case and control groups and this is mandatory to provide support for policy makers to design, modify and plan intervention activities for quality health care in the future. Moreover, the study serves as base line data and plays a major role in adding valuable information for interested researchers and academicians for further analysis in the sector.

2 .Literature review

A study done by Shaban MM et al., 2014 in Egypt, to investigate the association between H pylori infection and hyperemesis gravidarum, revealed that regarding maternal age, gestational age and socioeconomic status, there is no statistical difference between both groups. There is a marked statistical difference between both groups in terms of *Helicobacter pylori* seropositivity and frequency of vomiting(21).

In a prospective study done by Kazerooni T et al., 2002 in Iran, it was reported that serologically positive *Helicobacter pylori* infection was detected in 44 out of 54 patients with hyperemesis gravidarum (81.5%) whereas 29 out of 53 asymptomatic gravidas (54.7%) had positive antibody titers for *Helicobacter pylori*.The ratio of *Helicobacter pylori* seropositivity in pregnant women with hyperemesis gravidarum was significantly higher than asymptomatic pregnant women (22).

Another study done by Boltin D et al., 2014, in Arab Israeli women to examine if hyperemesis gravidarum is associated with H. pylori with ^{13}C-urea breath test showed that H. pylori infection was identified in 75.0% (18/24) of cases and 60.4% (29/48) of controls. H. pylori infection did not correlate with age, fetal sex, or the number of previous pregnancies(23).

A study done by Nashaat EH et al., 2010 in Egypt, to evaluate the role of HP in the pathogenesis of hyperemesis gravidarum, and the value of adding a non-teratogenic regimen for its treatment in intractable cases by using Serum test for H-pylori IgG antibody titre using (ELISA) method reported that 54 of the 62 HG cases were HP-positive and 20 out of the 62 controls were positive, six cases developed severe intractable vomiting. Two of them developed an attack of hematemesis(24).

The study conducted by Nanbakhsh F et al., 2012 in Urmia, Iran, to compare the rate of H. pylori infection between an Iranian sample of pregnant women who were suffering from HG in their first trimester and control pregnant women without HG, showed that no significant difference was seen between the two groups regarding age, gestational age and gravidity. Twenty-four patients (92.3%) in HG group had H. pylori infection, while this rate was only 7.7% (two patients) in control group. No correlation was detected between IgG titers and either maternal age or parity (25).

Another study done by Poveda GF et al., 2014 in Chile, to determine the prevalence of *Helicobacter pylori* infection on Chilean pregnant women and its relationship with the appearance and severity of hyperemesis and dyspepsia by using ImmunoComb II *Helicobacter pylori*IgG kit revealed that out of the total number of pregnant women**,** 68.6% showed infection by *Helicobacter pylori*. 79.6% of the total sample had symptoms of dyspepsia, and 72.5% of this group presented *Helicobacter pylori* infection. 12.4% showed pregnancy hyperemesis; among them, 79.4% were infected with *Helicobacter pylori*. 73.4% of the pregnant women that showed gastric discomfort during the first three months had *Helicobacter pylori* infection. 53.7% of them continued with gastric discomfort after the first three months; of those, 95.8% were infected. *Helicobacter pylori* infection was present only in 1.5% of pregnant women without gastric discomfort(26).

A similar Study done by Karaca C et al., 2004 in Istanbul, to investigate any possible association between infection with HP and hyperemesis gravidarum and between socio-economic status and HP infection in pregnant women with hyperemesis gravidarum, reported that serologically positive H. pylori infection was detected in 46 (82.1%) subjects of the hyperemesis gravidarum group and in 58 (64.4%) of the controls. With respect to the patients with lower socio-economic status, 40 (88.9%) of the 45 patients with hyperemesis gravidarum and 49 (68.1%) of the 72 controls were positive for H. pylori(27).

A case control study performed by Kazemzadeh M et al., 2014 in Tehran, Iran, on two groups of pregnant women who were in the first trimester of their pregnancies, showed that both groups had no statistically significant difference according to age, gestational age, gravidity, and body mass index. Fifty-one women out of 78 (65.4%) in HG group and 43 women (44.3%) in the control group were IgG positive for HP, which showed a significant difference. Also, mean serum level of IgG was higher in the HG group. Between the different variables of age, gestational age, gravidity and HP infection, only HP infection was found as a risk factor for HEG using logistic regression model (28).

The study done by Karadeniz RS et al., 2014 in Turkey, to investigate whether *Helicobacter pylori* is an etiologic factor in hyperemesis gravidarum, showed us *Helicobacter pylori* seropositivity was 67.7% in the patients with hyperemesis gravidarum and 79.3% in the control group ($\chi 2 = 1.02$, $P = .31$). *Helicobacter pylori* stool antigen (HpSA) was detected in 22.6% of

patients with hyperemesis gravidarum, but only in 6.9% of patients in the control group. The difference was not statistically significant ($\chi 2 = 2.89$, $P = .08$). In this study, no relation was found between *Helicobacter pylori* and hyperemesis gravidarum. The low social status of women in both groups could be one of the reasons for the high prevalence of HP infection(29).

A study conducted by Bezircioğlu İ et al., 2011 in İzmir, Turkey, to investigate the possible association between *Helicobacter pylori* infection and hyperemesis gravidarum revealed that eight HG patients (22.2%) and one control patient (2.8%) were established HpSA positive and it was statistically significant. There was no significant difference between HG and control subjects in terms of age, gestational week, parity, educational level, socioeconomic status and smoking. There was anemia in five HG patients, four of them were HPSA positive. HPSA positivity was more prevalent in HG patients with anemia. Severe vomiting (more than four times a day), heartburn, epigastric pain, duration of hospitalization (more than four days) and weight loss (≥5 kg) were not correlated to HPSA positivity (30).

A prospective cross-sectional study in Turkey done by Guven MA et al., 2011, to investigate the relationship between HP infection and HG during early pregnancy by using serologic and stool antigen tests, showed the rates of serology-specific HP IgG positivity were 80% (32 of 40) in patients with HG and 35% (14 of 40) in the control group. The difference between the two groups was significant. The rates of HPSA test positivity were 87.5% (35 of 40) in patients with HG and 62.5% (25 of 40) in control groups. The difference between the two groups was significant.(31).

A cross-sectional study in Uganda done by Baingana et al.2014 , to assess H. pylori infection by the stool antigen test revealed that, The overall prevalence of H. pylori infection was 45.2% but varied by geographical location from 18.2% to 60.5%(32).

Another study done by Nasr AA et al., 2012 in Cairo, Egypt, to detect the association between HP infection and HG among Egyptian women, showed that there was a highly significant difference in the HP IgG antibody titer between the cases (18.1–100U/ml) and the controls (0.9–62.7U/ml) .The number of cases found to be seropositive to HP IgG (100%) was higher than the number of the control individuals (86.67%), but the difference between both the groups was not statistically significant (P = 0.37)(33).

A cross-sectional study conducted in Butajira Hospital, Southern Ethiopia by Kibru D et al., 2014, showed that the overall prevalence of H. pylori infection was 52.4% and it was

significantly associated with age, presence of intestinal parasites, smoking habit, alcohol drinking habit and body mass index. The prevalence of anemia among H.pylori infected patients (30.9%) was significantly higher than uninfected patients (22.5%)(34).

Based on the above literature review, information concerning HG and HP is not well studied in the Ethiopian context. In particular, there were no data on the association of HG and HP using a case control study design among pregnant women.

3. Objectives

3.1 General Objective

- To investigate the possible association between *Helicobacter pylori* infection and hyperemesis gravidarum women visiting a selected Hospital and health centre in Kirkos Sub city, Addis Ababa, Ethiopia from July, 2016 to October, 2016.

3.2 Specific objectives

- To determine the prevalence of *H.pylori* infection among Hyperemesis gravidarum women in a selected Hospital and Health Centre, Kirkos sub city, Addis Ababa.
- To determine the prevalence of *H.pylori* infection among pregnant women without Hyperemesis gravidarum in a selected Hospital and Health Centre, Kirkos sub city, Addis Ababa.
- To assess *H.pylori* infection and its associated risk factors in a selected Hospital and Health Centre, Kirkos sub city, Addis Ababa.

3.3 Hypothesis

- There is no difference in prevalence of *H. pylori* infection between hyperemesis gravidarum pregnant women and pregnant women who did not develop hyperemesis gravidarum.

4. Materials and methods

4.1. Study design and period

A case control study was conducted from July to October 2016.

4.2. Study areas

The study was conducted in the Gandhi memorial Hospital, Kirkos health center and Kazanchis health cente in Kirkos sub city, Addis Ababa, Ethiopia. Kirkos subcity has 11 woredas (districts). The size of the sub city is about 16.26 km^2 and it is estimated that population density is 13501 people per km^2 .The total projected population of Kirkos sub-city is estimated to be 220,991 which is 8.07 % of the entire population of Addis Ababa for the year 2007 (source CSA) of which 103,334 (46.5%) are males and 117,677 (53.25%) are females. Women of childbearing age group constitute 34.64% of the total population.

The health office is authorized to organize, co-ordinate and regulate public health activities in the sub-city. Gandhi Memorial Hospital, Kirkos health center and Kazanchis health centers are the most visited health institutions in this sub-city. Gandhi Memorial Hospital was established by Mahatma Gandhi in 1948 E.C. and is dedicated primarily for maternal care, serving for 58,000 people annually. Gandhi Memorial Hospital has 384 staff. Labour and delivery room has seven waiting beds and two couches.

4.3. Study population:

4.3.1. Source population:

All pregnant women who attended antenatal care clinics in the selected locations during the study period.

4.3.2. Study population:

All pregnant women with hyperemesis gravidarum and pregnant women with the same gestational age but without hyperemesis gravidarum that fulfilled the inclusion criteria from the source population during the study period were involved.

4.4. Inclusion and exclusion criteria:

4.4.1. Inclusion criteria:

Inclusion criteria for the study group included hyperemsis gravidarium (vomiting three episodes per day without any obvious cause except for pregnancy, weight loss of 3 kg or 5 % and the presence of at least one positive ketonuria), age of 18 to 45 years, gestation between five and 16 weeks. The controls were matched by maternal age, gestational age, parity and women without history of HG who volunteered to participate in the study were included.

4.4.2. Exclusion criteria:

The study subjects were excluded if they had received antibiotics and women who had other causes of vomiting such as hyperthyroidism, multiple gestation and gestational trophoblastic disease.

4.5. Study variables

4.5.1. Dependent variables:

H.pylori infection rate (positivity of fecal Ag)

4.5.2. Independent variables:

Socio-demographic (age, educational level, employee status), gravidity, parity number of pregnancy, gestational period, some behavioral risk factor (smoking habit, drinking alcohol and khat chewing habit) and level of hemoglobin concentration and intestinal parasite.

4.6. Sample size and sampling technique

4.6.1. Sample size determination

A total of 150 pregnant women, 50 with hyperemesis gravidarum(cases) and 100 without hyperemesis gravidarum (by taking the given 1:2 case control ratio) was considered.

4.6.2 Sampling technique

Convenient sampling technique was employed consecutively to include study participants who met the inclusion criteria. Stool, urine and blood sample collection was continued until the achievement of the expected sample size from pregnant women at ANC department of the hospital and health centers.

4.7 Data collection and processing

4.7.1. Demographic characteristics and exposure to risk factors

Socio-demographic variables data like age, marital status, education level, occupation, and other relevant clinical data such as parity, gravidity, gestational period, history of hypermesis gravidum, history of gastrointestinal illness, water used for drinking, habits of hand wash before meals and after toilet use, habits of smoking, alcohol drinking, khat chewing were obtained using a predesigned questionnaire through interview by data collectors.

4.7.2. Biological data collection and processing

A well-trained laboratory technologist/technician collected stool, urine and blood specimens in order to ensure that appropriate stool specimen was obtained. Intestinal parasite identification was done by direct smear microscopy and Formol-Ether Stool Sedimentation Concentration technique, *H.pylori* stool antigen test, hemoglobin level determination was also performed (35).

4.7.2.1. Stool specimen collection and transportation

4.7.2.1.1. Stool specimen collection and handling: Stool samples were collected in a clean, dry stool cup. During the study, a total of 150 fresh stool samples were collected strictly following standard operational procedures with sterile stool cup (35). Proper stool specimen was taken from each woman to reduce the chance of occurrence of false negative and excess stool sample may lead to an invalid test result and to increase the chance of recovering the intestinal parasite. Transportation of specimens: following collection from women, specimens was transported by placing each a separate sterile stool cup to the laboratory within 30 minutes (36-38).

4.7.2.1.2. Stool sample processing for *H.pylori* test: following fecal sample collection, the *H.pylori* test strip was removed from the pouch and was placed on a clean, flat surface and the stool specimen and test components were brought to room temperature; the plastic dropper was filled with the stool specimen. Holding the dropper vertically one drop (about 30-45μL) of specimen was dispensed into the sample pad making sure that there was no air bubbles. Then add one drop (about 35 –50μL) of Sample diluents immediately. When an adequate volume of extracted faecal specimen was dispensed into the sample well of the test cassette, the specimen migrates by capillary action across the cassette. *H.pylori* antigens if present in the specimen was bind to the anti- *H.pylori* conjugates. The immunocomplex was then captured on the membrane by the precoated antibody, forming a burgundy colored T band, indicating an *H.pylori* positive

test result. Absence of the T band suggests that the concentration of *H.pylori* antigens in the specimen is below the detectable level, indicating an *H.pylori* negative test result(35, 36).

4.7.2.1.3.Stool sample processing for direct stool examination (wet smear): a drop of normal saline was dispensed on the cleaned microscope Slides, a small amount of stool specimen with a wooden stick was taken and mixed with saline and was examined as soon as possible (within 30 minutes of passage) and on soft/formed stool within 60 minutes of passage, Helminthes ova were examined using 10x objective and cysts and trophozoites were examined using 40×objectives, this aid to detect certain protozoa trophozoites retain their motility which may aid in their identification(39).

4.7.2.1.4.Stool sample processing for Formol ether concentration: a fresh stool sample was dispensed in to 10 ml of 10% formalin in a round bottom tube and the stool and formalin were mixed thoroughly and the mixture was stand for a minimum of 30 min for fixation. Strain a sufficient quantity through wet into a conical 15ml centrifuge tube to give the desired amount of sediment (0.5 to 1 ml), 10% formalin was added to the top of the tube and was centrifuged for 10 min at 500 x g. supernatant fluid was decanted and resuspend the sediment on the bottom of the tube, ethyl acetate was added and shaked vigorously by holding the tube so the stopper is directed away from your face then Centrifuged for 10 min at 500 x g. The sediment was examined using 10 X and 40 X microscopic examination (37, 38).

4.7.2.2. Blood specimen collection, handling, storage and transportation: by explanation of the blood drawing procedure to the client a total of 150 blood samples was collected strictly following standard operational procedures, disinfecting the phlebotomy site by swabbing the skin in small outward circles with 70% alcohol swab or cotton wool soaked in isopropyl alcohol from the study participants draw vein blood of approximately 4 or 5 ml in the EDTA vacutainer tube, mixed the blood properly by inverting the tube 6-8 times immediately after collection to avoid formation of small clots. The blood sample was not refrigerated and avoided extremes of temperature and arranged for immediate transport of the sample to testing laboratory sites to perform hematological tests for determination of Hemoglobin level by Humacount 30TS hematology analyzer(from Human p.l.c).

4.7.2.2.1. Blood sample processing: prior to running patient specimens, blank measurement was performed in case the instrument is not used for a specific time. The lysed sample dilution was measured by a photometric method. The reagent was lyses the red blood cell, which release

hemoglobin. The chemical process was form a stable form of methemogobin. This was measured by a photometer on the chamber. The Humacount 30TS are fully automated, bench top hematology cell counters. It implements the so called coulter method for counting cell passing through a small aperture, and measure the hemoglobin content of red blood cells.

4.7.2.3. Urine specimen collection, handling, Storage and transportation: biochemical testing of urine was performed using dry reagent strips, often called dipsticks. A urine dipstick consists of a white plastic strip with absorbent microfiber cellulose pads attached to it. Each pad contains the dried reagents needed for a specific test. The person performing the test dips the strip into the urine, lets it sit for a specified amount of time, and compares the color change to a standard chart (user's leaflet).

4.7.2.3. Urine sample processing

Fresh urine specimen in a clean dry container with labeling was collected and mixed well immediately before testing and completely immersed reagent areas of strip in urine and removed immediately to avoid dissolving out the reagent. While removing, run the edge of the entire length if strip against the rim of urine container to remove the excess urine. Then, hold the strip in a horizontal to prevent possible mixing of chemicals from adjacent reagent or contaminating the hands with urine. Reading was done visually by comparing the reagent areas to corresponding color chart on the bottle labeled at times specified (user's leaflet).

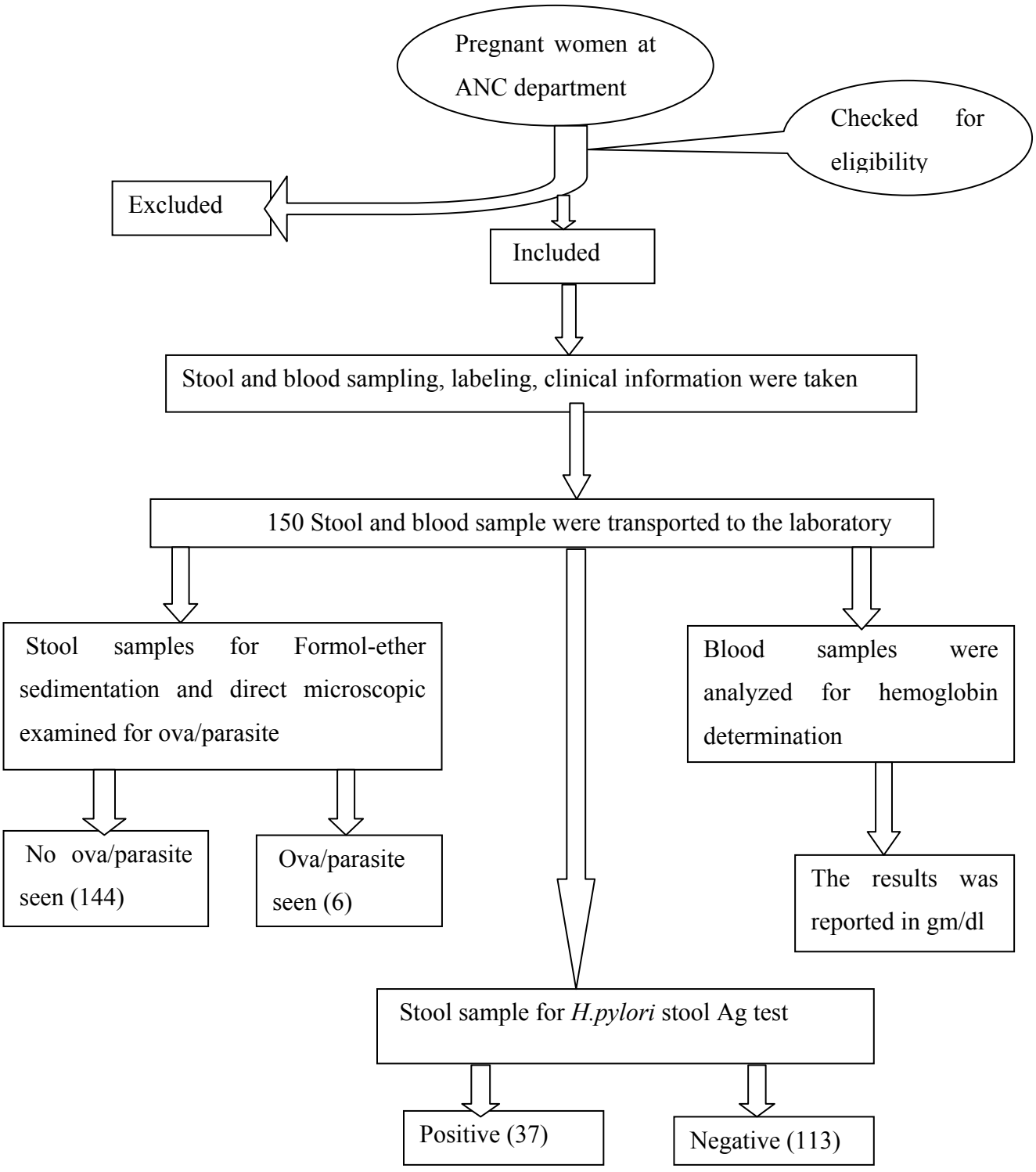

Fig.4.1. Work flow of stool and blood sample for *H.pylori* stool antigen test, direct microscopic stool examination, Formal-ether sedimentation and microscopic examination for ova/parasite and for hemoglobin determination

4.8. Quality assurance and quality control

Standard operating procedures were strictly followed and internal quality controls materials were included from the test kits were performed based on manufacturer 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 supervisor.

4.8.1. Pre-analytical phases

Stool samples were collected from the study subjects and properly labeled with their identification name or Id or card number. The blood sample was collected by the trained laboratory personnel and was mixed with EDTA very carefully. The laboratory personnel and principal investigator assured to collect good quality sample for analysis to produce reliable and valid data.

4.8.2. Analytical phases

The tests were done by trained laboratory personnel according to standard operational procedures of each test methods. The reagents, kits and the methods were assessed with known positive and negative controls materials, well-trained and experienced laboratory professionals were participated in the laboratory analysis procedure. Finally the results were checked by the investigator and supervisors.

4.8.3. Post-analytical phases

The results were recommended with patient's identification in order to avoid the error in the results of the test, repeatedly checked before reporting to the ordering physician/health officer if there was a need to treatment or support for patients and the results were documented properly.

4.9. Data management

Data quality was ensured through use of standardized data collection materials, pretesting of the questionnaires, proper training was given before starting of data collection and intensive supervision was followed during data collection by the principal investigator. Data was kept secret and the result was disclosed by code. The codes of the Hospital and health centers were notified for the participant facilities prior to completion of the study.

4.10. Data analysis and interpretation

Data were entered and analyzed using Excel, SPSS version 20, SAS version 9.4 and results were expressed using frequency and percentages. Chi square was done to identify factors associated with *H.pylori* followed by multi-variant analysis .Figures and tables were used for data presentation. Association between the prevalence of risk factors and *H.pylori* infection was assessed by $\chi 2$ tests. Quantitative data were expressed as mean ± standard deviation and range, using Student "t" test**.** Anemia was defined according to the WHO definition as a hemoglobin concentration of < 12 g/dL for adult women. Logistic regression was used to determine the effect of independent variables on the prevalence of *H.pylori* infection. In all case a 95% confidence interval was used and P-values less than 0.05 were considered as statistically significant.

4.11. Ethical considerations

This research project was approved by "Departmental Ethics and Research committee" of the Department of Medical Laboratory Sciences, Collage of Health Science, and School of Allied Health Science of Addis Ababa University, Addis Ababa public health research and emergency management core process. Permission was obtained from Addis Ababa City Administration Health Bureau to the participant Hospital and health centers. Moreover, privacy and confidentiality were assured for all the study participants. The right of any individual not to participate or withdraw from the study at any point was fully respected. Data collection from each study participants was started after they gave informed consent. Data collection was started after permission is obtained from each health centers of the study sites, no name and other identifier on the questionnaire. The Hospital and health centers have right to get the copy of the findings. Results were communicated with physician/health officer/Nurses for proper managements of the study participants.

4.12. Dissemination of results

The result of this study was disseminated to Addis Ababa public health research and emergency management core process, then will be presented to Department of Medical Laboratory Sciences, to the scientific community through scientific presentation and finally we will sent to publication on peer reviewed scientific Journals.

5. Results

5.1. Study subjects

A total of 150 adult women 33.3% (50) pregnant women with hyperemesis gravidarum and 66.7% (100) pregnant women without hyperemesis gravidarum participated in this study. The age of the study subjects were ranged from 18-40 years, with a mean (±SD) age of 28.0±4.5 years for HGM cases and18-38 years, with a mean (±SD) age of 27.0±4.4 for controls (Table 5.1.).

There was no significant difference between cases and controls according to marital status, education level, occupational status and number of people living house hold (Table 5.1.).

Table 5.1.Comparison between cases and controls according to sociodemographic data in a selected hospital and health centers from July to October, 2016.

Characteristics	Cases (n = 50)	Control (n = 100)	T	P value
Age (years)				
Min. - max.	18-40	18-38		
Mean±SD	28.0±4.5	27.0±4.4	1.438	0.153
Median	28	27		
Marital status	N, (%)	N, (%)	X^2	
Married	47 (33.6)	93 (66.4)		
Single	3 (30)	7 (70)	0.054	0.559
Education level				
Illiterate	4 (30.8)	9 (69.2)		
Primary school	11 (31.4)	24 (68.6)		
Secondary school	20 (30.8)	45 (69.2)	1.153	0.768
Higher education	15 (40.5)	22 (59.5)		
Occupational status				
Government	9 (31)	20 (69)		
NGO	4 (50)	4 (50)		
Private	19 (30.2)	44 (69.8)	2.446	0.650
house wife	18 (37.5)	30 (62.5)		
house maid	0 (0)	2 (100)		
Number of people in house hold				
Two	16 (30.8)	36 (69.2)		
Three	7 (24.1)	22 (75.9)		
Four	26 (41.3)	37 (58.7)	3.484	0.329
Greater than four	1 (16.7)	5 (83.3)		

T: Student t-test, Fisher's exact test used for cells less than 5, NGO: Nongovernmental organization,

There was no significant difference between patients and controls according to gestational age (p=0.225) but there was significant difference between both groups in gravidity (p=0.020) (Table 5.2.).

Table 5. 2. Comparison between the two groups according to obstetric history in a selected hospital and health centers from July to October, 2016.

Characteristics	Cases (n = 50)	Control (n = 100)	X^2	P value
Gravidity	N, (%)	N, (%)		
First pregnancy	20 (28.2)	51 (71.8)		
Second pregnancy	24 (48)	26 (52)	7.778	0.020*
Greater or equal to three	6 (20.7)	23 (79.3)		
Gestational period				
1-12 week	24 (28.8)	59 (71.1)		
13-2 4week	26 (38.8)	41 (61.2)	1.632	0.225

*statistically significant

5.2. Burden of *Helicobacter pylori* infection among HGM cases and control subjects

The overall rate of *H. pylori* infection among study subjects were 24.7 %(37/150). The rate of infection was higher in cases than controls, 56% (28/50) vs. 9% (9 /100), (X^2=39.626, P=000) (Table 5.3 and Fig.5.1).

Table 5.3.Prevalence of *H.pylori* infection among HGM Cases and control group in a selected hospital and health centers from July to October, 2016.

H. pylori positivity	Group			X^2&P-value	OR with 95 % CI
	Case N=50	Control N=100	Total		
Positive					
Count	28	9	37		12.869
% within Group	56%	9%	24.7%	39.626 & 0.000*	(5.318-31.318)

*statistically significant

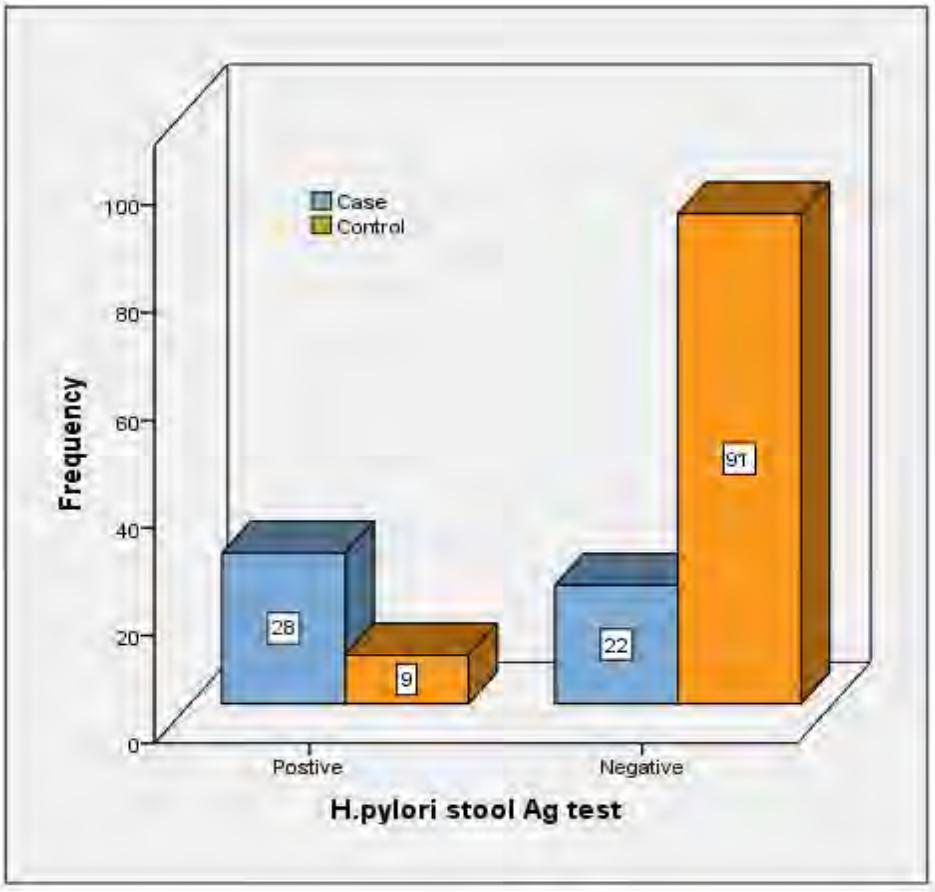

Fig.5.1. Prevalence of *H.pylori* infection among study participants in a selected hospital and health centers from July to October, 2016.

Regarding to behavioral characteristics & some expected associated risk factors like Smoking habit, khat chewing habit, History of hyperemesis gravidarum, History of gastrointestinal illness and Anemia status showed statistically significant difference between both groups. However the numbers of study participants that respond having Smoking habit and khat chewing habit were very low. There was no significant difference between cases and controls according to Habit of alcoholism, water used for drinking purpose and Intestinal parasite (Table5.4)

Table 5.4. Comparison between cases and controls according to behavioral characteristics and some expected associated risk factors in a selected hospital and health centers from July to October, 2016.

Characteristics	Cases (n = 50)	Control (n = 100)	X^2	P value
Habit of alcohol use	N, (%)	N, (%)		
Yes	4 (50)	4 (50)		
No	46 (32.4)	96 (67.6)	1.056	0.442
Smoking habit				
Yes	3 (100)	0 (0)		
No	47 (32)	100 (68)	6.122	0.036*
khat chewing habit				
Yes	4 (100)	0 (0)		
No	46 (31.5)	100 (68.5)	8.219	0.011*
Intestinal parasite				
Positive	4 (66.7)	2 (33.3)		
Negative	46 (31.9)	98 (68.1)	3.125	0.175
water for drinking purpose				
Tuncker	2 (100)	0 (0)		
pipe water	48 (32.4)	100 (67.6)	4.054	0.110
History of gastrointestinal illness				
Yes	28 (45.9)	33 (54.1)		
No	22 (24.7)	67 (75.3)	7.308	0.008*
History of hyperemesis gravidarum				
Yes	50 (96.2)	2 (3.8)		
No	0 (0)	98 (100)	141.3	0.000*
Anemia status				
Anemic	23 (71.9)	9 (28.1)		
Non anemic	27 (22.9)	91 (77.1)	27.191	0.000*

*statistically significant, Fisher's exact test used for cells less than 5.

Regarding the prevalence of *H. Pylori,* there was no statistically difference with age, gravidity, gestational period, marital status, education level, occupational status and number of people in house hold in cases group (Table 5.5).

Table 5.5.Relation between *Helicobacter pylori* with sociodemographic and obstetric history in cases group in a selected hospital and health centers from July to October, 2016.

	Helicobacter pylori status			
Characteristics	**Positive (n = 28)**	**Negative (n = 22)**	**T**	**P value**
Age (years)				
Min. - max.	18-40	18-35		
Mean±SD	28.1±4.76	27.33±4.293	0.572	0.570
Median	27	26		
Marital status	N, (%)	N, (%)	X^2	
Married	28 (59.6)	19 (40.4)		
Single	0 (0)	3 (100)	5.171	0.079
Education level				
Illiterate	1 (25)	3 (75)		
Primary school	6 (54)	5 (45.5)		
Secondary school	11 (55)	9 (45)	2.225	0.577
Higher education	10 (66.7)	5 (33.3)		
Occupational status				
Government	7 (77.8)	2 (22.2)		
NGO	2 (50)	2 (50)		
Private	12 (63.2)	7 (36.8)	4.275	0.237
house wife	7 (38.9)	11 (61.1)		
Number of people in house hold				
Two	10 (62.5)	6 (37.5)		
Three	2 (28.6)	5 (71.6)		
Four	16 (61.5)	10 (38.5)	3.822	0.294
Greater than four	0 (0)	1 (100)		
Gravidity				
First pregnancy	10 (50)	10 (50)		
Second	14 (58.3)	10 (41.7)		
Greater or equal to three	4 (66.7)	2 (33.3)	0.658	0.784
Gestational period				
1-12 week	14 (58.3)	10 (41.7)		
13-2 4week	14 (53.8)	12 (46.2)	0.102	0.783

T: Student t-test, Fisher's exact test used for cells less than 5, NGO: Nongovernmental organization,

5.3. Comorbidity of *H.pylori* infection and anemia in cases and controls groups

In this study we were interested to see a significant difference between HP infection and anemia; we found that HP infection is higher in cases than controls. Thirty two of our study participants, 21.3% were anemic, 71.9% (23/32) of anemic were cases. The remaining 28.1% (9/32) anemic were controls and 73.9% were infected with H.pylori in cases (X2= 5.547, p value=0.024 (Table 5.4 and table 5.6.).

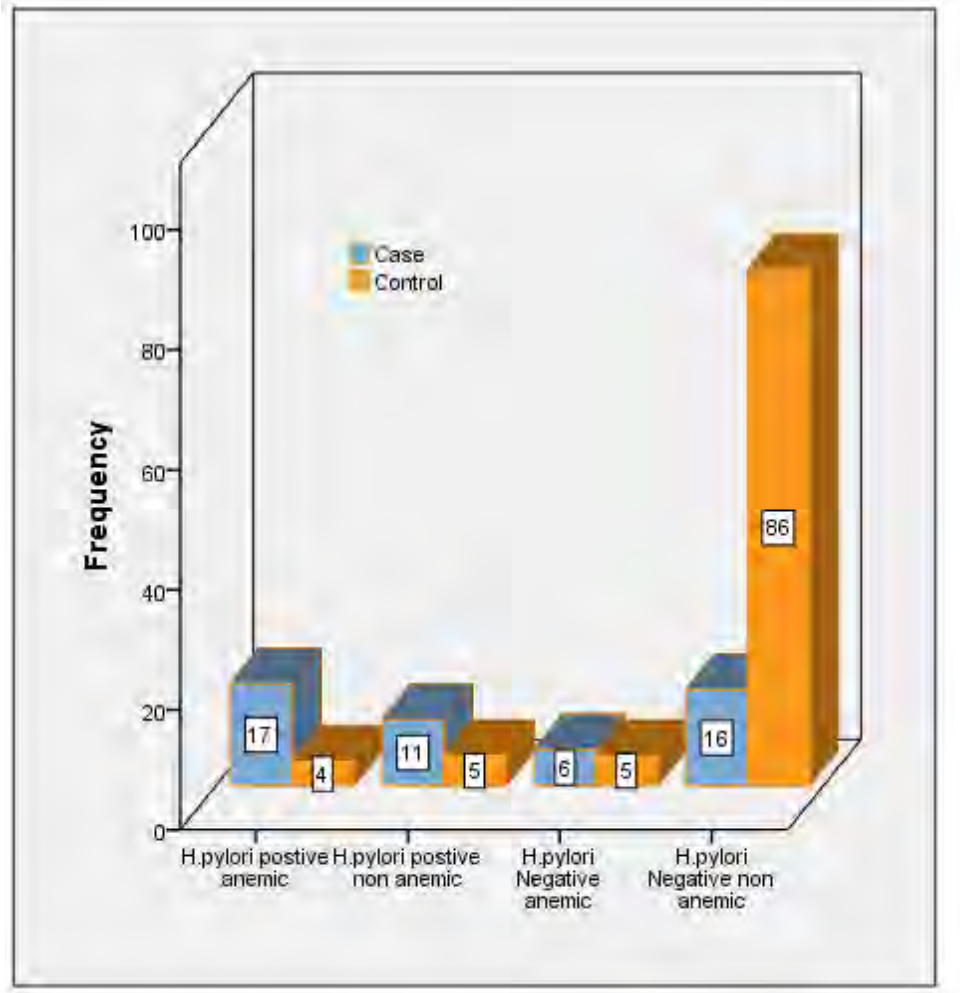

Fig.5.2. shows comorbidity between *H.pylori* infection and anemia among study participants in a selected Hospital and health centers from July to October, 2016.

There was also no statistically association between *H.pylori* and the expected risk factors like habits of drinking alcohol (P=1.00), cigarette smoking (P=1.00), khat chewing (0.621), using water for drinking (P=1.00), History of gastrointestinal illness (0.398) and Intestinal parasites (P=0.308). However, the study subjects who respond that had habits of drinking alcohol; cigarette smoking and chat chewing were very low in numbers (Table 5.6).

Table 5.6. Relation between *Helicobacter pylori* with behavioral characteristics and some expected associated risk factors in cases group in a selected hospital and health centers from July to October, 2016.

	Helicobacter pylori status				
Characteristics	**Positive (n = 28)**	**Negative (n = 22)**	**X^2**	**COR (95%CI)**	**P value**
Habit of alcohol use	N, (%)	N, (%)			
Yes	2 (50)	2 (50)	0.064	0.769	1.000
No	26 (56.5)	20 (43.5)		(0.100-5.944)	
Smoking habit					
Yes	2 (66.7)	1 (33.3)	0.147	1.615	1.000
No	26 (55.3)	21 (44.7)		(0.137-19.067)	
Chat chewing habit					
Yes	3 (75)	1 (25)	0.637	2.520	0.621
No	25 (54.3)	21 (45.7)		(0.244-26.065)	
Intestinal parasite					
Positive	1 (25)	3 (75)	1.696	0.235	0.308
Negative	27 (58.7)	19 (41.3)		(0.023-2.430	
water for drinking purpose					
Tuncker	1 (50)	1 (50)	0.030	0.778	1.000
pipe water	27 (56.2)	21 (43.8)		(0.046-13.178)	
History of gastrointestinal illness					
Yes	14 (50)	14 (50)	0.930	0.571	0.398
No	14 (63.6)	8 (36.4)		(0.182-1.790	
Anemia status					
Anemic	17 (73.9)	6 (26.1)	5.547	4.121	0.024*
Non anemic	11 (40.7)	16 (59.3)		(1.233-13.771)	

6. Discussion

It is estimated that HP infection might be present in two-thirds of the world population. However, the pathogenic relationship between HG and *H. pylori* is not self-evident because most of those infected with H. pylori do not complain of symptoms. In other words, the presence of *H. pylori* can be asymptomatic. Furthermore, the problems in diagnosis of HP infection are more complicated during pregnancy since HG can mask an active *H. pylori* infection or HG may be worsened by superimposed *H. pylori* infection (29).

To the best of our knowledge, information concerning the association of *Helicobacter pylori and hyperemesis gravidarum* among pregnant women obtained through case control, particularly in Ethiopia, is not available. In our study, we attempted to prove that there was an association between the severity of the disease and the positivity of HPSA.

Our data showed that according to maternal age (P=0.153), marital status (P=0.559), educational level (P=0.768), occupational status), (P=0.650), number of households (P=0.329), had no statistically significant difference between the hyperemesis gravidarum group and the control group. These results were in agreement with those reported by Kazemzadeh M et al., 2014 in Turkey (31). However, Shirin et al,2004,found that the women who had complaints of frequent vomiting in the first trimester and were positive for H. pylori IgG were significantly older than those who were negative for H. pylori IgG(40).

There was no significant difference between patients and controls according to gestational age (p=0.225) but there was significant difference between the two groups in number of pregnancies (gravidity) (p=0.020).On the other hand Elmhady M et al., 2016 and Samy MD et al., 2016, reported that there was no statistically difference regarding to gravidity (p=0.407, 0.270) between cases and controls respectively(28, 41).

Regarding to behavioral characteristics & some expected associated risk factors like Smoking habit (p=0.036), khat chewing habit (p=0.011), history of hyperemesis gravidarum (p=0.000), history of gastrointestinal illness (p=0.008) and Anemia status (p=0.000) showed statistically significant difference between the two groups. However the numbers of study participants that respond having Smoking and khat chewing habit were very low. Our result showed there was no significant difference between cases and controls according to Habit of alcoholism, water used for drinking purpose and presence of intestinal parasite. Interestingly, Vikanes A et al. 2010, reported that women who smoked daily (OR, 0.44; 95% CI, 0.32-0.60) or occasionally (OR,

0.64; 95% CI, 0.44-0.93) had lower risk of hyperemesis than non-smokers (42). A recent study done by MA Hassan et al.,2015 in Kenya which showed that khat chewing was associated with infection with *H. Pylori*, of the 93 cases, 58.1% were *H. Pylori* positive with a majority being khat chewers 67.2% and 32.8% non-khat chewers; the two groups were significantly different (p-value=0.007) which was contrary to our findings (43). However, in our study the number of study subjects who responded habit of chat chewing was very low in numbers.

According to our findings, regarding to the association of *H. Pylori,* there was no statistical difference with age, gravidity, gestational period, marital status, education level, occupational status and number of people in house hold in cases. This findings agreed with a study done by Aytac S et al., 2007 in Turkey that reported no statistically significant differences between the subjects and controls with regard to demographic characteristics and obstetric history(5).

The results of this study show that the HPSA positivity of H. pylori in the HG group was significantly higher than in control group, 56% in cases vs. 9% in controls. Recently, it was shown that H. pylori positivity was significantly high in pregnant women who suffer from HG. Our findings were in accordance with previous studies (22,30, 31). However, some recent studies could not find such an association. Thus this is one of the controversial issues in obstetric care [27, 28]. For example, Karadeniz RS et al., 2014.reported insignificant difference between HG patients (67.7%) and controls (79.3%) regarding the presence of H. pylori infection using H. pylori specific IgG(44).Nasr AA et al, 2012 in Egypt also reported statistically an insignificant difference seropositive to H. pylori IgG (100%) in cases and (86.67%) in control individuals (33).

Kazerooni T et al.,2002 in Iran, (22) reported a statistically significant differences between HG patients and asymptomatic ones regarding *H. pylori* infection (81.5 vs.54.7%). Guven MA et al,2011 in Turkey (31) also found comparable results (87.5% in HG patients vs. 54% in controls). Our result revealed significantly higher *H.pylori* positivity in HG patients than in control ones compared to a study done by Bezircioğlu İ et al, 2011 in Izmir, Turkey (22.2% vs. 2.8%) which was statistically significant and in their study they used HPSA test which was similar to ours (30).

Another study done by Samy MD et al., 2016 in Egypt, showed a significantly increased infection rate in patients with hyperemesis gravidarum than in controls 53.3% vs. 13.3% (28).

Elmahdy M et al., 2016 also reported *H.pylori* positivity of 75% in HG patients vs. 37.5% in controls(41).

Our results were in agreement with a recent meta-analysis carried out by Sandven I et al. 2009, in a total of 12 studies reviewed regarding H. pylori infection in HG patients, 11 relied on the serologic assay of H. pylori infection and the great majority of them showed significantly increased infection rate in HG patients than controls (45, 46). Interestingly, methods of laboratory diagnosis were detecting antibodies rather than antigen, unlike the present study which could not differentiate the active infection (47)

Our result had consistency with the sudy done by Hatziveis K et al.2007 in Greek, which was reported as HP infection was 56% (14 of 25) among patients with Hyperemesis gravidarum (48).

On the other hand Aytac et al. 2007 reported the overall prevalence of HpSA positivity appeared as 41.1%. Twenty-two of 52 (42.3%) HGM patients and 22 of 55 (40.0%) control pregnant women subjects were positive for HpSA. The difference was not significant(5).

A possible explanation for an association of *H.pylori* and hypermesisgravidum could be that an increased accumulation of fluid and a displacement of intracellular and extracellular volume occur as a result of increase in steroid hormones, and this condition results in a change of pH which could lead to the manifestation of a latent *H. pylori* infection in the gastrointestinal tract (32, 49). The increased level of steroid hormones & human chronic gonadotrophins (HCG) during pregnancy lead to changes in pH & motility of GI tract, this change favor activity of *H.pylori* infection (27, 34, 50).

In this study we observed a significant difference between HP infection and anemia; HP infection was higher in cases than controls. Thirty two of our study participants, (21.3%) were anemic, of these participants, 71.9% (23/32) of anemic pregnant women were from HGM cases. The remaining 28.1% (9/32) anemic pregnant women were from non HGM control group and 73.9% were infected with *H.pylori* among HGM cases (X2= 5.547, p value=0.024). In this study, *H.pylori* infection was associated with anemia (OR =4.121, 95% CI=1.233-13.771). Our findings agreed with other studies done by Bezircioğlu İ et al., 2011 who reported anemia in 5 Hyperemesis gravidarum patients, 4 of them (80%) were HpSA positive(30).

Some possible mechanisms by which *H. pylori* affects iron metabolism include (1) decreased absorption resulting from chronic gastritis, (2) decreased gastric juice ascorbic acid concentration

which is known to facilitate iron absorption by reduction of iron III to iron II (51), (3) increased hepcidine production associated with *H. pylori* gastritis (32), (4) uptake of iron by *H. pylori* for growth (49), and (5) decreased availability of iron by sequestration of iron in lactoferin in the gastric mucosa (34). Similar possible explanations in different studies have been hypothesized in *H. pylori* infection, some of which are decreased mucosal iron absorption capacity due to low gastric pH, reduction of stomach vitamin C levels, bacterium-host competition for dietary iron supply, Lactoferrin mediated iron sequestration by gastric *H.pylori*, increased hepatocytes hepcidin release in response to Interlukin-6 production associated with *H. pylori* gastritis (31).

Our study revealed higher *H. pylori* positivity in pregnant women with hyperemesis gravidarum , this result is similar to previous studies reporting a positive rate of more than 50%, the relationship between hyperemesis gravidarum and *H.pylori* infection as showing in (Table 6.1).

7. Strengths and limitation of the study

7.1. Limitation of the study

- Determination of *H.pylori* infection was done by only one method (*H.pylori* stool antigen test kit); even though the HPSA kit employed in this study have sensitivity 98.8% and specificity 100% (user leaflet of kits).The test should be confirmed by enzyme-linked immunosorbant assay (ELISA) stool Antigen test. Because the Linear *Helicobacter Pylori* Ag cassette is limited to the qualitative detection of H. Pylori antigen in human fecal specimen.

7.2. Strengths of the study

- We attempted to indicate the association of *Helicobacter pylori* infection with hyperemesis gravidarum women and associated risk factors in the Ethiopian situation.
- The determination of *H.pylori* infection in this study was *H pylori* stool antigen test kits which are more sensitive and specific for current *H.pylori* infection than rapid *H.pylori* antibody test kits that might be indicated current or past infection and could possibly overestimate the prevalence of infection in many different studies.

8. Conclusion and recommendation

8.1. Conclusion

Our study suggested that there was a strong association between *H.pylori* infection and HG.*H.pylori* infection was associated with a low hemoglobin value. *H pylori* infected HG pregnant women showed higher rates of anemia than pregnant women without HG. Some expected *H.pylori* associated risk factors like presence of intestinal parasites; smoking habit; khat chewing and habit of drinking alcohol do not have significant associations with *H.pylori* infection in this study.

8.2. Recommendation

- The investigation of *H.pylori* infection as a potential factor that might play a role in the managements of occurrence of anemia and hyperemesis gravidarum in pregnant women should be further explored.
- Further studies are required in the community using different diagnostic methods to explore the actual role of *H.pylori* and we recommend that a large scale study be conducted to further elucidate the role of *H. pylori* infection and warranted to consider whether screening for *H.pylori* infection during pregnancy could benefit the mother and the fetus.

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10. Lists of annexes

Annex I. English version of participant information sheet

1. Participant information sheet

Department of medical laboratory Science, College of health science, Addis Ababa University, Addis Ababa, Ethiopia

Title- evaluation of the possible association between *Helicobacter pylori* infection and Hyperemesis gravidarum women visiting a selected Hospital and health Center, Kirkos 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 unclarity before you decide to participate or not you can ask freely.

Purpose

The main purpose of this research is on the investigation of the possible association between *Helicobacter pylori* infection and Hyperemesis gravidarum women visiting a selected Hospital and health Centre in Kirkos Sub city, Addis Ababa. This finding will be helpful for us to identify the prevalence and the main associated factors to H.*pylori* in pregnant women who are following antenatal care.

Aim of the study: The objective of this study is to investigate the possible association between *Helicobacter pylori* infection and Hyperemesis gravidarum women visiting a selected Hospital and health Centre in Kirkos Sub city, Addis Ababa

Procedure for the sample collection: stool specimen will be collected for *H.pylori* stool antigen test and for stool intestinal parasite examination and blood sample will also be collected from the vein by cleaning with 70% alcohol and some parts of the blood specimen collected will be used for investigation of routine anti natal care (VDRL, B\g and Rh) the remaining parts of the sample will be used for research purpose after the client willing to participate the study is confirmed in their signature. The consent agreement will be made by the physician/health officer or nurse at ANC Clinic.

Benefits of the study participant

Study participant are not having any financial incentive or other inducements from participants on their study. However, their result will be given and will be treated by prescribing a physician\health officer\nurse based of HPSA test result.

Risks and complication: There is not considerable risk to the study participants in participating in the study.

Confidentiality: In order to maintain the confidentiality of participants' information, the name will not be given and the sample will be coded. Participant will not be prohibited to stop or with draw at any time from the study. Only interested participant will retrieve their own laboratory results using their code number. The physician/health officer or nurse will be responsible for the interpretation of the results and providing treatments.

Investigator contact address,

1. Name: Yilikal Assefa

Tel: +251-0911-82-55-69

E-mail:yisakmooz@gmail.com/Y.assefa@cgiar.org

2. Addis Ababa University, College of Health Sciences, School of Allied Health Sciences, Department of Medical Laboratory Sciences.

Tel: +251-112-75-51-70
Fax: +251-112-75-46-69
E-mail: SMLT@ethionet.et

Annex II. Amharic version of the participant information sheet

የተሳታፊዎች ሚጃ ቅፅ ፡- አዲስ አበባ ዩኒቨርሰቲ የጠፍ ሳይንስ ኮሌጅ የህክምና ላብራቶሪ ሳይንስ ዲፓርትመንት

አርዕስት ፡- ማንኛውም በመመለድ ዕድሜ ከልል ወስጥ ያሉ ነፍሰጠፎ እናቶችን ሊያቀ የሚችል ስለ ጩራ ባክቴሪያ ሄልኮባክተር ፓዮሎፂ እና ሀይፐርሚሲስ ግራቪዳረም ያላቸውን ግንኙነት ለማኮናት በኪርቆስ ክ/ከተማ ስር በሚኝ ሆስፒታልና ጠፍ ጧሆች አዲስ አበባ ኢትዮጵያ

አጠቃላይ ሚጃ ፡- በጥናቱ በማተፎ ከልብ እያማነንን ከመሰኖ በፊት ይህንን ቅፅ በትክክል አንብቡ ወይም ሲነበብልዎ በትክክል ያዳምጡ እንዲሁም ግልፅ ያልሆኖሎትን ነገር በማሉ በነፃነት ይጠይቁ፡፡

ስለጥናቱ ሚጃ ፡- የጩራ ባክቴሪያ ሄሊኮባክተር ፓዮሎፂ ህማማን በነፍሰጠፎ እናቶች ወስጥ ያለው ስርጭት እና በህማማተኖው ላይ የተለያዩ ችግሮችን ሊያስከትል ይችላል፡፡ ለምሳሌ ለጎማማተኖው ምቾትን ይነሳል፣ ሆስፒታል የመማለስ ጊዜዎትን ያራዝማል፡፡ ስለዚህም የጩራ ህማማን ሊያማጡ የሚችሉን ባክተሪያና አባባሽ ነገሮችን ማወቅና ለማላከል ይጠቅማል፡፡

የጥራቱ ዓላማ - የጩራ ህማም ሊያማጡ የሚችሉ ሄሊኮባክተር ፓዮሎፂ ባክቴሪያና አባባሽ ነገሮችን ማእትም ሃይፐርሚሲስ ግራቪዳረም በነፍሰጠፎች ላይ ያለውን ስርጭት እና ግንኙነት ሁኔታ ማኮናት ነው፡፡

በጥናቱ ለተሳታፊዎች ያለው ጥቅም፡ - በጥናቱ ለሚሳተፉ ፈቃደኛ ተሳታፊዎች ምንም ዓይነት የገንዘብ ክፍያ የለም ፤ ነገር ግን በምርመራ ወጤት ማረት የመታከም ዕድል ይኖሮታል፡፡ በተጩማሪም የጥናቱ ወጤት የጩራ ህማም ለመቆጣጠር እና ለማላከል ስለሚጩቅም በተዘዋዋሪ መንገድ ሌላ ህማማተኖ እንዲሁም ህብረተሰቡን የመጥቀም ዕድል ያገኖሉ፡፡

በጥናት ተሳታፊዎች ላይ ያለው ጉዳትና ተዛማጅ ችግር ፡ - በዚህ ጥናት በማተፍ ሊደርስቦ የሚችል አንድም ጉዳት አይኖርም ፡፡ ለዚህ ጥናት የሚለግል ናማ ደምና ሰገራ የሚወሰድ ሲሆን ከማጩኖ ስሜት በስተቀር በጠፍዎ ላይ ምንም አይነት ጉዳት አይደርስም ፡፡

የመጃ ሚጥራዊ አጠባበቅ ፡ - መጃ በሚጠቁት ወቅትም ሆነ ከዛ በኋላ ባሉት ጊዜያት ማሉ በማሉ ሚጥራዊነቱ የሚጠቅና መጃወም የሚዘው በስም ሳይሆን በማያ ቁጥር ይሆናል፡፡ በጥናቱ እያሉ በፈለጉት ጊዜ የማቆም ወይም የሚረጥ መቦት አለት፡፡ የላብቶሪ ወጩን ማወቅ ከፈለጉ የማያን ቁጥሮ በማጩም በሚጥዎት የቀጠሮ ጊዜ መወሰድ ይችላሉ፡፡

ጥናቱን የሚካሂደው ሰው ሚጋገጫ ፡- ለዚህ ጥናት ኃላፊነትን በመወሰድ ማኛቸውንም ጥናቱን የሚማከቱ ጉዳይ ከትትል ለሚድረግና ለሚማከተው አካል መለጫ ለማጠት በፊርሜ አረጋግጣለሁ፡፡

ፊርማ---------- ቀን----------

ማንኛውም ጥያቄ ማጠየቅ ለሚጩ የሚቋጥለወን አድራሻዬን ማጩም ይችላሉ፡፡

ስልክ ሞባይል: +251-0911-82-55-69

1. ኢሜል: *yisakmooz@gmail.com /Y.assefa@Cgiar.org*
2. *አዲስ አበባ ዩኒቨርሲቲ ፣ የጠፍ ሳይንስ ኮሌጅ፣በስክል ኦፍ ኦላይድ ሄልዝ ሳይንስ፣ ህክምና ላቦራቶሪ ሳይንስ ዲፓርትመንት*
   ሰ.ቁ.፡- +251-112-75-51-70

Annex III. English version of informed consent

I have been informed about the objective of the study entitled "association between *Helicobacter pylori* infection and Hyperemesis gravidarum pregnant women visiting a selected hospital and health Centre, Kirkos Sub city, Addis Ababa." I am also informed that all information contained within the questionnaire is to be kept confidential. Moreover, I have been well informed of my right to refuse information, decline to cooperate and drop out of the study if I want and none of my actions was have any bearing at all on my overall health care. Therefore, with full understanding of the situations I agree to give the entire necessary information blood and stool sample for laboratory analysis. I have had the opportunity to ask questions about the project and received clarification to my satisfaction in a language I understand. I was also told that results for the blood and stool analysis was given to the health facility and that I may ask the information if 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 of informed consent

የፈቃደኝነት ሚረጋገጫ ቅፅ

ስሜ ይልቃል አሰፋ ይባላል ፡፡ በአሁኑ ሰዓት በአዲስ አበባ ዩኒቨርሲቲ በክሊኒካል ላብራቶሪ ሳይንስ የ2ኛ ድግሪ ፕሮግራም በዲያግኖስቲክና ፕብሊክ ሄልዝ ሜይክሮ ባይሎጂ ስፔሻሊቲ ትራክ እየተከታተልኩ እገኛለሁ፡፡ የጨራ ባክቴሪያ ሄሊኮባክተር ፓዮሎሪ ህመምን ሃይፐርሞሲስ ግራቪዳረም በነፍሰጡር እናቶች ላይ ያለው ስርጭት ለሞት የዳሰሳ ጥናት በማካሄድ እንዲሁም ህመምን የሚባብሱ ነገሮችን ለመወቅ በሚል ርዕስ ላይ ለማጥናት በተመለከተ በሚረገው ጥናት ላይ ልሳተፍ መሆኑን የጥናቱ አላማና ጥቅም ተገልፆልኛል፡፡በማጠቃለያ ላይ ያለው መሉ መረጃም በሚጥር እንደሚዝ ተነግሮኛል፡፡ በተጨማሪም ጥናቱ ወስጥ አለመሳተፍ መቡቴ እንደሆነና በማንኛውም ጊዜ ከጥናቱ በራሴ ወሳኔ መውጣት እንደምችልና በዚህም ምክንያት ምንም ዓይነት መጉላላት እንደሚደርስብኝ በማባ ተረድቻለሁ፡ ፡

ስለሆነም ሁኔታውን በማባ ተረድቼ በፍቃደኝነት በምርምሩ ላይ ለመሳተፍ በተሞራሚው ፈቃደኝነቴን ሰጥቻለሁ፡፡ በተጨማሪም የምሳጠው የደምና ሰገራ ናሙና ለተጠቀሰው ጥናት ብቻ እንደሚወል ተነግሮኝ ተስማምቻለሁ፡፡ ማንኛውም ያልገባኝን ነገር የማጠየቅ እድል ተሰጥቶኝ በማባን ቋንቋ መልስ አግንቻለሁ፡፡

በተጨማሪም የሁሉም የላብራቶሪ ምርመራ ወጠቶች በጊዜው ከትትል ለሚደርግልኝ የጠፍ ባለሞያ እንደሚሰጡ እና ወጠቱን ማወቅ ከፈለጉ ማግኘት እንደሚቻል ተነግሮኛል፡ ፡

እኔ ----------የተባልኩ ግለሰብ ይህን ሁሉ በማንዘብ በምርምሩ ላይ ስለ እኔ መረጃና የደምና ሰገራ ናሙና ለማሰጠት ተስማምቻለሁ፡ ፡

ፊርማ ---------- ቀን ----------

Annex V: English version questionnaires

To evaluate association between *Helicobacter pylori* infection and Hyperemesis gravidarum pregnant women visiting a selected hospital and health Centre, Kirkos Sub city, Addis Ababa,2016.

Facility Name _____year_____

Participant code _____

Participants address _____sub city_____Tel._____sign_____

Data collectors name _____date_____Sign_____

Code	**A. Sociodemographic information(tick)of study participant**		
01	Age (in years) _____		
02	Marital status (circle one)	1. Single 2.Married 3. Divorced 4. widowed	
03	What is your levels of education (circle one)	1. Illiterate 2. Primary school 2. Secondary school 4. University	
04	What is your occupational status? (circle one)	1. Government 3. Private 2. Non government Organization 4. House wife 5. House maid	
05	No of people in household?	1. Two 3. Four 2. Three 4. Greater than four	
06	Gravidity?	1. First pregnancy 2. Second pregnancy 3. Greater or equal to three	
07	Gestational period in week _____		
	B. Associated risk assessment for *H.pylori* infection		
08	Have you been experienced for Consumption of alcohol?	1. Yes 2. No	
09	Have you been experienced for Smoking habits?	1. Yes 2. No	
10	Have you been experienced for chewing chat?	1. Yes 2. No	

11	History of hyperemesis gravidarum?	1. Yes 2. No	
12	Did you have Possible history of Gastrointestinal illness?	1. Yes 2. No	
13	**C. Some hygienic Applications habits**		
Code	Washing hands before meals?	1. Yes 2. No	
14	Washing hands after toilet?	1. Yes 2. No	
15	Water use for drink (circle one)?	1. Tuncker water 2. Wheel water 3. Water source 4. Pipe water	
16	Final Helicobacter pylori Stool Antigen test result?	1. Positive 2. Negative	

Comments_____

Name of principal Investigator _____

Date_____

Annex VI: Amharic version questionnaires

በአዲስ አበባ ማስተዳደር ቂርቆስ ክ/ከተማ ጠፍ ጽ/ቤት ስር ባሉ ሆስፒታልና ጠፍ ጣቢያዎች ሄሊኮባክተር ፓዮሎሪ ባክቴሪያ እና ሀይፐርማህሰ ግራቪዳረም ነፍሰጠፎ እናቶች ላይ ለው ስርጭትና አባባሽ ነገሮችና ለማጥናት ነው፡፡

እባክዎን ለጥናቱ መሳካት ያግዘን ዘንድ ጥያቄዎችን በትክክል እንዲሞሉልን በትህትና እንጠይቃለን፡፡

የጠፍ ተቋሙ ስም..... የተጠየቁበት ቀን.....

የጥናቱ ተሳታፊ ማለያ ቁጥር.....

አድራሻ ፡- ከተማ ----------ክ/ክ----------ስልክ----------

ኮድ	ሀ. ማህበራዊዳታዎችእና ፣ሌሎችሚጃዎችንበተመለከተ	
01	እድሜበዓመት----------	
02	የጋብቻሁኔታ ሀ.ያገባችለ .ያላገባችሐ.የፈታችመ የሞተባት	
03	የትምህርትደረጃሀ .ያልተማረ ለ .የመጀመሪያደረጃ ት/ቤት ሐ.ሁለተኛደረጃ ት/ቤትመ ኮሌጅ/ዩኒቨርሲቲ	
04	ሥራ ?ሀ .የ መንግስትለ.መንግስታዊያልሆነ ድርጅት ሐየ ግልመ የቤትእመቤትሠ.የ ቤትሰራተኛ ረ.ሌላ (ይገለጽ)----------	
05	የ ቤተሰብአባላትብዛት ሀ.2 ለ.3 ሐ.4 መ >4	
06	ስንተኛ እርግዝናሽ ነው ሀ.አንደኛ ለ.ሁለተኛ ሐ.ሦስተኛናከዚያበላይ	
07	የእርግዝናሽ ጊዜ ስንት ነው? ሀ. 1-12 ሳምንት ለ. 13-24 ሳምንት ሐ. 25-40 ሳምንት	
	ለ.የሄልኮ ባክተር ፓይሎሪን ባክቴርያ እንፈክሽ ሊያባብሱ የሚችሉ ችግሮች	
08	አልኮል ማጠጥ ይጠጣሉ? ሀ. አዎ ለ. የለም	
09	የሜጨስ ልምድ አለት? ሀ. አዎ ለ. የለም	
10	ጫትይቅማሉ? ሀ. አዎ ለ. የለም	
11	በመጀመሪያ ወራት አከባቢ ህመማና መቅለሽለሽ ነበር? ሀ. አዎ ለ. የለም	
12	1. የ ጨጓራ ህመም ታመው ያወቃሉ? ሀ. አዎ ለ. የለም	
	የንጽህና አጠባበቅ ሁኔታን በተመለከተ	

13	ሀ. ምግብ ከመበላቴ በፊት እጇን እታጠበለሁ? ሀ. አዎ ለ. የለም	
14	ሽንት ቤት ከ በኋላ እጇን እታጠበለሁ? ሀ. አዎ ለ. የለም	
15	ለማጠጥ የምትጠቀሚው ውሃ? ሀ. የታንከር ውሃ ለ. የጉድጓድ ውሃ ሐ. የምንጭ ውሃ መ. የቧንቧ ውሃ	
16	የሜጪሻ የሄልኮ ባክተር ፓይሎሪ ባክቴሪያ ምርመራ ወጠት? ሀ. ፖዜቲቭ ለ. ነገቲቭ	

አስተያየት ----------

ማጠይቁን የሞላወላለሞያስም ----------

ቀን----------

Annex VI I. Standard operational procedure for laboratory investigation

Well-trained laboratory technologist were collect stool and blood specimens in order to ensure that appropriate stool specimen is obtained and for quality test of *H.pylori* stool antigen test and hemoglobin level determination.

9.1 SOP for Helicobacter pylori Stool Antigen test.

9.1.1 Purpose

The *H.pylori* Ag rapid test is a lateral flow chromatographic immunoassay for the qualitative detection of *H.pylori* antigen in human faecal specimen. It is intended to be used by professionals as a screening test and as an aid in the diagnosis of infection with *H.pylori*. Any reactive specimen with the *H.pylori* Ag Rapid test must be confirmed with alternative testing method(s) and clinical findings. The *H.pylori* Ag Rapid test uses a colloid gold conjugated monoclonal anti-*H.pylori* antibody and another monoclonal anti-*H.pylori* antibody to specifically detect *H.pylori* antigen present in the faecal specimen of an infected patient. The test is user friendly, accurate, and the result is available within 15 minutes.

9.1.2 Test principle

The *H.pylori* Ag rapid test is a sandwich lateral flow chromatographic immunoassay. The test strip consists of: a burgundy colored conjugate pad containing monoclonal anti- H.pylori antibody conjugated with colloid gold (anti-H.P conjugates) and a nitrocellulose membrane strip containing a test band (T band) and a control band (C band). The T band is pre-coated with another monoclonal anti-H.P antibody, and the C band is pre-coated with goat anti-mouse IgG antibody. When an adequate volume of extracted faecal specimen is dispensed into the sample well of the test cassette, the specimen migrates by capillary action across the cassette. H.pylori antigens if present in the specimen was bind to the anti-*H.pylori* conjugates. The immunocomplex is then captured on the membrane by the pre-coated antibody, forming a burgundy coloured T band, indicating an *H.pylori* positive test result. Absence of the T band suggests that the concentration of *H.pylori* antigens in the specimen is below the detectable level, indicating an H. pylori negative test result.

Reagents and materials provided

1. Individually sealed foil pouches containing:

- One cassette test device.
- One desiccant.

2. Sample extraction tubes, each containing 2ml of extraction buffer.

3. Plastic droppers for transferring watery stool.

4. One package inserts (instruction for use).

10.1.3 Test procedure

1. Bring the specimen and test components to room temperature if refrigerated or frozen. Mix the specimen well prior to assay once thawed

2. When ready to test, open the pouch at the notch and remove the test strip. Place the strip on a clean, flat surface.

3. Fill the plastic dropper with the specimen. Holding the dropper vertically, dispense 1 drop (about 30-45 μL) of specimen into the sample pad making sure that there are no air bubbles.

Then add 1 drop (about 35 – 50 μL) of Sample Diluent immediately and wait for 15 minutes

4. Set up timer

5. Results read within 15 minutes.

10.1.4 Test quality control

The test contains an internal control (C band) which should exhibit a burgundy coloured band of the immunocomplex of goat anti-mouse IgG/mouse IgG-gold conjugate regardless of the colour development on the T band. If the C band does not develop, the test result is invalid and the specimen must be retested with another device.

Interpretation of assay result of *H.pylori* test

1. Negative result: If only the C band is developed, the test indicates that no detectable *H.pylori* antigen is present in the specimen. The result is negative.

2. Positive result: If both C and T bands are developed, the test indicates the presence of *H.pylori* antigen in the specimen. The result is positive.

3. I**nvalid:** If no C band is developed, the assay is invalid regardless of any colour development on the T band as indicated below. Repeat the assay with a new test device. Excess faecal specimen can lead to invalid test results; if this is the cause, re-sample and re-test (see instructions for collection of specimen).

Safety precaution

Consider any materials of human origin as infectious and handle them using standard biosafety procedures.

9.2 SOP for hematological test (Complete blood count) for determination of Hemoglobin

9.2.1 Purpose

To perform hematological tests for determination of Hemoglobin level by Humacount 30TS hematology analyzer (Complete Blood cell Count, CBC).The Humacount 30ts are fully automated, bench top hematology cell counters. They implement the so called coulter method for counting cell passing through a small aperture, and measure the hemoglobin content of red blood cells.

9.2.2 Principle of hemoglobin concentration measurement

The lysed sample dilution can be measured by a photometric method. The reagent lyses the red blood cells, which release hemoglobin. The chemical process forms a stable form of methemogobin. This is measured by a photometer on the chamber.

9.2.3 Procedure

1. Prior to running patient specimens, perform blank measurement in case the instrument is not used for a specific time.
2. When the blank ok on the Screen, the instrument is ready to run specimens. Enter sample or patient data
3. With the cap tightly secured on the specimen tube, slowly invert the tube 10 to 15 times
4. Remove the cap from the pre-mixed specimen tube
5. Insert the sample to be analyzed in to sample holder tube
6. Press the start Plate to activate the run.
7. Then the sample has been taken in side of the analyzer, after aspirated from the tube, was move back. Remove the specimen tube and Recap the tube
8. After the cycle is completed, run results are displayed on screen and the current run data is saved to the data log.
9. Press [print report] to obtain a copy of the results. The print report format is the only method to be used for reporting patient results

9.2.4 Quality control procedures

Quality control feature allows tracing the operation and reliability of the analyzer in time. The best practice is to run a control sample every morning.

9.3 SOP for direct stool examination

9.3.1 Purpose of the test

For detection and identification of parasites in wet mount preparation of stool.

9.3.2 Principle of the test

The value of wet preparations lies in the fact that certain protozoa trophozoites retain their motility which may aid in their identification. Definitive identification however may not be possible, especially for amoeba, since the nuclei of trophozoites and cysts are often not clearly visible. Wet preparations on fresh unpreserved liquid stool should be performed and examined as soon as possible (within 30 minutes of passage) and on soft/formed stool within 60 minutes of passage provided that prior arrangements have been made with the lab.

9.3.3 Test procedure

1. Place a drop of fresh physiological saline on one a slide, to avoid contaminating the fingers and stage of the microscope, do not use too large a drop of saline

2. Using a wire loop or piece of stick, mix a small amount of specimen, about 2 mg, (matchstick head amount) with the saline .Make smooth *thin* preparations. Cover preparation with a cover glass. Sample from different areas in and on the specimen or preferably mix the faeces before sampling to distribute evenly any parasites in the specimen. Do not use too much specimen otherwise the preparations will be too thick, making it difficult to detect and identify parasites.

3. Examine systematically the entire saline preparation for larvae, ciliates, helminth eggs, cysts, and oocysts. Use the 10x objective with the condenser iris closed sufficiently to give good contrast. Use the 40x objective to assist in the detection and identification of eggs, cysts, and Oocysts. Always examine several microscope fields with this objective before reporting 'No parasites found'.

4. Report the number of larvae and each species of egg found in the entire saline preparation.

9.4 SOP for stool sedimentation concentration technique

9.4.1 Purpose of the test

Sedimentation methods (using centrifugation) lead to the recovery of all protozoa, oocysts, spores, eggs, and larvae present; however, the preparation contains more debris. If one technique is selected for routine use, the sedimentation procedure is recommended as being the easiest to perform and least subject to technical error.

9.4.2 Principle

By centrifugation, this concentration procedure leads to the recovery of all protozoa, eggs, and larvae present; however, the preparation contains more debris than is found with the flotation procedure. Ethyl acetate is used as an extractor of debris and fat from the feces and leaves the parasites at the bottom of the suspension. The formol ether sedimentation concentration is recommended as being the easiest to perform, allows recovery of the broadest range of organisms, and is least subject to technical error.

9.4.3 Test procedures

1. Using a rod or stick, emulsify an estimated 1g (pea size) of faeces in about 4 ml of 10% formol water contained in a screw cap bottle or tube from the surface and several places in the specimen.
2. Add a further 3–4 ml of 10% v/v formol water, cap the bottle, and mix well by shaking.
3. Sieve the emulsified faeces, collecting the sieved suspension in a beaker.
4. Transfer the suspension to a conical (centrifuge) tube made of strong glass, copolymer, or polypropylene. Add 3–4 ml of diethyl ether or ethyl acetate.
5. Stopper the tube and mix for 1 minute. If using a Vortex mixer, leave the tube unstoppered and mix for about 15 seconds (it is best to use a boiling tube). * Do not use a rubber bung or a cap with a rubber liner because ether attacks rubber.
6. With a tissue or piece of cloth wrapped around the top of the tube, loosen the stopper (considerable pressure will have built up inside the tube).
7. Centrifuge immediately at 750–1 000 g (approx. 3000 rpm) for 1 minute. After centrifuging, the parasites will have sedimented to the bottom of the tube and the faecal debris will have collected in a layer between the ether and formol water
8. Using a stick or the stem of a plastic bulb pipette, loosen the layer of faecal debris from the side of the tube and invert the tube to discard the ether, faecal debris, and formol water.

9. 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 resuspend and mix the sediment. Transfer the sediment to a slide, and cover with a cover glass.
10. Examine the preparation microscopically using the 10objective with the condenser iris closed sufficiently to give good contrast. Use the 40 objective to examine small cysts and eggs. To assist in the identification of cysts, run a small drop of iodine under the cover glass

9.4.4 Microscopic result interpretation

1. No ova of parasite seen, if there is no finding.

2. Examine systematically the entire saline preparation for larvae, ciliates, helminthes eggs, cysts, and oocysts.

9.5 Urine biochemical tests

Biochemical testing of urine is performed using dry reagent strips, often called dipsticks. A urine dipstick consists of a white plastic strip with absorbent microfiber cellulose pads attached to it. Each pad contains the dried reagents needed for a specific test. The person performing the test dips the strip into the urine, lets it sit for a specified amount of time, and compares the color change to a standard chart.

Procedure

1. Collect fresh urine specimen in a clean dry container with labeling
2. Mix well immediately before testing and enter in routine record book and give lab number too.
3. Open the cover and remove the one strip from strip bottle and replace cap.
4. Completely immerse reagent areas of strip in fresh urine and remove immediately to avoid dissolving out the reagent
5. While removing, run the edge of the entire length if strip against the rim of urine container to remove the excess urine.
6. Hold the strip in a horizontal to prevent possible mixing of chemicals from adjacent reagent or contaminating the hands with urine.
7. If reading visually compare the reagent areas to corresponding color chart on the bottle labeled at times specified.
8. Hold the strip close to color blocks and match carefully.
9. Avoid the layering of the strip directly on color chart as this will result on urine flow in the chart.

Declaration:

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

Yilikal Assefa _____ _____

Principal investigator Signature Date

Place: Addis Ababa University, school of medical laboratory science

This thesis has been submitted with my approval as University advisor

Kassu Desta (MSc, PhD fellow, Assistant professor) _____ _____

Advisor Signature Date

Place: Addis Ababa University, school of medical laboratory science