ADDIS ABABA UNIVERSITY SCHOOL OF ALLIED HEALTH SCIENCES

DEPARTMENT OF MEDICAL LABORATORY SCIENCES

PREVALENCE OF *HELICOBACTER PYLORI* INFECTIONS AND ASSOCIATED RISK FACTORS AMONG WOMEN OF CHILD BEARING AGES IN SELECTED HEALTH CENTERS, KOLFE KERANIO SUB CITY, ADDIS ABABA

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A THESIS SUBMITTED TO DEPARTMENT OF MEDICAL LABORATORY SCIENCES, COLLEGE OF HEALTH SCIENCES, ADDIS ABABA UNIVERSITY, IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR MASTER OF DEGREE IN CLINICAL LABORATORY SCIENCES (DIAGNOSTIC AND PUBLIC HEALTH MICROBIOLOGY).

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ADDIS ABABA, ETHIOPIA

Declaration

As thesis advisor, I hereby certify that I have read and evaluated this thesis prepared under my guidance, by Kumera Terfa; entitled: ' **Prevalence of *H.Pylori* Infections and Associated Risk Factors among child bearing ages of women in a selected Health Centre Kolfe Keranio Sub City, Addis Ababa**

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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 Kumera Terfa 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).

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Acronyms /Abbreviation

Ab: Antibody

Ag: Antigen

ANC: Antenatal care

ELISA: Enzyme linked immunoserbentassay

EQA: External Quality Control

FGR: Fetal growth restriction

GI: Gastrointestinal

Hb: Hemoglobin

HCl: Hydrochloric acid

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

SMLS: School of medical laboratory Sciences

SOP: Standard operational procedures

WHO: World Health Organization

I. 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.
- **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.

Abstract

Background: *H.pylori* is a gram-negative, spiral shaped, flagellated organism uniquely adapted to colonize the gastric mucous layer. It affects approximately one half of the world population and it is more prevalent in developing countries. The increased risk of infection is especially high among those living in the developing world due to precarious hygiene standards, crowded households and deficient sanitation associated with this part of the world.

Methods: A cross-sectional study was conducted in kolfe keraniyo sub city in two health centers (Wereda-5 & Woreda- 9) Addis Ababa, Ethiopia among child bearing ages of women, 195 pregnant and 137 non pregnant women matched for aged (16-40 years). A structured questionnaire was used to collect sociodemographic data of the study participants. Biological data such as blood samples were analyzed for determination of hemoglobin concentration and Stool samples were assessed for the presence of *H.pylori* infection by *H.pylori* stool antigens test kit and the presence of intestinal parasites were also assessed by direct stool examination (wet smear) and formol- ether concentration techniques.
Data were summarized in frequencies (%) and mean (SD) as appropriate. Chi-square tests, logistic regression was used in the analysis as needed. In all cases P-value <0.05 was considered as statistically significant.

Results: The overall prevalence of *H. pylori* infection among study subjects of child bearing ages of women was 29 %(96/332).The prevalence of *H.pylori* infection was higher in pregnant women than non pregnant women, 33.8% (66/195) among pregnant women and 21.9% (30 /137) among non pregnant women (X^2= 5.589 P value = 0.020).

In this study there was statistically association between some association of risk factors and *H.pylori* infection like pregnancy status (OR1.825, 95%CI=1.105-3.014, P=0.020), history Hypermesisgravidum in pregnant women (OR=1.652 95%C.I=1.262-2.162, P=0.00), history of gastrointestinal illness and low hemoglobin value, (OR=5.259, 95% CI=2.978–14.449, p=0.003).

There was no statistically significance between *H.pylori* stool antigen test positive results and sociodemographic characteristics of the study participants including like Maternal age (X^2 =3.670, P=0.435), marital status (X^2 =0.634, P=0.996), educational level (X^2 =3.033, P=0.387),

occupational status,),(X^2 =3.708, P=0.447), number of pregnancies (gravidity) (X^2 =8.314, P=0.140), number of people in house hold (X^2=2.479, P=0.140)**,** gestational age (age of pregnancy) (X^2 =7.879, P=0.069) and number of children (parity) (X^2=0.437,P=0.933).
And some expected risk factors like habits of drinking alcohol (P=0.677), cigarette smoking (P=0.468), chat chewing (p= 0.818), drinking tea and coffee (P=0.757), using water for drinking (P=1) and Intestinal parasites (P=0.306) between *H.pylori* stool antigen test positive results, however, the study subjects who had a habits of drinking alcohol; cigarette smoking and chat chewing were very few in numbers.

Conclusion: This study showed high prevalence of *H.pylori* infection among pregnant than non pregnant women. *H.pylori* infection was associated with a low hemoglobin value, history of gastrointestinal illness and presence of Hypermesisgravidum. *H.pylori* infected pregnant women showed high rate of anemia than non infected pregnant women. Some expected *H.pylori* associated risk factors like presence of intestinal parasites, smoking habit, chewing chat and drinking alcohol habit do not have significant association with *H.pylori* infection in this study.

Key words: Anemia**,** *H.pylori*, pregnancy, Hemoglobin concentration, *H. pylori* Stool Antigen test

1. Introduction

1.1 Background

Helicobacter pylori is a gram negative, spiral shaped, microaerophilic bacteria. It is one of the commonest bacterial infections worldwide and accepted as the cause of chronic active gastritis. [1]. Helicobacter pylori infection has been recognized as one of the most common chronic bacterial infections in humans and infecting more than half of the population of the world. The overall prevalence is high in developing countries [2]. It is a worldwide problem but the prevalence varies from country to country [2]. *H. pylori* infection is acquired in early childhood and becomes a chronic infection if left untreated [3]. The majority of infected people remain asymptomatic, and only small portions develop illness, usually in adulthood [4]. H. pylori cause upper gastrointestinal disease such as gastritis, peptic ulcer disease and also increase the risk of gastric cancer [2, 4].

H.pylori is most commonly found in the gastrointestinal tract and its existence is associated with chronic gastritis, gastro duodenal ulcer, gastric adenocarcinoma and Mucosa Associated Lymphatic Tissue Lymphomas (MALT). Moreover, the World Health Organization (WHO) has included *H.pylori* as a class I carcinogen due to its strong correlation with gastric cancer. Each year approximately 1million people lose their lives due to *H.pylori* infection [1, 2]. *H.pylori* is transmitted through the following routes: person-to-person, oral-oral or fecal-oral, and consumption of contaminated water and vertical transmission of *H.pylori* through breastfeeding may also occur. Most individuals infected with *H.pylori* remain asymptomatic. [3] The prevalence of *H.pylor*i closely tied to socioeconomic conditions and accordingly, this infection is more common in developing countries than in developed countries [4].

World Health Organization (WHO) estimates indicated that approximately 50% of the world's population is infected with *H. pylori*. As high rates of *H. pylori* infection are associated with low socioeconomic status and educational levels, poor housing and personal hygiene, overcrowding and unhygienic sources of drinking water, its prevalence is significantly higher in the developing countries than in the developed countries. *H. pylori* infection is predominantly acquired in early childhood, with the prevalence curve rising with increasing age, and persists throughout life, unless specific treatment is applied. Early detection and eradication of the organism can lead to long term healing of all *H.pylori* related infection [5].

Different studies point out that *H.pylori* prevalence can be related with a low socioeconomic status, low family income, educational levels, living in crowded homes, and having contaminated sources of drinking water and lifestyle habits, smoking and alcohol consumption are risk factors for *H.pylori* infection. For this reason it is important for every pregnant woman to be aware of the frequency of the infection and to participate in eradication [5].

Variation in the prevalence of infection between and among populations suggest that parameters such as age, cultural background, genetic predisposition, socioeconomic status and environmental factors all play a role in the acquisition and transmission of the organism. An improvement of living conditions like proper sanitation, drinking water and improving basic hygiene as well as balance diet and avoid overcrowding conditions are play a role in preventing against *H.pylori* infection [6].

H. pylori has been found in the stomachs of humans in all parts of the world. In developing countries, 70 to 90% of the population carries *H. pylori*; almost all of these acquire the infection before the age of 10 years (reviewed in reference. In developed countries, the prevalence of infection is lower, ranging from 25 to 50%. The data from developed countries also suggest that most infections are acquired in childhood. Most studies suggest that males and females are infected at approximately the same rates, although in at least one study, male sex was a significant risk factor for infection. In developed countries, persons of higher socioeconomic status have lower infection rates, although among certain ethnic minorities, high rates persist despite economic advancement [7].

The prevalence of *H. pylori* infection in women varies according to geographic area, socioeconomic conditions and method used to detect H. pylori infection. It's prevalence among pregnant women is about 20%-30% in most European countries, Japan and Australia, while it is 50%-70% in Turkey, Mexico and in Texas, US, more than 80% in Egypt and Gambia[6 ,7]

Serologic testing is the most common noninvasive diagnostic method for *H .pylori* and is relatively inexpensive and convenient; in my opinion a test that shows an active gastrointestinal colonization was more appropriate in diagnosis of the study subjects. *H.pylori* measured by *H. pylori* stool antigen test (HPSA) which is an enzymatic immunoassay detects bacterial antigen of actual ongoing infection in stool. It is a reliable noninvasive marker in the primary diagnosis and in the monitoring of post treatment out come in the *H.pylori* infection [8].

1.2 Statement of the problem

H.pylori plays a major etiologic factor in the pathogenesis of chronic gastritis, peptic ulcer disease, asymptomatic gastric adenocarcinoma, and mucosa associated lymphoid tissue lymphoma. It is one of the commonest bacterial infections worldwide and accepted as the cause of chronic active gastritis. Most patients continue through life with a chronic superficial gastritis while some develop either duodenal or gastric ulcer [9].

The putative/supposed pathogenic determinants of *H.pylori* can be divided into two major groups: virulence factors, which contribute to the pathogenic effects of the bacterium, and maintenance factors, which allow the bacterium to colonize and remain within the host. Virulence factors will contribute to the major pathogenic effects of *H.pylori*: gastric inflammation, disruption of the gastric mucosal barrier, and alteration of gastric physiology[10].

Infections with this bacterium cause considerable morbidity, and impose a major burden upon health care systems worldwide [5]. They are of particular concern in developing countries, where the prevalence of infection is often markedly higher than seen in the developed world. In various regions of sub-Saharan Africa, for example, 61–100% of the population may harbour the pathogen; young children have the highest prevalence [11].

Infection with *H.pylori* has been recognized as a public health problem worldwide (affecting approximately 50% of the world population and more prevalent in developing than the developed countries [11].

H. pylori seroprevalence of 20% has been reported in pregnant women and is associated with nausea and vomiting. *H. pylori* infection has been associated with both gastrointestinal and non-gastroenterological conditions such as chronic active gastritis, peptic ulcer, gastric Adeno carcinoma; mucosa associated lymphoid tissue lymphoma (MALT), and Cardiovascular disease [12]. *Helicobacter pylori* infection in pregnancy is also causes iron deficiency anemia, fetal malformations, miscarriage, and pre-eclampsia and fetal growth restriction. These pregnancy related-disorders are potentially life threatening for both mother and fetus/neonate. *H. pylori* are actually a causal factor, the public health implications would be important since the infection is treatable [13].

Generally, once *H.pylori* infection established it is persist thoughout life resulting in chronic gastritis, which is a risk factor for development of atrophic gastritis, reduced gastric acid output, and gastric cancer. Gastric acid is considered to be one of the most important luminal factors necessary for optimal nonheme iron absorption. Thus, it is possible that *H. pylori* infections can result in a reduced ability to absorb iron due to gastric atrophy and achlorhydria, or due to a more transient hypochlorhydria during active infections. Both mechanisms would result in an increased susceptibility to iron deficiency anemia. Pervasive occult gastrointestinal bleeding as a result of chronic active gastritis has also been considered as a cause for iron deficiency anemia in *H. pylori* infected subjects [14].

To the best of our knowledge, there is no study conducted in this area in particular and in Ethiopia in general, about *H. pylori* infection in pregnant and or child bearing ages of women hence conducting this study and address this issue may fill the existing gap.

1.3 Significance of the study

H.pylori chronically infects billions of people worldwide and is one of the most genetically diverse of bacterial species. Infection with the bacterium which leads to chronic gastritis, peptic ulceration, gastric cancers and gastric MALT lymphoma has been reported, however, there is no data available about the infection rate and the incidence of morbidity caused by the infection in study populations.

So that this study provides the current prevalence of *H.pylori* infection and its associated risk factors among the study subjects. The study also helps to explain the association between *H.pylori* infection, intestinal parasite and anemia among child bearing age of women and used to plan intervention activities in the future. Lastly the study serves as base line data for the upcoming researchers in this area.

2. Literature Review

Study in Chilean done by Hemiptera et al., 2015, shows that gastric discomfort of pregnant women and the continuity of severe symptoms of dyspepsia and hyperemesis are significantly correlated with *H.pylori* infection**.** Out of the total number of 274 pregnant women, 68.6% showed infection by *H .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 *H.pylori*. 73.4% of the pregnant women that showed gastric discomfort during the first three months had *H .pylori* infection. 53.7% of them continued with gastric discomfort after the first three months; of those, 95.8% were infected. *H.pylori* infection was present only in 1.5% of pregnant women without gastric discomfort [17].

A study conducted by Alvarado-Esquivel C et al., 2013, in US-Mexico border revealed the seroprevalence of *H.pylori* infection in pregnant women 33.3% among municipalities. In contrast, the seroprevalence was comparable among women regardless their age, educational level, occupation, socioeconomic status, foreign travel, contact with soil, crowding, sanitary conditions at home and educational level of the head of their families. The seroprevalence of *H.pylori* infection increased significantly with the number of pregnancies and rural pregnant women in Durango had a lower seroprevalence of *H.pylori* infection than those from populations in developing countries [18].

A Cross sectional Study done in France by Kalach et al., 2012, shows that *H.pylori* seroprevalence in asymptomatic pregnant women varies according to geographic origin. *H.pylori* seroprevalence is very low in Western European countries (10.6% in Finland and 15.5% in Belgium in comparison to those in other countries (44.8% in Turkey, 62.5% in the United States, and 88% in Egypt. *H.pylori* seroprevalence in the present study is approximately 21.5%, closely comparable to that of other Western European countries. *H.pylori* seroprevalence decreased significantly in French pregnant women from the first period to the second one: 18.7% (30 of 160) versus 11.2% (25 of 223) [19].

Study in Brussels, Belgium conducted by Lanciers et al., 1999, Shows 61/229 (26.6%) of the pregnant women had recently been infected with H. pylori, compared with 11% of the healthy, non pregnant population [20].

Study done in Izmir, Turkey by Bezircioğlu I et al., 2011, Shows that the pregnant women with Hyperemesis gravidarum have a significantly higher prevalence of *H. pylori* compared to pregnant women without Hyperemesis gravidarum subjects, (22.2%) and pregnant women without Hyperemesis gravidarum (2.8%) were established HpSA positive. But there was no significant difference between Hyperemesis gravidarum and control subjects in terms of age, gestational week, parity, educational level, socioeconomic status and smoking. There was anemia in 5 Hyperemesis gravidarum patients, 4 of them were HpSA positive. HpSA positivity was more prevalent in Hyperemesis gravidarum patients with anemia [21].

Study conducted in Ankara, Turkey, by Sinan et al., 2013, revealed us *H.pylori* Seropositive is high in both study and control groups, 67.7% and 79.3%, respectively. There was no find an association between Hp and Hypermesisgravidum. The low social status of women in both groups could be one of the reasons for the high prevalence of Hp infection [22].

Studies conducted in Turkey by Karaca, N et al., 2014, Shows that the seropositivity of *H .pylori* in the Hypermesisgravidum group was significantly higher than in controls and this difference was more pronounced in the groups of lower socioeconomic status. It was shown that the incidence of transmission is high because the childbearing tendency in families with low socioeconomic status in Turkey is high [23].

Study done in Iran by Kazemzadeh M.et al., 2014, shows that out of 175 pregnant women; 78 women with Hypermesisgravidum and 97 without, both groups had no statistically significant difference according to age, gestational age, and gravidity. 51 women out of 78 (65.4%) in Hypermesisgravidum group and 43 women (44.3%) in the control group were IgG positive for *H.pylori*, which shows a significant difference between the different variables of age, gestational age, gravidity and HP infection, only *H.pylori* infection was found as a risk factor for Hypermesisgravidum [24].

Study in Jeddah, Saudi Arabia, by Ahmed R. et al., 2014, Shows a significantly high prevalence of *H.pylori* among pregnant with hyperemesis gravidarum compared to control group (80% vs. 15 %,) and anemia (Hb level <11gm/dl) were found to have significantly higher in HPSA positive compared to control group [25].

Study done in Iraq, Baghdad by Meroj et al., 2011, Found that pregnant women infected with *H.pylori* had lower mean hemoglobin (Hypermesisgravidum) level, the blood loss in chronic gastritis, and bleeding from duodenal or gastric ulcers related to *H.pylori* infection, plays an important role in the development of iron deficiency [26].

Another Study done in India by Ria Malik et al., 2011, Shows that high prevalence of *H .pylori* infection was seen in pregnant women with anemia compared to pregnant. A routine Hb screening at the first visit was adopted in the hospital, according to which 57.05% had mild to moderate anemia (Hb 7 to 10.9 g %) and 1.26% had severe anaemia (Hb <7 g %). Pure iron deficiency in mild to moderate anemic women was found to be 39.8 %. In the present study, 62.5% of these subjects had infection with *H.pylori*, indicating a high prevalence in our pregnant population with anemia [27].

Studies conducted in different parts in Africa by Tanih N et al., 2008, showed that a the majority of study subjects are infected with the organism (61-100%), more than 50% of children are infected by the age of 10 years, the prevalence rising to more than 80% in adults. Recently, report show that an *H .pylori* prevalence of 50.6% in their study in Venda, North of South Africa. In the D. R.Congo, 62.4% of participants tested positive for *H .pylori* antibody, while a prevalence of 93% was detected in Ethiopia [28].

Study Conducted in Egypt by Samir B. et al., 1999, showed that *H.pylori* prevalence was of the 169 mothers tested 148 (88%) were seropositive for *H.pylori* IgG antibodies. Although maternal age, number of pregnancies, and number of past deliveries were not associated with the serostatus of the mother, maternal level of education was related. 93% of the educated mothers were infected with *H.pylori* compared with 79% of the mothers without Formal education. The significant relationship between maternal education and *H.pylori* serostatus remained after adjusting for age of the mother [29].

In Africa other different studies demonstrated by Tamer H. et al, 2007.Out of examining 857 pregnant women aged 20–40 years living on Pemba Island, Zanzibar, Tanzania. They show that a low proportion of pregnant women (17.5%) had an active *H. pylori* infection. Typical prevalence rates among a variety of groups on the African mainland are≥80%; prevalence data from pregnant woman typically reflect *H.pylori* prevalence among the general population. [30].

Study done in four districts of Uganda by Rhona K et al, 2014, shows that the prevalence of H. pylori infection in pregnant women was 45.2% but varied by geographical location from 18.2% in Apac District to 60.5% at Kawempe Health Centre. At 18.4%, the Langi ethnic group, who were enrolled exclusively in Apac District, had the lowest prevalence of H pylori infection while the Gisu had the highest prevalence (58.4%). H. pylori was independently associated with enrollment at clinics not in Apac and with using water from public wells, boreholes or springs and from rivers, lakes or streams Urban residence and no formal education were also independently associated with H .pylori infection [31].

In other Study conducted in Sudan, Khartoum, by Mubarak N.et al, 2014.Shows that among 179 women, of these women, 42/179 (24.3%), 50/179 (28.9%) and 19/179 (11%), respectively, were anemic (hemoglobin <11 g/dl) or iron deficiency anemia. There was no association between *H. pylori* infection and anemia, iron deficiency or thrombocytopenia there was no association between the expected risk factors (age, parity, and education) and *H.pylori* seropositive [32].

The study conducted by D. Kibru et al 2014, at Butajira hospital, Butajira town, Gurage Zone, Southern Nations Nationalities, and People's Region, Ethiopia shows that the overall prevalence of *H.pylori* infection was 52.4% and it was significantly associated with age, presence of intestinal parasites, smoking habit, and alcohol drinking habit. The prevalence of anemia among *H.pylori* infected patients (30.9%) was significantly higher than uninfected patients (22.5%). Intestinal parasitic infection in this study was significantly associated with *H.pylori* infection [33].

❖ **Hypothesis**

The prevalence of *H. pylori* infection was higher in pregnant women than non pregnant women of child bearing ages.

3. Objectives

3.1 General objective:

- To determine the prevalence of *H.pylori* and its associated risk factors among women of child bearing age who attend antenatal care and non pregnant women visiting in a selected health Centre, Kolfe Keranio Sub city, Addis Ababa.

3.2 Specific objectives:

- ❖ To determine the prevalence of *H.pylori* infection among women of child bearing age in a selected Health Centre, Kolfe Keranio sub city, Addis Ababa.
- ❖ To assess *H.pylori* infection and its associated risk factors among women of child bearing age.

4. Methods and Materials

4.1 Study design and period

A cross-sectional prospective study was conducted from April to June 2015.

4.2 Study Area

The study was conducted in a selected health centers (Woreda 5 & Woreda 9 health centers) in Kolfe Keranio sub city, Addis Ababa, Ethiopia, which were among the most patients visiting health centers in Addis Ababa.

Moreover, the health centers were delivering service for about 100-150 clients per day, the average a women of child bearing age following antenatal care and Visiting outpatient department were averagely 20-30 and 50-65per a day respectively.

The health centers laboratory providing services in the health centers compromises all disciplines except cultures, molecular techniques & electrolyte analysis In besides to participating in quality related issues

4.3 Study population:

4.3.1 Source population:

All women of child bearing ages who were attended antenatal care clinics and visiting outpatient department (OPD) in a selected health centers (Woreda -5 and Woreda-9) during study period

4.3.2 Study population:

All women of child bearing ages who were attended antenatal care clinics and visiting outpatient department (OPD) from the source population during the study period were involved.

4.4 Inclusion and exclusion criteria:

4.4.1 Inclusion criteria:

Child bearing ages of pregnant and non-pregnant women who were voluntary to participate in the study was included.

4.4.2 Exclusion criteria:

Women were with triple therapy in the past two weeks.

Non pregnant women above forty five years of age and below sixteen years of ages were excluded

4.5 Study variables:

4.5.1 Dependent variables:

H.pylori prevalence

4.5.2 Independent variables:

Sociodemographic (Age, Educational Level, Employee, gravidity, parity number of Pregnancy, gestational period, some behavioral risk factor(Smoking Habit, Drinking Alcohol and Chat Chewing Habit) and Level of Hemoglobin Concentration, Intestinal Parasite,.

4.6 Sample size and sampling technique

4.6.1 Sample size determination

There is no study conducted in our country, therefore, the sample size determination was done by taking 86% prevalence method because of different study in Africa shows that the prevalence of *H.pylori* infection in pregnant Women is greater than or equal to 68%.

So for the determination of sample size, previous prevalence was used, 68% [5, 15].

So that determination of sample size is calculated by single population proportion

$n = \underline{z (a/2)^2 p (1-p)/ d^2}$

Sample size =n

Level of significance =0.05

Marginal of error (d) = 5%

P = 68%

Z a/2 = Z-score at 95%

Confidence interval; which is 1.96

$$n = \frac{z (a/2)^2 p (1-p)}{d^2}$$

$$n = \frac{(1.96)^2\ 0.68\ (1-0.68)}{(0.05)^2}$$

10% non response rate = (10*309)/ 100 =309+23 = **332**

The minimum sampler size for study subjects were = **332**

4.6.2 Sampling Technique

Consecutive sampling technique was employed to include study participants who met the inclusion criteria. Stool and blood sample collection was continued until the achievement of the expected sample size from pregnant women at ANC and non pregnant women in OPD Department of the health centers from March to June 2015.

4.7 Data collection and processing

I. 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 hypermesisgravidum, History of gastrointestinal illness, water used for drinking, habits of hand wash before meals and after toilet used, habits of smoking, Alcohol drinking, Chat chewing, drinking tea and coffee) were obtained using a predesigned questionnaire through interview by data collectors.

II. Biological data Collection and Processing

Well-trained laboratory/technologist/technician was collected stool and blood specimens in order to ensure that appropriate stool specimen was obtained and intestinal parasite identification was done by direct smear microscopy and Formol-Ether Stool Sedimentation Concentration technique, *H.pylori* stool antigen test, hemoglobin level determination were also performed [35, 36].

A. Stool Specimen collection and transportation

Stool specimen collection and handling: Stool sample was collected in a clean, dry clean stool cup. During the study, a total of 332 fresh stool samples were collected strictly following standard operational procedures with sterile stool cup [36, 37]. 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 [38, 39, 40].

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 was 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 are no air bubbles. Then add one drop (about 35 –50μL) of Sample diluents immediately. 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 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, 38].

Stool sample processing for direct stool examination (wet smear): a drop of normal saline was put 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 was examined using 10x objective and cysts and trophozoites was examined using 40×objectives, this aid to detect certain protozoa trophozoites retain their motility which may aid in their identification[40,41,42].

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 was mixed thoroughly and let 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 centrifuge 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. Centrifuge for 10 min at 500 x g. The sediment were examined using 10 X and 40 X microscopic examination [40, 41, 42].

B. Blood specimen collection ,handling, Storage and transportation:

By explanation of the blood drawing procedure to the client a total of 332 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, mix the blood properly by inverting the tube 6-8 times immediately after collection to avoid formation of small clots. the blood sample was not refrigerate or freeze and avoid extremes of temperature so that specimens was not freeze or get heated above 32 ^{0}C, Arrange for immediate transport of the sample to testing laboratory sites to perform hematological tests for determination of Hemoglobin level by Humacount 30^{TS} hematology analyzer (Complete Blood cell Count, CBC).

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 lysing the red blood cells, which release hemoglobin. The chemical process was form a stable form of methemogobin. This is measured by a photometer on the chamber. The Humacount 30^{TS} 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. Quality control was run a control sample every morning.

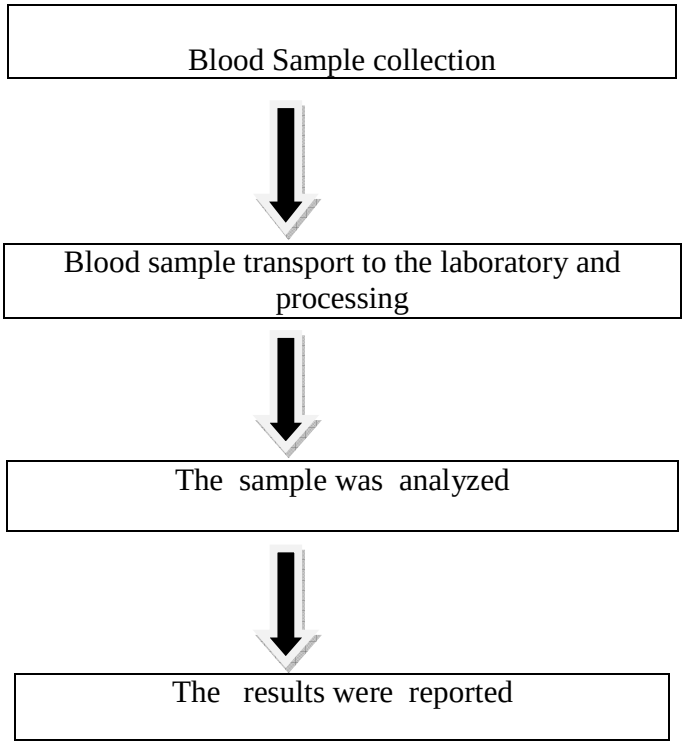

Fig 4.1 Work flow for complete blood count

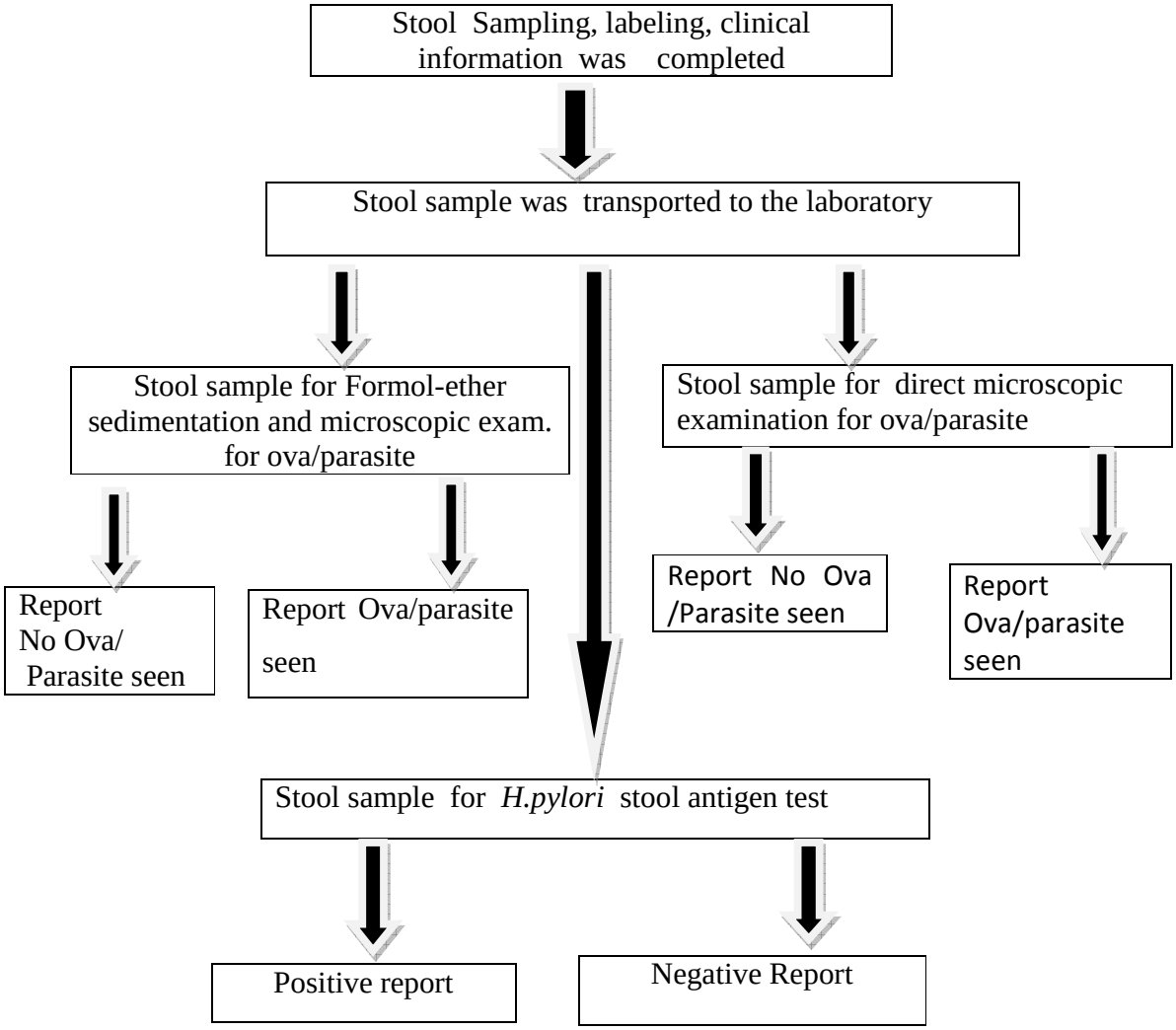

Fig 4.2 Work flow of stool sample for *H.pylori* stool antigen test, direct microscopic stool examination and Formol-ether sedimentation and microscopic examination for ova/parasite

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 investigators and supervisors.

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 principal investigator extremely was tried to collect good quality sample and analyzed it and produced 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 was assessed with known positive and negative controls materials, well-trained and experienced laboratory professionals was participated in the laboratory analysis procedure. Finally the result was checked by the supervisors.

4.8.3 Post analytical phases

The results was 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 it was need 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 and intensive supervision was followed during data collection by the principal investigator .The confidentiality of this data was kept secret and the result was disclosed by code. The codes of the health centers were notified for the participant facilities prior to completion of the study. The health centers have right to get the copy of the findings.

4.10 Data Analysis and Interpretation

Data was entered and analyzed using Excel, SPSS version 19.0 and results was 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. Anemia was defined according to the WHO definition as a hemoglobin concentration of < 12 g/dL for adult women [27].

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 consideration

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. Permission was obtained from Addis Ababa City Administration Health Bureau; Kolfe Keraniyo Health Office to the participant health centers. Moreover, privacy and confidentiality was 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 subjects was started after they gave informed consent. Data collection was started after permission was obtained from each health centers of the study sites, no name and other identifier on the questionnaire. Results were communicated with physician/health officer/Nurses for proper managements of the study subjects.

4.12 Dissemination of results

The result of this study was presented first to Department of Medical Laboratory Sciences, then to the scientific community through scientific presentation and finally we will sent to publication on peer reviewed scientific Journals. Participant results were communicated to the attending health personnel in the health centers.

5. Results

5.1. Study subjects

In this study a total of 332 women in the child bearing age were involved to investigate the prevalence of *Helicobacter pylori* infection and its association risk factors. About 58.7 % (195 women) were pregnant. The age of the study subjects were ranged from 16-40 years, with a mean (± SD) age of 27.3± 4.7 years.

Majority of the study participants were married (80.5%) and few of them (2 %) were divorced. From the study subjects 10.8 % (36/ 332) were illiterate and 38 % (126/322) working in private institute. Most child bearing women (75.9 %) live with a family number of four or more. (**Table 5.1)**

Table 5.1 Sociodemographic characteristics of the study participants in a selected health centers from April 2015 to July2015 (n=332)

Characteristics	**Frequency, N**	**Percentage, (%)**
Age group		
16-20	42	12.6
21-25	119	35.8
26-30	95	28.6
31-35	50	15.2
36-40	26	7.8
Marital status		
Single	58	17.5
Married	267	80.5
Divorced/widowed	7	2.0
Education level		
Illiterate	36	10.8
Primary school	105	31.7
Secondary school	85	25.6
Higher education	106	31.9
Occupational status		
Government	62	18.7
NGO	22	6.6
Private	126	38
house wife	114	34.3
house maid	8	2.4

Number of people in house hold		
Two	4	1.2
Three	76	22.9
Four	138	41.6
Greater than four	114	34.3
Pregnancy status		
Pregnant	195	58.7
Non pregnant	137	41.3

Among the pregnant women, 46.7 % (91/ 195) and 30 .7 % (60/195) were in the first and second trimester respectively and 41.5 % (81/195) and 39.5 % (77 /195) pregnant women were within a gestational period of 13-24 weeks and 1-12 weeks respectively .

A few numbers of study participants had a habit of drinking alcohol, cigarette smoking, chat chewing .Majority of them had an experience of drinking tea & coffee (81.3 %) and almost all women used pipe / tap water for drinking purpose and wash their hands before meals and after toilet used (99.7%).

One hundred ninety of, 332 (57 %) of the study participants had experience of gastrointestinal illness and among pregnant women study participants, 74.4 % (145/195) had history of hypermesisgravidum while the remaining 50 (25.6%) had no history of hypermesisgravidum. Intestinal parasites were identified in 71 (21.4%) of all the study participants and 42.3 % (30 /195) of pregnant women had intestinal parasites **Table5.2**.

Table 5.2 Behavioral characteristics & some expected associated risk factors of the study participants in a selected health centers from April 2015 to July2015(n=332)

Characteristics	Frequency, N	Percentage, (%)
Habit of alcoholism		
Yes	6	1.8
No	326	98.2
Smoking habit	1	0.3
Yes	331	99.7
No		
Chat chewing habit		
Yes	2	0.6
No	330	99.4
Drinking coffee & tea		
Yes	270	81.3
No	62	18.7
Intestinal parasite		
Negative	261	78.6
Positive	71	21.4
Sources of water for drinking		
pipe water	331	99.7
Tuncker	1	0.3
wheel	0	0
water source	0	0
History of gastrointestinal illness		
Yes	190	57.2
No	142	42.3

5.2. Prevalence of *Helicobacter pylori* infection among Women of child bearing age

The overall prevalence of *H.pylori* infection among study participants was 29 % (96/332). *Helicobacter pylori* prevalence was higher among pregnant women than non pregnant women 33.8 % (66/195) versus 21.9 % (30/137) respectively (X2=5.589 p value=0.020). Women with age group of 31- 35 years had the highest prevalence of *H.pylori* infection 18 / 50 (36%).

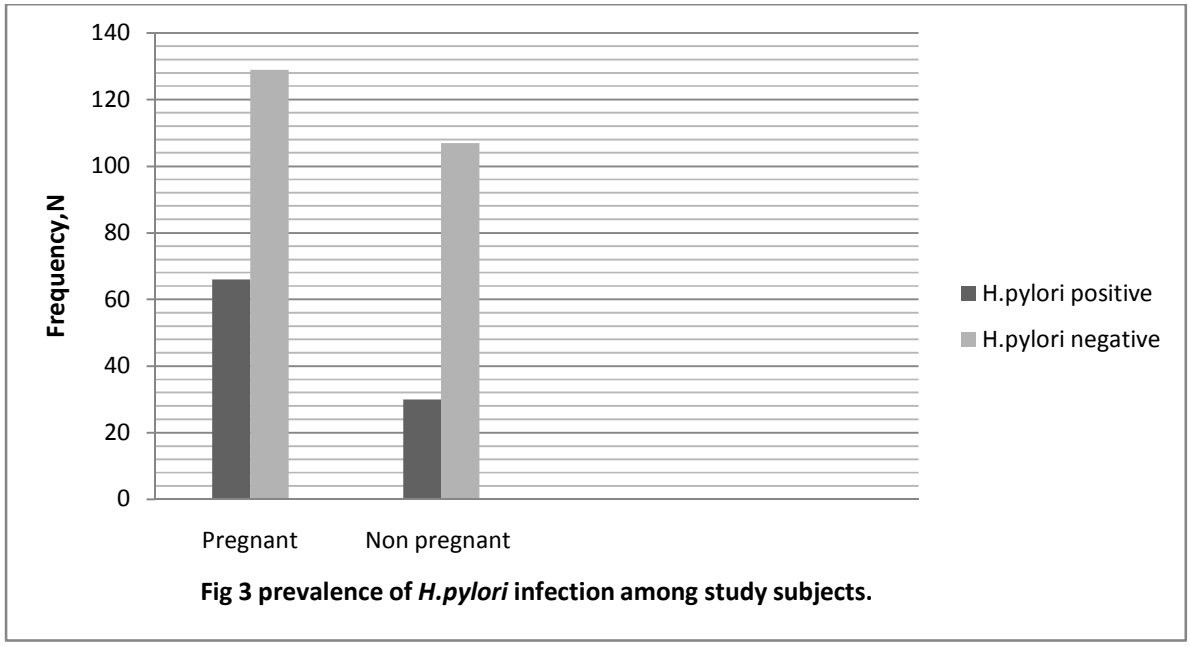

Fig 3 prevalence of *H.pylori* infection among study subjects.

There was no difference between prevalence of *H.pylori* infection and, some sociodemographic characteristics like marital status, educational background, and occupational status of the study subjects.

Women with third trimester gestational age had the highest prevalence of HPSA 43.2% (16 / 37) and lowest in second trimester with prevalence of 29.6 % (24/81). The prevalence of HPSA was lowest among women without children than those with two or more children (13.6% versus 39.4 % respectively). There is a slight increment of *H.pylori* infection as the number of people living in house hold increased **Table 5.3**.

Table 5.3 Prevalence of *H.pylori* infection by socio demographic features among study participants in a selected health centers from April 2015 to July2015. (n=332)

Variables	HPSA		X^2	P value
	Positive	Negative		
Age group				
16-20	8	34		
21-25	33	86	3.670	0.453
26-30	30	65		
31-35	18	32		
36-40	7	19		
Marital status				
Single	17	190	0.063	0.996
Married	77	41		
Divorced/widowed	2	5		
Education level				
Illiterate	7	29		
	30	75	3.033	0.387
Primary School	23	62		
Secondary school	36	70		
Higher education				
Occupational status				
Government	22	40		
NGO	4	18	3.708	0.447
Private	35	91		
house wife	34	80		
house servant	1	7		
Gestational week				
1-12 week	26	51		
13-2 4week	24	57	7.879	0.069
25-40 week	16	21		
No. of people in house				
Two	2	2		
Three	26	50	2.479	0.140
Four	36	102		
Greater than four	32	82		
Gravidity / no. of pregnancy				
One	28	63	8.314	0.140

Two	24	37		
Greater than three or equal to three	14 30	29 107		
Number of children (parity)				
No children	53	9		
one	35	22	0.437	0.933
two	29	26		
Three and above	12	9		

HPSA: Helicobacter pylori stool antigen

The Prevalence of *H.pylori* infection among the pregnant women and non pregnant women study participants were 33.8% (66/195) and 21.9% (30/137) respectively. Majority of the study participants had no the experiences of habits of drinking alcohol, smoking habits, chat chewing habits. But they were frequently drinking tea and coffee, So that there was no association between the expected risk factor and *H.pylori* infection in this study. Almost all of the study subjects had a habit of hand washing before meal and after toilet used, and was used tap/pipe water for drinking purpose.

Ninety six of 332(29.0%) *H.pylori* infected study participants had experience of gastrointestinal illness and the study participated pregnant women *H.pylori* stool antigen positive also revealed that 61/145(42%) had a history of hypermesisgravidum while only, 5/45(10%) *H.pylori* stool antigen positive had no history of hypermesisgravidum.

Intestinal parasites were identified in 25% (24/96) *H.pylori* infected study participants. While 75% (74/96) *H.pylori* infected study subjects were negative for Intestinal parasites. (**Table 5.4)**

Table 5.4 Prevalence of *H.pylori* among some associated risk factors of study participants in a selected health centers from April to July, 2015

Variables	HPSA		X^2	P value
	Positive	Negative		
Pregnancy				
Pregnant	66	129	5.589	0.020*
Non pregnant	30	107		
Habit of alcoholism				
Yes	1	5	0.446	
No	5	321		0.677
Smoking habit				
Yes	0	1	0.468	1
No	96	235		
Chat chewing habit				
Yes	0	2	0.818	1
No	96	234		
Drinking coffee & tea				
Yes	77	193	0.111	0.757
No	19	43		
Washing hands before meal and after used toilet				
Yes	96	236	0.498	1
No	0	0		
History of gastrointestinal illness				
Yes	66	124	7.323	0.007*
No	30	112		
water for drinking purpose				
pipe water	96	235		
Tuncker	0	1	0.408	1
wheel	0	0		
water source	0	0		
Intestinal parasite				
Negative	72	189	1.049	0.306
positive	24	47		
Anemia status				
Anemic	44	17	68.61	0.000*
Non anemic	52	219		

*Statistically significant, HPSA -H.Pylori Stool Antigen test, NGO-Nongovernmental organization

5.3. Comorbidity of *H.pylori* infection and Anemia in Women of child bearing age

It is interesting to see a significant difference between HP infection and anemia, HP infection is more common in pregnant women than non pregnant women. The overall prevalence of anemia in women of child bearing age is 18.4% (61/332) [Hemoglobin value < 12 gm/dl). The prevalence of anemia is highest among pregnant women than non pregnant women, 24.6% (48/195) versus 9.5 % (13/137) respectively (X2= 68.61, p value=0.000). About 55.7 % (34/61) of anemic women were also infected with *Helicobacter pylori*, 64.6%, (31/48) versus 23% (3/13) were pregnant and non pregnant women respectively.. In this study, *H.pylori* infection was associated with anemia (OR = 5.259, 95% CI=2.978–14.449, p=0.003).

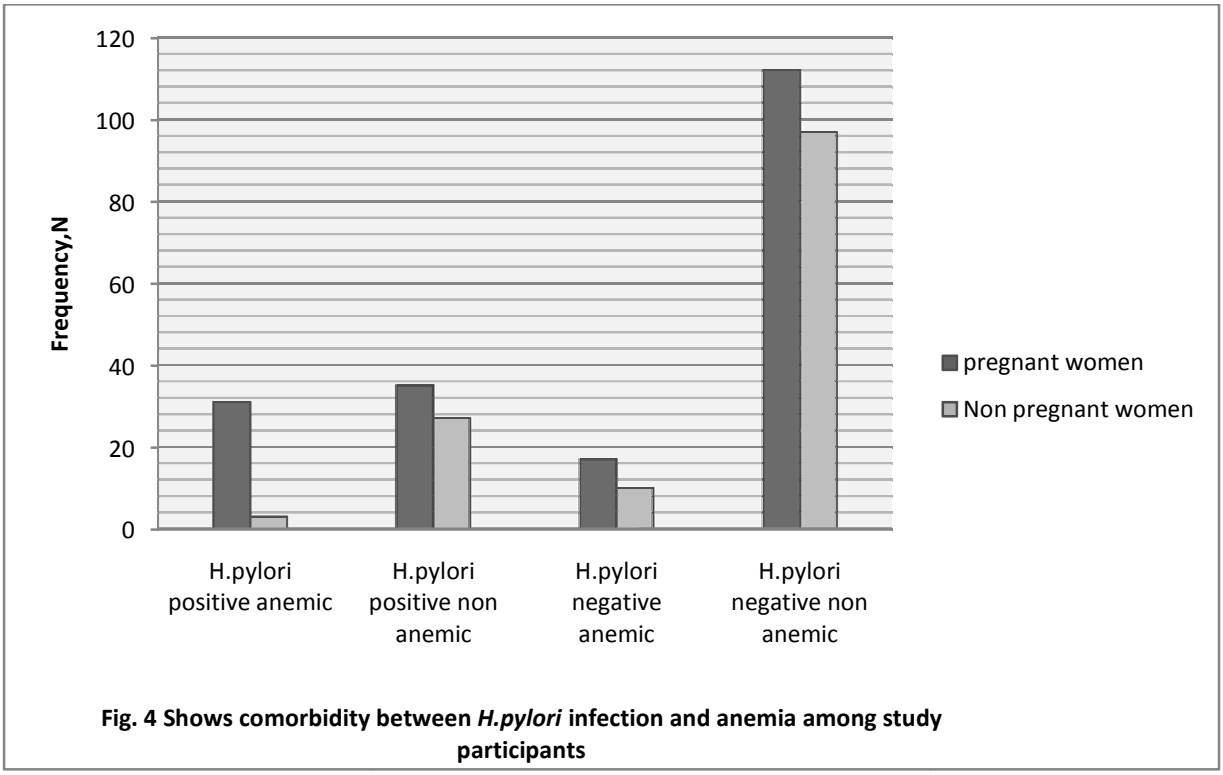

Fig. 4 Shows comorbidity between *H.pylori* infection and anemia among study participants

5.4 Association between *H.pylori* infection and Hypermesisgravidum among pregnant women

It was shown that *H pylori* infection was significantly high in the pregnant population with hyperemesis gravidarum, 74.3% (145/ 195) of study participants pregnant women had a history of hypermesisgravidum while only, 10%(5/50) of study participant had no history of hypermesisgravidum. Among pregnant women *H.pylori* stool antigen positive, 94.2% (61/66) of them were *H.pylori* infected with hypermesisgravidum.

Table 5.5 Prevalence of *H.pylori* among pregnant women with and without hyperemesis gravidarum in selected health centers from April 2015 to July, 2015

characteristics	HP positive		HP negative		X2	P- value
	No.	%	No.	%		
Pregnant women with hyperemesis gravidarum	61	42	84	58	22.416	0.000*
Pregnant women without hyperemesis gravidarum	5	10	45	90		

*Statistically significant, HP –Helicobacter Pylori

5.5. Risk factors

There was no statistically significance association between *H.pylori* infection and sociodemographic characteristics of the study participants including maternal age (X^2 =3.670, P=0.435), marital status (X^2 =0.634, P=0.996), educational level (X^2 =3.033, P=0.387), occupational status,), (X^2 =3.708, P=0.447), number of pregnancies (gravidity) (X^2 =8.314, P=0.140), number of people in house hold (X^2=2.479, P=0.140)**,** gestational age (age of pregnancy) (X^2 =7.879, P=0.069) and number of children (parity) (X^2=0.437, P=0.933).

There was also no statistically significant association between the expected risk factors, like habits of drinking alcohol (P=0.677), cigarette smoking (P=0.468), chat chewing (p= 0.818), drinking tea and coffee (P=0.757), water source (P=1) and presence of Intestinal parasites (P=0.306), however, the study subjects who responds that had a habits of drinking alcohol; cigarette smoking and chat chewing were very low in numbers that make comparison difficult.

While, in this study there was statistically significant association between *H.pylori* infection and pregnancy status (OR= 1.825, 95%CI=1.105-3.014, P=0.020), history of hypermesisgravidum in pregnant women (OR=1.652 95%C.I=1.262-2.162, P=0.00), history of gastrointestinal illness and anemia status (OR=5.259, 95% CI=2.978–14.449, p=0.003).

In this finding, 96 pregnant women (49.2%) had History of gastrointestinal illness and 41 (62.0%) of them were also had *Helicobacter pylori* infection .There is significant association between *H.pylori* infection and gastrointestinal disease (OR=1.987, 95 %CI=1.203-3.281, P=0.007).

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Table 5.6 Prevalence of HPSA and some associated risk factors among women of child bearing ages in selected health centers from April to July, 2015.

Variables	Number of tested, N	HPSA positive N (%)	AOR(95%CI)	P-value
Pregnancy status				
Pregnant	195	66 (68.75)	1.825 (1.105-3.014)	0.020
Non pregnant	137	30 (31.25)	1	
History of gastrointestinal illness				
Yes	190	66(68.75)	1.987(1.203-3.283)	
No	142	30(31.25)	1	0.007
History of Hypermesisgravidum				
Yes	145	61 (92.4)	1.652(1.262-2.162)	
No	50	5 (7.6)	1	0.00
Level of hemoglobin determination				
Anemic	61	44(46.0)	5.259(2.978–14.449)	0.003
Non anemic	271	52 (54.0)	1	

HPSA -H.Pylori Stool Antigen test

6. Discussion

To the best of our knowledge, information concerning prevalence of *Helicobacter pylori* among women of child bearing age and pregnant women in particular is not available in Ethiopia. In this study we found, an overall prevalence of 29 % (96/332). Prevalence among pregnant women (33.8%, 66/195) is higher than non pregnant women (21.8 %, 30/137). This finding is similar with study conducted in US-Mexico by Alvarado-Esquivel et al., 2003, and In Nigeria by Ugwuja et al, 2010, that reported seroprevalence of 33.3 % and 24.1% among pregnant women respectively. However, the laboratory methods is quite different as they used serological test were, past infection could be dominant [17, 18].

A higher level of *Helicobacter pylori* prevalence has been reported in Belgium, Brussels by Lanciers et al., 1999, and in Uganda by Baingana et al., 2014, that reported prevalence of 52.4 %, and 45.2% respectively. The difference might be due to difference in sample size, socio demography, and especially laboratory methods they used (The former used *H.pylori* antibody tests and the later used H.pylori stool antigen tests) [20, 31].

Lower prevalence of *Helicobacter pylori* has been reported in study conducted in Zanzibar, by Farag et al, 2007, and in France by Kalach et al, 2002, with a prevalence of 17.5 % and 21.5% respectively. The most probable difference could be differences in laboratory method used and sociodemographic conditions, study populations (child bearing age group women in general), irrespective of dyspeptic complaint [19, 30].

The highest HpSA prevalence was in the age group of 31–55 years relatively, 18/50(36%) while the lowest number were in the age group of 16-20 years, 8/42(19.0%). Our findings are in accordance with the previous studies. These could be due to persistency of earlier acquired infection during childhood by higher *H.pylori* infection prevalence in older age groups [34].

In this study, 96 (49.2%) pregnant women showed that had History of gastrointestinal illness, of these 41 (42.7%) pregnant women were *H.Pylori* infected. This study was comparable with Study in Chilean done by Hemiptera et al, 2015, and in Jamshoro by Ansari et al, 2014 [17]. This indicates that one of the major consequences of *H. pylori* infection is its effect on acid production in the stomach. The bacteria affect the stomach cells that control stomach acid secretion. This can lead to overproduction of hydrochloric acid, paving the way for ulceration and the acid producing

cells themselves are affected and less acid is secreted, causing hypochlorhydria or low stomach acid. The consequence of low stomach acid is low B12, since it is difficult to assimilate the nutrient from animal protein if you do not have adequate stomach acid, lead to gastritis or stomach inflammation always accompanies infection [34, 36].

The results of this study showed that *H.pylori* infection was significantly high in the pregnant population with hypermesisgravidum, 145/195 (74.4%), of these, 61/66 (92.3%) of *H.pylori* infected pregnant women with a history of hypermesisgravidum. this is agree with study in Jeddah, Saudi Arabia, by Ahmed R et al, 2014 [25]. however, this is a case control study with HG and without HG. But this study shows higher result than the Study done in Izmir, Turkey by Bezircioğlu I et al, 2011, which shows pregnant women with Hyperemesis gravidarum have prevalence of *H.pylori* (22.2%) were established HpSA positive [24, 28]. The 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 [31, 32]. The increased level of steroid hormones & human chronic gonadotrophins (HCG) during pregnancy also lead to changes in PH & motility of GI tract, this change favor activities of *H.pylori* infection [29, 30].

The highest prevalence of *H.pylori* infection was seen in anemic pregnant women 31/48 (64.6%) compared to not *H.pylori* infected, 17/48(35.4%) whereas 3/13(32.8%) anemic was non pregnant who were *H.pylori* infected. Our findings agree with other studies done in Baghdad by Meroj A, 2011, having lower mean hemoglobin level. [26] Study in Jeddah, Saudi Arabia, by Ahmed R et al, 2014 [25] , in Zambia by Farag et al, 2007, in India by Ria Malik et al, 2011 that showed who had infection with *H.pylori*, indicating a high prevalence in pregnant population with anemia [29].. Showed a significantly high prevalence of *H .pylori* among pregnant with anemia (Hb level <11gm/dl) were found to have significantly higher in HPSA positive compared to non anemic group [30]. This result Some Possible mechanism by which *H.pylori* affects iron metabolism by: (1) decreased absorption resulting from chronic gastritis [29], (2) decreased gastric juice ascorbic acid concentration which is known to facilitate iron absorption [30], (3) increased hepcidine production associated with *H.pylori* gastritis [31], (4) uptake of iron by *H.pylori* for growth [32], and (5) decreased availability of iron by sequestration of iron in lactoferin in the gastric mucosa

and bacterium host competition for dietary iron supply. Another explanation most commonly offered for this relationship is based upon the development of *H.pylori* associated chronic gastritis with resultant achlorhydria and reduced ascorbic acid secretion leading to reduced intestinal iron absorption. Other potential explanations for an association between anemia and *H. pylori* include occult blood loss from erosive gastritis and sequestration and utilization of iron by the organism [32, 33,34].

The most important point Showed that significantly higher prevalence of *H.pylori* infection among pregnant with hyperemesis gravidarum,145/195 (74.4%), of these, 61/66 (92.3%) of *H.pylori* infected pregnant women also had a history of hypermesisgravidum. this is agree with study in Jeddah, Saudi Arabia, by Ahmed R et al, 2014. (80%) found to have significantly higher in HPSA positive [25] however, this study shows higher result than the Study done in Izmir, Turkey by Bezircioğlu I et al, 2011, which shows pregnant women with Hyperemesis gravidarum have prevalence of *H.pylori* (22.2%) were established HpSA positive [24]. but this study not agrees with Study conducted in Ankara, Turkey, R. Sinan et al.,2013, *H.pylori* Seropositive is high in study and control groups, 67.7% and 79.3%, respectively, That means there was no find an association between *H.pylori* infection and hypermesisgravidum, however, this is a case control study with HG and without HG. [28]. the results of this study showed that *H.pylori* was significantly high in the pregnant population with hypermesisgravidum [29, 30]. This finding is also in accordance with 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 [31, 32]. The increased level of steroid hormones & human chronic gonadotrophins (HCG) during pregnancy lead to changes in PH & motility of GI tract, this change favor activities of *H.pylori* infection [33,34,35].

7. Strengths and Limitation of the study

7.1 Limitation of the study

- The cross sectional nature of this study has a limitation to show cause and effect associations between *H.pylori* infections with some associated factors like anemia , hypermesisgravidum and gastrointestinal illness in pregnant women needs to be investigated in cohort type of studies.
- Another limitation also the determination of *H. pylori* infection was with only one method by (*H.pylori* stool antigen test kit); even though the HpSA kit employed in this study have sensitivity 98.8%, specificity 100% and Accuracy 98.9%.(user leaflet of kits)

7.2 Strengths of the study

- We attempt to indicate the prevalence of *Helicobacter pylori* infection and associated risk factors in 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

This study showed high prevalence of *H.pylori* infection among pregnant than non pregnant women relatively and *H.pylori* infection was associated with a low hemoglobin value, history of gastrointestinal illness and presence of Hyperemesis gravidarum. *H.pylori* infected pregnant women had low hemoglobin concentration than compared to *H.pylori* infected non pregnant women. Sociodemographic characteristics and some expected association risk factors like presence of intestinal parasites, smoking habit, and alcohol drinking habit showed statistically no significant association with *H.pylori* infection in this study.

8.2 Recommendation

- The 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 and anemia in pregnant women would be further explored.
- Further studies are required in the community using different diagnostic methods to explore the actual role of *H.pylori* in and We recommend that a large scale study to be conducted to further elucidate the role of *H. pylori* infection and warranted to consider weather screening *H.pylori* infection during pregnancy could benefit the mother and the fetus

9. Reference

1. Ehab H. Nashaat, MD, Ghada M. Mansour, MD *Helicobacter pylori* and Hyperemesis Gravidarum. Department of Obstetrics and Gynecology, *Journal of Nature and Science* 2010; 8(8): 22-26.
2. Naous A, Al-Tannir M, Naja Z, Ziade F, El-Rajab M. Fecoprevalence and determinants of *Helicobacter pylori* infection among asymptomatic children in Lebanon. *Lebanon Journal of Medicine* 2007; 55 (3)
3. Cosme Alvarado-Esquivel. Seroepidemiology of *Helicobacter Pylori* Infection in Pregnant Women in Rural Durango, Mexico. *International journal of Biomedical science* 2013; 9: 4-8
4. Wasiam D. Benjamin C.Y. Wong, M.D., Ph.D. American College of Gastroenterology Guideline on the Management of *Helicobacter pylori* Infection. *American Journal of Gastroenterology* 2007;102
5. Cardaropoli S, Rolfo A, Todros T. *Helicobacter pylori* and pregnancy-related disorders. *World Journal of Gastroenterol*2014; 20(3): 654-664
6. Leonardo H. Eusebi, Rocco M. Zagari and Franco Bazzoli Epidemiology of Helicobacter pylori Infection, Italy. John *Wiley & Sons Ltd, Helicobacter* 2014, 19 (1): 1–5
7. ShayestehParashi ,ShohrehBahasadri, and MansourehAlirezaiei. Assessing the Association between Iron Deficiency Anemia and *H. Pylori* Infection among Pregnant Women referring to a Busy Antenatal Clinic in Tehran-Iran.*Shiraz E-Medical Journal* 2013;14(3).153-161
8. Meroj A. Jasem, Alia A. AlUbaidi, Najah M. Daood, et al. Iron deficiency in Helicobacter pylori infected patients in Baghdad.*Journal of Microbiology and Infectious Diseases*2011; 1 (3):.
9. Amal N, Mohamad T, Ziad N, et al.Fecoprevalence and determinants of *helicobacter pylori* infection among asymptomatic children in Lebanon. *Lebanese Medical Journal* 2007, 55 (3)123-128.
10. I Gede Arinton.Adjustment of Cut-off Values in ELISA for Detection of *Helicobacter pylori* Infection, Indonesia.Acta Med Indones-Indones J Intern Med, 2011; 43: 2.
11. Roland N. Ndip, Alertia E. MalangeTakang, Juliet E. A. Ojongokpoko1, Henry N. Luma *Helicobacter pylori* isolates recovered from gastric biopsies of patients with gastro-duodenal

pathologies in Cameroon: current status of antibiogram. *Tropical Medicine and International Health 2008; 13 (6) 45-56.*

12. Emmanuel Ike Ugwuja and Nicholas C.Ugwu. Plasma lipids in *Helicobacter pylori* infected pregnant women, Abakaliki, Nigeria. *International Journal of Medicine and Medical Sciences* 2009; 1(5):4-11.
13. Elena M, Natale F, Giulia C, Antonio P. Can *Helicobacter pylori* infection influence human reproduction? Italy.World Journal of Gastroenterology 2014 May 21; 20(19): 5567-5574
14. Biniam Mathewos, BeyeneMoges and MulatDagnew, Seroprevalence and trend of *H. pylori* infection in Gondar University Hospital among dyspeptic patients, Gondar, North West Ethiopia, Mathewos et al. *BMC Research Notes* 2013, 6:346
15. Ahmed S Rahman, Shafiqul A Sarker, Tahmeed A, Rafiqul Islam, Mohammad A Wahed, David A et al . Relationship of Intestinal Parasites, H. Pylori Infection with Anemia or Iron Status among School age Children in Rural Bangladesh. *Journal of Gastroenterology and Hepatology Research*2014; l 3(11):
16. Tanih N. F., Clarke A. M. , Mkwetshana N., Green E., Ndip L. M. and Ndip R. N. *Helicobacter pylori* infection in Africa: *Pathology and microbiological diagnosis in African Journal of Biotechnology*2008; 7 (25): 4653-4662.
17. Hemiptera, Pentatomida and Capricornia. *Helicobacter pylori* infection and gastrointestinal symptoms on Chilean pregnant women. *Saudi medical Journal* 2015; 8 (45): 1408-1411
18. Alvarado-Esquivel C, Seroepidemiology of *Helicobacter pylori* infection in pregnant women in rural Durango, Mexico. *International journal of biomedical science*.2013; 9:4 224-9
19. Kalach N, Desramé,C. Bonnet, P. Commegeille,D. Couturier, S. Chaussade, P. Hance, C. Dupont, and J. Raymond, *Helicobacter pylori* Seroprevalence in Asymptomatic Pregnant Women in France, *clinical and diagnostic laboratory immunology*, 2002; 9(3):736–737.
20. Lanciers, B. Despinasse, D.I. Mehta, and U. Blecker, Increased Susceptibility to *Helicobacter pylori* Infection in Pregnancy Brussels, Belgium. *Infectious Diseases in Obstetrics and Gynecology*1999*;* 7:195-198
21. İncim Bezircioğlu, Hatice Barın Elveren, Ali Baloğlu, Merve Biçer:The positivity of *H. pylori* Stool Antigen in patients with Hyperemesis gravidarum: İzmir, Turkey. *J Turkish-German of Gynecology Association 2011; 12: 71-4*

22. Sinan R Karadeniz, Ozlem Ozdegirmenci,M.Metin Altay, Ayse Solaroglu, Serdar Dilbaz, Nedret Hızel, and Ali Haberal. *Helicobacter pylori* Seropositivity and Stool Antigen in Patients with Hyperemesis Gravidarum, Ankara, *Turkey Infectious Diseases in Obstetrics and Gynecology. 2006; 73(73): 11–23*
23. Karaca, N. Güler, A. Yaza C, et al. Is lower socio-economic status a risk factor for *Helicobacter pylori* infection in pregnant women with hyperemesis gravidarum? *Turk Journal of Gastroenterology* 2004; 15 (2): 86-89
24. Kazemzadeh M, Kashanian M, Baha B, Sheikhansari N. Evaluation of the relationship between *Helicobacter Pylori* infection and Hyperemesis Gravidarum. *Medical Journal of Islam Republic of Iran* 2014; (21 July): 28-42.
25. Ahmed R, Abd Alwahed ,Hossam M. Elsaadany,Ahmed M.Radwan, Mohammed A.Noureldin and Roobina K.Kumar. *Research Journal of Obstetrics and Gynecology2014; 7(1): 6-13*
26. Meroj A. Jasem1, Alia A. Al-Ubaidi, Najah M. Daood, Jenan A. Muhsin. Iron deficiency in *Helicobacter pylori* infected patients in Baghdad. *Journal of Microbiology and Infectious Diseases* 2011; 1: 3.
27. Ria Malik, Kiran Guleria, Iqbal Kaur, Meera Sikka, and Gita Radhakrishnan Effect of *Helicobacter pylori* eradication therapy in iron deficiency anaemia of pregnancy. *Indian Journal Medicine and Research.2011; 134(2): 224–231.*
28. Tanih N. F.,Clarke A. M., Mkwetshana N., Green E., Ndip L. M. and Ndip R. N. *Helicobacter pylori* infection in Africa: *Pathology and microbiological diagnosis African Journal of Biotechnology 2008*; 7 (25): 4653-4662,
29. Samir B., Robert W. Frenck, Emad W. et al .seroprevalence of *Helicobacter pylori* among Egyptian newborns and their mothers: a preliminary report. *American Journal of Tropical Medicine and Hygine.*1999; 61(1):37–40
30. Tamer H. Farag, Rebecca J. Stoltzfus, Sabra S. Khalfan, and James M. Tielsch. *Helicobacter pylori* infection is associated with severe anemia of pregnancy on Pemba Island, Zanzibar. *American Journal of Trop. Med. Hyg.2007; 76 (3):541–548.*
31. Rhona K. B, John K. E and Lena D. *Helicobacter pylori* infection in pregnant women in four districts of Uganda: role of geographic location, education and water sources. *BMC Public Health* 2014; 14:915.

32. . Mubarak N, Gasim GI, Khalafalla KE, Ali NI, Adam I. *Helicobacter pylori*, anemia, iron deficiency and thrombocytopenia among pregnant women at Khartoum, Sudan. *Trans R Soc Tropical Medicine of Hygiene*. 2014; 108(6):380-4.
33. D. Kibru, B. Gelaw, A. Alemu and Z. Addis. *Helicobacter pylori* infection and its association with anemia among adult dyspeptic patients attending Butajira Hospital, Ethiopia, *Bio Med Central Infectious Diseases* 2014; 14:656
34. Çetin Karaca, Nilay Güler, Aziz Yazar, Hakan Çamlica, Kadir Demir, Gonca Yildirim Is lower socio-economic status a risk factor for *Helicobacter pylori* infection in pregnant women with hyperemesis gravidarum? *Turk J Gastroenterol 2004; 15 (2): 86-89*
35. Fariba Nanbakhsh, Hamideh Mohaddesi, Fatemeh Bahadory, Javad Amirfakhrian and Pouya Mazloomi Comparison of *Helicobacter Pylori* Infection between Pregnant Women with Hyperemesis Gravidarumand **,** Urmia, Iran *World Applied Sciences 2013 ;(12): 1918-1922.*
36. Shimoyama T, KatoC, et al. Applicability of a monoclonal antibody-based stool antigen test to evaluate the results of *Helicobacter pylori* eradication therapy. 2009; 62(3): 225
37. Lambert IR, Lin SK, and Aranda Michel. J, *Helicobacter pylori* Scan. *J. Gastroenterology*. 1995; 208: 33-46
38. M.Rafeey and S.Nikvash. Detection *Helicobacter pylori* Antigen in stool samples for diagnosis of infection in children, *Eastern Mediterranean Health Journal*, 2007;13: 5.
39. Garcia LS, Bruckner DA. Diagnostic Medical Parasitology. 3rd Edition. ASM Press, Washington DC. 1997; 45-56
40. Neva FA, Brown HW. Basic Clinical Parasitology.6th Edition. Appleton and Lange, *Norwalk Connecticut.* 1994; 78-82.
41. Ash LR, Oreil TC. Atlas of Human Parasitology. 4th Edition. ASCp Press, Chicago.1997.
42. Yang, J., Scholten, T.H. A fixative for intestinal parasites permitting the use of concentration and permanent staining procedures. *Am. J. Clin. Pathol*. 1977; 67:300- 304

10. Lists of annexes

Annex I: Standard operational Procedure for Laboratory investigation

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

10.1 Standard operational procedures (SOP) for Helicobacter pylori Stool Antigen test.

10.1.1 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.

10.1.2 Test Principle

The *H.pylori* Ag Rapidest 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.

10.2 SOP for Hematological test (Complete blood count) for determination of Hemoglobin

10.2.1 Purpose

To perform hematological tests for determination of Hemoglobin level by Humacount 30^{TS} 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.

10.2.2 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.

10.2.3 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

10.2.4 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.

10.3 SOP for Direct stool examination

10.3.1 Purpose of the test

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

10.3.2 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.

10.3.3 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.

10.4 SOP for Stool Sedimentation Concentration technique

10.4.1 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.

10.4.2 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.

10.4.3 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

10.4.4 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.

Annex II. English version of Participant information sheet

1. **Participant information sheet**

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

Title- The prevalence and associated factors of *H.pylori* infection among child bearing ages of women at a selected health center in kolfe keraniyo sub city, Addis Ababa, Ethiopia.

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 prevalence of *H.pylori* infection and associated factors among child bearing ages of women at a selected health facility (Woreda-5 and Woreda-9 health centers). This finding was helping us to identify the prevalence and the main associated factors to H.*pylori* in pregnant women these who following antenatal care.

Aim of the study: The objective of this study is to determine the prevalence and associated factors of *H.pylori* among child bearing ages of women at selected health centre, kolfe keraniyo sub city, Addis Ababa, Ethiopian.

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

Benefits of the study participant

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

Risks and complication

There is not considerable risk to the study subjects in the participating in the study.

Confidentiality

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

Investigation contact address,

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Annex III: Amharic Version of the participant information sheet, Consent and questionnaire.

የተሳታፊዎችየመረጃ ቅፅ ፡አዲስ አበባ ዩኒቨርሲቲ የጤና ሳይንስ ኮሌጅ የህክምና ላብራቶሪ ሳይንስዲ ፓርትመንት

<u>አርዕስት</u>፡ ማንኛውንም በመዉለድ እድሜ ክልል ዉስጥ ያሉ እናቶችን ሊያጠቃ የሚችል ስለጨገራ ባክተሪያ ሄልኮባክተር ፓዮሎሪ እና ከሱ ጋር ተያያዥነት ያላቸውን ነገሮችን ለማጥናት በኮልፌቀራኒዮ ክ/ከተማ ሥር በሚገኙ ጤና ጣቢያዎች (ወረዳ-5 እና ወረዳ-9) አዲስ አበባ ኢትዮጵያ

አጠቃላይ መረጃ፡ በጥናቱ በመሳተፍዎ ከልብ እያመሰገንን ከመወሰንዎ በፊት ይህን ቅፅ በትክክል አንብቡ ወይም ሲነበብልዎ በትክክል ያዳምጡ፤ እንዲሁም ግልጽያ ልሆነልዎትን ነገር በሙሉ በነፃነት ይጠይቁ፡፡

ስለጥናቱ መረጃ፡ የጨገራ ባክተሪያ ሄልኮባክተር ፓዮሎሪ ህመምን በመዉለድ እድሜ ክልል ዉስጥ ያሉ እናቶችን ላይ ያለው ስርጭት እና በህመምተኛው ላይ የተለያዩ ችግሮችን ሊያስከትል ይችላል፡፡ ለምሳሌ ለህመምተኛው ምቾት ይነሳል፣ ሆስፒታል የመመላለስ ጊዜዎን ያራዝማል;; ስለዚህም የጨጋራ ህመምን ሊያመጡ የሚችሉ ባክተሪያና አባባሽ ነገሮችን ማወቅ ህመሙን ለመቆጣጠርና ለመከላከል ይጠቅማል፡፡

የጥናቱ አላማ፡ የጨጋራ ህመም ሊያመጡ የሚችል ሄልኮባክተር ፓዮሎሪ ባክተሪያና አባባሽ ነገሮችን በመዉለድ እድሜ ክልል ዉስጥ ያሉ እናቶች ላይ ያለው ስርጭት ሁኔታ ማጥናት ነው፡፡

ጥናቱ ለተሳታፊዎች ያለው ጥቅም፡ በጥናቱ ለሚሳተፉ ፍቃደኛ ተሳታፊዎች ምንም አይነት የገንዘብ ክፍያ የለም፣ ነገርግን በምርመራው ውጤት መሰረት የመታከም እድልይኖሮታል፡፡ በተጨማሪም የጥናቱ ውጤት የጨጋራ ህመም ለመቆጣጠርና ለመከላከል ስለሚጠቅም በተዘዋዋሪ መንገድ ሌላ ህመምተኛ እንዲሁም ህብረተሰቡን የመጥቀም እድል ያገኛሉ፡፡

<u>በጥናቱ ተሳታፊዎች ላይ ያለው ጉዳትና ተዛማጅ ችግር</u> በዚህ ጥናት በመሳተፍ ሊደርስብዎ የሚችል አንድም ጉዳት አይኖርም ለዚህ ጥናት የሚያገለግል ናሙና ደምና ሰገራ የሚወሰድ ሲሆን ከመጠነኛ ስሜት በስተቀር በጤናዎ ላይ ምንም ጉዳት አይደርስም፡፡

<u>የመረጃሚ ስጥራዊ አጠባበቅ</u> መረጃ በሚሰጡበት ወቅትም ሆነ ከዛ በኋላ ባሉት ጊዜያት ሙሉ በሙሉ ሚስጥራዊነቱ የሚጠበቅና መረጃውም የሚያዘው በስም ሳይሆን በመለያ ቁጥር ይሆናል፡፡ በጥናቱ ላይ እያሉ በፈለጉት ጊዜ የማቆም ወይም የማቋረጥ መብትአልዎት፡፡ የላብራቶሪ ውጤትዎን ማወቅ ከፈለጉ የመለያ ቁጥሮን በመጠቀም በሚሰጣቸው የቀጠሮ ጊዜ መውሰድ ይችላሉ፡፡

<u>የጥናቱን የሚካሄደው ሰው ማረጋገጫ፡</u> ለዚህ ጥናት ሃላፊነቱን ለመውሰድና፣ ማናቻውንም ጥናቱ የሚመለከቱ ጉዳይ ክትትል ለማድረግና ለሚመለከተው አካል መግለጫ ለመስጠት በፊርማዬ አረጋግጣለሁ፡፡

ፊርማ ---------- ቀን ----------

ማንኛውም ጥያቄ መጠየቅ ለሚሹ የሚቀጥለውንአድራሻዬንመጠቀምይችላሉ፡፡

ስልክ ሞባይል +251-922-879-233 / +251-912-058-200

ኢሜል *<u>kumerat2012@gmail.com/kumerat@yahoo.com</u>*

IV. English version of Informed consent

I have been informed about the objective of the study entitled "Prevalence of *H.pylori* infection and its associated risk factors among child bearing ages of women in a selected health centers (Woreda-5 & Woreda-9) in kolfe keraniyo sub city .Addis Ababa, Ethiopia." 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: _____

V. Amharic version of Informed consent

የፈቃደኝነትማረጋገጫቅጽ

ስሜ ኩመራ ተርፋ ይባላል፡፡ በአሁኑ ሰዓት በአዲስ አባባዩኒቨርስቲ በክሊኒካል ላቦራቶሪ ሳይንስ የሁለተኛ ዲግሪ ፕሮግራም በዲያግኖስቲክ እና ፐብሊክ ሄልዝ ማይክሮባዮሎጂ ትምህርት ክፍል እየተከታተልኩ እገኛለሁ፡፡ የጨገራ ባክተሪያ ሄልኮባክተር ፓዮሎሪ ህመምን በነፍሰጡር እናቶችና ነፍሰጡር ያልሆኑ እናቶች ላይ ያለው ስርጭት ለማየት የዳሰሳ ጥናት ለማካሄድ እንዲሁም ህመምን የሚያባብሱ ነገሮች በሚልርእስ ላይ ለማጥናት በተመለከተ በሚደረገው ጥናት ላይ ልሳተፍ መሆኑን የጥናቱ አላማና ጥቅም ተገልፆልኛል፡፡ በመጠይቁ ላይ ያለው ሙሉ መረጃም በሚስጥር እንደሚያዝ ተነግሮኛል፡፡ በተጨማሪም ጥናቱ ውስጥ አለመሳተፍ መብቱ እንደሆነና በማንኛውም ጊዜ ከጥናቱ በራሴ ውሳኔ መውጣት እንደምችልና በዚህም ምክንያት ምንም አይነት መጉላላት እንደማይደርስብኝ በሚገባ ተረድቻለሁ፡፡

ስለሆነም ሁኔታውን በሚገባ ተረድቼ በፍቃደኝነት በምርምሩ ላይ ለመሳተፍ ለተመራማሪው ፍቃደኝነቴን ሰጥቻለሁ፡፡ በተጨማሪም የምሰጠው የደምና ሰገራ ናሙና ለተጠቀሰው ጥናት ብቻ እንደሚውል ተነግሮኝ ተስማምቻለው፡፡ ማንኛውም ያልገባኝን ነገር የመጠየቅ እድል ተሰጥቶኝ በሚገባኝ ቋንቋ መልስ አግኝቻለሁ፡፡

በተጨማሪም የሁሉም የላብራቶሪ ምርመራ ውጤቶች በጊዜው ክትትል ለሚያደርግልኝ የጤና ባለሙያ እንደሚሰጡ እና ውጤቱን ማወቅ ከፈለጉ ማግኘት እንደምችል ተነግሮኛል፡፡

እኔ----------የተባልኩ ግለሰብ ይህን ሁሉ በመገንዘብ በምርምሩ ላይ ስለ እኔ መረጃና የደምና ሰገራ ናሙና ለመስጠት ተስማምቻለሁ፡፡

ፊርማ ---------- ቀን ----------

Annex VI: English Version Questionnaires

To determine the prevalence of *H.pylori* infection and its association factors among pregnant women attend ANC and non pregnant women visiting in a selected health centers in kolfe keraniyo sub city, Addis Ababa,Ethiopia,2015.

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	Have you been experienced for Consumption of tea and coffee?	1. Yes 2. No	

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

Comments_____

Name of principal Investigator _____

Date_____

Annex VII: Amharic Version Questionnaires

በአዲስ አበባ ከተማ መስተዳደር ኮልፌ ቀራኒዮ ክፍለ ከተማ ጤና ፅ/ቤት ሥር ባሉ ወረዳ-9 እና ወረዳ-5 ጤና ጣቢያዎች የሄልኮ ባክተር ፓዮሎሪ ባክተሪያና አባባሽ ነገሮችን በመዉለድ እድሜ ክልል ዉስጥ ያሉ እናቶች ላይ ያለው ስርጭትንና አባበሽ ነገሮችን ለማጥናት ነው፡፡

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

የጤና ተቀሙስም _____የተጠየቁበት ቀን _____

የጥናቱ ተሳታፊመለያቁጥር_____

አድራሻ ከተማ_____ክ/ከ _____ስልክ_____

ኮድ	ሀ. ማህበራዊዳታዎችእና 'ሌሎችመረጃዎችንበተመለከተ	
01	እድሜበዓመት----------	
02	የጋብቻሁኔታሀ. ያገባችለ . ያላገባችሐ . የፈታችሙ. የሞተባት	
03	የትምህርትደረጃሀ . ያልተማረ ለ . የመጀመሪያደረጃ ት/ቤት ሐ . ሁለተኛደረጃ ት/ቤትመ. ኮሌጅ/ዩኒቨርሲቲ	
04	ሥራ ሁኔታ ሀ.የመንግስትለ.መንግስታዊያልሆነድርጅት ሐየግልመ. የቤትእመቤትሠ.የቤትሰራተኛ ረ.ሌላ(ይገለጽ)----------	
05	የቤተሰብአባላትብዛት ሀ. <5 ለ.>6 ሐ. ሌሎች_____	
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	
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08	አልኮል መጠጥ ይጠጣሉ? ሀ. <u>አዎ</u> ለ. <u>የለም</u>	
09	የማጨስ ልምድ አሎት? ሀ. <u>አዎ</u> ለ. <u>የለም</u>	
10	ጫትይቅማሉ? ሀ. <u>አዎ</u> ለ. <u>የለም</u>	
11	ሻይና ቡና ይጠቀማሉ? ሀ. <u>አዎ</u> ለ. <u>የለም</u>	
12	በመጀመሪያ ወራት አከባቢ ህመምና መቅለሽለሽ ነበር? ሀ. <u>አዎ</u> ለ. <u>የለም</u>	

13	1. የጨንራ ህመም ታመው ያዉቃሉ? ሀ. አዎ ለ. የለም	
	የንጽህና አጠባበቅ ሁኔታን በተመለከተ	
14	ሀ. ምግብ ከመብላቱ በፊት እጁን እታጠባለሁ? ሀ. አዎ ለ. የለም	
15	ሽንት ቤት ከ በኋላ እጁን እታጠባለሁ? ሀ. አዎ ለ. የለም	
16	ለመጠጥ የምትጠቀሚው ውሃ? ሀ.የታንከር ዉሃ ለ. የጉድጓድ ዉሃ ሐ. የምንጭ ዉሃ መ. የቧንቧዉሃ	
17	የመጨረሻ የሄልኮ ባክተር ፓይሎሪ ባክቴሪያ ምርመራ ውጤት? ሀ. ፖዜቲቭ ለ. ነገቲቭ	

አስተያየት ----------

መጠይቁን የሞላውባለሞያስም ----------

ቀን----------

ስለትብብርዎ እናመሰግናለን፡፡