



## Frequency of *H.pylori* and associated risk factors among out patients in Keren referral military hospital of Eritrea

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### Abstract

*H. pylori* infection is a major cause of chronic gastritis and peptic ulcer disease. The pathogenetic mechanisms underlying *H. pylori* infection are not completely understood. *H. pylori* infection can be confirmed by invasive and/or noninvasive methods. Invasive tests require esophageal gastroduodenal endoscopy. A cross sectional study was conducted on 125 subjects consisting primarily of adult males and females for about one month. The subjects were selected by stratified random sampling method. Five to ten ml venous blood was collected from each informed and consenting adult dyspeptic patients and non-dyspeptic patients. These sera were analyzed using One Step *H. pylori* Test Strip. Data were entered and analyzed using SPSS for windows version 18.0. Pearson  $\chi^2$  and Fisher exact test was used to assess the univariate association between *H. pylori* infection and the possible risk factors. The study showed that the overall 65(52%) of the patients had *H. pylori* infection in both symptomatic and asymptomatic patients. The frequency of *H. pylori* infection was higher in men (54.1%) than women (37.5%), but statistical level of significance was not reached ( $P=0.220$ ,  $OR=1.967$ ,  $CI=0.0668-5.791$ ).

Key Words: Helicobacter pylori, H. pylori Test Strip, Seropositivity

## Introduction

### Helicobacter pylori infection

*Helicobacter pylori* (*H. pylori*) was isolated from gastric mucosa and bacteriologically identified in 1982 (1). Since then, it has been reported that ulcer recurrence rates have decreased after the eradication of *H. pylori* from the stomach of peptic ulcer patients (2). It has been established that *H. pylori* infection is a major cause of chronic gastritis and peptic ulcer disease.

The genus *Helicobacter* belongs to a subdivision of *Proteobacteria*, order *Campylobacterales*, family *Helicobacteraceae*, and consists of over 20 recognized species which are all microaerophilic organisms. Most of the *Helicobacter* species are catalase and oxidase positive, and many but not all species are also ureases positive. They can be subdivided into two major lineages: the gastric *Helicobacter* species and the enterohepatic (nongastric) *Helicobacter* species. *H. pylori* is a gram-negative bacterium which usually appears spiral-shaped or rod shaped with several flagella (3). *H. pylori* is genetically heterogeneous, suggesting a lack of clonality. These results in every *H. pylori* positive subject carrying a distinct strain, although differences within relatives may be small (4). The pathogenic mechanisms underlying *H. pylori* infection are not completely understood. *H. pylori* is sheltered from gastric acidity in the mucus layer. The majority of *H. pylori* in colonized hosts are free-living, but approximately 20% bind to gastric epithelial cells (5). Colonization of the gastric epithelium by *H. pylori* is specific *in vivo* and when *H. pylori* is found in the duodenum, it overlays gastric metaplasia (6). Colonization of the gastric mucosa by *H. pylori* evokes local inflammatory responses, which result in further mucosal injury but are not able to clear the infection (3, 7). *H. pylori* infection thus escapes the natural gastrointestinal defences, which allows it to induce chronic infection. It has been suggested that the differences in the outcome of the disease are due to various virulence factors present in different *H. pylori* strains. Virulence factors help bacteria to invade the host, cause disease and evade host defences.

Several virulence factors of *H. pylori*, such as production of urease, a vacuolating cytotoxin, and the cytotoxin-associated gene A encoded protein, are associated with injury to the gastric epithelium (8).

*H. pylori* infection can be confirmed by invasive and/or noninvasive methods. Invasive tests require esophageal gastroduodenal endoscopy. During endoscopy, biopsy specimens of the stomach and duodenum are obtained, and the diagnosis of *H. pylori* is generally made by urease testing, histology and/or culture. All of these biopsy-based methods are unpleasant for patients, carry a small but definite risk of complications and are subject to sampling error since infection is patchy (9). Non-invasive tests are based on peripheral samples, and the most commonly used ones are the urea breath test, fecal antigen tests and serology. There is no single golden standard for the diagnosis of *H. pylori*. In research settings, a combination of at least two methods is often applied, as compared to clinical practice, where it is common to use a single test (3). Several other tests, such as whole-blood rapid tests, saliva 22 and urine antibody tests, and PCR, are also available, both from biopsy and from stool samples (10).

Several molecular methods based on hybridization or amplification techniques have been developed to detect wide range of *H. pylori* infection (11, 12). PCR is regarded as the most sensitive technique for the detection of the organism in the gastric biopsy and in the gastric aspirates with 95% of sensitivity and specificity (13, 14). This test is particularly useful in post-treatment diagnosis and in the detection of antimicrobial resistance to *H. pylori* (15).

Approximately 50% of all *H. pylori* strains secrete VacA, a highly immunogenic 95-kDa protein that induces massive vacuolization in epithelial cells *in vitro* (16). The VacA protein plays an important role in the pathogenesis of both peptic ulceration and gastric cancer (17, 18, 19).

One of the striking features of *H. pylori* is that it is able to colonize the acidic gastric environment, although the bacterium is not an acidophile. The pH of the gastric mucosa is thought to vary between 4

and 6.5, but occasional acid shocks may occur (20). *H. pylori* thus require mechanisms to protect itself from acute acid shocks and mechanisms to grow at pH values around 5.5 (21, 22).

## Materials and Methods

**Study design:** A cross sectional study was conducted on 125 subjects for about one month from July 15-2011 up to August 10-2011 to determine the frequency of *H. pylori* and associate risk factors among the outpatients.

**Study area:** The study was conducted in (KEREN REFERAL MILITARY HOSPITAL OF ERITREA) KRMH which provide health services for all the Eritrean defense forces.

**Study population:** The study population consisted primarily of adult male and female referrals from regional Military Hospitals for further investigation of their problems, attended in the outpatient department for routine health examinations from July 15-2011 through August 10-2011 at KRMH. None of the patients had been on any therapy since four weeks that might have affected their *helicobacter* status.

### Sampling Method

The subjects were selected by stratified random sampling method, to make the sample more representative and scientific using the formula

$$n = Z^2 p(1-p) / d^2$$

The patients were stratified in to group of dyspeptic or symptomatic and group of none dyspeptic or asymptomatic categories according to their symptoms and medical history taken by the O.P.D attending physician. From group of symptomatic fifty eight subjects every two patients were randomly selected, similarly from symptomatic sixty seven subjects every eight patients were randomly

selected. Dyspeptics were patients with the actual symptoms of dyspepsia who were referred to the gastro-enterology department of KRMH for further health services from all over the country regional Military Hospitals. The non-dyspeptics were patients with no complain of dyspepsia attending at KRMH for different medical reasons. However, some of the non-dyspeptic patients gave positive answer for the symptoms of dyspepsia on the interview.

### Inclusion criteria

Patients who consented to participate in the study of all ages and sex with symptoms of dyspepsia and asymptomatic patients from the out patient department of KRMH were recruited. Understanding the purpose of the study written informed consent was obtained from all participants of the study.

### Exclusion criteria

Patients who had previously been treated for *H. pylori* or had received any antimicrobial treatment or proton pump inhibitor within the period of two or three weeks were excluded.

## Data collection methods

### Questionnaire

After informed consent was obtained the investigator conducted a face to face interview with each volunteer and completed a detailed questionnaire. The individuals were questioned regarding the presence of symptoms referable to the upper gastrointestinal tract including indigestion, heartburn, epigastric pain, nausea, vomit, bloating, hematemesis (blood in vomite), and melena (black stool). Demographic data focused on the environment during the subject's childhood (zoba and rural/urban), age, sex, and ethnicity was recorded. Living conditions (number of persons residing in a single room). Household hygiene questions concerned the water source used for drinking, cooking,

and bathing (i.e., tap, well, or river), volume of water available (large, moderate and small), excreta disposal facilities (indoors or outdoors), and other sanitation practices. Sanitation practices questions included details about frequency of bathing, and boiling of water before drinking or use of bottled water. All appropriate information (demographic, crowding, source of water, hygiene practices and laboratory data) was recorded on the questionnaire prepared for this study and the data was entered in to a computer.

### Laboratory Methods

The participants interviewed and gave whole blood specimen for the serologic study of anti *H. pylori* antibodies. Five to ten ml venous blood was collected from each informed and consenting adult dyspeptic patients and non-dyspeptic patients. The sera were obtained from the blood by centrifugation (3000 RPM for 10 minutes). Serum storage at -20 degree centigrade was not required, because the sera were analyzed soon after collection in the same day.

These sera were analyzed using One Step *H. pylori* Test Strip. It is a simple one step immunochromatographic assay for the rapid, qualitative determination of anti *H. pylori* anti-bodies of all isotypes (IgG, IgA, IgM etc.) in human serum or plasma. The test kit was the product of ACON 4108 Sorrento Valley Boulevard San Diego, CA 92121, USA.

Specificity and interference study was carried out by the manufacturers to determine the performance of the test kit. In the specificity study, the ability of the *H. pylori* test to specifically detect *H. pylori* was challenged through cross reaction studies on serum samples containing known other closely related microorganisms such as *Campylobacter fetus*, *Campylobacter jejunii*, and *E. coli*. Serum samples that are negative to *H. pylori* were spiked with various concentration levels of the above microorganisms. These samples were then tested on the *H. pylori* test kit. Each micro organism had 10 runs of the *H. pylori* test. All 30 test results

indicated that no cross reactivity with the *H. pylori* test.

In the interference study the potentially interfering chemicals such as pain medications, lipids, hemoglobin, Bilirubin, and glucose were supplemented to negative normal serum specimen and were analyzed in the same way as *H. pylori* positive samples. All interference studies indicated none of the above substances interfere with the *H. pylori* procedure.

### Ethical issue

This study was approved by the ethical clearance committee of Asmara College of Health Sciences and permission was given to carry out the study in KRMH from Officers Office Ministry of Defense. All subjects participating in this study signed a consent justifying their will to participate in the study. Participation in the study was voluntary. Information collected from all participants was entered into a computer and confidentiality maintained.

### Method of data analysis

Data were entered and analyzed using SPSS for windows version 18.0. Pearson  $\chi^2$  and Fisher exact test was used to assess the univariate association between *H. pylori* infection and the possible risk factors. Odds ratios (OR) and the corresponding 95% confidence interval (CI) were calculated to measure the strength of association using SPSS version 18 and EPO INFO. P value of  $<0.05$  were required for significance.

### Result

#### Socio-Demographic Characteristics of the subjects

This cross sectional study was conducted from 15 June 2011 to 10 August 2011 among the out patients in KRMH. A total of 125 subjects were included in the study. The socio-demographic characteristics of the study population are shown in Table 1. Of the

125 participants 58 individuals were dyspeptic and 67 were non dyspeptic patients. The median age of the subjects was 32 years (Range, 17-67 years). A substantial majority 45(36%) of the patients were young adults aged 20-29 years. Male constituted 109(87%) of the patients and 16(13%) were females. From the nine ethnic groups in Eritrea, 89(71 %) of the subjects were predominantly Tigrigna ethnic group, followed by Tigre 23(17%), and Bilen 7(6%) and rest of ethnic groups were represented by less than 2%.

The distribution of the different age category was, 5(4%) of the patients were below 20 years of age, 45(36%) "Between" 20-29 of age, 40(32%) "Between" 30-39, 29(23%) "Between" 40-49 of age, 4(3%) of them were "Between" 50-59 and the remaining 2 patients (2%) were older than 60 years of age.

The participants from Zoba Anseba were 39(31%) followed by Zoba Maekel 36(29%), Debub 32(26%), Gash barka 9(7%), Northern Red sea 8(6%) and Southern Red Sea 1(1%). Out of the 125 participants 71(57%) were rural dwellers during their growing up and the rest 54(43%) were from urban area.

see Table 1.

#### **Overall frequency of *H. pylori* among the 125 study subjects**

The frequency of *H. pylori* infection among the 67 asymptomatic patients was 34(50.7%) and out of the 58 symptomatic patients was 31(53.4%). The overall frequency of the infection among the O.P.D patients was 65(52.0%). (Table2)

see Table 2.

#### **Frequency of *H. pylori* with respect to sociodemographic characteristics**

When patients were compared with respect to their residence during their childhood the frequency of the infection was slightly higher in urban than rural. That is, 29(53.7%) in urban, and 36(50.7%) in

rural, but it was not statistically significant level was reached ( $p=0.740$ )

We also compared the frequency of the infection among the males and females subjects. The positive rate was found to be higher in males than in females. It was 59(54.1%) in males, and 6(37.5%) in females. There was no significant relationship between gender and *H.pylori* infection ( $P=0.220$ ).

The infection rate in relation to different age category was, 2(40.0%) in subjects <20 years of age, 22(48.9%) in subjects 20-29 years, 23(57.5%) in subjects 30-39 years of age, 15(51.7%) in subjects 40-49 years of age, 20(50.0%) in subjects 50-59 years of age and there was one out of the two patients was infected with the organism in subjects above 60 years old. As a whole, the frequency of infection did not increase significantly with age ( $p=0.996$ ). The highest frequency was observed between age 30-39(57.5%), followed by age group 20-29. Of this age group 22(48.9%) were seropositive for *H. pylori* antibodies.

see Table 3.

see Table 4.

#### **Frequency and seropositivety of *H. pylori* antibodies in relation to lifestyle**

Out of the 125 study subjects 118(94.4%) for hand washing after toilet and 121(96.8%) for hand washing before food reported yes when they questioned during the study period. Out of these subjects also 7(5.6%) fore hand washing after toilet and 4(3.2%) for hand washing before food were reported no. In the habit of frequency of bathing, 49(39.2%) of the subjects were taking shower more than 2-3 times per week, 59(47.2%) of the subjects were taking bath 2-3 times per week, and 17(13.6%) were also taking shower once or less per week. In the study of excecerta disposal practices 65(52.0%) people use indoor toiles and 60(48.0%) use out door toilets. The avelability of water per person was also assessed as large (60liter per day), moderate (40 liter per day), and small (20 liter per day). Subjects

large volume of water were 61(48.8%), moderate 46(36.8%), and small 18(14.4%). Among lifestyle characteristics, only crowding was significantly associated with the infection ( $P=0.036$ ).

see Table 5.

### **Frequency and sero positivity of *H. pylori* in relation to major symptoms of dyspepsia**

The major clinical presentation is shown in table 6 below. Out of the seven major clinical symptoms studied in the current investigations epigastric pain and chest burning were the commonest symptoms that were found epigastric pain 83(66.4%) and chest burning 83(66.4%) out of the overall 125 patients, followed by bloating 71(56.8%), food indigestion 70(56.0%), nausea 61(48.8%), hematemesis 14(11.2%) and melena was found in 9(7.2%) subjects. However, only nausea [ $P=0.024$ ,  $OR=2.317(0.469-11.727)$ ] and food indigestion [ $P=0.006$ ,  $OR=2.740(1.322-5.678)$ ] were significantly associated with *H. pylori* infection [ $P=0.024$ ,  $OR=2.317(0.469-11.727)$ ].

see Table 6.

## **Discussion**

### **General information**

*H. pylori* is estimated to infect 50% of the world's population. It is acquired early in childhood, usually before 5 years of age in both industrialized and developing countries. It is reported that there is a 3% to 10% increase of *H. pylori* infection per year in children aged 2 to 8 years old in developing countries<sup>16</sup>. Frequency of *H. pylori* differs remarkably among developed and developing countries. There is a lack of information about the frequency of the infection among the Eritrean defense army. Besides, a nationwide community based study to assess the prevalence and associated risk factors of *H. pylori* was not conducted in all regions of the country.

The current study tried to determine the frequency of *H. pylori* and associated risk factors of *H. pylori* infection among the OPD patients in KRMH.

Our population of interest for the present study were OPD patients, from whom 125 samples were drawn using simple random sampling method from both dyspeptic symptom ( $n=58$ ) and non dyspeptic symptom ( $n=67$ ) patients. The dyspeptic and non dyspeptic patients were categorized by the OPD attending physician depending on the patients reason for referral and their medical history of the patients. Patients who had taken antibiotics or proton pump inhibitors within three weeks prior to study were excluded.

After signing a consent form, the subjects were interviewed by the investigator using self administered standardized questionnaire. The questionnaire contained three categories of questions; one category gathered demographic information (age, gender, residence, region, and ethnicity). The second one included questions related to hygiene, sanitation practices, living conditions/crowding, and source of water for drinking, cooking, and bathing. The third category included questions related to symptoms of dyspepsia (epigastric pain, chest burning, bloating, nausea/vomits, hematemesis and melena). All the data was recorded on the questionnaire prepared for the purpose of this study.

A blood sample was taken from each of the 125 participants, for the serological study of anti *H. pylori* antibodies. The sera were isolated and soon analyzed after collection.

### **Sero status of *H. pylori* with respect to associated risk factors**

The study showed that the overall 65(52%) of the patients had *H. pylori* infection in both symptomatic and asymptomatic patients. This is consistent with the world population prevalence of *H. pylori* infection, but lower than the resource deprived developing countries. For example, the incidence of *H. pylori* infection in Kenya is staggeringly high. An investigation into the prevalence of *H. pylori* in patients with dyspepsia and asymptomatic controls from the same population, showed high prevalence levels in both groups. Sero positivity for *H. pylori*

was found in 98 (71%) symptomatic patients and in 70 (51%) asymptomatic participants. (14)

However, the current study showed that frequency of 31(53.4%) of *H. pylori* infection among patients with dyspepsia. Among the patients those do not have symptoms of dyspepsia 34(50.7%) had *H. pylori* infection. There was no statistically significant difference in seroprevalence of *H. pylori* infection between dyspeptics and non-dyspeptics ( $p=0.763$ ,  $OR=1.114$ ,  $95\%CI$  0.551-2.253) patients. In the present study association of some risk factors such as, age, sex, hand washing after toilet, hand washing before food, frequency of bathing, excreta disposal, volume of water available per person and source of water fore drinking and bathing with *H. pylori* was not statistically significant( $P>0.05$ ). However, number of people sharing the same room showed significant association with *H. pylori* infection ( $\chi^2_{(4)}10.279$ ,  $P= 0.036$ ).

The frequency of *H. pylori* infection was higher in men (54.1%) than women (37.5%), but statistical level of significance was not reached ( $P=0.220$ ,  $OR=1.967$ ,  $CI=0.0668-5.791$ ).

There was also no significant difference observed among various sources of water regarding the frequency of infection ( $P =0.727$ ). This is probably due to the fact that virtually most of the participants in our study consumed water from tap (49%) and well (67%). This shows that the municipality water treatment and protected well construction was effective in the protection of *H. pylori* infection.

### Conclusion and Recommendations

The present study conclude that no significant difference among the dyspeptic and non dyspeptic patients regarding the frequency of *H. pylori* infection was observed. The *H. pylori* infection was evenly distributed across all the study subjects. From the study results, crowding was found to be the major risk factor for *H. pylori* infection. The symptoms of Nausea and food indigestion were also associated with *H. pylori* infection. People who have such symptoms, most likely have a chance to

become seropositive for *H. pylori* infection.

The authors recommend that further studies should be conducted with increased sample size and extended period of investigation, performed with appropriate sampling techniques to include all age category, sex, and ethnic groups from all regions of the country among the defence forces, including the general population. Furthermore the laboratory method used in this study was a one step *H. pylori* test stripe. It would be worthwhile to include other available diagnostic methods for the validation of the test methods, such as stool antigen, urea breath test, endoscope and PCR (polymerase chine reaction).

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<i>Variables</i>		<i>Dyspeptic</i>	<i>Non dyspeptic</i>	<i>Total</i>
		<i>n(%)</i>	<i>n(%)</i>	<i>n(%)</i>
<b><i>Age</i></b>				
	<20	5(9)	0(0)	5(4)
	20-29	18(31)	27(40)	45(36)
	30-39	21(36)	19(28)	40(32)
	40-49	11(19)	18(27)	29(23)
	50-59	3(5)	1(1)	4(3)
	60-69	0(0)	2(3)	2(2)
	Total	58(100)	67(100)	125(100)
<b><i>Sex</i></b>				
	Male	51(88)	58(87)	109(87)
	Female	7(12)	9(13)	16(13)
	Total	58(100)	67(100)	125(100)
<b><i>Zoba</i></b>				
	Maekel	18(31)	18(27)	36(29)
	Debub	10(17)	22(33)	32(26)
	Gash Barka	8(14)	1(1)	9(7)
	Anseba	16(28)	23(34)	39(31)
	Nor.Red Sea	5(9)	3(4)	8(6)
	Sou.Red Sea	1(2)	0(0)	1(1)
	Total	58(100)	67(100)	125(100)
<b><i>Residence</i></b>				
	Rural	30(52)	41(61)	71(57)
	Urban	28(48)	26(39)	54(43)
	Total	58(100)	67(100)	125(100)
<b><i>Ethnic Group</i></b>				
	Tigrigna	39(67)	50(75)	89(71)
	Tigre	10(17)	13(19)	23(18)
	Saho	1(2)	2(3)	3(2)
	Bilen	5(9)	2(3)	7(6)
	Afar	1(2)	0(0)	1(1)
	Kunama	0(0)	0(0)	0(0)
	Nara	1(2)	0(0)	1(1)
	Rashida	0(0)	0(0)	0(0)
	Hidarb	1(2)	0(0)	1(1)
	Total	58(100)	67(100)	125(100)

Table 1: Socio demographic Characteristic of the subjects

<i>H. pylori</i> status	Symptomatic n(%)	Asymptomatic n(%)	Total
Positive	31(53)	34(50.7)	65(52)
Negative	27(46)	33(49.3)	60(48)
Total	58(100%)	67(100%)	125(100%)

Table 2. Frequency of *H. pylori* infection among the overall dyspeptic and none dyspeptic subjects

Characteristics		Sero status of <i>H. pylori</i> positivity		Total(100%)
		Positive n(%)	Negative n(%)	
Sex	Male	59(54.1)	50(45.9)	109(100)
	Female	6(37.5)	10(62.5)	16(100)
Age	<20	2(40.0)	3(60.0)	5(100)
	20-29	22(48.9)	23(51.1)	45(100)
	30-39	23(57.5)	17(42.5)	40(100)
	40-49	15(51.7)	14(48.3)	29(100)
	50-59	2(50.0)	2(50.0)	4(100)
	>60	1(50.0)	1(50.0)	2(100.)
Residence	Rural	36(50.7)	35(49.3)	71(100)
	Urban	29(53.7)	25(46.3)	54(100)
Zoba	Anseba	17(43.6)	22(56.4)	39(1000)
	Debub	19(59.4)	13(40.6)	32(100)
	Gashbarka	4(44.4)	5(55.6)	9(100)
	Maekel	20(55.6)	16(44.4)	36(100)
	N/Red Sea	4(50.0)	4(50.0)	8(100)
	S/Red Sea	1(100.0)	0(0.0)	1(100)
Ethnicity	Tigrigna	46(51.7)	43(48.3)	89(100)
	Tigre	9(39.1)	14(60.9)	23(100)
	Bilen	6(85.7)	1(14.3)	7(100)
	Saho	3(100)	0(0.0)	3(100)
	Nara	0(0.0)	1(100.0)	1(100)
	Hidarb	0(0.0)	1(100.0)	1(100)
	Afar	1(100.0)	0(0.0)	1(100)
n(%) = number(percent)				

Table 3. Frequency of *H. pylori* seropositivity in relation with sociodemographic characteristics

n(%) = number(percent)

		pos	neg	N(%)	OR(95%CI)	P value
<b>Age</b>					1.16(0.83-3.15)	0.966
	<20	2	3	5(4)		
	20-29	22	23	45(36)		
	30-39	23	17	40(32)		
	40-49	15	14	29(23)		
	50-59	2	2	4(3)		
	>60	1	1	2(2)		
<b>Gender</b>					1.967(0.668-5.791)	0.220
	Male	59	50	109(87)		
	Female	6	10	16(13)		
<b>Residence</b>					0.88(0.436-1.802)	0.740
	Rural	36	35	71(57)		
	Urban	29	25	54(43)		
OR= odds ratio,95% CI= 95% confides interval of odds ratio						

Table 4. Relationship between socio-demographic characteristics Vs H. pylori infection

OR= odds ratio,95% CI= 95% confides interval of odds ratio

Risk factors		positive	negative	n(%)	OR(95% CI)	P.value
<b>Hand washing After toilet</b>					0.167(0.019-1.427)	0.102
	Yes	59	59	118(94.4)		
	No	6	1	7(5.6)		
<b>Hand washing Before food</b>					1.086(0.148-7.963)	0.935
	Yes	63	58	121(96.8)		
	No	2	2	4(3.2)		
<b>Frequency of Bathing</b>					0.794(0.197-3.196)	0.626
	>2-3times Per week	23	26	49(39.2)		
	2-3times Per week	32	27	59(47.2)		
	Once or less Per week	10	7	17(13.6)		
<b>Excreta disposal</b>					0.612(0.302-1.243)	0.174
	In door	30	35	65(52.0)		
	Out door	35	25	60(48.0)		
<b>Volume of water</b>					0.577(0.136-2.445)	0.553
	Large	29	32	61(48.8)		
	Moderate	25	21	46(36.8)		
	Small	11	7	18(14.4)		
<b>Crowding</b>					7.530(1.112-51.015)	0.036
	0-2	16	13	29(23.2)		
	3-5	33	20	53(42.4)		
	6-8	12	13	25(20.)		
	9-11	0	5	5(4.0)		
	>11	4	9	13(10.3)		
<b>Source of water</b>					1.190(0.182-7.790)	0.727
	Bottled water	2	1	3(2.4)		
	Tap water	24	25	49(39.2)		
	Boiled water	0	1	1(0.8)		
	From wells	35	28	63(50.4)		
	From river	4	5	9(7.2)		
OR= odds ratio, CI= confidence interval of odds ratio						

Table 5. Frequency of *H. pylori* in relation with major risk factors (lifestyle)

OR= odds ratio, CI= confidence interval of odds ratio

Symptoms		Positive	Negative	OR	P value
<b>Epigastric pain</b>				0.977(0.465-2.054)	0.952
	Yes	43	40		
	No	22	20		
<b>Chest burning</b>				1.505(0.714-3.173)	0.282
	Yes	46	37		
	No	19	23		
<b>Nausea</b>				2.317(0.469-11.727)	0.024
	Yes	38	23		
	No	27	37		
<b>Food indigestion</b>				2.740(1.322-5.678)	0.006
	Yes	44	26		
	No	21	34		
<b>Bloating</b>				1.011(0.498-2.052)	0.977
	Yes	37	34		
	No	28	26		
<b>Hematemesis</b>				1.768(0.557-5.610)	0.334
	Yes	9	5		
	No	56	55		
<b>Melena</b>				1.932(0.461-8.098)	0.361
	Yes	6	3		
	No	59	57		

Table 6. H. pylori infection Vs major symptoms of dyspepsia

**Appendix 1**

ASMARA COLLEGE OF HEALTH SCIENCES  
SCHOOL OF ALLIED HEALTH PROFESSIONS  
DEPARTMENT OF CLINICAL LABORATORY SCIENCE

Interview on Frequency of *H. Pylori* and Associated Risk Factors Among Out Patients in Keren Rereral Military Hospital

Sample identification no: \_\_\_\_\_

1. Age \_\_\_\_ 2. Sex: M  F  3. Zoba: 3. Origin: Rural:   
Maekel:  Debub:  Urban:   
G/Barka:  Anseba:   
D/Keyih bahir:  S/Keyih bahri:
4. Ethnic group \_\_\_\_\_
5. Personal hygiene practices:
- a) Hand washing after toilet: Regularly:  Some times:  Not at all:
- b) Had washing before food: Regularly:  Sometimes:  Not at all:
- c) Taking shower: Regularly every  
<1day  1---3 days  4---6days  7---9 days  >9days
6. How many people are sharing the same room?  
0—2:  3—5:  6—8:  9—11:  >11:
7. Source of drinking water  
a. Clean bottled water b. Tap water c. Boiled water d. Water from wells
8. Have ever such symptoms:
- a. An ach or burning pain in the abdomen behind the ribs . Yes:  No:
- b. Nausea Yes:  No:
- c. Frequent Chest burning Yes:  No:
- d. Food indigestion Yes:  No:
- e. Bloating Yes:  No:
- f. Vomite with blood Yes:  No:
- g. Black stool Yes:  No:
9. Laboratory results: Positive:  Negative:

**Appendix 2**

Consent form

ASMARA COLLEGE OF HEALTH SCIENCES  
SCHOOL OF ALLIED HEALTH PROFESSIONS  
Department of clinical laboratory science

**Title: FREQUENCY OF H. PYLORI AND ASSOCIATED RISK FACTORS AMONG OUT PATIENTS IN KEREN REFERAL  
MILITARY HOSPITAL**

Purpose of the study is to assess the frequency of H. pylori and associated risk factors. You are randomly selected for this study. We kindly ask you to participate in this study and we also expect you to give reliable information to the interview questions, in order to make this study a fruitful one.

To maintain confidentiality your name will not be recorded on the interview paper. After you finish the interview you will be asked to give blood for the study. Appropriate procedure will be used to minimize the risk and pain of drawing blood sample. Furthermore, the result of this test will be kept confidential.

Any subject has the right to withdraw from this study at any stage.

Signature: Understanding the purpose and importance of the study, I participate in this study on my own will.

Name: \_\_\_\_\_

Signature: \_\_\_\_\_

Date: \_\_\_\_\_