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DEPARTMENT OF MEDICAL BIOCHEMISTRY

EVALUATION OF LIPID PROFILE AND INFLAMMATORY MARKER IN PATIENTS
WITH GASTRIC *HELICOBACTER PYLORI* INFECTION

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TABLE OF CONTENTS

TABLE OF CONTENTS.....	IV
ABBREVIATIONS AND ACRONYMS	VI
LIST OF TABLES	VII
LIST OF FIGURES	VIII
ABSTRACT.....	IX
1. INTRODUCTION	1
1.1 Backgrounds:.....	1
1.2. Statement of the problem	3
1.3. Significance of the study	5
2. LITERATURE REVIEW	6
3. OBJECTIVES	19
3.1. General objective.....	19
3.2. Specific objectives.....	19
4. MATERIALS AND METHODS.....	20
4.1. Study area and period.....	20
4.2. Study design	20
4.3. Population.....	20
4.3.1. Source population	20
4.3.2. Study population.....	20
4.4. Eligibility Criteria	20
4.4.1. Inclusion criteria	20
4.4.2. Exclusion criteria.....	21
4.5. Sampling method and sample size determination	21

4.6. Variables.....	21
4.6.1. Dependent variables	21
4.6.2. Independent variables	22
4.7. Blood sample and data collection procedures	22
4.8. Laboratory test principles.....	22
4.9. Data processing and analysis.....	26
4.10. Ethical consideration	27
4.11. Operational definitions	27
5. RESULT	28
6. DISCUSSION.....	37
7. CONCLUSION	45
8. STRENGTHS AND LIMITATIONS OF THE STUDY	46
9. RECOMMENDATION.....	47
10. REFERENCES	48
11. ANNEXES:.....	57
11.1. Annex i: English version information sheet.....	57
11.2. Annex II: English Version Consent form.....	58
11.3. Annex III: English version questionnaires	59
11.5. Annex V: አማርኛ የስምምነት ማረጋገጫ ቅጽ	62
11.6. Annex VI: አማርኛ መጠይቅ	62

ABBREVIATIONS AND ACRONYMS

ADMA:	Asymmetric dimethylarginine
CAD:	Coronary artery disease
CAG:	Chronic atrophic gastritis
CagA:	Cytotoxin associated gene A
CVD:	Cardiovascular disease
DMHC:	Debre Markos Health Center
DMRH:	Debre Markos Referral Hospital
EGFR:	Epidermal growth factor receptor
FMHACA:	Food, Medicine and Healthcare Administration and Control Authority
HDL:	High-density lipoprotein
HHC:	Hidassie Health Center
<i>H. pylori:</i>	<i>Helicobacter pylori</i>
hs-CRP:	High sensitivity C reactive protein
ICAM-1:	Intracellular adhesion molecule-1
LDL:	Low-density lipoprotein
MAPK:	Mitogen-activated protein kinase
TC:	Total cholesterol
TG:	Triglyceride
TNF- α :	Tumor necrosis factor- α
VacA:	Vacuolating cytotoxin gene A

LIST OF TABLES

Table 1: The summary of the reference range for serum lipid profile and hs-CRP in adults.....	26
Table 2 Socio-demographic characteristics of dyspeptic patients with and without <i>H. pylori</i> infection at DMRH, DMHC, and HHC, Debre Markos, Ethiopia (N=100).....	29
Table 3 Dyspepsia risk factors among dyspeptic patients at DMRH, DMHC, and HHC, Debre Markos, Ethiopia (N=100).....	30
Table 4 The comparison of serum lipid profile and hs-CRP between <i>H. pylori</i> -positive and <i>H. pylori</i> -negative dyspeptic patients for the same gender at DMRH, DMHC, and HHC, Debre Markos, Ethiopia (N=100).....	32
Table 5 The comparison of serum lipid profile and hs-CRP levels among different age group in dyspeptic patients at DMRH, DMHC, and HHC, Debre Markos, Ethiopia (N=100)	33
Table 6 The comparison of serum lipid profile and hs-CRP levels between male and female dyspeptic patients at DMRH, DMHC, and HHC, Debre Markos, Ethiopia (N=100)	35
Table 7 The correlation of age with serum lipid profile and hs-CRP levels in <i>H. pylori</i> -positive dyspeptic patients at DMRH, DMHC, and HHC,Debre Markos, Ethiopia (n=50)	36

LIST OF FIGURES

Figure 1 How <i>H. pylori</i> infection induces endothelium dysfunction and increased risk of CVD.	12
Figure 2 The potential mechanism of <i>H. pylori</i> infection towards cardiovascular disorders	14
Figure 3 Mean serum values of lipid profile in <i>H. pylori</i> -positive and negative dyspeptic patients at DMRH, DMHC, and HHC, Debre Markos, Ethiopia (each of 50 HP+ and HP- participants)	31
Figure 4 Mean serum values of LDL and TC in <i>H. pylori</i> -positive dyspeptic patients based on age categories at DMRH, DMHC, and HHC, Debre Markos, Ethiopia (N=100).	34

ABSTRACT

Introduction: *H. pylori* are gram-negative, microaerophilic helical-shaped bacteria with multiple flagella and commonly exist in the stomach. This infection may cause significant mucosal inflammation and damage, leading to ulcers in the stomach. It can also affect organ systems external to the gastrointestinal tract. To assess cardiovascular risk factors and to predict cardiovascular disorders, we are evaluating and comparing lipid profile and hs-CRP levels between *H. pylori*-positive and negative patients.

Objectives: To evaluate lipid profile and hs-CRP levels in dyspeptic patients with and without *H. pylori* infection.

Methods: Hospital-based comparative cross-sectional study was conducted from September 2020 – January 2021 at Debre Markos town. Fifty *H. pylori*-positive and 50 *H. pylori*-negative patients were included. Socio-demographic data were assessed by using a structured questionnaire and it was checked for completeness and entered into SPSS version 25.0 Software. Both descriptive and inferential statistics were used in our study. The association between variables was determined by the chi-square test and Pearson correlation. Serum lipid profiles were compared by using a t-test and one-way ANOVA between groups but, hs-CRP levels were compared by using Mann-Whitney U-test between groups since the data wasn't follow normal distribution. A p-value < 0.05 was considered statistically significant.

Result: The mean serum HDL values were 37.54 ± 7.98 mg/dL and 43.12 ± 7.86 mg/dL for *H. pylori*-positive and negative dyspeptic patients, respectively, and the median of serum hs-CRP levels were 6.29 mg/L and 3.35 mg/L for *H. pylori*-positive and negative dyspeptic patients, respectively, with the p-value < 0.05 for both HDL and hs-CRP levels.

Conclusion: Serum HDL levels were significantly lower in *H. pylori*-positive dyspeptic patients than negative dyspeptic patients, but serum hs-CRP levels were significantly higher in *H. pylori*-positive dyspeptic patients than negative dyspeptic patients. Therefore, *H. pylori*-positive dyspeptic patients are potentially at higher risk for cardiovascular diseases than the negative.

Keywords: *H. pylori*, Dyspepsia, Lipid profile, hs-CRP, Inflammation, Cardiovascular diseases

1. INTRODUCTION

1.1 Backgrounds:

The term dyspepsia was derived from the two Greek words dys (bad) and Pepsis (digestion). Therefore, dyspepsia means bad digestion with prolonged and recurrent upper abdominal pain most commonly located at the epigastric area (William *et al.*, 2014). There is no globally accepted definition for dyspepsia. Instead, dyspepsia refers to persistent symptoms within the upper gastrointestinal tract (Torlutter *et al.*, 2003).

Dyspepsia is a broad term that incorporates several symptoms that are initiated from the upper gastrointestinal tract and have many causes. It is not a disease rather it is a collection of symptoms, which are relatively nonspecific and may present with one symptom or a combination of symptoms (Koduru *et al.*, 2018). The most common symptoms are epigastric pain, nausea, vomiting, bloating, anorexia, early satiety, and heartburn (Mahmoud *et al.*, 2016).

Patients presenting with a complaint of central epigastric pain or poor digestion who have not performed endoscope, laboratory, and X-ray examination are called uninvestigated dyspepsia. On the other hand, patients with dyspeptic symptoms who are performing investigation can be caused by 5 major causes: gastro-esophageal reflux, medications, functional dyspepsia, chronic peptic ulcer disease, and malignancy (William *et al.*, 2014).

Gastroesophageal reflux diseases can be described as a reflex of gastric contents towards the esophagus which results in epigastric pain, heartburn, and regurgitation. In dyspeptic patients, the occurrences of gastroesophageal reflux diseases are 25%. Peptic ulcer disease is the most common cause of dyspepsia which is characterized by chronic or recurrent upper abdominal pain that may be associated with meals and is often aggravated at night-time (Torlutter *et al.*, 2003). From the total dyspeptic patients, 5-15% of patients having chronic peptic ulcer disease. The majority of chronic peptic ulcer diseases are caused by *H. pylori* infection but NSAIDs has also a contributor to peptic ulcer diseases. This bacterium causes 90% of duodenal ulcers and 70% of gastric ulcers. Gastric and esophageal cancer is also a little contribution to dyspeptic symptoms.

Among dyspeptic only 2% of patients having gastric or esophageal adenocarcinoma (Harmon and Peura, 2010).

Functional dyspepsia can be demarcated as patients with a history of dyspeptic symptoms for at least 3-month without the presence of any organic, systemic, or metabolic diseases (Connors *et al.*, 2010). Factors involved in the disease process of functional dyspepsia are *H. pylori* infection, delayed gastric emptying, hypersensitivity, enteric pathogen, impaired accommodation of stomach, diet, lifestyle factors including smoking, alcohol drinking, and khat chewing, and psychosocial factors like stress, anxiety, and depression (Koduru *et al.*, 2018).

Another cause of dyspepsia is drug/medication such as aspirin and nonsteroidal anti-inflammatory drugs (e.g. cyclooxygenase-2-selective NSAIDs) can cause dyspepsia (Harmon and Peura, 2010).

The minor causes of dyspepsia are: autoimmune (Kulnigg-dabsch *et al.*, 2016), organisms other than *H. pylori* such as mycobacterium aviumintracellulare, enterococcal infection, herpes simplex, Crohn's disease, and cytomegalovirus (Sugano *et al.*, 2015), and eosinophilic gastritis (Nayak *et al.*, 2017).

H. pylori are a spiral-shaped, gram-negative, microaerophilic rod with 4–7 flagella. The flagella help in the settlement of the bacterium to the gastric mucosa layer (Lacy & Rosemore, 2001). *H. pylori* bacterium, which was first designated by Professor W. Jaworski at Cracow in Jagiellonian University, Poland. Next to him, Marshall and Warren grew the bacteria from dyspeptic patients, but that was not accurately similar to any previous species. Rather, it possibly belonged to the *Campylobacter* genus, therefore, they were naming it “*pyloric campylobacter*” next, they change the name to *Campylobacter pylori*. Finally, it had been termed *Helicobacter pylori* due to its distinct shape and genomic structures (Jamkhande *et al.*, 2016).

There are numerous strains of *H. pylori* and this multiplicity of *H. pylori* strains is because of the presence of several bacterial genome arrangements. *H. pylori* are the most commonly genetically wide-ranging species of bacteria, that produce different kinds of toxins such as urease, VacA, and CagA. Urease allows *H. pylori* to degrade urea down into ammonia and bicarbonate, then after bicarbonate degraded into water and carbon dioxide. Ammonia neutralizes the gastric acidic

environment that surrounds the bacteria and the bacteria use this ammonia as a protective device from acidic attack (Lacy & Rosemore, 2001).

Even though *H. pylori* infection is the central cause of chronic gastritis, the higher prevalence of *H. pylori* infection may also have a contribution to the pathogenesis of extra gastric diseases like that of cardiovascular diseases. Different studies have shown the possible mechanisms of *H. pylori* bacteria that could affect the gastric and extra gastric areas. Among other mechanisms, *H. pylori* induce inflammatory and immunological activity that probably disturb the normal function of body organs such as the heart, and blood vessels. *H. pylori* infection has a direct or indirect consequence on cardiovascular functions. The direct consequence can be blood vessels injury, cardiac damage by circulating lipopolysaccharides, abnormal growth of smooth muscle, and indigenous inflammation, and also had indirect consequence such as induction of proinflammatory cytokines, blood clotting, formation of fatty deposits in the arteries, fabrication of cross-reactive antibodies, LDL oxidation, auto-immunity, and nutrient malabsorption and metabolic consequences for example high ammonia production (Jamkhande *et al.*, 2016).

1.2. Statement of the problem

H. pylori produce different virulence factors that can cause gastric inflammation, disruption of the gastric mucosal barrier, and extra gastric effect (Dunn and Cohen, 2000). The critical component of the disease process of *H. pylori* infection is a chronic inflammation which is the initial step starting from superficial gastritis to chronic gastritis, intestinal metaplasia to dysplasia, and until invasive adenocarcinoma (He *et al.*, 2016).

Because of high virulent factors produced by *H. pylori*, it has been linked with many diseases inside and outside of the gastric area. These virulent factors mainly include vacuolating cytotoxic A (VacA) and cytotoxic-associated genes (CagA). CagA is more infectious than VacA and has greater roles in the formation of fatty deposits in the arteries, stimulation of autoimmunity, and highly activate the immune system. About half of *H. pylori* strains are CagA positive, which has been highly associated with cardiac conditions. These toxigenic molecules of *H. pylori* cause inflammation to host cells, cellular damage, and induce host factors such as interleukins, interferon-gamma, tumor necrosis factor, T and B lymphocytes, and phagocytic cells as a result the probability of the occurrence of cardiac diseases are increased (Jamkhande *et al.*, 2016).

The prevalence of *H. pylori* had great geographical variation in which higher prevalence occurs in developing countries than in developed countries (Agbor *et al.*, 2018).

H. pylori infections have been tested globally and attack all age groups. On average half of the world's populations are infected by these bacteria (Correa *et al.* 2016). The prevalence of *H. pylori* infection was 79.1% in Africa, 63.4% in Latin America and the Caribbean, 54.7% in Asia, 37.1% in Northern America, and 24.4% in Oceania (Sjomina *et al.*, 2018). This variation probability has been related to topography, age, race, and socioeconomic status (Melese *et al.*, 2019). Aging-associated cellular and molecular alteration can increase the risk of the gastric and extra gastric effect of *H. pylori* infection (Zendehdel, 2020).

In Ethiopia, the pooled prevalence of *H. pylori* infection was categorized by region which was the lowest (39.9%) in the Oromia and the highest (71%) in the Somalia region. Other regional pooled prevalence rates were 48.1% in Addis Ababa, 54.6% in Amhara, 48.7% in Benishangul Gumuz, and 53.6% in SNNPR (Melese *et al.*, 2019).

H. pylori infection not only cause gastric problem but also induce extra gastric diseases. Common extra gastric diseases related to *H. Pylori* include iron deficiency anemia, Henoch Schönlein Purpura, immune thrombocytopenic purpura, chronic urticaria, hepatocellular carcinoma, laryngeal cancer, insulin resistance, metabolic syndrome, cardiovascular diseases, and asthma (Testerman and Morris, 2014; Aksoy and Sebin, 2015).

The association between *H. pylori* and coronary artery disease was first proposed in 1994. The presence of CagA seropositivity leads to strengthening associations between *H. pylori* infection and cardiac diseases including atherosclerosis, unstable angina, and cardiac syndrome (Hamrah *et al.*, 2018). However, there are different kinds of controversial pieces of evidence that show the interrelationship of *H. pylori* infection and predictive risk factors of cardiovascular disorders such as lipid profile and hs-CRP levels. This research was conducted to fill the gap of earlier findings and to check those interrelations in our country that would help to inpatient management and complication prevention of *H. pylori*-positive dyspeptic patients.

1.3. Significance of the study

Majority of the world population are infected by *H. pylori* and this infection are associated with different complication including gastric and extra gastric problems. But different researchers demonstrated various data regarding the interrelation of *H. pylori* infection with lipid profile and inflammatory markers. In Ethiopia, chronic disorders are highly increased partially due to *H. pylori* infection, which contributes to gastric and extra gastric chronic complications. This study determines the burden of *H. pylori* infection and its association with lipid profile and serum hs-CRP levels. Therefore, the study will have significance for better management of *H. pylori*-infected patients to reduce further complications and help to perform a different kind of preventive and therapeutic measurement by the health professionals and insight to pay attention to laboratory investigation of lipid profile and hs-CRP in *H. pylori*-infected patients.

2. LITERATURE REVIEW

2.1. Epidemiology: In various developing countries, the majority of the population is *H. pylori*-positive, even at young ages. The prevalence of *H. pylori* in developed countries was generally lower than in underdeveloped countries, which indicates the inverse correlation of *H. pylori* infection with socioeconomic status. In developing countries, the prevalence of *H. pylori* infection relatively remains constant, but it is rapidly decreasing in the industrialized world due to improved hygiene and sanitation and the active elimination of carrier ship by antimicrobial treatment. In unindustrialized countries, *H. pylori* infection rates rise rapidly in the first 5 years of life and remain constantly high which indicating that *H. pylori* are acquired early in childhood. Reversely, in industrialized countries the prevalence of *H. pylori*, infection is low early in childhood and slowly rises with increasing age (Sjomina *et al.*, 2018).

A study conducted in the Korean population showed that 70.9% of study participants were *H. pylori*-positive. Study subjects in their 50s and 60s years had the highest (77.2%) seropositivity rate and the lowest (54.9%) rate in participants younger than 30 years old and more males (73.2%) were infected by *H. pylori* than females (66.7 %) (Sunga *et al.*, 2005).

The prevalence of functional dyspepsia was about 16% in Iran and it was to some extent greater among females and age group 25- 34 years old were highly affected. The occurrence of dyspepsia was high among subjects who were cigarette smokers, alcohol drinkers, and those who drink tea and coffee frequently compared with other subjects (Seyedmirzaei *et al.*, 1970). The prevalence of *H. pylori* infection in Cameroon was 47.4%. The distribution of this infection in terms of sex showed that the prevalence of *H. pylori* infection in females was 47.5% and 47.0% in males. Most (56.7%) of the seropositive samples were from patients less than 50 years old (Agbor *et al.*, 2018).

In our country Ethiopia, the prevalence of *H. pylori* infection was 71.0 % in Jigjiga, and the prevalence was high in females (75.5%) than males (68.5%). The majority of dyspeptic patients were chewing khat, drinking alcohol, and smoking a cigarette (Alebie, 2017). Five-year movements of *H. pylori* prevalence among dyspeptic patients conducted at Bahir Dar Felege Hiwot Referral Hospital showed that among all the study subjects (41.6%) were found to be

seropositive. The prevalence was significantly higher in males (43.2%) than in females (39.9%). Concerning age groups of the patients, high rates of *H. pylori* were found among the study subjects above 60 years (57%) (Workineh & Andargie, 2016).

The average age of dyspeptic patients was 37.84 ± 12.41 years at Dessie Referral Hospital. Among dyspeptic patients more than half (54.4%) were females, 46.9% were in the age group 30 to 49 years old, 67.4% were married, 61% were living in the urban area and 50% of dyspeptic patients had a habit of drinking alcohol (Seid *et al.*, 2018).

A study conducted at Yirga Cheffee Primary Hospital on dyspeptic patients showed that 52.98% were males and 47.02% were females, the average age of the subjects was 25 years, 66.7% of the patients were urban residents and 33.3% were from rural, the majority (46.4%) was a high school level educated, and *H. pylori* infection was maximum and minimum in patients, whose age was from 21 to 30 years and above 40 years, respectively (Ayele and Molla, 2017).

2.2. Microbiology of *H. pylori*

The Genus *Helicobacter* has different species but *H. pylori* can survive only in human beings. Other species of *helicobacter* can survive in different animals and some example of species includes: *H. felis* its natural hosts are Cat, and dog, *H. nemestrinae* it's the natural host is Primates, *H. mustelae* it's the natural host is Ferret, and *H. acinonyx* it's the natural host is Cheetah (Lee, 1994).

H. pylori bacterium is curved shaped, microaerophilic, gram-negative bacteria commonly present at the gastric antrum, which is the preferred habitat for it. The size of *H. pylori* bacterium is 2.5 to 5.0 μm long and 0.5 to 1.0 μm wide. *H. pylori* also have four to six unipolar sheathed flagella structure used for motility (Dunn and Cohen, 2000).

In genomic maps, *H. pylori* have multiple genes indifferent location that indicates that wide-ranging rearrangement of the *H. pylori* genome occurs. Those genes encoding for urease, a structural protein, accessory proteins, flagella, vacuolating cytotoxin, and CagA have significant sequence diversity (Thorell *et al.*, 2017).

H. pylori produce outer membrane vesicles that contain different virulence factors encompassing cell-bound components, secreted molecules, and antigens that could be injected into a host cell by the type IV secretion system (T4SS) or direct secretion of soluble compounds to the gastric area. Outer membrane vesicles are described as 20–300 nm blebs, which are secreted during the logarithmic phase of Gram-negative bacteria growth. Outer membrane vesicles can be crossing the cellular barrier which is the effective way of *H. pylori* antigens and virulence factors translocation via the gastric mucosa towards the immune cells. *H. pylori* outer membrane vesicles contain phospholipids (phosphatidylglycerol, phosphatidylethanolamine, lyso phosphatidylethanolamine, phosphatidylcholine, lysophosphatidylcholine, cardiolipin, and lipopolysaccharide) and proteins such as VacA, CagA, blood group antigen-binding adhesin, sialic acid-binding adhesin, outer inflammatory protein A, *H. pylori* neutrophil-activating protein, adherence associated lipoprotein, and urease. Those proteins and lipids are involved in the pathogenesis of *H. pylori* infection (Chmiela *et al.*, 2018).

2.3. Pathophysiology of *H. pylori* infection in the gastric area

The critical component of the disease process of *H. pylori* infection is a chronic inflammation which is the initial step starting from superficial gastritis to chronic gastritis, intestinal metaplasia to dysplasia, and until invasive adenocarcinoma (He *et al.*, 2016). *H. pylori* pathogenic determinants can be generally categorized into two major groups: the first one is virulence factors, which have a contribution to the disease process and the second one is maintenance factors, which are important for settlement and stability of bacterium within the host (Dunn and Cohen, 2000).

2.3.1. The virulence factors:

The virulence factors of *H. Pylori* have a greater contribution to the disease process via gastric inflammation and disruption of the gastric mucosal barrier. The first one is *H. pylori* induce gastric inflammation through a different mechanism: *H. pylori* capable of inducing IL-8 secretion from gastric cell more specifically VacA and CagA positive strains produced significantly more IL-8 than VacA and CagA negative strains; *H. pylori* can induce neutrophil-endothelial cell interactions and increase adherence of white blood cell through CD11a/CD18 and CD11b/CD18

interactions with intercellular adhesion molecule type 1; *H. pylori* induce inflammation through activation of platelet-activating factor by metabolizing the non-ulcerogenic precursor lyso-PAF (platelet-activating factor) into PAF. The activated PAF may induce mucosal injury directly or indirectly; the lipopolysaccharide of *H. pylori* can disrupt the gastric mucus coat via interfering with the interaction between mucin and its receptor and induce inflammation; the other mechanism of gastric inflammation is via urease enzyme which is secreted by *H. pylori* and a powerful stimulus of mononuclear phagocyte activation and inflammatory cytokine production (Richard *et al.*, 1997; Dunn and Cohen, 2000; Costa *et al.*, 2009).

The second virulence factor is via disruption of the gastric mucosal barrier these include: *H. pylori* express phospholipases A2 and C which disrupt apical membrane of mucus cells which is protective phospholipid-rich layer; *H. pylori* express mucinase like gene which has a contribution on the disruption of the gastric mucosal barrier; most of *H. pylori* secrete vacuolating cytotoxin and this cytotoxin induces acidic vacuoles in the cytoplasm of eukaryotic cells and disrupt mucosal barrier; *H. pylori* increase expression of reactive oxygen species which leads to mucosal injury; *H. pylori* also induce programmed cell death and induce gastric mucosal injury (Dunn and Cohen, 2000; Bohr *et al.*, 2007).

2.3.2. Maintenance factors

Maintenance factors are those factors involved for colonization and stability of *H. pylori* in the gastric area, some of the factors involved in this phenomena are: *H. pylori* have two to six flagella which is important for colonization of gastric and for motility of bacterium in the gastric area; a large quantity of urease is produced by *H. pylori* which is an essential colonization factor of *H. pylori* by maintaining gastric environment; *H. pylori* express the enzymes catalase superoxide dismutase which is homologs to intracellular microorganism and protect the bacteria from leukocytes attack; *H. pylori* has multiple adhesion molecules that can bind to the receptor in gastric epithelium. Commonly identified lipid receptor in the gastric mucosa that *H. pylori* can bind are Phosphatidylethanolamine and ganglio-tetraosyl-ceramide; another mechanism that *H. pylori* increase its stability in gastric through immune evasion in which *H. pylori* produce

ammonia that disrupts phagosome membrane and prevents itself from killing by phagocytes (Science *et al.*, 1997; Dunn and Cohen, 2000; Costa *et al.*, 2009).

2.4. Transmission of *H. pylori* infection

The transmission means of *H. pylori* infection is not known but can be transmitted from human to human or from contaminated environment to human. Most of the time person to person transmission occurred in the same family probability due to close interpersonal contacts. Person-to-person transmission can be happening probably through three major roots these are: gastro oral transmission which is the transmission of *H. pylori* infection through gastric juice during vomiting in early childhood; Oral-Oral transmission which is the transmission of *H. Pylori* infection from the oral carrier to another person via saliva commonly from mother to child transmission due to close contact; Fecal-Oral transmission is the transmission of the bacteria from face to mouth. *H. Pylori* also transmitted by the contaminated environment through contaminated water and food (Dunn and Cohen, 2000; Delpont, 2007).

2.5. Diagnosis of *H. pylori* infection

The diagnostic methods for *H. pylori* can be categorized into two groups. The first one is non-invasive methods which include: serological test, the urea breath test, urine test, and stool antigen test. The second method of detection of *H. pylori* is invasive methods which require endoscopy and biopsy such as histological examination, polymerase chain reaction (PCR), culture, and rapid urease test (Ricci and Holton, 2007; Talebi and Abadi, 2018).

2.6. Treatment of *H. pylori* infection

According to the Ethiopian treatment guideline, the First Line of *H. pylori* eradication therapy is triple treatments which include: Amoxicillin one gram per mouth two times a day, Clarithromycin 500mg per mouth two times a day, and proton pump inhibitor (PPI). Alternative treatment for *H. pylori* infection for those penicillin-allergic patients is Clarithromycin 500mg per mouth two times a day, *Metronidazole* 500mg per mouth two times a day, and proton pump inhibitor (PPI) (FMHACA, 2014).

2.7. The role of *H. Pylori* infection in extra gastric areas

***H. pylori* and endothelial dysfunction:** Infected with *H. pylori* induces chronic vascular inflammation and may lead to endothelial dysfunction through the following mechanism. The first one was, infected with *H. pylori* leads to an increase in inflammatory adhesion molecules such as intracellular adhesion molecule-1 and inflammatory mediator cytokines such as hs-CRP, IL-1, IL-6, and tumor necrosis factor- α . These molecules affect the microvascular vasomotor functions and resulting in vasoconstriction and endothelial dysfunction (Rasmi *et al.*, 2016; Coskun *et al.* 2008).

The second one was, *H. pylori* infection causes malabsorption of nutrition such as vitamin B12 and folic acid which lead to an increase in serum homocysteine levels. The relationship between vitamin B12, folate, and homocysteine levels and *H. Pylori* infection in dyspeptic patients showed that B12 deficiency was 23.8% and 22% in the *H. pylori*-positive and negative patients, respectively, and hyperhomocysteinemia was 46.2% and 44% in *H. pylori*-positive and negative patients, respectively (Rasool *et al.*, 2012).

Thirdly, *H. pylori* infection cause alteration of asymmetric dimethylarginine (ADMA) which plays a crucial role at the beginning of *H. pylori*-induced endothelial dysfunction. ADMA is a competitive inhibitor of endothelial NOS which reduces nitric oxide production from L-arginine by nitric oxide synthase in the endothelium to carries out its fundamental role in the regulation of vascular tone. ADMA is an endogenous competitive inhibitor of endothelial NOS, which reduces its fabrication and bioavailability (He *et al.*, 2014).

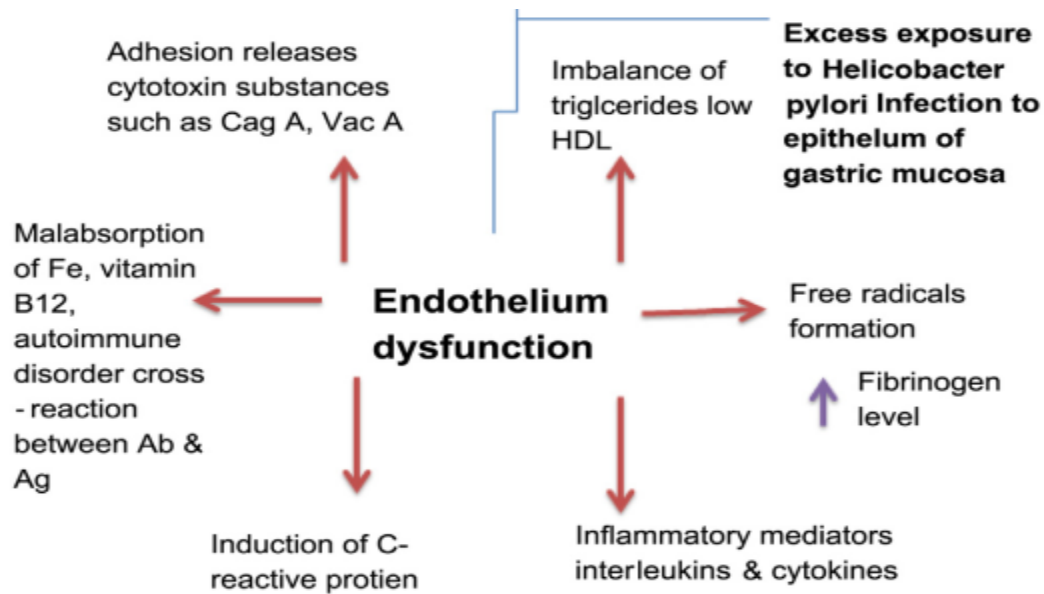


Figure 1 How *H. pylori* infection induces endothelium dysfunction and increased risk of CVD (Adapted from Jamkhande *et al.*, 2016).

***H. pylori* and chronic inflammation:** Chronic *H. pylori* infection provoke a pro-inflammatory condition, which causes an increase in cytokines levels such as TNF- α , interleukins (IL-1, IL-6, IL-8), gamma interferon, coagulant factors - fibrinogen, thrombin and soluble adhesion molecules such as intercellular adhesion molecule (ICAM-1), vascular cell adhesion molecule (VCAM-1) all of these have offered the central role for pathogenesis of CVD (Jamkhande *et al.*, 2016, and Rasmi *et al.*, 2016). Cytokines activate the T lymphocytes and macrophages, the activated lymphocytes and macrophages are inducing the proliferation of smooth muscle cells and extracellular matrix, which are playing a crucial role in the pathogenesis of cardiovascular disorders (Shin *et al.*, 2018; Kanbay *et al.*, 2005).

H. pylori infection also induce an immune-inflammatory reaction through activation of cyclooxygenase enzyme-2 (COX-2), which lead to an increment of production of prostaglandin (PGE2) and nitric oxide (NO). *H. pylori* cell wall lipopolysaccharide (LPS) triggers toll-like receptor-4, which activates different secondary mediators such as mitogen-activated protein kinase (MAPK), extracellular signal-regulated kinase, c-Jun N-terminal kinase (JNK) and p38 kinase resulting in boosting stimulation of NOS and COX-2 gene expression. LPS-induced

activation of MAPK cascade is also associated with epidermal growth factor receptor (EGFR) transactivation which is a vital protein regulating cellular proliferation, differentiation, migration, and modulation of apoptosis (Zhao *et al.*, 2017; Yonetamari *et al.*, 2012; Slomiany & Slomiany, 2013).

***H. pylori* and hyperhomocysteinemia:** *H. pylori*-infection causes atrophic gastritis, which is resulting in malabsorption of vitamin B12 and folic acid. Shortage of these vitamins leads to hyperhomocysteinemia because of disturbance of the re-methylation pathway (Rasool *et al.*, 2012; Evrengul *et al.*, 2007). Atrophic gastritis and low vitamin B12 were predictors of hyperhomocysteinemia possibly through vitamin B12 malabsorption (Santarelli *et al.*, 2004).

***H. pylori* and dyslipidemia:** Lower HDL and higher (TC, LDL, and TG) levels are convinced in *H. pylori* infection in some study (Satoh *et al.*, 2010), but a study conducted by Guzmán *et al.*, showed that gastric *H. pylori* infection did not have a significant relationship with the presence of dyslipidemia. The changes of the serum lipid profile (TC, TG, and LDL) are discreetly greater in the patients infected by *H. pylori*, but they are not statistically significant (Guzmán *et al.*, 2016). A study conducted in Jimma, Ethiopia, showed that except HDL all serum TC, TG, and LDL levels were significantly higher in *H. pylori*-positive dyspeptic patients than *H. pylori*-negative patients, but serum HDL levels were insignificantly different (Abdu *et al.*, 2020).

***H. pylori* and oxidative stress:** *H. pylori* infection resulting not only in inflammation but also causes the accumulation of reactive oxygen species (ROS) and oxidative DNA damage in the gastric mucosal layer. Therefore, the accumulation of ROS has been resulting to initiate multiple disease processes which have a contribution in the pathogenesis of cardiovascular disorders like that of atherosclerosis, through the expression of adhesion molecules, stimulation of vascular smooth muscle proliferation and migration, apoptosis in the endothelium, and oxidation of lipids (He *et al.*, 2014).

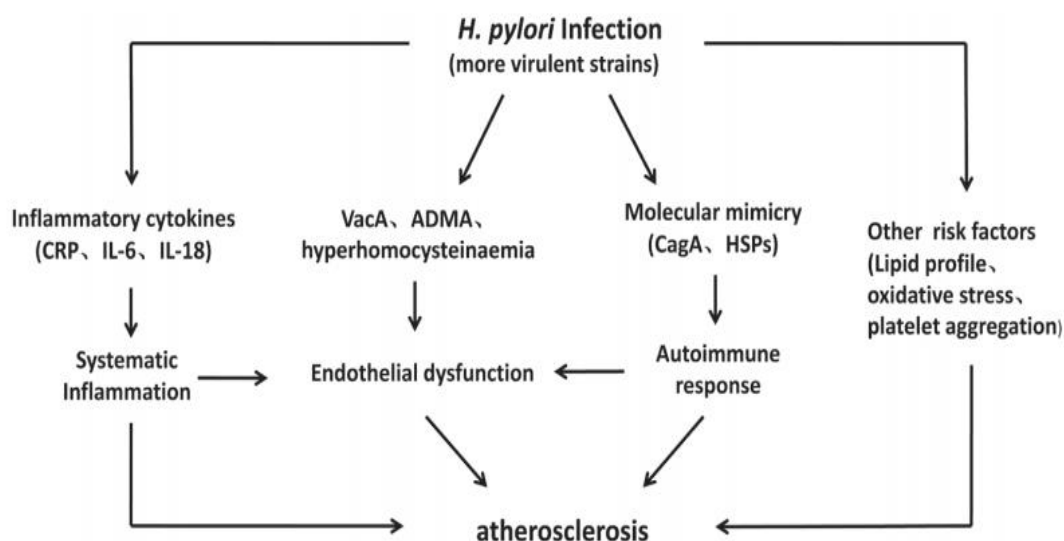


Figure 2 The potential mechanism of *H. pylori* infection towards cardiovascular disorders (Adapted from He *et al.* 2014)

2.8. The association of *H. pylori* infections with lipid profile and inflammatory markers

The potential risk of cardiovascular disorders following *H. pylori* infection is related to inflammation and immunity. The virulent strains *H. pylori* infection induces endothelial dysfunction, which is the primary step of the disease process, then atherosclerosis and other CVD will occur because of the effects of vacuolating cytotoxin A (VacA), asymmetric dimethylarginine (ADMA) modification, and nutritional deficiencies (Jamkhande *et al.*, 2016). Besides, following infection, the up-regulation of inflammatory cytokines, such as hs-CRP, IL-6, and IL-18, and induction of systematic inflammation were occurred which leads to cardiovascular disorders. The autoimmune responses triggered by cross-reactions between *H. pylori* antigens, such as cytotoxin-associated gene A (CagA), heat shock proteins (HSPs), and self-antigens, also help the role of *H. pylori* in the pathogenesis of atherosclerosis (Ishida *et al.*, 2008).

H. pylori infection is also linked with an atherogenic modified lipid profile. These alterations are may be mediated by cytokines, particularly TNF- α , which inhibits lipoprotein lipase and thus results in the mobilization of lipids from tissues as well as increase serum TG and reductions in

HDL. An altered lipid profile, including an increased LDL level and decreased HDL level, is one of the most important risk factors for atherosclerotic disease (Yang and Lu, 2014).

In *H. Pylori* positive patients, serum levels of TG (159 ± 125 mg/dL) and HDL (46.4 ± 12.1 mg/dL) were found to be significantly higher and lower, respectively, in comparison to *H. Pylori* negative patients with serum levels of TG (128 ± 71.4 mg/dL) and HDL (50.1 ± 12.3 mg/dL). However, no significant difference between seropositive and negative dyspeptic patients in terms of TC and LDL (Sayilar *et al.*, 2015).

The average age of *H. Pylori* positive (70.2 ± 7.8 years) subjects was significantly higher than the seronegative subjects (68.9 ± 7.2 years), but serum lipids, including TG, HDL, and LDL in patients with *H.pylori* positive and negative were similar (Sotuneh *et al.*, 2014).

The relationship between gastric *H. Pylori* infection and lipid levels according to Guzman *et al.*, showed that the levels of TC were 196.6 ± 42.1 mg/dL and 191.7 ± 29.5 mg/dL in the *H. pylori*-positive and negative patients, respectively, but the difference was not significant. In addition, the values of TG (164.4 ± 89.1 mg/dL vs. 139.2 ± 69.1 mg/dL), LDL (119.2 ± 33.8 vs. 115.9 ± 27.4 mg/dL), and HDL (44.4 ± 9.1 vs. 42.5 ± 10.7 mg/dL) for *H. Pylori*-positive and negative patients, respectively, but all of these values were statistically insignificant difference between infected and non-infected patients (Guzmán *et al.*, 2016).

Effect of *H. pylori* infection on serum lipid profile according to Haeri *et al.*, showed that the amount of LDL was significantly greater among *H. pylori*-infected patients as compared with those patients without the infection, but higher levels of TG and lower levels of HDL among *H. pylori* seropositive cases, however, there was no significant difference between the infected and non-infected cases concerning the levels of TG and HDL (Haeri *et al.*, 2018).

A comparative study conducted by Moutar *et al.*, showed that lipid profile in *H. pylori*-positive subjects were significantly higher for LDL (99.89 mg/dL), TC (169.71 mg/dL), and TG (132.29 mg/dL) than in *H. pylori*-negative subjects with (LDL 83.06 mg/dL, TC 153.37 mg/dL, and TG 112.62 mg/dl). But, the average HDL levels were significantly lower in *H. pylori*-positive patients (43.51 mg/dL) than *H. pylori*-negative patients (54.43 mg/dL) (Moutar *et al.*, 2016).

The average LDL, TG, TC, and HDL serum levels in the *H. pylori*-positive group was not significantly different with age groups, but the maximum levels of HDL were 52.7 mg/dL and the minimum levels were 40.69 mg/dL in the age group of 40-49 years and <20 years, respectively. The serum values of TG were highest and lowest in the age group of 40-49 years and < 20 years, respectively. The greatest and the lowest serum levels of TC was in the age group of < 20 years (177.83 mg/dL) and ≥ 50 years (162.14 mg/dL), respectively. There were no significant alterations in mean serum levels of LDL, HDL, TG, and TC between males and females in *H. pylori*-positive dyspeptic patients. LDL average serum values were lower in females (97.7 mg/dL) as compared to males (102.93mg/dL). Whereas HDL average serum levels were the same in both genders. TG average serum levels were higher in females (134.67 mg/dL) than in males (128.97 mg/dL). But, the average serum levels of TC were lower in females (167.85 mg/dL) than in males (172.3 mg/dL)(Moutar *et al.*, 2016).

The influence of *H. pylori* infection on serum lipid profile studied by Ibrahim *et al.*, indicated that there was a significant change of serum lipid profile between *H. pylori*-infected and non-infected dyspeptic patients. Serum TC, LDL, and TG levels were found to be significantly greater in *H. pylori*-infected cases than non-infected cases, but serum HDL levels were significantly lower in *H. pylori*-infected cases (Ibrahim *et al.*, 2014).

Another study conducted by Hassan *et al.*, also showed that the average serum levels of TC, TG, and LDL were 143.1 mg/dL and 115.61 mg/dL, 135.97 mg/dL and 92.23 mg/dL, 136.45 mg/dL and 66.38 mg/dL for *H. pylori*-positive and negative patients, respectively, and these values were significantly increased in *H. pylori*-positive dyspeptics, but HDL levels were decreased significantly (Hassan *et al.*, 2010). But, according to Kim *et al.*, serum levels of TC (184.6 ± 29.6 vs. 177.4 ± 31.0 mg/dL) and LDL (138.5 ± 33.0 vs. 121.3 ± 30.6 mg/dL) were significantly higher in the subjects with *H. pylori*-positive relation to those with *H. pylori*-negative patients, but there was no significant variation in other lipid profiles. Based on that research *H. pylori* infection can be associated with increased LDL levels, the greatest key risk factor for atherosclerosis. So, eradication therapy of *H. pylori* infection has a vital role in preventing atherosclerosis (Kim *et al.*, 2011).

The effect of *H. pylori* eradication therapy on lipid profile conducted by Iwai *et al.*, showed that HDL levels were (61.2 ± 14.7 mg/dL before treatment against 63.3 ± 15.8 mg/dL at post-eradication therapy, $P < 0.01$) were significantly improved, however the TC (206.0 ± 32.5 mg/dL against 205.1 ± 30.8 mg/dL), LDL (121.2 ± 28.7 mg/dL against 119.0 ± 27.6 mg/dL), and TG (98.1 ± 50.9 mg/dL against 103.5 ± 58.0 mg/dL) before and after treatment, respectively, but the change was significant. So that, giving the *H. pylori* eradication therapy can elevate the HDL levels in the *H. pylori*-infected chronic gastritis patients. This indicates that *H. pylori* eradication may have an anti-atherogenic role in the infected patients with chronic gastritis (Iwai *et al.*, 2019).

The relationship between *H. pylori* infection and lipid profile as studied by Kim *et al.*, showed that *H. pylori*-positivity was significantly correlated with higher levels of TC, LDL, and lower HDL levels. But, there was no positive correlation between *H. pylori*-positivity and TG levels. Therefore, *H. pylori* infection was significantly associated with dyslipidemia (Kim *et al.*, 2016).

The metabolic consequences of *H. pylori* infection as described by Buzás *et al.*, *H. pylori* infection could activate metabolic disturbances mediated by the cytokines produced in the inflamed gastric mucosa and elevate the TC and LDL and reduce HDL levels (Buzás *et al.*, 2014). Serum HDL levels have atheroprotective function via their ability to limit the expression of proinflammatory cytokines and to decrease the expression of leukocyte adhesion molecules when stimulated by inflammatory cytokines. Besides, HDL can induce the production of IL-10 which is an anti-inflammatory and atheroprotective cytokine (Fantadis, 2009).

The prevalence of CAD was 70% and 59% in *H. pylori*-positive and negative patients, respectively. High-sensitive-CRP levels (0.5 mg/dL) were significantly higher in patients with CAD than without CAD. The prevalence of CAD was significantly increased gradually depending on *H. pylori*-positivity and elevated hs-CRP levels i.e., the prevalence of coronary artery disease were 52% in seronegative patients without elevated hs-CRP levels, 56% in the subcategory with *H. pylori* seropositive, 61% in the subcategory with increased hs-CRP levels only, and 75% in the subcategory with joined seropositivity and elevated hs-CRP levels (Zhu *et al.*, 2002).

H. pylori infection had a greater prevalence in patients with CAD, the majority of patients with multiple vessel diseases (76.3%) and single-vessel diseases (70%) had *H. pylori* infection, but in patients without CAD, its prevalence was 50% and this variation was statistically significant. At the same time, patients with CAD had significantly higher hs-CRP levels compared to patients without CAD. HDL levels were significantly lower in the CAD group than the normal control group (Vafaeimanesh *et al.*, 2014).

A study conducted in Japan on the association between *H. pylori* infection and serum hs-CRP levels indicated that the serum mean levels of hs-CRP were 0.047mg/dL and 0.035mg/dL for *H. pylori*-positive and negative dyspeptic patients, respectively. This difference was statistically significant, but serum hs-CRP levels were not significantly different among different age groups and between gender (Ishida *et al.*, 2008).

3. OBJECTIVES

3.1. General objective

- ✓ To evaluate serum lipid profile and inflammatory marker among dyspeptic patients with *H. pylori* infection.

3.2. Specific objectives

- ✓ To measure lipid profile (HDL, TC, LDL, and TG) in *H. pylori*-positive and negative dyspeptic patients.
- ✓ To compare lipid profile (HDL, TC, LDL, and TG) between *H. pylori*-positive and negative dyspeptic patients.
- ✓ To assess and compare hs-CRP levels in *H. pylori*-positive and negative dyspeptic patients.

4. MATERIALS AND METHODS

4.1. Study area and period

The study was conducted from September 2020 to January 2021. The blood samples were collected from dyspeptic patients at the outpatient department of Debre Markos Referral Hospital, each of 20 *H. pylori*-positive and negative, Debre Markos Health Center, each of 15 *H. pylori*-positive and negative, and Hidassie Health Center, each of 15 *H. pylori*-positive and negative 15 samples in Debre Markos town, Ethiopia. The Hospital provides health services to more than 3.5 million populations. It has 140 beds with 152 staff for inpatient and outpatient services. Each health center is also providing health services for more than one hundred thousand populations and has 6 beds with 35 staff members. Debre Markos, the zonal town of East Gojjam, is located 300Km North West of Addis Ababa. The experimental serum samples were analyzed at the Ethiopia Public Health Institute (EPHI) Addis Ababa.

4.2. Study design

A comparative hospital-based cross-sectional study was conducted to evaluate the serum levels of lipid profile and inflammatory marker among clinically diagnosed dyspeptic patients with and without *H. pylori*-infection.

4.3. Population

4.3.1. Source population

The source population was all dyspeptic patients attending Debre Markos Referral Hospital, Debre Markos Health Center, and Hidassie Health Center.

4.3.2. Study population

The study population was all dyspeptic patients attending Debre Markos Referral Hospital, Debre Markos Health Center, and Hidassie Health Center during the study period.

4.4. Eligibility Criteria

4.4.1. Inclusion criteria

All dyspeptic patients attending at Debre Markos Referral Hospital, Debre Markos Health Center, and Hidassie Health Center during the data collection period were included in the study.

4.4.2. Exclusion criteria

- ✓ Subjects with a history of *H. pylori* eradication therapy.
- ✓ Patients who receive the antihyperlipidemic drug.
- ✓ Patients with rheumatoid arthritis, gouty arthritis, and other known inflammatory disorders.
- ✓ Subjects with liver disease, renal dysfunction, obesity, diabetes, cardiovascular disorders, and malignancy.

4.5. Sampling method and sample size determination

The non-probability purposive sampling technique was used to incorporate the desired dyspeptic patients during the study period. We were using a well-structured questionnaire to obtain appropriate information from study participants. For studies where the plan is to estimate the difference in means between two independent populations, the formula for determining the sample sizes required in each comparison group is given below:

$$n_i = \frac{2 (Z_{\alpha/2} + Z_{\beta})^2 SD^2}{d^2}$$
$$n_i = \frac{2(1.96 + 0.84)^2 * 9^2}{5^2}$$
$$n_i = 50.8$$

Where n_i is the sample size required in each group, $Z_{\alpha/2}$ is the value from the standard normal distribution reflecting the confidence level, usually, 95% confidence level is used = 1.96; Z_{β} is 80% power, usually 0.84; d is the effect size and SD reflects the standard deviation from previous studies. We have used the standard deviation (9 mg/dL) and effect size 5 mg/dL of TC from Haeri *et al.*, (2016) study. According to the above calculation, approximately 50 *H. pylori*-positive and 50 *H. pylori*-negative dyspeptic patients have used in our study.

4.6. Variables

4.6.1. Dependent variables

- ✓ Serum TC, TG, HDL, LDL, and hs-CRP.

4.6.2. Independent variables

- ✓ Socio-demographic factors.
- ✓ Risk factors for dyspepsia (chewing, smoking, and drinking).

4.7. Blood sample and data collection procedures

After we obtain informed consent from the study participants, about 5 mL of blood was drawn by qualified health care professionals. Blood collected in appropriate tubes was allowed to stand for 30 minutes at room temperature for complete clotting and clot retraction. Then the samples were centrifuged at 3000 rpm for 15 min to extract serum. The serum sample was stored at -70 °C before the day of analysis.

4.8. Laboratory test principles

4.8.1. Test principles of *H. Pylori* Ag cassette

The linear *H. Pylori* Ag cassette is a chromatographic immunoassay for the qualitative detection of *H. Pylori* antigen in human fecal specimen. It is used in the diagnosis of infection with *H. Pylori* and it 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 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. pylori* antibody, and the C band is pre-coated with goat anti-mouse IgG antibody.

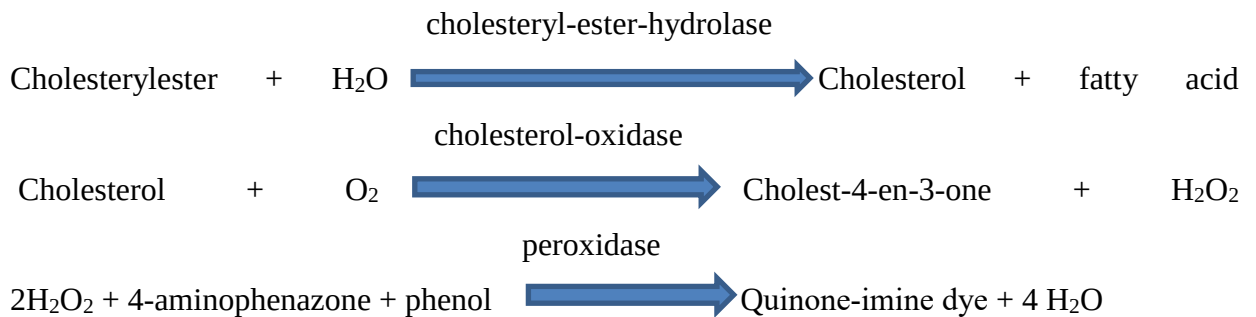
The fecal specimen is distributed into the sample well of the test cassette, the specimen migrates by capillary action across the cassette. If *H. pylori* antigen is present in the specimen, it will bind to the anti-*H. pylori* conjugates. The immune-complex is then captured on the membrane by the pre-coated antibody, forming a reddish-purple colored T band, indicating a *H. pylori*-positive test result. Absence of this band suggests that the concentration of *H. Pylori* in the specimen is below the detectable level or negative result. The detection limit for linear *H. Pylori* Ag is 5 ng/mL of *H. pylori* lysate. Fecal specimen containing *H. pylori* lysate ≥ 5 ng/mL routinely test positive. (Shimoyama *et al.*, 2009).

4.8.2. Test principles of the laboratory analytes

Materials: The common materials used in our laboratories test were syringes, serum separator gel tube, Nung tubes, refrigerator, centrifuge, Cobas® C 501 Chemistry Analyzer, micropipettes, micropipette tips, mixer, and substrate kits.

A. Total Cholesterol

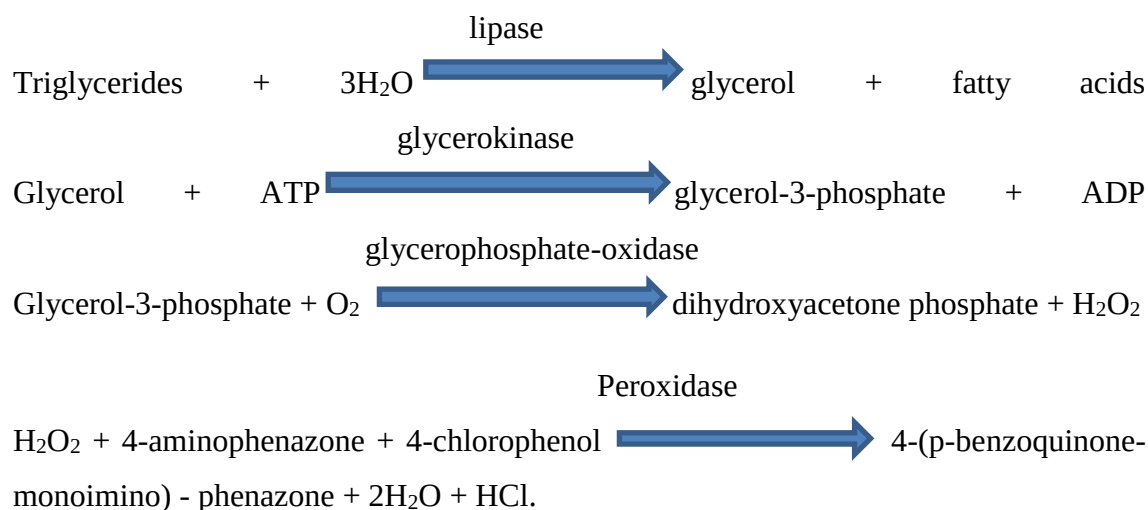
Cholesterol is measured enzymatically in serum in a series of reactions that hydrolyze cholesteryl esters and oxidize the 3-OH group of cholesterol. The byproducts, H_2O_2 are measured quantitatively in a peroxidase catalyzed reaction that produces color, and the color intensity is proportional to cholesterol concentration and the normal range is < 200 mg/dL (CDC, 2004). Absorbance is measured at 500 nm. The reaction sequence is as follows:



The increased serum values of total cholesterol enhance the potential risk of cardiovascular diseases, hence, TC measurement is important to assess CVDs risk status and to follow the progress of the patient's treatment to lower serum cholesterol concentrations (Kim *et al.*, 2016).

B. Triglycerides

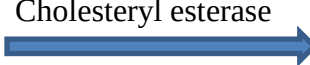
Triglycerides are also measured enzymatically in serum using a sequence of reactions in which triglycerides are hydrolyzed to produce glycerol. Glycerol is then oxidized using glycerol oxidase. The byproducts, H_2O_2 are measured quantitatively in a peroxidase catalyzed reaction that produces color, the color intensity is proportional to triglycerides concentration, and normal range is < 150 mg/dL. Absorbance is measured at 500 nm (CDC, 2004). The reaction sequence is as follows:



C. High-density lipoprotein (HDL) cholesterol

There are different methods used to determine serum HDL levels such as ultracentrifugation, electrophoresis, HPLC, precipitation, and direct methods. Among these methods, direct assay methods are commonly used. The assay uses detergents, cholesterol esterase, cholesterol oxidase, and peroxidase to form a colored pigment that is measured optically. Absorbance is measured at 600 nm and the normal range is > 40 mg/dL (CDC, 2004).

The apoB containing lipoproteins are reacted with a blocking reagent that renders them non-reactive with the enzymatic cholesterol reagent under conditions of the assay. The apoB containing lipoproteins are thus effectively excluded from the assay and only HDL is detected under the assay conditions. The method uses sulfated alpha-cyclodextrin in the presence of Mg^{+2} , which forms complexes with apoB containing lipoproteins, and polyethylene glycol-coupled cholesteryl esterase and cholesterol oxidase for the HDL measurement (CDC, 2004). The reactions are as follows:

1. ApoB containing lipoproteins + α -cyclodextrin + Mg^{+2} + dextran SO_4  soluble non-reactive complexes with apoB-containing lipoproteins.
2. HDL-cholesteryl esters  HDL-cholesterol + fatty acid.
3. HDL-cholesterol + O_2  Δ^4 -cholestenone + H_2O_2 .
4. $2H_2O_2$ + 4-amino-antipyrine + EMSE + H_2O + H^+  quinoneimine dye + $5H_2O$

D. LDL-cholesterol

Most of the circulating cholesterol is found in three major lipoprotein fractions: very-low-density lipoproteins (VLDL), LDL, and HDL.

$$TC = VLDL + LDL + HDL$$

LDL is calculated from measured values of TC, TG, and HDL according to the relationship:

$$LDL = TC - HDL - TG/5$$

where $TG/5$ is an estimate of VLDL and all values are expressed in mg/dL.

Adult normal range is < 130 mg/dL (CDC, 2004).

E. High sensitive-CRP

There are different assay methods available for determination of CRP levels such as nephelometry and turbidimetry. The Roche CRP assay method is based on the principle of particle-enhanced immunological agglutination. Human CRP agglutinates with latex particles coated with monoclonal anti-CRP antibodies. The precipitate is determined turbidimetrically. Particle-enhanced assays are based on the reaction between a soluble analyte and the corresponding antigen or antibody bound to polystyrene particles. Elevated values can be found among people with certain chronic inflammatory diseases, i.e. rheumatoid arthritis, juvenile chronic arthritis, ankylosing spondylitis, and Crohn's disease; in diagnosis and therapy of infections, and premature rupture of membranes or prediction of chorioamnionitis; differential diagnosis of pyelonephritis versus cystitis, bacterial versus viral infections, necrotizing pancreatitis versus edematous interstitial pancreatitis; and suspected renal allograft rejection. High sensitive-CRP has been of increasing interest because of the current availability of

quantitative assays and it provides a direct measurement of a serum protein that rises and falls rapidly in response to acute inflammation and/or tissue destruction. Although hs-CRP is still a nonspecific indicator, increasing numbers of investigators advocate its quantification for early detection of bacterial infections in a wide variety of clinical settings and for following disease activity and therapy in several chronic diseases. The concentrations of hs-CRP have been explored as risk factors for cardiovascular diseases (Hutchinson, 2001). The reference interval for hs-CRP: Adult, < 5mg/L; Neonates (0-3 weeks), 0.1-4.1 mg/L; Children (2 months-15 years), 0.1-2.8 mg/L (CDC, 2019).

Table 1: The summary of the reference range for serum lipid profile and hs-CRP in adults

Testes	Reference range
TC	< 200 mg/dL
TG	< 170 mg/dL
HDL	40-60 mg/dL
LDL	< 130 mg/dL
Hs-CRP	< 5 mg/L

4.9. Data processing and analysis

Data obtained from laboratory analyses of the serum samples and questionnaires were analyzed by entering the data into excel and SPSS software version 25.0 package and the different variables were tested. Simple descriptive statistics are used to present the socio-demographic and risk factors of the study subjects. While Chi-square (χ^2) tests are used to describe the association of categorical variables, lipid profiles are presented as mean $\pm$ standard deviation and are compared using the student t-tests and ANOVA test and hs-CRP levels were presented as medians and are compared by Mann-Whitney U-test since the data was not normally distributed. The associations between continuous variables were performed by Pearson correlation test. A p-value of < 0.05 at a 95% confidence level is considered to be statistically significant.

4.10. Ethical consideration

Before starting data collection, ethical clearance with reference number (SOM/BCHM/092/2012) was obtained from the Departmental Research and Ethics Review Committee, Department of Biochemistry, College of Health Sciences, Addis Ababa University. The aim of the research was clearly explained for every study subject, before incorporating all of the appropriate study subjects. After we were signing informed consent, blood samples and data were collected from the study participants. Privacy, impartiality, liability, and academic honesty were preserved during the study, for example, by using codes.

4.11. Operational definitions

Dyspepsia: comprises a wide and common clinical entity that presents in one of the three ways: Epigastric pain/burning (epigastric pain syndrome), Postprandial fullness, or Early satiety (EFMHACA, 2014).

Inflammatory markers: are used to detect acute inflammation that might indicate specific diseases/ as the markers of treatment response. Most commonly used inflammatory markers include hs-CRP, erythrocyte sedimentation rate, and plasma viscosity. In the present study, we have tested hs-CRP because hs-CRP levels are considered to be particularly useful in detecting bacterial infection(Watson, 2012).

High-sensitive-CRP levels: < 1 mg/L are desirable and lower atherosclerotic risk; levels between 1- 3 mg/L indicate moderate vascular risk; levels > 3 mg/L indicate higher vascular risk (Wang *et al.*, 2017).

Functional dyspepsia: is a clinical syndrome of gastrointestinal tract for at least 3-month without the presence of any organic, systemic, or metabolic diseases (Connors *et al.*, 2010).

Serum Paraxonase-1: a major antiatherogenic component of HDL, which has the following roles: protection of oxidation/ lipid peroxidation, induce natural immunity, and detoxification (Ceron *et al.*, 2014).

***H. pylori* lipopolysaccharides:** are the main surface antigens which are essential for the physical integrity and function of the bacterial outer membrane (Richard *et al.*, 1997).

5. RESULT

5.1. Socio-demographic data of dyspeptic patients

This study was conducted on 100 dyspeptic patients with 50 *H. pylori*-positive and 50 *H. pylori*-negative dyspeptic patients. The mean age of *H. pylori*-positive and negative dyspeptic patients was 43.63 ± 13.31 and 35.54 ± 12.59 years, respectively. In *H. pylori*-positive dyspeptic patients, 26 participants were males and 24 participants were females. The age category of this study indicated that in *H. pylori*-infected patients 4 participants were ages ≤ 20 years, 16 participants were ages from 21-40 years, and 30 participants were ages from 40-60 years. *H. pylori* status is significantly associated with Age category of dyspeptic patients at p-value < 0.05 . The socio-demographic variables are summarized in the following Table 1.

Table 2 Socio-demographic characteristics of dyspeptic patients with and without *H. pylori* infection at DMRH, DMHC, and HHC, Debre Markos, Ethiopia (N=100)

Variables	Categories of variables	HP+ (n=50)		HP- (n=50)		P-value
		Frequency	%	Frequency	%	
Age category	≤20 years	4	8%	6	12%	0.019
	21-40 years	16	32	28	56	
	40-60 years	30	60	16	32	
Sex	Males	26	52	17	34	0.069
	Females	24	48	33	66	
Marital status	Single	7	14	9	18	0.66
	Married	38	76	39	78	
	Divorced	2	4	1	2	
	Windowed	3	6	1	2	
Educational status	Illiterate	12	24	5	10	0.165
	Primary education	19	38	16	32	
	Secondary education	7	14	11	22	
	College/university	12	24	18	36	
Occupational status	Housewife	9	18	10	20	0.409
	Farmer	19	38	11	22	
	Gov't employee	6	12	10	20	
	Private employee	5	10	4	8	
	Merchant	6	12	5	10	
	Student	5	10	10	20	
Residency	Urban	21	42	26	52	0.316
	Rural	29	58	24	48	
Income level (in Ethiopian birr)	Below 2000	7	14	10	20	0.589
	From 2000-5000	33	66	33	66	
	Above 5000	7	14	10	20	

Keynote: All variables are categorical, and determined by number and percent, p-value was determined by using Pearson chi-square test. *H. pylori*, *helicobacter pylori*; HP+, *H. pylori* positive; HP-, *H. pylori* negative; DMRH, Debre Markos Referral Hospital; DMHC, Debre Markos Health Center; HHC, Hidassie Health Center.

5.2. Dyspepsia risk factors

The majority (70 participants) of dyspeptic patients were alcohol drinkers. Thirty-nine of the 70 alcohol drinkers were *H. pylori*-positive dyspeptic patients. Only 8 of the 100 dyspeptic patients had a history of cigarette smoking, but 6 of the 8 smokers were *H. pylori*-positive dyspeptic patients. Similarly, only 7 of the 100 dyspeptic patients were khat chewers, however, 6 of the 7 khat chewers were *H. pylori*-positive and *H. Pylori* is significantly associated with chewing khat with p-value of 0.026. Dyspepsia risk factors are summarized in the following Table 2.

Table 3 Dyspepsia risk factors among dyspeptic patients at DMRH, DMHC, and HHC, Debre Markos, Ethiopia (N=100)

Risk factors	Risk category	HP+		HP-		P-value
		Frequency	%	Frequency	%	
Alcohol use	Current alcohol users	39	78	31	62	0.165
	Ex-alcohol users	0	0	1	2	
	Never used	11	22	18	36	
Cigarette smoking	Current smokers	0	0	1	2	0.093
	Ex-smokers	6	12	1	2	
	Never smokers	44	88	48	96	
Khat chewing	Current chewers	0	0	1	2	0.026
	Ex-chewers	6	12	0	0	
	Never chewers	44	88	49	98	

Keynote: All variables are categorical, and determined by number and percent. P-value was determined by using Pearson chi-square test.

5.4. The comparison of serum lipid profile and hs-CRP levels between *H. pylori*-positive and negative dyspeptic patients

An independent t-test was conducted to compare the mean lipid profiles such as TG, TC, HDL, and LDL between *H. pylori*-positive and negative dyspeptic patients. Among other lipid profiles, serum HDL levels were significantly different between *H. pylori*-positive and negative dyspeptic patients. Serum HDL ($t(97.978) = -3.52, p < 0.05$) in scores for *H. pylori*-positive patients

(M=37.54, SD =7.98 mg/dL) were lower and for *H. pylori*-negative patients (M =43.12, SD =7.86 mg/dL).

An independent non-parametric Mann-Whitney U-test was conducted to compare serum hs-CRP levels between *H. pylori*-positive and negative dyspeptic patients. Serum hs-CRP levels were significantly different between *H. pylori*-positive and negative dyspeptic patients with the median of *H. pylori*-positive and negative dyspeptic patients was 6.29 mg/L and 3.35 mg/L, respectively with p-value < 0.01.

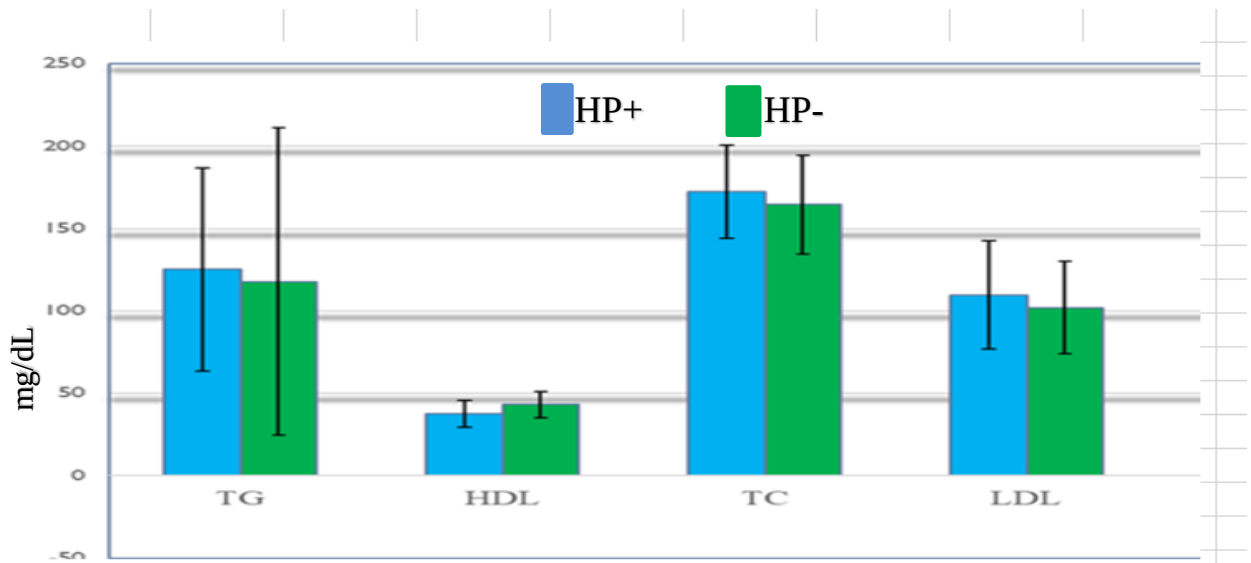


Figure 3 Mean serum values of lipid profile in *H. pylori*-positive and negative dyspeptic patients at DMRH, DMHC, and HHC, Debre Markos, Ethiopia (each of 50 HP+ and HP- participants)

Keynote: HDL, high-density lipoprotein; TC, total cholesterol; LDL, low-density lipoprotein; TG, triglyceride.

5.5. The comparison of serum lipid profile and hs-CRP levels between *H. pylori*-positive and negative dyspeptic patients for the same gender.

An independent t-test was conducted to compare the mean lipid profile of TG, TC, HDL, and LDL between *H. pylori*-positive and negative dyspeptic patients for the same gender. In males, serum HDL levels were insignificantly different between *H. pylori*-positive and negative dyspeptic patients, but in females, HDL levels were significantly different between *H. pylori*-positive and negative dyspeptic patients.

To evaluate serum hs- CRP levels between *H. pylori*-positive and negative dyspeptic patients for the same gender Mann-Whitney U-test was conducted. The test showed that, in males' serum hs-CRP levels were significantly different between *H. pylori*-positive and negative dyspeptic patients with median of 6.79 mg/L and 3.42 mg/L, respectively ($p = 0.004$), in females hs-CRP levels also significantly different between *H. pylori*-positive and negative dyspeptic patients with median 5.78 mg/L and 3.33 mg/L, respectively ($p = 0.001$). The following table summarizes it.

Table 4 The comparison of serum lipid profile and hs-CRP between *H. pylori*-positive and *H. pylori*-negative dyspeptic patients for the same gender at DMRH, DMHC, and HHC, Debre Markos, Ethiopia (N=100)

Variables	Sex	<i>H. pylori</i> status		P-value
		HP+	HP-	
Serum TG in mg/dL	Males	120.673 $\pm$ 64.468	148.859 $\pm$ 83.918	0.378
	Females	130.275 $\pm$ 59.45	101.836 $\pm$ 50.83	0.057
Serum HDL in mg/dL	Males	39.242 $\pm$ 7.763	42.654 $\pm$ 8.854	0.19
	Females	35.7 $\pm$ 7.952	43.355 $\pm$ 7.428	0.001
Serum TC in mg/dL	Males	173.476 $\pm$ 28.589	162.6 $\pm$ 30.077	0.239
	Females	171.159 $\pm$ 28.367	165.564 $\pm$ 30.43	0.484
Serum LDL in mg/dL	Males	110.226 $\pm$ 31.633	96.065 $\pm$ 31.304	0.157
	Females	109.196 $\pm$ 34.65	105.133 $\pm$ 26.32	0.617
Serum hs-CRP in mg/L	Males	6.74	3.42	0.04
	Females	5.78	3.33	0.001

Keynote: Serum lipid profiles are presented as mean $\pm$ SD and serum hs-CRP levels are presented as median. The p-value represents the significant level of lipid profile and hs- CRP between *H. pylori*-positive and negative patients for each male and female.

5.6. Comparison of serum lipid profile and hs-CRP among different age groups in dyspeptic patients.

One-way ANOVA tests were conducted to compare the mean serum levels of lipid profile among different age groups in dyspeptic patients. In *H. pylori*-positive patients, the mean serum LDL and TC levels were significantly different among different age categories, but in *H. pylori*-negative patients all serum lipid profile were insignificantly different among different age categories.

To compare serum hs-CRP levels among different age categories, Kruskal-Wallis-test was conducted. Both in *H. pylori*-positive and negative dyspeptic patients, serum hs-CRP levels were insignificantly different among different age categories. The following table summarizes it.

Table 5 The comparison of serum lipid profile and hs-CRP levels among different age group in dyspeptic patients at DMRH, DMHC, and HHC, Debre Markos, Ethiopia (N=100)

Variables	<i>H. pylori</i> status	Age categories			P-value
		≤ 20 years	21-40 years	41-60 years	
TG in mg/dL	HP+	97.0 ± 12.405	121.963 ± 69.137	130.823 ± 61.559	0.578
	HP-	115.267 ± 77.0	109.732 ± 71.813	132.944 ± 129.307	0.735
HDL in mg/dL	HP+	32.25 ± 4.009	38.7 ± 9.385	37.63 ± 7.461	0.357
	HP-	45.667 ± 11.676	43.801 ± 8.127	40.963 ± 5.379	0.367
LDL in mg/dL	HP+	85.85 ± 37.236	97.49 ± 35.76	119.445 ± 27.416	0.027
	HP-	94.913 ± 24.379	102.039 ± 28.956	104.744 ± 29.135	0.773
TC in mg/dL	HP+	137.5 ± 32.734	160.931 ± 30.583	183.11 ± 19.779	0.001
	HP-	163.633 ± 38.344	160.329 ± 29.245	172.3 ± 28.637	0.453
hs-CRP in mg/L	HP+	20.37	4.32	6.32	0.44
	HP-	1.27	3.49	3.35	0.053

Keynote: Serum lipid profiles are presented as mean ± SD and hs-CRP levels are presented as median. The p-value represents the significant level of lipid profile and hs- CRP in different age categories for *H. pylori*-positive as well as negative dyspeptic patients.

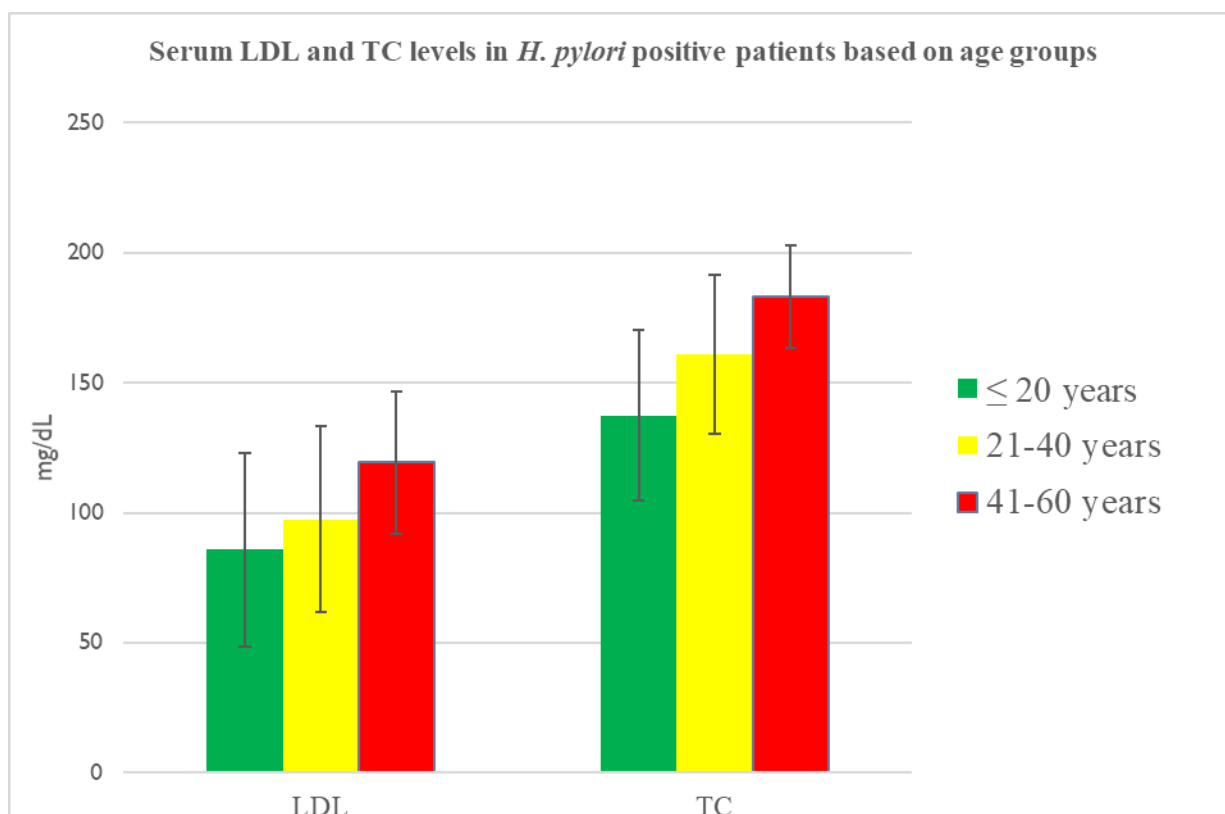


Figure 4 Mean serum values of LDL and TC in *H. pylori*-positive dyspeptic patients based on age categories at DMRH, DMHC, and HHC, Debre Markos, Ethiopia (N=100).

5.7. Comparison of serum lipid profile and hs-CRP levels between male and female dyspeptic patients.

An independent t-tests were conducted to compare the mean serum levels of lipid profile between male and female dyspeptic patients. There is no significant difference between males and females in serum levels of lipid profile both in *H. pylori*-positive and negative patients.

To compare hs- CRP levels between males and females' dyspeptic patients Mann-Whitney U-test was conducted. The test showed that hs-CRP levels were insignificantly different between males and females both in *H. pylori*-positive and negative patients The following table summarizes it.

Table 6 The comparison of serum lipid profile and hs-CRP levels between male and female dyspeptic patients at DMRH, DMHC, and HHC, Debre Markos, Ethiopia (N=100)

Variables	<i>H. pylori</i> status	Sex of participants		P-value
		Males	Females	
Serum TG in mg/dL	HP+	120.673 ± 64.468	130.275 ± 59.454	0.0588
	HP-	148.859 ± 83.918	101.836 ± 50.832	0.091
Serum HDL in mg/dL	HP+	39.242 ± 7.763	35.7 ± 7.952	0.118
	HP-	42.654 ± 8.854	43.355 ± 7.428	0.768
Serum LDL in mg/dL	HP+	110.226 ± 31.633	109.196 ± 34.652	0.913
	HP-	96.065 ± 31.304	105.133 ± 26.317	0.285
Serum TC in mg/dL	HP+	173.476 ± 28.589	171.159 ± 28.367	0.775
	HP-	162.6 ± 30.077	165.564 ± 30.433	0.745
Serum hs-CRP in mg/L	HP+	6.74	5.78	0.69
	HP-	3.42	3.33	0.68

Keynote: Serum lipid profiles are presented as mean ± SD and hs-CRP levels are presented as median. The p-value represents the significant level of lipid profile and hs- CRP between males and females for each *H. pylori*-positive and negative dyspeptic patients.

5.8.The correlation of age with serum lipid profile and hs-CRP levels among *H. pylori*-positive and negative dyspeptic patients.

Pearson correlation was conducted to assess the relationship between age with serum TG, HDL, LDL, TC, and hs-CRP levels among *H. pylori*-positive and negative dyspeptic patients. In *H. pylori*-positive dyspeptic patients, serum LDL and TC were found a statistically significant positive relationship with age ($r = 0.364$, $p < 0.05$) and $r = 0.485$, $p < 0.05$), respectively. This shows that in *H. pylori*-positive dyspeptic patients increase in age would lead to an increase in serum LDL and TC levels, but in *H. pylori*-negative dyspeptic patients' all lipid profiles and hs-CRP levels have an insignificant correlation with age ($p > 0.05$).

Table 7 The correlation of age with serum lipid profile and hs-CRP levels in *H. pylori*-positive dyspeptic patients at DMRH, DMHC, and HHC, Debre Markos, Ethiopia (n=50)

Variables	Pearson correlation	Age in year
Serum TG in mg/dL	r	0.096
	P-value	0.508
Serum HDL in mg/dL	r	0.086
	P-value	0.553
Serum TC in mg/dL	r	0.485
	P-value	0.001
Serum LDL in mg/dL	r	0.364
	P-value	0.009
serum hs-CRP in mg/L	r	0.137
	P-value	0.173

Keynote: Correlation is significant at p- value < 0.05 level (2-tailed).

6. DISCUSSION

H. pylori infection was associated with biochemical alteration in the human body (Moutar *et al.*, 2016; Guzmán, 2015). Our study was focusing on the evaluation and comparing of serum lipid levels (HDL, LDL, TG, and TC) and serum hs-CRP levels between *H. pylori*-positive and negative dyspeptic patients. In this study, each of 50 *H. pylori*-positive and negative patients was involved. The study participants were selected from the outpatient Department at Debre Markos Referral Hospital, Debre Markos Health Center, and Hidassie Health Center at Debre Markos town, Ethiopia. The average age of dyspeptic patients was 43.63 ± 13.31 years and 35.54 ± 12.59 years for *H. pylori*-positive and negative dyspeptic patients, respectively. The majority (60%) of *H. pylori*-positive dyspeptic patients were within the age group 40-60 years. Serum HDL levels were significantly lower in *H. pylori*-positive dyspeptic patients than negative dyspeptic patients, but serum hs-CRP levels were significantly higher among *H. pylori*-positive dyspeptic patients than negative dyspeptic patients. The rest of serum LDL, TG, and TC values were higher among *H. pylori*-positive dyspeptic patients than negative patients, but difference was not significant. The findings in sociodemographic, dyspepsia risk factors, serum lipid profile, and serum hs-CRP levels among *H. pylori*-positive and negative dyspeptic patients are discussed.

A. The role of sociodemographic factors in dyspeptic patients

Different studies have shown that age was one of the factors for the occurrence of dyspepsia (Harmon and Peura, 2010; Chul *et al.*, 2005). We have found that the age of *H. pylori*-positive dyspeptic patients (43.63 ± 13.31 years) was higher than the ages of *H. Pylori*-negative dyspeptic patients (35.54 ± 12.59 years). The same result was reported from studies conducted in Jimma (Abdu *et al.*, 2020). Besides, we have found that *H. Pylori*-positivity was significantly associated with the age group of dyspeptic patients, and significantly higher in the age group 40-60 years than the other categories. Similar findings were reported in studies conducted in Taiwan (Chen *et al.*, 2014); China (Wang *et al.*, 2019). This association of age with *H. Pylori* infection might be due to the reduction of the immune system when age is increasing, as a result, susceptibility to infectious diseases is increasing. On one hand, the immune system cannot protect the body from the foreign body efficiently because increasing age is resulting in dysregulation of multiple

constituents of the immune process. Besides this, living long increasing the chance of acquiring *H. pylori* infection due to prolonged exposure to the bacteria (Castelo-Branco and Soveral, 2014).

In the different studies, there was a gender difference in susceptibility to *H. pylori* infection (Ali, 2018; Mabeku, 2018). In our study, males (52%) are slightly more infected than females (48%). A similar finding was also reported in studies conducted in Jimma (Abdu *et al.*, 2020), Yemen (Ali, 2018), and China (Wang *et al.*, 2019). However, the exact mechanism was not clear, but the probability related to the hormonal difference between males and females (Zhu *et al.*, 2014). In males, androgen suppresses the phagocytic activity of macrophages and increases the susceptibility of males for infection than females (Klein and Flanagan, 2016). Our study revealed that from *H. pylori*-positive dyspeptic patients, males (52%) are slightly more infected than females (48%). However, the studies were done in Taiwan (Chen *et al.*, 2014), and Hosanna (Kahase and Haile, 2020) showed that females were more infected than males. This variation might due to differences in study participants. In our study, approximately the number of both genders was equal (47 females and 53 males), but in Taiwan and Hosanna studies, the majority of study participants were females.

The occupational and educational status also associated with *H. pylori* infection (Chen *et al.*, 2014). Our result indicated that farmers and primary education school completed are more infected than the others. This was in agreement with the study done in Jimma (Abdu *et al.*, 2020), and Hosanna (Kahase and Haile, 2020). This might be due to a lack of knowledge, attitude, and practice about the prevention, route of transmission, and treatment of *H. pylori* infection (Delpont, 2007).

The marital status was linked with acquiring *H. pylori* infection (Wang *et al.*, 2019), this might due to increasing in the family sizes are enhancing the transmission of bacteria from person to person because of close contact of family members (Dunn and Cohen, 2000; Delpont, 2007). In our result, the majority (78%) of *H. pylori*-positive dyspeptic were married. This was in agreement with studies conducted in Dessie Referral Hospital (Seid *et al.*, 2018), China (Wang *et al.*, 2019), Taiwan (Chen *et al.*, 2014), Jimma (Abdu *et al.*, 2020), and Hosanna (Kahase and Haile, 2020).

In our study, the majority (58%) of *H. pylori*-positive dyspeptic patients were rural residence. This might due to a lack of adequate health services, and other facilities such as poor water supply systems, and poor waste disposal systems that enhance the prevalence of *H. pylori* infection. Besides, lack of knowledge about the prevention, transmission, and treatment of the bacteria in rural areas increases its incidence. This was supported by studies done in Hosanna (Kahase and Haile, 2020), and Italy (Dore *et al.*, 2002). However, in contrast with a study done in Jimma (Abdu *et al.*, 2020), which showed the majority (61.8%) of *H. pylori*-positive dyspeptic patients were urban dwellers. This might be because of majority (58%) of study participants were comes from the urban area during that study, but in our study approximately the urban (47 participants), and rural (53 participants) residences were equal.

B. The role of alcohol drinking, cigarette smoking, and khat chewing in dyspeptic patients

Alcohol drinking increases the susceptibility to *H. pylori* infection and rises the incidence of dyspepsia through different ways. First, alcohol damages the gastric mucosal membrane and increases the mucosa's permeability, which is resulting in inflammation. Following inflammation, macrophages and neutrophils are releasing cytokine (IL-8), which combines with its receptor to facilitate more inflammation. Interleukin-8 is associated with HP0638, which is an outer inflammatory protein virulence factor that increases the adherence properties of *H. pylori* and thus facilitates bacterial colonization (Zhang *et al.*, 2009). Our study revealed that the majority (70 participants) of dyspeptic patients were current alcohol drinkers. Among these, 36 of the 70 alcohol drinkers were *H. pylori*-positive. The same findings were reported from studies were done in West Africa (Ofori *et al.*, 2019), Hosanna (Kahase and Haile, 2020), Dessie Referral Hospital (Seid *et al.*, 2018), and Yirga Cheffie Primary Hospital (Ayele and Molla, 2017), but in contradict with a study done in Jimma (Abdu *et al.*, 2020) and the variation might be due to socio-cultural difference between Jimma population and Debre Markose population on alcohol drinking.

Cigarette smoking increases the incidence of *H. pylori* infection due to the active molecule nicotine, which is present in cigarettes is reducing the synthesis of prostaglandins, as a result, the normal function of prostaglandins such as induction of mucosal bicarbonate secretion, induction of mucus secretion, increased blood flow, keeping gastric mucosal barrier integrity, increase cell

proliferation, and stimulation of cellular ionic transport chain are reduced. This causes the mucus layer susceptible to erosion and disruption by the acid and leading to gastric or peptic ulcers (Cope, 2013). Besides that, nicotine alters gastric mucosal blood flow, mucus secretion, and epidermal growth factor secretion that might facilitate *H. pylori* colonization (Zhang *et al.*, 2009). We have found that eight dyspeptic patients have a history of a smoking habit. Six of the eight smokers are *H. pylori*-positive. Similar findings were reported in Taiwan (Chen *et al.*, 2014), China (Wang *et al.*, 2019), Jigjiga (Alebie, 2016), and Cameroon (Mabeku *et al.*, 2018).

In our study, there is a significant association between *H. pylori* infection and history chewing Khat. This significant association might due to the negative effect of chemical constituents of khat such as cathinone, alkaloids, terpenoids, flavonoids, sterols, glycosides, and tannins on the body system (Gashaw and Getachew, 2014). Among these chemical components, cathinone is the major active ingredient of khat and has amphetamine-like properties that affect the central nervous system (Hassan *et al.*, 2015). Ninety percent of these ingredients are absorbed during chewing through the oral mucosa and affect appetite centrally, by acting in the hypothalamus. In addition to the central effect, cathinone enhances sympathomimetic activity leading to a delay in gastric emptying and enhance colonization of microorganisms including *H. pylori* bacteria in the stomach (Gashaw and Getachew, 2014). Other ingredients tannins in khat induce periodontal disease, stomatitis, esophagitis, and gastritis (Ali, 2018). This was supported by studies conducted in Yemen (Ali, 2018), Kenya (Hassan *et al.*, 2015), Yirga Cheffie primary hospital (Ayele and Molla, 2017), and Jigjiga (Alebie, 2017).

C. The role of *H. pylori*-infection in lipid metabolism

Earlier studies had indicated that *H. pylori* might be implicated in the alteration of serum lipid profile concentration and increase the potential risk of cardiovascular disorders (Satoh *et al.*, 2010, Sayılar *et al.*, 2015, Haeri *et al.*, 2018, Moutar *et al.*, 2016, Niemela *et al.*, 2016, Hassan *et al.*, 2014, Ibrahim *et al.*, 2014, and Kim *et al.*, 2011). But, some studies have reported that *H. pylori* infection did not significantly correlate with lipid profile (Guzmán *et al.*, 2016).

We have found that the mean serum TG (125.28 ± 61.67 mg/dL), TC (172.36 ± 28.22 mg/dL), and LDL (109.73 ± 32.78 mg/dL) levels were higher among *H. pylori*-positive dyspeptic patients

than the mean serum TG (117.82 ± 93.16 mg/dL), TC (164.56 ± 30.04 mg/dL), and LDL (102.05 ± 28.13 mg/dL) levels of *H. pylori*-negative patients. But, the difference was not statistically significant. This was in agreement with (Aarabi, 2010; Guzman *et al.*, 2016), but in contrast with (Moutar *et al.*, 2016; Abdu *et al.*, 2020). According to Moutar *et al.*, the mean serum LDL, TC, and TG levels of *H. pylori*-positive dyspeptic patients were significantly higher than *H. pylori*-negative dyspeptic patients. The increment of TG, LDL, and TC levels in *H. pylori*-positive dyspeptic patients might due to alteration of lipid metabolism following *H. pylori* infection (He *et al.*, 2014). The changes in lipid metabolism are related to inflammation caused by *H. pylori* infection. Chronic *H. pylori* infections cause inflammation via increasing expression of inflammatory cytokines, specifically by TNF- α , that inhibits lipoprotein lipase and resulting in the mobilization of lipids from tissues to the blood and causing an increase of serum TG, TC, and LDL levels (Aksoy and Sebin, 2015).

We have found that serum HDL levels of *H. pylori*-positive dyspeptic patients (37.54 ± 7.98 mg/dL) were significantly lower than negative dyspeptic patients (43.117 ± 7.858 mg/dL). A similar result was reported in studies conducted in Iraq (Moutar *et al.*, 2016), Iran (Hassan *et al.*, 2010), and a meta-analysis was done in Japan (Shimamoto *et al.*, 2020). This significant difference of HDL between *H. pylori*-infected and non-infected might be due to metabolic and inflammatory effects of *H. pylori* infection. Inflammation caused by *H. pylori* infection is bringing significant changes in the protein (apoA-I), and lipid content of HDL, as well as reduces the concentration of HDL (Fantidis, 2009). Inflammation causes an increase in reactive oxygen species such as Myeloperoxidase and superoxide anion radical, that resulting in the generation of oxidized and modified lipoproteins and reduce the concentration of HDL (Bonnin *et al.*, 2020). The metabolic effects of *H. pylori* infection are mediated by cytokines, predominantly TNF- α , which inhibit lipoprotein lipase and that results in the mobilization of lipids from tissues along with an increase in serum TG and decrease in HDL levels (He *et al.*, 2014).

Serum HDL has an atheroprotective function through the following mechanisms. The first one is, HDL has an antiatherogenic component called paraoxonase-1 (Chmiela *et al.*, 2015), which is an enzyme produced in the liver and has a various biochemical function in the body including protection of oxidation/ lipid peroxidation, induce natural immunity, and detoxification (Ceron

et al., 2014). It has also the ability to limit the expression of proinflammatory cytokines and to decrease the expression of leukocyte adhesion molecules when stimulated by inflammatory cytokines. Besides, HDL can induce the production of IL-10 which is an anti-inflammatory and atheroprotective cytokine (Fantadis, 2009). However, in our study, *H. pylori*-positive dyspeptic patients' serum HDL levels were significantly reduced due to the metabolic and inflammatory effect of *H. pylori* infection as a result they are potentially at higher risk for atherosclerosis and other cardiovascular diseases.

Except for the levels of HDL, the serum TG, TC, and LDL were found higher in males when compared with females. This was mainly due to the positive effect of estrogen on lipid profile in females and the negative effect of androgen on lipid profile in males (Kolovou *et al.*, 2011). In females' estrogen increases hepatic cell surface LDL receptors and consequently rapid clearance of LDL particles (Abdrabo *et al.*, 2013). Estrogen suppresses lipase activity in the liver as a result HDL levels increase. It also increases lipoprotein lipase activity, and lipoprotein lipase is responsible for hydrolyzing TG (Ariadi *et al.*, 2019). In males' testosterone increases the degradation of HDL molecule by stimulating the expression of genes encoding for hepatic lipase and scavenger receptor B. On the other hand, testosterone reduced the expression of LDL receptors and the activity of lipoprotein lipase. Therefore, HDL decrease, and LDL, TG and TC levels increased in males (Kolovou *et al.*, 2011). But, in our finding, there was no significant difference in lipid levels between males' and females' dyspeptic patients, and this might due to the effect of *H. pylori* infection, inflammation, and other confounding factors such as alcohol consumption, smoking, and khat chewing on lipid profile.

We have found that among *H. pylori*-infected patients' serum LDL and TC levels were significantly correlated with the age of dyspeptic patients and significantly higher among age group > 40 years old. This was inconsistent with (Chul *et al.*, 2005; Haeri *et al.*, 2018)). This significant association might be due to hormonal changes following increasing in age. In females, estrogen levels are decreasing after menopause which is resulting in the reduction of hepatic cell surface LDL receptors and consequently slowing the catabolism of LDL (Abdrabo *et al.*, 2013). Besides, the reduction of estrogen causes dysregulation of lipoprotein lipase activity and resulting in to increase in LDL and TC levels (Ariadi *et al.*, 2019).

D. The role of *H. pylori*-infection in inflammation

H. pylori-infection increases gastric and systemic inflammation through a different mechanism: The first one is, *H. pylori* capable of inducing inflammatory cytokines such as IL-8 secretion from gastric cells more specifically VacA and CagA positive strains produced significantly more IL-8 than VacA and CagA negative strains. The second one is, *H. pylori* induce neutrophil-endothelial cell interactions and increase adherence of white blood cell through CD11a/CD18 and CD11b/CD18 interactions with intercellular adhesion molecule type 1. The third one is, *H. pylori* activates platelet-activating factor by metabolizing the non-ulcerogenic precursor lyso-PAF (platelet-activating factor) into PAF. The activated PAF may induce mucosal injury directly or indirectly. The fourth one is, the lipopolysaccharide of *H. pylori* can disrupt the gastric mucus coat via interfering with the interaction between mucin and its receptor and induce inflammation, and the other mechanism of gastric inflammation is via urease enzyme which is secreted by *H. pylori* and a powerful stimulus of mononuclear phagocyte activation and inflammatory cytokine production (Richard *et al.*, 1997; Dunn and Cohen, 2000; Costa *et al.*, 2009).

H. pylori infection also induce an immune-inflammatory reaction through activation of cyclooxygenase enzyme-2 (COX-2), which lead to an increment of production of prostaglandin (PGE₂). *H. pylori* cell wall lipopolysaccharide (LPS) triggers toll-like receptor-4, which activates different secondary mediators such as mitogen-activated protein kinase (MAPK), extracellular signal-regulated kinase, c-Jun N-terminal kinase (JNK) and p38 kinase resulting in boosting stimulation COX-2 gene expression (Zhao *et al.*, 2017; Yonetamari *et al.*, 2012; Slomiany & Slomiany, 2013).

Ishida *et al.*, reported that the mean hs-CRP levels were significantly higher among *H. pylori* seropositive than those seronegative dyspeptic patients (Ishida *et al.*, 2008). This was in agreement with the present study with hs-CRP levels for *H. pylori*-positive (6.29 mg/L) and *H. pylori*-negative (3.35 mg/L; $p < 0.05$). This significant increment of hs-CRP levels among *H. pylori*-positive dyspeptic patients might be due to the inflammatory effect of *H. pylori* bacterium since the bacterium contain various virulent factors such as CagA and VacA. CagA, a highly virulent factor that is produced by *H. pylori* bacterium that has a greater role in *H. pylori*-induced

inflammation. *H. pylori* cause gastric and extra gastric low-grade systemic inflammation. One of the possible mechanism that promoting inflammation is via activation of the nuclear factor Kappa-B (NF- κ B) which mediates inflammation through activation of pro-inflammatory cytokines such as IL-6, TNF-alpha and IL-18. Finally, IL-6 stimulates acute phase reactant protein production such as hs-CRP from the liver (Aksoy and Sebin, 2015).

An increase in hs-CRP levels among *H. pylori*-infected dyspeptic patients has been a vital predictor of atherosclerosis and other cardiovascular diseases. This might due to hs-CRP provoke a wide array of pro-atherogenic effects such as stimulation of leukocyte-endothelium interactions, induction of a prothrombotic state, and activation of the complement system (He *et al.*, 2014). Besides, hs-CRP also has a great contribution to atherosclerotic plaque formation, activating endothelial cells, and increasing coagulation cascade. *H. pylori*-induced inflammation cause increase in serum fibrinogen levels resulting in thrombotic complication such as atherosclerosis, and other cardiovascular disorders (Aksoy and Sebin, 2015).

According to Satoh *et al.*, serum hs-CRP levels were significantly different between male and female subjects in *H. pylori*-positive dyspeptic patients (Satoh *et al.*, 2010). This variation of serum hs-CRP levels between males and females might probability due to the effects of androgen hormone on inflammatory mediators. Androgen hormone has an immunosuppressive effect and reduces the concentration of inflammatory markers including hs-CRP levels in males. The immunosuppressive effect of androgens could be either a direct effect on the expression of inflammatory genes or an indirect effect via inhibition of nuclear factor-kB activation (Tsilidis *et al.*, 2014). But in our study, serum hs-CRP levels were slightly higher in males than females in *H. pylori*-positive and negative dyspeptic patients, but the difference was not statistically significant and this variation might be due to difference in test statistics since we used non-parametric test statistics in our study.

In the present study, mean serum hs-CRP levels were significantly different between *H. pylori*-positive and negative males' dyspeptic patients. Similarly, mean serum hs-CRP levels were significantly different for *H. pylori*-positive and negative females' dyspeptic patients. This significant difference might be due to the inflammatory effect of *H. pylori* bacteria both in males and females (Aksoy and Sebin, 2015).

7. CONCLUSION

From our result, we can conclude that serum HDL levels were significantly lower in *H. pylori*-positive dyspeptic patients than negative dyspeptic patients, but serum hs-CRP levels were significantly higher among *H. pylori*-positive dyspeptic patients than negative dyspeptic patients. In *H. pylori*-positive dyspeptic patients, serum LDL and TC values were significantly different among different age groups and significantly higher in the age group ≥ 40 years old. Besides, chewing khat significantly increases the risk of *H. pylori* infection, and patients whose age greater than 40 years are highly susceptible to *H. pylori*-infection than the others. From this study, it is possible to conclude that *H. pylori*-infected dyspeptic patients will be potentially at high risk for atherosclerosis, endothelial dysfunction, and other cardiovascular disorders. Hence, reduction in serum HDL levels and increase in serum hs-CRP levels could be used as vital predictors of inflammation and atherogenesis.

8. STRENGTHS AND LIMITATIONS OF THE STUDY

The present study has the following strengths: In this study, we tried to exclude different disorders/ factors that can affect serum lipid profiles and hs-CRP levels, and have tried to include several socio-demographic characteristics and other factors that can be associated with dyspepsia and *H. pylori* infection. The study was conducted on participants who had volunteered to participate in the project, and as a result non-respondent rate was zero and finally *H. pylori* were detected by stool examination rather than serum. Therefore, false positivity was reduced. Besides, this research was conducted with struggles associated with Covid-19 pandemic.

Even though this study has substantial strength as mentioned above, it has some limitations as well. In our study endoscopic examinations were not conducted as a result different gastric malignancies could not be excluded; stool bacterial load was not assessed; the strain of *H. pylori* was not identified so that the exact effect of virulence factors of *H. pylori* could not be followed.

9. RECOMMENDATION

In developing countries, like that of our country Ethiopia, the prevalence of dyspepsia is high. However, the majority of dyspeptic patients are treated symptomatically without conducting a *H. pylori* test or other investigation. But, *H. pylori* infection were also associated with different gastric and extra gastric disorders, as a result, the following recommendations are being made keeping in mind the immediate needs for Ethiopian population:

- ☞ Chronic dyspeptic patient need to be tested for *H. pylori* bacterium to prevent further complications of the infection.
- ☞ *H. pylori*-positive chronic dyspeptic patient could be treated by *H. pylori* eradication therapy.
- ☞ *H. pylori*-positive chronic dyspeptic patient could have a health checkup for chronic disorders like cardiovascular diseases.
- ☞ During the diagnostic test, the strain of *H. pylori* bacterium needs to be identified among dyspeptic patients because the virulence strain (CagA⁺ and VacA⁺) could be associated with gastric and extra gastric complications.
- ☞ Lipid profile and inflammatory markers need to be tested in *H. pylori*-infected patients to predict future complications.

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11. ANNEXES:

11.1. Annex i: English version information sheet

Research project: evaluation of lipid profile and inflammatory marker in *H. pylori* infected dyspeptic patients at DMRH, DMHC, and HHC, Debre Markos, Ethiopia.

Principal Investigator: Gelagey Baye (BSc)

Advisors: Dr. Menakath Menon and Dr. Solomon Tebeje Gizaw

Sponsoring organization: Debre Markos and Addis Ababa Universities

Introduction: Good morning/afternoon dear participant! My name is _____. I am working as a data collector for the study conducted at DMRH, DMHC, and HHC by Gelagey Baye who is studying for MSc degree in Medical Biochemistry at Addis Ababa University, College of Health Sciences. I kindly request you to lend me your attention to explain about the study and being you selected as the study participant.

Study Objective: The purpose of this study is to evaluate the lipid profile and inflammatory marker in *H. pylori* positive and negative dyspeptic patients.

Procedures: If you agree to take part in this study, you will be given the consent form to sign, and interviewed by a health professional to assess whether you qualify to participate in the study or not. If you are fit for the study, the data collector will ask some questions which are important for the study like socio-demographic, and 5mL of the blood sample will be also collected for laboratory examination.

Possible risks: The study has no health risk except minimum pain associated with the blood withdrawal procedure.

Possible Benefit: Any incentive to you during participation in this study will not be given as compensation. But you will have the chance to know your general health status from the project without any payment because the cost will be covered by the project.

Confidentiality: The information you provide for us will be confidential and only used for research purposes. There will be no information that will identify you in particular. The findings of the study will be general for the study area and will not reflect anything particular of individual persons. The questionnaire will be coded to exclude showing names. No reference will be made in oral or written reports that could link participants to the study.

Rights: To protect the participant from any risk and discomfort which may result due to the procedure of the study, we have written an ethical clearance and cooperation letter obtained from Addis Ababa University College of Health Sciences, Department of Biochemistry. Participation in this study is fully voluntary. You have the right to declare to participate or not in the study. If you decide to participate, you have the right to withdraw from the study at any time.

Finally, if you faced any problem related to the study or if you have any question about the study, you can contact me via phone: 0918479703

11.2. Annex II: English Version Consent form

I am volunteering to participate in a research project conducted by Gelagey Baye from Addis Ababa University. I understand that the project is designed to gather information about the investigation of lipid profile and inflammatory marker among *H. pylori* positive and negative dyspeptic patients at DMRH, DMHC, and HHC, I will be one of the participants being selected for this research. I understand that I will not be paid for my participation. I may withdraw and discontinue participation from the study at any time without penalty. I understand that the researcher will not identify me by name in any reports using information obtained from this interview and that my confidentiality as a participant in this study will remain secure. Subsequent uses of records and data will be subject to standard data use policies which protect the privacy of individuals and institutions. I have been given a copy of this consent form and I have clearly understood the explanation provided to me. So, I approve my consent with my signature to take part in the study.

Participant's code number

Date

Signature

11.3. Annex III: English version questionnaires

Subject code number: _____ Card number _____

Instruction: Dear participants, you are generously requested to give correct information accordingly and Please choose and encircle for closed ended questions and fill the provided blank space for open ended questions.

Part I: Socio demographic variables				
S.No	Question	Response		
1	Age of participant	_____year		
2	Sex of participant	1. Male 2. Female		
3	Marital status	1.Single	2.Married	3.Divorced 4.Widowed
4	Educational status	1. Illiterate 2. Primary school 3. Secondary school 4. College/university		
5	Occupation	1. Housewife 2. Merchant 3. Farmer 4. Government employee 5. Private employee 6. Others _____?		
6	Residence	1. Rural 2. Urban		
7	Income level	1. <2000 birr 2. 2000-5000 birr 3. >5000 birr		
Part II: Risk assessing questions				
1	Smoking	1. Current smoker 2. Ex-smoker 3. Never smoking		
2	Alcohol drinking	1. Current alcoholic 2. Ex-alcoholic 3. Never drinking		
3	Chewing khat	1. Current chewer 2. Ex-chewer 3. Never chewing		

11.4. Annex IV አማርኛ ለተሳታፊዎች ስለጥናቱ መረጃ

ጤና ይስጥልኝ! እኔ_____ እባላለሁ። በአሁኑ ሰአት በአዲስ አበባ ዪኒቨርሲቲ ጤና ሳይንስ ኮሌጅ ሕክምና ት/ቤት በሜዲካል ባዮኬሚስትሪ ት/ክፍል በማስተርስ ድግሪ የሚያጠናው አቶ ገላጋይ ባዩ ለሚያደርገው የመመረቂያ ጥናት በመረጃ ሰብሳቢነት እየሰራሁ እገኛለሁ። እናም እርስዎ በዚህ የመመረቂያ ጥናት ላይ እዲሳተፉ ተመርጠዋል። እባክዎ በዚህ ጥናት ለመሳተፍ ከመስማማትዎ በፊት ከዚህ ቀጥሎ የሚገኘውን ምንባብ በጥሞና ያንብቡና ግልጽ ያልሆነልዎትን ማንኛውም ሃሳብ ይጠይቁ።

የጥናቱ ርዕስ፡- ኤች ፓይሎሪ ባለባቸው እና በሌላባቸው የጨንጓራ ህመምተኞች ደም ውስጥ ያለውን የስብ መጠን እና የሰውነትን መቆጣት የሚያሳይ ንጥረ ነገር መመርመር።

የጥናቱ ተሳታፊ ለመሆን የሚጠበቅበዎት፡ በዚህ ጥናት ለመሳተፍ የሚስማሙ ከሆነ የደም ናሙና እንደሚወሰድና ለጥናቱ እንዲሟወል መስማማት ይጠበቅብዎታል። ከተወሰደው ናሙና ላይ የሚገኙ መረጃዎች ከዚህ ጤና ተቋም ውጭ ለሚገኙና ለስራው አግባብነት ላላቸው ሰዎች ቢነገር የማይቃወሙ መሆኑን መስማማት ይጠበቅብዎታል። ይሁን እንጂ ይህ አይነቱ መረጃ የርስዎን ማንነት የሚገልጡ መረጃዎችን ማለትም ስም አድራሻና የስልክ ቁጥር የመሳሰሉትን መረጃዎችን አይጨምርም። ይልቁንም ለዚህ አገልግሎት ብቻ የሚወልድ እርስዎን ለማወቅ የሚያስችል መለያ ቁጥር ጥቅም ላይ እንዲወልድ ይደረጋል። በተጨማሪም ስለርስዎ አጠቃላይ የጤና ሁኔታ ለሚቀርቡ አንዳንድ ተጨማሪ ጥያቄዎች መልስ መስጠት።

በዚህ ጥናት መሳተፍ ሊያስከትላቸው የሚችሉ ችግሮች፡ ናሙና በሚሰበሰብበት ወቅት ምንም አይነት የከፋ ችግር አያጋጥምዎትም። ነገር ግን ደም ሲወሰድ መጠነኛ የህመም ስሜት ሊያስከትል ይችላል። ሆኖም ግን ናሙናውን ለመሰብሰብ ልምድ ያለው ባለሙያ ስለሚመደብና አስፈላጊው የጥንቃቄ እርምጃ ስለሚወሰድ የህመም ስሜት አይኖርም።

በዚህ ጥናት መሳተፍ ሊገኙ የሚችሉ ጥቅሞች፡ ይህ ጥናት የማስተርስ ዲግሪ መመረቂያ እንደመሆኑ መጠን በዚህ ጥናት በመካፈልዎ በገንዘብ የሚያገኙት ጥቅም ባይኖርም ከጥናቱ በሚገኘው ውጤት ግን ተጠቃሚ ነዎት። የእርሶዎ ተሳትፎ የእርስዎንና የወገንዎን ኤች ፓይሎሪ ባለባቸው የጨንጓራ ህመም እና

ሊያስከትላቸው የሚችለውን ተዛማጅ በሽታዎች ቀድሞ ለማወቅ ከፍተኛ ጥቅም ይኖረዋል።

የመረጃ በሚስጥር አጠባበቅ ሁኔታ: ስለራስዎ የሰጡት ማንኛውም መረጃና ከተወሰደው ናሙና ላይ የተገኘው የለቦራቶሪ ውጤት የሚዉለዉ ለጥናቱ አላማ ብቻ ነዉ። የጥናቱ ውጤት የተሳታፊዎችን አጠቃላይ ሁኔታ እንጂ የአንድን ግለሰብ ምንም ነገር አያንጸባርቅም።የተሳታፊዎችን ስም ላለማሳየት የራሳችንን መለያ ቁጥር የምንጠቀም ይሆናል።ይህን ማህደር ሊያገኙ የሚችሉት የተወሰኑ የጥናቱ ተባባሪ ሰዎች ብቻ ናቸው።ከዚያም በላይ ስለ እርስዎ ያለውን ማንኛውንም መረጃ የተለየ የይለፍ ቃል ባለው የኮምፒውተር የመረጃ ማህደር ውስጥ እንዲቀመጥ ይደረጋል።

በዚህ ጥናት ላለመሳተፍ ያለዎት መብት: በዚህ ጥናት መሳተፍ ሙሉ በሙሉ በእርስዎ ፈቃደኝነት የተመሰረተ በመሆኑ በማንኛውም ሰዓትና ቦታ የማቋረጥ ሙሉ መብት የተጠበቀ ከመሆኑም በላይ እራስዎን ከጥናቱ በማግለልዎ ምክንያት የሚቀርብዎት ምንም አይነት የጤና አገልግሎት አይኖርም ። ከዚህም በተጨማሪ ጥናቱን በተመለከተ ማንኛውንም አይነት ጥያቄ የመጠየቅና ገለጻ የማግኘት መብት አለዎት።የላብራቶሪ ምርመራ ውጤቱንም በነጻ ማግኘት ይችላሉ። ነገር ግን እርስዎ በሚሰጡን መረጃ የችግሩን ስፋት ለማወቅ ጠቃሚ ስለሆነ ለሚቀርብልዎት ጥያቄ ቀጥተኛ መልስ ይሰጡን ዘንድ በታላቅ አክብሮት እንጠይቃለን። ጥያቄ ካልዎት ወይም ችግር ካጋጠመዎት፡ ይህንን ጥናት በተመለከተ ወይም ከዚህ ጥናት ጋር በተዛመደ መልኩ ችግር ከገጠመዎት ወይምጥያቄ ካለዎት በስልክ ቁጥር፡ 0918479703 ይደውሉ።

11.5. Annex V: አማርኛ የስምምነት ማረጋገጫ ቅጽ

የዚህ ጥናት መሰረታዊ ዓላማ እና ሌሎች መረጃን በሚገባ ተገንዝቢያለሁ። ጥናቱ በደ/ማ/ረ/ሆ፣ ደ/ማ/ጤ/ጣ እና ህ/ጤ/ጣ በሚገኙ በጨጓራ ህመምተኞች ላይ በደም ውስጥ ያለውን የስብ መጠን እና የሰውነትን መቆጣት የሚያሳይ ንጥረ ነገር ለመለካት እንደሆነ በሚገባ ተገንዝቢያለሁ። ተሳትፎ በፍቃደኝነት ላይ ብቻ የተመረኮዘ እንደሆነም ተረድቻለሁ። ማንኛውም ሰብዓዊም ሆነ ህጋዊ መብቱ ሳይነካ ከጥናቱ ራሴን ማግለል እንደምችልም እንደሆነ። ስለ ጥናቱ ዝርዝር ጉዳይ በግልፅ ከተረዳሁት ባሻገር በተጨማሪ ማብራሪያ ብፈልግ መጠየቅ እንደምችልም አዉቄያለሁ። የጥናቱም ባለቤት የዚህ የጥናቱ መረጃ ይፋ የሚሆነው ለእኔ ብቻ እንደሆነ እና ስለሚወሰደው ማንኛውም መረጃዎች ሆነ የጥናት ውጤት ለማሰራጨት በስም ሳይሆን በሚስጥር(ኮድ) እንደሆነም ተረድቻለሁ። በመሆኑም በፈቃዴ የዚህ ጥናት አካል እንድሆን ስፈልግ የምጠብቅብኝን ሁሉ ለሚድረግ በመወሰን መሆኑን በፊርማዬ አረጋግጣለሁ።

የተሳታፊ የምስጥር ቁጥር ቀን ፊርማ

11.6. Annex VI: አማርኛ መጠይቅ

የሚስጥር ቁጥር: _____ ካርድ ቁጥር _____

አቅጣጫ: ውድ ተሳታፊ ቀጥሎ ያለውን መጠይቅ ለመሙላት ስለተባበሩን እናመሰግናለን በመቀጠል ለምርጫ ጥያቄዎች መልሱን አክብቡ፤ ለፃፍ ጥያቄዎች ደግሞ ፊትለፊት ካለው ክፍት ቦታ ላይ መልሱን ይፃፉ።

ክፍል 1: ማህበራዊና ዲሞክራሲያዊ መጠይቆች		
ተ.ቁ	ጥያቄ	መልስ
1	ዕድሜ	_____ (በዓመት)
2	ፆታ	1. ወንድ 2. ሴት
3	የጋብቻ ሁኔታ	1. ያላገባ 2. ያገባ 3. የፈታ/ች 4. ባለቤቷ/ቱ በህይወት የሌለ
4	የትምህርት ደረጃ	1. ያልተማረ 2. የመጀመሪያ ደረጃ 3. ሁለተኛ ደረጃ 4. ኮሌጅ/ዩኒቨርሲቲ
5	የስራ ሁኔታ	1. የቤት እመቤት 2. አርሶ አደር 3. ነጋዴ 4. የመንግስት ሰራተኛ 5. የግል መስሪያ ቤት ሰራተኛ 6. ሌላ _____ (ካለ ጥቀስ)
6	የመኖሪያ ቦታ	1. ከተማ 2. ገጠር
7	የገቢ መጠን	1. ከ 2000 ብር በታች 2. ከ 2000-5000 ብር 3. ከ 5000 ብር በላይ
ክፍል 2: በሽታ አጋለጫ ከሆኑ ነገሮች ጋር ተያያዥነት ያላቸው ጥያቄዎች		
1	ሲጋራ ያጨሳሉ ?	1. አዎ አሁንም አጨሳለሁ 2. በፊት አጨስ ነበር 3. አጭሽ አላውቅም
2	አልኮል ይጠጣሉ ?	1. አዎ አሁንም እጠጣለሁ 2. በፊት እጠጣ ነበር 3. ጠጥቼ አላውቅም
3	ጫት ይቅማሉ?	1. አዎ አሁንም እቅማለሁ 2. በፊት እቅም ነበር 3. ቅሜ አላውቅም