A LINK BETWEEN *Helicobacter pylori* INFECTION AND CORONARY HEART DISEASE

PhD Thesis

Submitted by

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I hereby humbly declare that this Thesis entitled "A link between *Helicobacter pylori* infection and coronary heart disease" is based on work carried out by me and no part of it has been presented previously for any higher degree.

The research work was carried out in the Department of Cardiology, NICVD, Dhaka, under the guidance of Prof. MA Malek, Institute of Nutrition and Food Science, and Prof. MA Khaled, Department of Nutritional Sciences, University of Alabama, Birmingham, United States of America.

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Certified that Dr Md Afzalur Rahman has completed his Thesis entitled "A link between *Helicobacter pylori* infection and coronary heart disease" at the Department of Cardiology, NICVD under our guidance.

His work is genuine and up to our full satisfaction.

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Prof MA Malek,
Institute of Nutrition and Food Science,
Dhaka University

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ABBREVIATIONS

Ab	Antibody
BMI	Body Mass Index
CAG	Coronary angiogram
CHD	Coronary heart disease
DM	Diabetes Mellitus
ELISA	Enzyme linked immunosorbant assay
HDL-C	High density lipoprotein-cholesterol
HTN	Hypertension
Ig	Immunoglobulin
LDL-C	Low density lipoprotein-cholesterol
MI	Myocardial Infarction
NICVD	National Institute of Cardiovascular Diseases
R	Correlation coefficient
P	Probability
SD	Standard deviation
SE	Standard error
TC	Total cholesterol
TG	Triglyceride

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ABSTRACT

Abstract

Several studies have shown the association of *Helicobacter* pylori infection with coronary heart disease (CHD). Although there are reports of very high *H pylori* infection rate in Bangladesh, its association with CHD has not yet been explored.

Aim: To investigate the relationship between *H pylori* antibody status and angiographically defined CHD.

Methods: Using an analytical cross-sectional (Case-Comparison) design we studied 112 subjects who underwent coronary angiography for the first time for suspected or known CHD at the Department of Cardiology, NICVD between July and December 2002. Arterial stenosis of 50% or more was taken as significant. Among the study subjects 86 (77%) have CHD (Cases) and 26 (23%) did not have CHD (Comparison group). Demographic data, history and physical examination findings were recorded. Major risks factors considered: family history of CHD, diabetes mellitus, smoking, dyslipidemia and hypertension. Anti- *H pylori* IgG antibody titers were determined by specific ELISA method.

Results: Fifty-eight (67%) of CHD patients and 14 (54%) of the comparison group were seropositive for *H pylori*. Logistic regression analysis revealed that sex (β= -16.375, P=0.002), LDL-C (β=0.209, P=0.007), history of diabetes (β= -6.067, P=0.013), and anti *H pylori* antibody status (β= -4.015, P= 0.028) are significant predictors of development of CHD irrespective of age, BMI, smoking status, serum total cholesterol, HDL-C and tryglyceride levels. There was a significant association (P= 0.039) between *H pylori* infection and serum total cholesterol controlling for other risk factors. *H pylori* antibody positive subjects had around 20 mg/dL higher serum total cholesterol level than *H pylori* antibody negative subjects. Also, a significant association was found between *H pylori* infection and serum HDL-cholesterol level (P <0.001) controlling for other risk factors. It was notable that, *H pylori* positive subjects had lower level of HDL-C than *H pylori* negative subjects. But no significant association was found between *H pylori* infection and other lipid parameters (serum LDL-C or fasting triglyceride levels).

Conclusions: The conclusions of the study are:

a) There is a significant association between *H pylori* infection and increased risk of CHD irrespective of age, sex, BMI, history of hypertension and diabetes, smoking and serum total

cholesterol, HDL-C, LDL-C and triglycerides levels.

b) There is a significant association between *H pylori* infection and serum total cholesterol. *H pylori* antibody positive subjects had higher serum total cholesterol level than *H pylori* antibody negative subjects. Also, a significant association was found between *H pylori* infection and serum HDL-cholesterol level. *H pylori* antibody positive subjects had lower serum total cholesterol level than *H pylori* antibody negative subjects. But no significant association was found between *H pylori* infection and other lipid parameters (serum LDL-C or fasting triglyceride levels).

c) Further large-scale, population-based and prospective studies are needed to define the role of *H pylori* infection in the pathogenesis of CHD.

INTRODUCTION

Introduction:

Atherosclerotic vascular disease and its manifestations remains the scourge of the modern world. Conventional risk factors, such as smoking, diabetes, hypertension, dyslipidemia and life style, do not fully explain the diversity of this disease and why interventions have not reduced its epidemiologists have predicted. One should therefore think laterally [1].

In the pathogenesis of thrombosis, Virchow's triad is satisfied by three broadly independent factors- slowing of blood flow (viscosity), changes in blood constituents (abnormal clotting factors and platelet activation, leading to a hypercoagulable state), and changes in the vessel wall endothelial damage or dysfunction). Thrombogenesis is intimately related to atherogenesis, and the abnormalities described above have been examined by many workers to explain how risk factors lead to atherosclerosis, for example, smoking contributes to endothelial damage, hypercoagulability, and platelet activation.

Chronic infections can increase hypercoagulability, by including hyperfibrinogenemia (and therefore also hyper viscosity), and if particular infections also cause endothelial damage or dysfunction, the key components leading to atherogenesis are present. Chronic

infections and atherogenesis also have uncanny similarities to a chronic inflammatory process, with activation of macrophages and increased cytokine production [1,2].

Chlamydia pneumoniae is an intracellular organism that has been shown in case-control studies to be associated with coronary heart disease (CHD), atherosclerotic carotid disease, and stroke. [1,2]

The possibility of more than one organism for example, *C pneumonia* and *Helicobacter pylori* being guilty in a synergistic manner also cannot be excluded [2,3]. *H pylori* is a lifelong bacterial infection of the stomach that is largely acquired in childhood, and affects nearly half the adult population in the developed world [10,11,12]. Both *H pylori* and *C pneumoniae* infections have been shgown to be associated with coronary heart disease. These relations are not explained by a wide range of confounding factors. Possible mechanisms include an increase in risk factor levels due to a low-grade chronic inflammatory response. [2,3]

Fibrinogen concentrations were significantly raised, to a similar degree, in association with each infection. In addition, *H pylori* but not *C pneumoniae* was associated with a significant increase in total leukocyte count, whereas *C pneumoniae* but not *H pylori* was

associated with higher concentrations of factor VII antigen and malondialdehyde. [4]

The systemic effects of *H pylori* infection on fibrinogen concentration and total leukocyte count parallel those seen in chronic dental infection, which has also been linked to coronary heart disease. The effect of *H pylori* gastritis on markers of inflammation such as fibrinogen concentration, circulating leukocyte count, C reactive protein and silica acid concentrations may be mediated via certain cytokines, including tumor necrosis factor α and interleukin σ, whose concentrations are increased in the gastric mucosa of *H pylori* in fetched patients. [4]

It was observed that those who were *H pylori* positive had significantly higher concentration of serum triglycerides than those who were *H pylori* negative: the trend among the cases was similar, but non-significant. The concentration of HDL cholesterol tended to be lower in those who where *H pylori* positive than in those who were *H pylori* negative, among both the cases and the controls. [5,6,7]

The impact of *H pylori* infection as an independent risk factor for CHD seems to be minor; On the other hand the results are consistent with the hypothesis that *H pylori* infection might modify the serum

lipid concentrations in a way that could increase the risk of CHD. [5,6,7]

H pylori infection and coronary heart disease (CHD) are common conditions in late middle and old age. *H pylori* usually causes a lifelong infection of the gastric mucosa. In addition, *H pylori* has been shown to cause systemic responses related to CHD. [8]

After adjustment for the effect to age, gender, and smoking by the analysis of covariance, those who were *H pylori* positive had higher serum concentrations of triglycerides and lower HDL cholesterol than those who were not. Findings in the CHD patients were similar. [5,6,7]

The proportion of *H pylori*-positive individuals was higher among the cases with two or three vessel disease than in the other CHD patients. [5]

Fibrinogen has emerged as an important risk factor for coronary heart disease (CHD). They have shown an association between *Helicobacter pylori* and hyperfibrinogenemia [10,11,12].

Hyperhomocysteinemia and *H pylori* infection have recently been implicated in the pathogenesis of coronary artery disease [3]. These two risk factors, though they seem unrelated, could be linked by a

deficiency of vitamins caused by chronic gastritis in *H pylori* infection (Figure 1.1). This nutritional defect could lead to failure of methylation by 5 methyl-tetrahydrofolic acid and thus exacerbate the accumulation of homocysteine in susceptible patients. Homocysteine is toxic to endothelial cells and results in coronary artery disease [3].

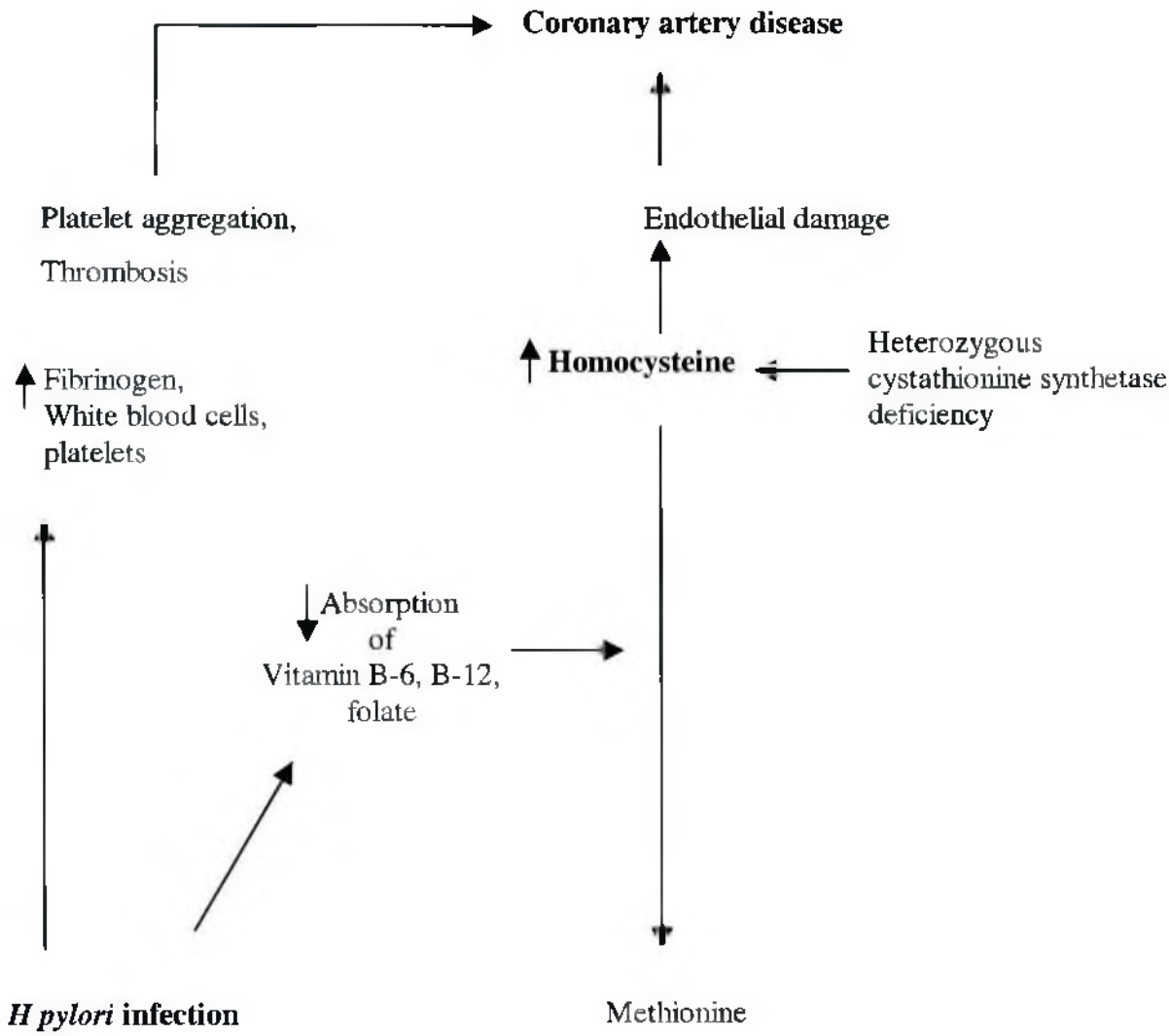

Figure 1.1 *H pylori* infection, hyperhomocysteinemia, and Coronary artery disease

Both the conditions are highly prevalent in our country. Therefore, we investigated the association of *H pylori* infection with angiographically documented CHD.

Hypothesis

There is an association between *Helicobacter pylori* infection and coronary heart disease (CHD).

AIMS and OBJECTIVES:

GENERAL OBJECTIVE: To investigate the relationship between *Helicobacter pylori* infection and coronary heart disease (CHD) which in the long run may create a scope for curative and preventive treatment of CHD.

SPECIFIC OBJECTIVES:

1. To find out whether *H pylori* infection is an independent risk factor for CHD in our country.
2. To find out whether *H pylori* infection modifies serum lipid concentrations in a way that may increase the risk of CHD.

REVIEW OF LITERATURE

Review of literature:

Coronary Heart Disease (CHD) is a major health problem throughout the world and the most common cause of premature morbidity and mortality. Despite steady progress in diagnosis and management of CHD, people are still dying of this disease, although at later ages. By the year 2020, CHD will hold first place, in the World Health Organization's list of leading causes of disability [18].

The major cause of CHD is atherosclerotic disease of the epicardial arteries. Luminal narrowing due to the atherosclerosis, resulting in hemodynamically significant obstruction of blood flow is the major cause of symptoms of coronary ischemia. Although atherosclerotic disease of the coronary arteries is the more important cause of luminal narrowing and CHD, there are multiple nonatherosclerotic (congenital and acquired) causes of severe luminal narrowing and subsquent clinical events – (angina pectoris, acute myocardial infraction and sudden death). This accounts for 4 to 7 percent of all patients with acute myocardial infarction.

Atherosclerosis is a complex inflammatory – fibroproliferative response to retension of plasma derived atherogenic lipoproteins in the arterial intima. The lipid related component destabilizes plaques and this is responsible for the great majority of all the life threatening

complications of human atherosclerosis: plaque disruption with superimposed thrombosis.

The great majority of acute coronary syndrome and ischemic strokes originate from atherosclerotic lesions that, prior to the acute events, only were mild to moderately stenotic; i.e. they were hemodynamically insignificant and probably asymptomatic. Although the risk of occlusion, or myocardial infarction or stroke, increases with stenosis severity, the great majority of coronary occlusions (71 percent) in the Coronary Artery Surgery Study and myocardial infarctions (86 percent) studies originated from lesions that caused less than 70 to 80 percent angiographic stenosis prior to acute events [19].

Gastric mucosal damage caused by *Helicobacter pylori* involves various bacterial and host-dependent toxic substances that have been recently associated with increased risk of CHD.

However, the exact nature of pathophysiological link between the organisms and CHD remains to be elucidated. Future antibiotic interventional studies may help to further clarify the role of chronic infection and inflammation in CHD. The pathophysiology of atherosclerosis, the role of infectious agents (cytomegalovirus, *Chlamydia pneumoniae* and *H pylori*) in atherogenesis and studies

supporting the potential beneficial effects of antibiotics or antiviral agents in the management of atherosclerotic disease.

Inflammation as the major factor for Atherosclerosis

Inflammation plays an important role in the initiation and progression of atherosclerosis [20]. It is evidenced by the presence of inflammatory systemic blood markers such as C-reactive protein, fibrinogen and amyloid A. These are emerging as powerful predictors of coronary events [21,22]

Chronic infection and Atherosclerosis

The role of infection as a CHD risk factor has not been established like others as hypertension, smoking, diabetes mellitus, dyslipidemia, and hyperhomocysteinemia. But many evidence regarding the association of infections and atherosclerosis have come up. Chronic infection with various microorganisms, particularly, *H pylori*, *C pneumoniae*, and Cytomegalovirus (CMV) may play a role in etiological factors, linking inflammation and atherogenesis. Results from epidemiological studies, pathology of atherosclerotic plaques, animal studies, molecular biology and clinical antibiotic trials indicated a positive association between *H pylori* and *C pneumoniae* infection with CHD [23].

Chronic infection can increase hypercoagualability, by inducing hyperfibrinogenemia and also causing the endothelial damage or dysfunction, leading to atherogenesis. Chronic infection and atherogenesis have similarities to a chronic inflammatory process, with the activation of macrophages and increased cytokine production [1,2].

Mechanism

Infection may directly initiate and aggravate endothelial damage, which might initiate or perpetuate atherosclerosis. There are several other mechanisms, which may directly or indirectly play the role in atherosclerosis.

Metabolic and biologic consequences of infection:

- Endothelial cell damage by bacterial lipopolysacharides
- Lipoprotein disturbances and endotoxin-lipid complex formation
- Monocyte activation and triggering of cytokine production
- Increase synthesis of acute phase proteins and inflammatory markers
- Enhanced activity of hemostatic and procoagulant mediators

- Heat shock protein expression and immune cross-reactivity
- Other – electrolyte disturbances, glucose intolerance, platelet activation [24].

HELICOBACTER PYLORI

History

Barry Marshall and Robin Warren of Perth, Western Australia, discovered *H pylori* in 1983. Originally, the organism was named *Campylobacter pyloridis* because it was structurally similar to other *Campylobacter* species, such as *C jejuni*. *C jejuni* is a gut pathogen which has the ability to colonize the gastric mucosa. *C pyloridis* was renamed *C pylori* to fit in with the names of other enteric pathogens. In 1989, it was finally named *Helicobacter pylori* based on functional and enzymatic properties [25].

GENERAL INFORMATION

H pylori is a spiral shaped bacterium that lives in the stomach and duodenum (section of intestine just below stomach). It has a unique way of adapting in the harsh environment of the stomach [26].

The inside of the stomach is bathed in about half a gallon of gastric juice every day. Gastric juice is composed of digestive enzymes and concentrated hydrochloric acid, which can readily tear apart the toughest food or microorganism. Bacteria, viruses, and yesterday's steak dinner are all consumed in this deadly bath of chemicals.

Previously it was thought that the stomach contained no bacteria and was actually sterile, but *H pylori* changed that [27].

The stomach is protected from its own gastric juice by a thick layer of mucus that covers the stomach lining. *H pylori* takes advantage of this protection by living in the mucus lining.

Routes of transmission

Once *H pylori* is safely nested in the mucus, it is able to fight the stomach acid that does reach it with an enzyme it possesses called urease. Urease converts urea, of which there is an abundant supply in the stomach (from saliva and gastric juices), into bicarbonate and ammonia, which are strong bases. This creates a cloud of acid neutralizing chemicals around the *H pylori*, protecting it from the acid in the stomach. The reaction of urea hydrolysis is important for diagnosis of *H pylori* by the breath test [28].

Another defense *H pylori* has is that the body's natural defenses cannot reach the bacterium in the mucus lining of the stomach. The immune system will respond to an *H pylori* infection by sending white cells, killer T cells, and other infection fighting agents. However, these potential *H pylori* eradicators cannot reach the infection, because they cannot easily get through stomach lining [29].

They do not go away either, though, and the immune response grows and grows. Polymorphs die, and spill their destructive compounds (superoxide radicals) on stomach lining cells. Extra nutrients are sent to reinforce the white cells, and the *H pylori* can feed on this. Within a few days, gastritis and perhaps eventually a peptic ulcer results. It may not be *H pylori* itself which causes peptic ulcer, but the inflammation of the stomach lining; i.e. the response to *H pylori*. *H pylori* causing a neutrophilic reaction (active chronic gastritis) in the lining mucosa of the stomach [30].

H pylori is believed to be transmitted orally. Many researchers think that H, pylori is transmitted orally by means of fecal matter through the ingestion of waste tainted food or water. In addition, it is possible that *H pylori* could be transmitted from the stomach to the mouth through gastro-esophagal reflux (in which a small amount of the stomach's contents is involuntarily forced up the esophagus) or belching, common symptoms of gastritis. The bacterium could then be transmitted through oral contact.

Epidemiology

Prevalence of *H pylori* infection correlates best with socio-economic status rather than race. In the United States, probability of being infected is greater for older persons (>50 years = > 50%), minorities

(African Americans 40-50%) and immigrants from developing countries (Latino> 60%, Eastern Europeans> 50%). The infection is less common in more affluent Caucasians (< 40 years = 20%).

Persons in the infected group develop duodenal ulcer at the rate of about 1 % per annum so that approximately one third eventually have peptic ulcer disease. Nearly all persons with duodenal ulcer are infected. Conversely, it is very unlikely that persons without *H pylori* will ever develop duodenal ulcer. Gastric ulcer is usually caused by *H pylori*, but about 30% of gastric ulcers in the United States occur in persons without *H pylori* and can be related to aspirin and other nonsteroidal anti-inflammatory drugs (NSAIDs). Most gastric adenocarcinomas and lymphomas occur in persons with current or past infection with *H pylori*. In developing countries the ulcer groups are smaller and the gastric cancer group may be larger. For example, in northern Brazil, gastric cancer is the most common malignancy in men [31].

Western Countries

In general, the following statements can be made to summarize prevalence of *H pylori* in Western countries:

- *H pylori* affects about 20% of persons below the age of 40 years, and 50% of those above the age of 60 years.

- *H pylori* is uncommon in young children.
- Low socio-economic status predicts *H pylori* infection.
- Immigration is responsible for isolated areas of high prevalence in some Western countries.

Studies of sera from epidemiologists and Californians show that a 50% decline in the prevalence of *H pylori* has occurred in the United States since 1968 [31].

Developing Countries

In developing countries most adults are infected. Acquisition occurs in about 10% of children per annum between the ages of 2 and 8 years so that most are infected by their teens. It is evident from careful surveys that the majority of persons in the world are infected with *H pylori*.

H pylori can be cultured from the stools in most infected persons (using special techniques). This is evidenced that spread by fecal oral contact with infected persons is likely. In addition, polymerase chain reaction (PCR) can detect *H pylori* in dental plaque from 30% of persons with the gastric infection. This may be a less common source of transmission.

Colonization

The antrum of the stomach is a region of moderate acidity where *H pylori* usually prefers to colonize first. The bacterium uses its flagella and spiral shape to drill through the mucus layer in the stomach. *H pylori* produces adhesins which bind to membrane associated lipids and carbohydrates, Electron microscopy shows the adherence of *H pylori* to plasma membranes of surface epithelial cells. Some times an adhesion pedestal is present, which involves tight adhesions associated with local effacement of the microvilli. Adhesins have been identified based on the characterization of bacterial proteins or identification of cellular receptors. There has been some evidence of tissue tropism in *H pylori* colonization. The patterns of tropism are not yet understood, but the classification of adhesins and receptors could lead to an explanation [32].

The enzyme urease plays a role in the bacterium's ability to colonize the acidic gastric environment. The enzyme is found readily on the surface and in the cytoplasm of the bacterium. Urease helps digest urea to produce ammonia and bicarbonate. Ammonia generated around the bacterial cells neutralizes gastric acid. This process benefits the bacterium, but is toxic to gastric epithelial cells [33].

Pathogenicity

Ammonia production from urease activity is toxic to mammalian cells. Epithelial cells undergo vacuolation because of urease activity. In addition, urease and other products of *H pylori,* including protease, catalase, and phospholipases A_2 and C, cause weakening of the mucus bicarbonate layer of the gastro-intestinal tract and damage to surface epithelial cells [26].

Lipopolysacharides (LPS) seems to inhibit glycosylation of mucus leading to a significant change in the macromolecular structure of mucus from high molecular weight to low molecular weight form. LPS may interfere with protective function of mucus layer and make epithelial cells at the surface vulnerable to acid. LPS from some strains of *H pylori* stimulates secretion of pepsinogen. Lastovica et al showed that *H pylori* is the only bacterium that stimulates pepsinogen secretion. There are high levels of pepsinogen in ulcer patients. The Lipid A component of LPS in *H pylori* has an unusual fatty acid substitution and is under-phosphorylated compared to LPS in other Gram negative bacteria. Lipid A modifications are known to reduce immunological activity (antigenicity) of LPS. Studies have shown *H pylori* does have reduced immunological activity. This lowered

activity may allow *H pylori* to persist in its host as compared to a more aggressive pathogen [34].

Linkage to gastrointestinal disease

Studies have linked *H pylori* to gastrointestinal disease in humans. Even though *H pylori* was discovered in 1983, it was noted over 60 years ago that bacteria are associated with damage of the gastric mucosa. *H pylori* is the most common cause of gastritis in man. Gastritis is an infiltration of the tissue with lymphocytes and plasma cells. Electron microscopy shows damage to the plasma membrane, vacuolation of the cytoplasm, and ingested bacteria. Gastric epithelial cells acquire features of activated macrophages. Cytokines are produced that attract and activate inflammatory cells. In patients with duodenal ulcers, gastritis is restricted to the antrum [35].

Duodenal ulcers are associated with chronic superficial gastritis in the gastric antrum. Greater then 90% of duodenal ulcer patients are infected with *H pylori*. Ulcer patients without *H pylori* infection are typically those who have taken non-steroid anti-inflammatory drugs such as aspirin and indomethacin, which can commonly cause ulcers.

This proposed method involves an increase in gastrin release caused by *H pylori* infection. The increased gastrin level causes increased

acid delivery to the duodenum. Ulceration can result from the levels of acid. Eradication of the bacterium from a person greatly reduces the recurrence of ulcers [36].

There is increased prevalence *of H pylori* association with gastric cancer. There is a known stepwise progression from chronic superficial gastritis to intestinal type cancer. It is estimated that *H pylori* infection increases the risk of gastric cancer 6 times. There have also been studies of gastric MALT lymphoma which is caused by B-cells stimulated by T-cells stimulated by *H pylori*. Research has shown that 5 of 6 early lymphomas regressed after eradication of *H pylori* [35,36].

Immune responses

Cultured cells in contact with *H pylori* produce Interleukin-8 (IL-8). IL-8 recruits neutrophils and leukocytes by chemotaxis and activates them. IL-8 is stimulated directly by bacterial factors. Cytotoxin is not required for stimulation of IL-8 production. A specific immune response is generated by continued exposure of the bacterium to the gastric mucosa. Almost all patients with chronic gastritis have a specific response to *H pylori* by the gastric mucosal IgA. IgG plasma cells are increased with gastritis. Almost all infected patients have IgG antibodies in the serum. A systemic IgA response may be found

in some people, but measuring these antibodies is not a reliable method of diagnosis [35].

Linkage to coronary heart disease

H pylori is an agent of chronic infection of the human stomach, and more than half of the adult populaton has serological evidence of infection with *H pylori*. Recently *H pylori* seropositivity has been shown to be associated with CHD.

Proposed mechanisms for how *H pylori* might increase risk of coronary heart disease include- increased plasma fibrinogen, C-reactive protein, blood leucocyte count, homocystein level and decreased serum folate level in seropositive subject. Although, direct infection may not cause atherosclerosis but *H pylori*. might act in concert with traditional risk factors to accelerate atherosclerosis or cause complication or aggravation of existing atheroma.

Diagnosis

For diagnosis, tests for *H pylori* can be divided into two groups

1. Non-invasive test

a 13c or 14c urea breath test.

b Serology

2. Invasive (endoscopic biopsy based)

a Biopsy urease test.

b Histology

c Culture

d Detection of *H pylori* DNA from lesion by PCR.

Non invasive

a. Carbon-14-urea breath test

In the C14- urea breath test the subject has to fast for about 6 hours (from midnight). The test is usually performed in the morning. The subject swallows a capsule or drink water, which contains one micro Curie of C14-urea. The subject provides the breath sample usually by blowing up a small balloon or blowing bubbles in a small bottle of collection liquid. Samples of breath are then taken between 10 and 20 minutes after the capsule is given (the exact details may vary from place to place). The C-14-urea contains a tiny amount of radioactive material, which passes out of your body in a day or so in the urine and breath. The amount of radioactive exposure from the test is less than you will normally receive in one day from nature. The test is quick and simple to perform, and much less expensive than endoscopy [26].

b. Carbon-13-urea Breath Test

In the C13-urea breath test the subject has to fast for about 6 hours (from midnight). A baseline breath sample is collected (the subject blows into a bag or tube), then the subject eats a small, high calorie, meal. Then the subject drinks a solution of Carbon-13-urea in water. Then breath samples are taken at intervals, usually 20, 40 and 60 minutes later (it varies). The samples may be mailed to a testing lab. If *H pylori* is present in the subject's stomach the C13-urea will be broken down and C13 will appear in the subject's breath [26].

C. Serology– Blood Tests

After cure of *H pylori* infection, serologic titers for anti-*H pylori* antibodies, as measured in currently available commercial tests, decline gradually over several months to years but do not always become negative. Therefore, the presence of a positive serologic test does not necessarily establish a current infection with viable organisms. Nevertheless, in seropositive patients with a history of proven peptic ulcer, there is a statistically sound reason to conclude that *H pylori* infection is active and playing a causative role. Treatment is therefore warranted. In most situations an empiric course of anti-*H pylori* treatment is much less expensive than the cost of an endoscopy-based diagnosis.

Chronic *H pylori* infection elicits local and systemic immunologic responses leading to the production of IgG and IgA antibodies. [15,16,17] In general, measurement of the serum IgG level is the preference test basis, as levels of this antibody are more accurate of infection status. Diagnostic tests that detect *H pylori* antibodies are inexpensive, global tests with typically high specificity and sensitivity. Modalities that are presently available use separated serum for either quantitative enzyme-linked immunosorbent assays (ELISAs) or qualitative in office immunoassays. [15,16,17] Whole blood immunoassays, salivary antibody tests, urine serology tests, and latex agglutination test are being developed.

Immunoassay and whole blood are qualitative tests will remain positive in the post treatment period and therefore cannot be used to determine bacterial eradication. Quantitative ELISA tests may be used to follow the fall in antibodies 4-6 months after treatment to confirm eradication.

Table I Comparison of different diagnostic tests for *H pylori*

Parameter	Sensitivity (%)	Specificity (%)	Predictive Value	
			Positive (%)	Negative (%)
Noninvasive				
Urea breath test	90.2	95.8	97.5	84.3
Serum 1gH	91.3	91.6	95.2	85.3
Serum 1Ga	85.3	85.3	89.8	61.8

Invasive

a. Specimen collection (endoscopy based)

For specimen collection, one should be off all antibiotics and/or antacid for one month, omeprazole or sucralfate for one week and H_2 blockers.

In this test one do not eat or drink for up to six hours. In the endoscopy room the subject is given an injection of a sedative drug into a vein in the arm. A blood sample may be taken from the vein at this time. The subject's throat is sprayed with a local anesthetic spray.

The subject then swallows a narrow, flexible tube. The tube is only about the thickness of the subject's little finger and although most patients have a little discomfort during the first five seconds of the test, once the tube has passed the back of the throat, very little discomfort occurs. Through this tube (the endoscope), the doctor examines the inside of the esophagus (food pipe), stomach and duodenum. While in the stomach, it is usual to take up to ten small biopsy samples from the lining of the duodenum, stomach, and esophagus. The complete endoscopy examination takes 15 minutes.

b. Biopsy urease test:

It is most simple, quick and convenient endoscopy based biopsy test. In this test two antral biopsy specimens are put into a gel containing urea and an indicator. The presence of *H pylori* urease elicits a color change, which often takes place within minutes but can require upto 24 hours [36].

c. Histology:

Histologic examination of biopsy specimen is accurate, provided that a special stain (e.g. a modified Giemsa or silver stain) permitting optimal visualization of *H pylori* is used. Histologic study yields

additional information including the degree and pattern of inflammation, atrophy, metaplasia, and dyspalsia [37].

d. **Microbiologic culture:**

It is most specific but may be insensitive due to difficulty with *H pylori* isolation. Once cultured, the identity of H.pyloei can be confirmed by its typical appearance on Gram's stain and its positive reaction in oxidase, catalase and urease test. Antibiotic sensitivity also can be determined [38].

e. *H pylori* DNA can be detected by PCR method from a atherometous lesion. In this method one should be careful to prevent contamination of the specimen.

Most of the available *H pylori* diagnostic tests have good sensitivity and specificity although none works perfectly [15].

Helicobacter pylori treatment

Treatment of *Helicobacter* pylori is usually simple but occasional patients need repeated endoscopies; biopsies, and several courses of treatment with antibiotic drugs.

After treatment of *H pylori*, it is necessary to repeat one of these tests to see if the germ has been killed. Only breath tests or endoscopy with

biopsy can be used to prove that the bacterium has been eradicated. The blood test may remain positive for months or even years after successfully killing the *H pylori*.

At present, the only clear indications for treatment are *H pylori* related duodenal and gastric ulceration and the rare low-grade B-cell MALT lymphoma. *H pylori* should be eradicated in patients with documented ulcer disease, whether or not the ulcers are currently active, to reduce the likelihood of relapse. At present treatment is not recommended for nonulcer dyspepsia or for prophylaxis against ulcers or gastric adenocarcinoma. Reasons for avoiding treatment for this other potential indications include expense, the induction of morbidity in otherwise healthy people, the risk of inducing widespread antibiotic resistance in *H pylori* and in other colonizing bacteria, and the risk of inducing or worsening GERD (Gastro Esophageal Reflux Disease) [39].

H pylori is susceptible to a wide range of antibiotics *in vitro*, but monotherapy has been disappointing *in vivo*, probably because of inadequate antibiotic delivery to the full locus of colonization. Failure of monotherapy led to the development of multi-drug regimens, the most successful of which are triple and quadruple combinations that achieve *H pylori* eradication rates of >90% in many trials and >75%

susceptibilities. When this information cannot be obtained, the recommended course is quadruple therapy without clarithromycin (if a clarithromycin-containing regimen was given first) or triple therapy with omeprazole/clarithromycin/amoxicillin (if clarithromycin has not been used previously) [40].

Table II. Recommended 7-14 day regimens for the eradication of *H pylori*.

Name	Drug 1[a]	Drug 2	Drug 3	Drug 4
OCA[b]	Omeprazole (20mg bid)	Clarithromycin (500mg bid)	Amoxicillin (500mg bid)	
OCM[b]	Omeprazole (20mg bid)	Clarithromycin (250mg bid)	Metronidazole (500mg bid)	
OBTM[d]	Omeprazole (20mg bid)	Bismuth sub salicylate (2tabs qid)	Tetracycline HCL (500mg qid)	Metronidazole[c] (500mg bid)

a. In any of the three regimens, omeprazole may be replaced by lansoprazole (30 mg bid), pantoprazole (40 mg bid), ranitidine bismuth citrate (400 mg bid), or possibly ranitidine.

b. These regimes may be given for 7 to 14 days; meta-analysis suggests that 14 days regimens are slightly more effective.

c. The optimal dose of metronidazole is not known. Tinidazole (500 mg bid) can be given in place of metronidazole.

d. Data for this regimen are mainly from Europe and are based on bismuth subcitrate, Omeprazole is given for 10 days, and the other three agents are given on days through 10.

Given the high efficacy of treatment regimens, it is unclear whether the success of attempted *H pylori* eradication should be checked. For gastric ulceration, the opportunity to retest for *H pylori* is present in the repeat endoscopy, which is performed to evaluate healing. For duodenal ulceration, although many clinicians prefer to retest only with symptoms recur, a urea breath test or endoscopy should be performed no sooner than 1 month after treatment. This test will provide reassurance if treatment has been successful and will prompt re-treatment in cases of persistence.

With any triple-drug regimen, compliance is difficult, especially when side effects develop. The secret of success with these regiments lies in having a physician who is knowledgeable about the effects of the drugs spend sufficient time with patients in advance of therapy. The physician needs to educate the patient about possible side effects, advise the patient when to continue and when to terminate therapy, and be alert to the need to switch to an alternative regimen, if necessary. In the approximately 70% of cases who continue triple therapy for 2 weeks, eradication is accomplished in most.

Nevertheless, compliance is sub optimal in up to one third of patients in practice. Because of problems with compliance, there has been a major search for effective eradication therapy regimens that are simpler and well tolerated. Several studies suggest that 1 week courses of triple therapy are almost as effective as 2 week courses.

Prevention

Carriage of *H pylori* has public health significance in developing countries, where gastric adenocarcinoma is a common cause of cancer death. However, *H pylori* has co-evolved with its human host over millennia, and there may be disadvantages in preventing or eliminating colonization. For example, as has been mentioned, the absence of *H pylori* appears to increase the risk of developing GERD and esophageal adenocarcinoma. If mass prevention were contemplated, vaccination would be preferred, and experimental immunization of animals has give promising results. However, in the United States and other developed countries, the incidences of *H pylori* carriage, peptic ulceration, and gastric adenocarcinoma are dropping. Thus, prevention of colonization in these countries may be unnecessary or even unwise.

H pylori Vaccine

Vaccination against *H pylori* are mainly on trial phase. Given the generally positive results in small animal models, the more modest results of therapeutic vaccine trials in monkeys and humans are disappointing, but illustrate the difficulties in extrapolating from mice to men.

Virtually all vaccines in use today are prophylactic; they are designed to prevent acquisition of infection. There is growing interest, however, in therapeutic vaccines, which might be used to treat pre-existing infection. Owing to the large number of people who are infected with *H pylori*, the absence of simple, single drug treatment protocol, and the documented high probability for the development of drug resistant *H pylori*, *H pylori* infection is a good candidate for therapeutic vaccination.

Resistant Infections

If two therapies fail (*H pylori* persists by breath test or biopsy) then expert advice is required. There may have antibiotic resistant *H pylori*. In this case, endoscopy and biopsy with culture to check sensitivities should be done and then can treat appropriately when the results are known (after 21 days).

CORONARY HEART DISEARE

Definition

This is a condition in which fatty deposits (Cholesterol) accumulate in the wall of the coronary artery and restrict blood flow through them.

Epidemiology

The variable occurrence and severity of CHD among individuals and groups may provide important clues to its pathogenesis. Epidemiologic data are expressed largely in terms of the incidence of or the number of deaths caused by CHD [41].

Deaths from cardiovascular disease in the United States rose from 14% of all deaths in 1937 to 54% in 1968, almost all cases being related to atherosclerosis. Happily, they appeared to plateau in the late 1960s, and by 1975, for the first time, the rate showed a statistically significant decline, which has been maintained since. The downward trend is believed to be mediated largely by a reduction in atherosclerosis influenced by changes in diet and lifestyle better control of hypertension, and improved therapy for myocardial infarction and other implication of CHD [42].

Nevertheless, the death rate related to atherosclerosis in the United States is still among the highest in the world, lower than that of

Finland and Scotland, but above that of other well-developed, affluent countries, such as Canada, France, and the other Scandinavian countries. The rates are remarkable low in Asia, Africa, and South and Central America. For example, death rates from CHD in Japan are one-sixth of those in the United States. Japanese who migrate to the United States, however, and adopt the lifestyles and dietary customs of their new home acquire the predisposition to atherosclerotic diseases of the American population [43].

Risk factors

During the first half of the twentieth century, animal experiments and clinical observation linked certain variables, such as hypercholesterolemia, to the risk of atherosclerotic events. The systematic study of risk factors in humans, however, began approximately mid-century. The prospective, community-based Framingham Heart Study provided rigorous support for the concept that hypercholesterolemia, hypertension, and other factors correlated with cardiovascular risk. Similar observational studies performed in the United States and abroad provided independent support for the concept of "risk factors" for cardiovascular disease. Numerous studies, including the Seven Countries Study performed by Keys and colleagues, suggested a link between dietary habits and

Cardiovascular risk based upon population studies [44].

Risk Factors for Atheroselerosis

A. Modifiable risk factors

By Life-style

Smoking

Obesity

Physical inactivity

By Pharmacotherapy and or life-style

Lipid disorders

Hypertension

Diabetes mellitus

B. Unmodifiable risk factors

Age

Male gender

Genetics

From a practical viewpoint, it is useful to group the cardiovascular risk factors that have emerged from different studies into two categories: those modifiable by lifestyle and/or pharmacotherapy, and those that are essentially unmodifiable. The weight of evidence supporting various risk factors differs. For example,

hypercholeserolemia and hypertension indubitably predict coronary risk, but other so-called nontraditional risk factors, such as levels of homocystine, lipoprotein or infection, remain controversial. It is worth distinguishing further between factors that actually participate in the pathogenesis of atherosclerosis and those that may merely serve as markers of risk without themselves playing a primary role in pathogenesis. The sections below will consider some of these risk factors and approaches to their modification [45].

Lipid Disorders

Abnormalities in plasma lipoproteins and derangements in lipid metabolism rank as the most firmly established and best under stood risk factors for atherosclerosis. Current national guidelines recommend cholesterol screening in all adults. The screen should include a fasting lipid profile. Dietary measures, including specific consultation by practitioners with training in nutrition, should be offered to all patients with hyperlipidemia as defined by the National Cholesterol Education Project Adult Treatment Panel II [46].

Hypertension

The preponderance of epidemiologic data supports a relationship between hypertension and atherosclerotic risk. Clinical trial evidence

available since the 1970s established that pharmacologic treatment of hypertension can reduce the risk of stroke and heart failure. However, clinical trial evidence demonstrating reduced risk of coronary events due to antihypertensive therapy has lagged. At present, the combined weight of the evidence supports a reduction in coronary risk by antihypertensive therapy. Some of the difficulty in demonstrating this benefit may derive from the potentially adverse effects of certain classes of antihypertensive drugs on the lipid profile, notably, thiazide diuretics and beta-blocking agents. Indeed, studies of patients with previous myocardial infarction or reduced left ventricular function have shown that treatment with angiotensin-converting enzyme (ACE) inhibitors can reduce the risk of coronary events, an unanticipated outcome. Therefore "lipid neutral" antihypertensive agents such as ACE inhibitors or adrenergic blocking agents merit consideration in patients with other risk factors for coronary artery disease or with established atherosclerosis [47].

Diabetes Mellitus and Insulin Resistance

Most patients with diabetes mellitus die of atherosclerosis and its complications. Secular trends towards aging of the population and increased girth will make type 2 diabetes mellitus an increasing public health problem in the coming years. The criteria for diagnosis

of diabetes have recently undergone revision. Currently, a fasting plasma glucose level of 7.0 mmol/L establishes the diagnosis of diabetes. In the intermediate range, plasma glucose levels between 6.1 and 6.9 mmol/L indicate impaired fasting glucose. Thus fasting glucose >6.1 mmol/L indicates abnormal glucose tolerance. These definitions based on fasting plasma glucose alone obviate the trend for performing glucose tolerance tests. A major feature of elevated cardiovascular risk in patients with type-2 diabetes probably related to the abnormal lipo-protein profile associated with insulin resistance known as Diabetic Dyslipidemia [43,48].

Male Gender/Postmenopausal State

Decades of observational studies have verified excess coronary risk in males compared with premenopausal females. After menopause, however coronary risk accelerates in women. At least part of the apparent protection against coronary heart disease in premenopausal women derives from their relatively higher HDL levels compared with those of men. After menopause, HDL values fall in concert with increased coronary risk. Estrogen therapy lowers LDL cholesterol and raises HDL cholesterol, changes that should decrease coronary risk. A multitude of observational studies has suggested that estrogen-replacement therapy (ERT) reduces coronary risk. Substantial

experimental data support the biologic plausibility of a beneficial effect of estrogen in reducing atherosclerotic events, but a number of potential confounding factors render clinical trials necessary to establish the cardiovascular benefits of ERT. In men, high-does estrogen treatment caused excess mortality, probably due to increased thromboembolic complications [46].

Dysregulated Coagulation or Fibrinolysis

Thrombosis ultimately causes the gravest complications of arteriosclerosis. The propensity to form thrombi and/or to lyse clots once they form could clearly influence the manifestations of atherosclerosis, Thrombosis provoked by atheroma rupture and subsequent healing may promote plaque growth. Certain individual characteristics can influence thrombosis or fibrinolysis and have received attention as potential coronary risk factors. For example, fibrinogen levels correlate with coronary risk and provide information regarding coronary risk independent of the lipoprotein profile. Elevated fibrinogen levels might promote a thrombotic diathesis. Alternatively, fibrinogen, an acute-phase reactant, may serve as a marker of inflammation rather than directly participating in the pathogenesis of coronary events [43].

Homocysteine

A large body of literature suggests a relationship between hyperhomocysteinemia and coronary events. Several mutations in the enzymes involved in homocysteine accumulation correlate with thrombosis and in some studies, coronary risk [44].

Infection/Inflammation

Recent years have witnessed a resurgence of interest in the possibility that infections may cause or contribute to atherosclerosis. A spate of recent publications has furnished evidence in support for a role of *Chlamydia pneumoniae*, cytomegalovirus, or other infectious agents in atherosclerosis and restenosis following coronary intervention. Some microorganisms exist in human antherosclerotic plaques. However, seroepidemiologic evidence for an association between infection with various agents and therosclerosis remains inconclusive. Several ongoing large trials of antibiotic treatment in survivors of myocardial infarction may provide support for an etiologic or contributory role of microbial infection in recurrent coronary events. Even if positive, however, such clinical trials would neither inculpate any particular microorganisms nor even prove that a benefit derived from the antimicrobial action of the agent employed.

Although direct infection may not cause atherosclerosis, the infectious agents and the host defenses against these invaders might potentiate atherogenesis, acting as inflammatory stimuli. Just as inflammation may mediate some of the altered arterial biology in response to hyperlipoproteinemia, so might infectious agents incite an inflammatory response that could promote atherosclerosis and its complications. Thus, microbial pathogens might act in concert with traditional risk factors to accelerate atherogenesis or cause complication or aggravation of existing atheroma.

In this regard, evidence is accumulating that markers of inflammation correlate with coronary risk. For example, elevated plasma levels of C-reactive protein (CRP) can prospectively predict risk of myocardial infarction and correlate with outcome of patients with acute coronary syndromes. As in the case of fibrinogen, elevated levels of the acute-phase reactant CRP may merely reflect ongoing inflammation rather than a direct etiologic role for CRP in coronary artery disease. It remains uncertain whether elevations in acute-phase reactants such as fibrinogen or CRP serve as a marker for the overall atherosclerotic burden, and hence of coronary events. Alternatively, the elevation in acute-phase reactants could reflect extravascular inflammation that could potentiate atherosclerosis or its complications. In all likelihood,

both factors contribute to elevation of inflammatory markers in patients at risk for coronary events. These observations raise the possibility that anti-inflammatory therapies might reduce atherosclerotic events. Indeed, lipid-lowering therapy may reduce coronary events in part by reducing the inflammatory aspects of the pathogenesis of atherosclerosis [47,49,50,51].

Pathogenesis

An infectious etiology of atherosclerosis has received considerable attention recently. This theory was initially formulated in the first two decades of this century, but did not receive much attention until the late 1970s, when Fabricant et al. showed that chickens infected with avian Herpes virus developed vascular lesions similar to those found in human atherosclerosis. Since then a number of infectious agents have been implicated in human atherosclerosis and include *Chlamydia pneumoniae*, *Helicobacter* pylori, herpes simplex virus and cytomegalovirus (CMV), on the basis of finding these infectious agents in the atherosclerotic segments and by positive serology [49].

Helicobacter pylori and *Chlamydia* organisms, as well as CMV, induce production of several cytokines, including TNF-alpha, IL-1 and IL-2. These cytokines have a variety of actions, including stimulation of fibroblasts and smooth muscle cell proliferation. TNF-

alpha inhibits the action of lipoprotein lipase, leading to altered lipid metabolism, accumulation of serum triglycerides and a decrease in serum high density lipoprotein cholesterol (HDL-C). Lipopolysaccharide, a bacterial component, binds in human serum to both HDL-C and low density lipoprotein cholesterol (LDL-C) and makes LDL-C immunogenic or toxic to endothelial cells.

It has been postulated that, infection and inflammation play an *important* role early in the initiation of endothelial injury. The dysfunctional endothelium permits monocyte deposition and infiltration of lipid-laden macrophages into the subendothelial layers. This is associated with a decrease in constitutive nitric oxide (NO) formation and activity. NO may be rapidly broken down by release of large amounts of free radicals, resulting in focal vasospasm. Dyslipidemia, altered folate metabolism (and resultant increased homocysteine levels) and growth factor release cause smooth muscle cell (SMC) proliferation. Deposition and activation of inflammatory cells lead to release of procoagulant cytokines and thrombosis. Increased release of free radicals and a relative deficiency of endogenous antioxidant pool may oxidize lipids, inactivate NO and enhance thrombosis and prevent thrombolysis.

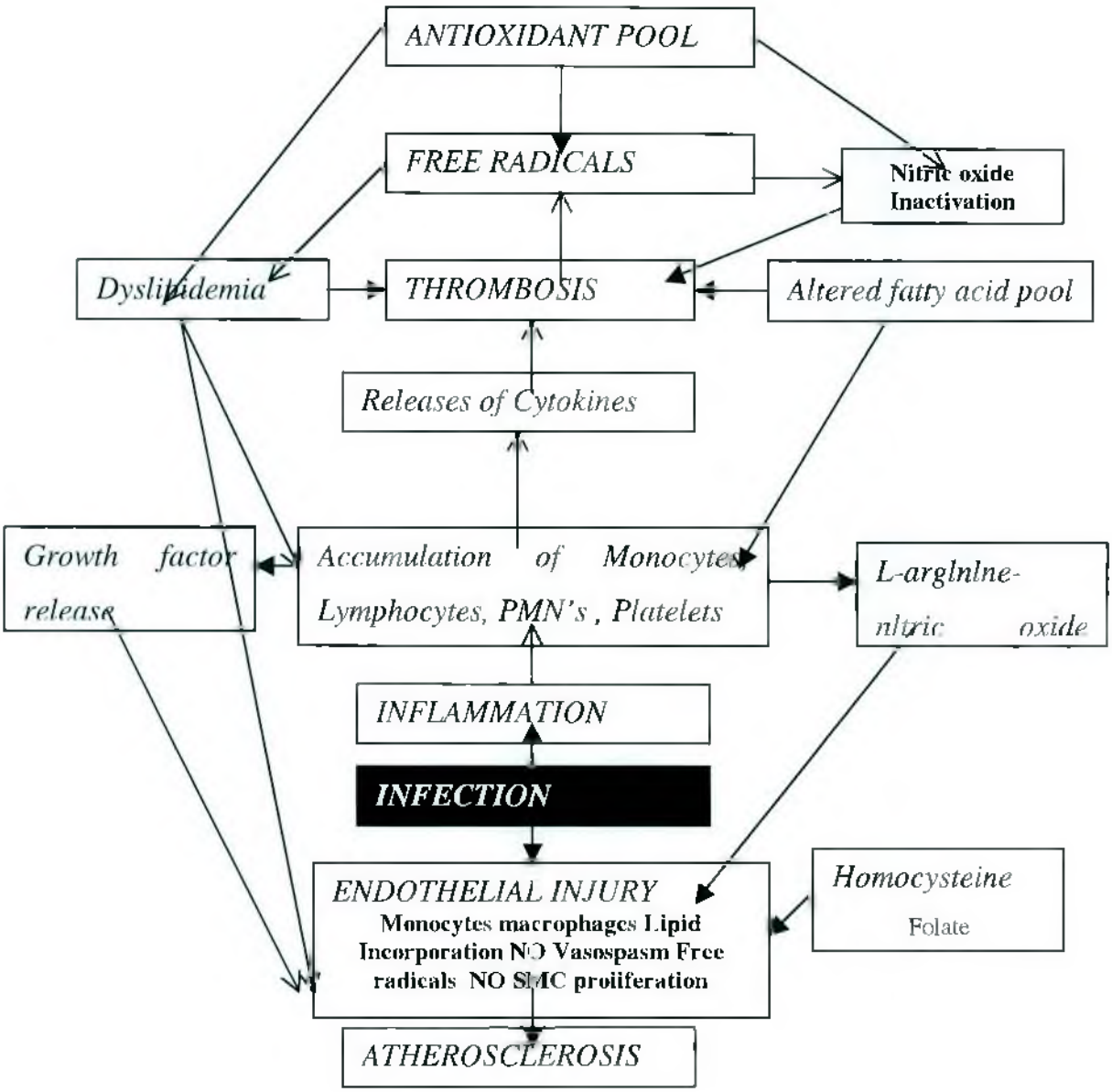

Figure 2.1 Postulated steps in the pathogenesis of atherosclerosis.

In this postulate, abnormalities in lipid profiles, folate metabolism and other traditional risk factors (e.g. diabetes mellitus and hypertension) play a rather *peripheral* role and serve to amplify the atherosclerotic process initiated by persistence of infection and inflammation. This postulate is not designed to be operative in all patients with atherosclerosis, but only in those with genetic predisposition. Major abnormalities are listed in block letters [50].

Cytokines are potent inducers of a neutrophil free radical generation, which may facilitate oxidation of LDL-C, a key event in atherogenesis, and attract monocytes and other inflammatory cells to the area of endothelial injury. Coupled with the fibroblast and smooth muscle cell proliferating effects, leukocyte activation may lead to propagation of the atherosclerotic process [51].

Free radicals also stimulate platelet activation and leukocyte chemotaxis and may participate in the formation of thrombus in atherosclerotically narrowed arteries. Cytokines also influence the coagulation cascade by stimulating formation of endogenous tissue plasminogen activator and its fast acting inhibitor, plasminogen activator inhibitor-1, the overall effect being stimulation of thrombus formation. Plasma levels of both tissue-type plasminogen activator and plasminogen activator inhibitor-1 have been shown to be

increased in unstable coronary syndromes. Cytokines decrease the activity of constitutive nitric oxide synthase, a hallmark of atherosclerosis, and the loss of release of constitutive nitric oxide may predispose to vasospasm and *in situ* platelet aggregation and thrombosis [52].

Bacterial lipopolysaccharide is a potent stimulus for inducible nitric oxide synthase activity leading to the formation of large amounts of nitric oxide, which could cause endothelial dysfunction and disruption followed by deposition of monocytes and platelets on the vessel wall, release of growth factors and migration of smooth muscle cells [51].

Other well known risk factors, such as smoking, hypertension, homocyteinemia and altered fatty acid pool may play a variable, but important, role in the development of atherosclerosis in susceptible people and precipitation of an acute ischemic event. It is possible to develop a postulate wherein infection and inflammation play a very important role in atherogenesis in some subjects.

Clinical syndromes of atherosclerosis

Atherosclerotic lesions occur ubiquitously in western societies. Most atheroma produce no symptoms, and many never cause clinical

manifestations. Numerous patients with diffuse atherosclerosis may succumb to unrelated illnesses without ever having experienced a clinically significant manifestation of atherosclerosis. What accounts for this variability in the clinical expression of atherosclerotic disease?

Arterial remodeling during atheroma formation represents a frequently overlooked but clinically important feature of lesion evolution. During the initial phases of atheroma development, the plaque usually grows outward, in an abluminal direction. Vessels affected by atherogenesis tend to increase in diameter, a phenomenon known as compensatory enlargement, a type of vascular remodeling. The growing atheroma does not encroach upon the arterial lumen until the burden of atherosclerotic plaque exceeds approximately 40% of the area encompassed by the internal elastic lamina. Thus, during much of its life history, an atheroma will not cause stenosis that can limit blood flow [51].

Flow-limiting stenosis commonly form later in the history of the plaque. Many such plaques manifest themselves by stable syndromes such as demand-induced angina pectoris or intermittent claudication in the extremities. In the coronary and other circulations, even occlusion due to atheroma does not invariably lead to infarction. The

hypoxic stimulus of repeated bouts of ischemia characteristically induces formation of collateral vessels in the myocardium, mitigating the consequences of an acute occlusion of an epicardial coronary artery. On the other hand, we now appreciate that many lesions that cause acute or unstable atherosclerotic syndromes, particularly in the coronary circulation may arise from atherosclerotic plaques that do not produce a flow-limiting stenosis. Such lesions may produce only minimal luminal irregularities on traditional angiorgrams and often do not meet the traditional criteria for "significance" by arteriography. Instability of such nonocclusive stenoses may explain the frequency of myocardial infarction as an initial manifestation of CHD (in about a third of cases) in patients who report no prior history of angina pectoris, a syndrome usually caused by flow-limiting stenoses [52].

Pathologic studies afford considerable insight into the microanatomic substrate underlying "instability" of plaques that are not critically stenotic. A superficial erosion of the endothelium or a frank plaque rupture or fissure usually produces the thrombus that causes episodes of unstable angina pectoris or the occlusive and relatively persistent thrombus that causes acute myocardial infarction. In the case of carotid atheroma, a deeper ulceration that provides a nidus for

formation of platelet thrombi may underlie the unstable syndromes that cause transient ischemic attacks [53].

Rupture of the plaque's fibrous cap permits contact of coagulation factors in the blood with highly thrombogenic tissue factor expressed by macrophage foam cells in the plaque's lipid-rich core. If the ensuing thrombus is nonocclusive or transient, the episode of plaque disruption may not cause symptoms or may result in ischemic symptoms such as rest angina. Occlusive thrombi that endure will often cause acute myocardial infarction, particularly in the absence of a well-developed collateral circulation supplying the affected territory. Repetitive episodes of plaque disruption and healing provide one likely mechanism of transition of the fatty streak to a more complex fibrous lesion. The healing process in arteries, as in skin wounds, involves the laying down of new extracellular matrix and fibrosis [54].

Not all atheroma exhibit the same propensity to rupture. Studies of the pathology of culprit lesions that have caused acute myocardial infarction reveal several characteristic features. Plaques that have proven vulnerable tend to have thin fibrous caps, relatively large lipid cores, and a high content of macrophages. Morphometric studies of such culprit lesions show that macrophages and T lymphocytes

predominate at the site of plaque rupture. On the other hand, sites of plaque rupture contain relatively few smooth-muscle cells. The cells that concentrate at sites of plaque rupture bear markers of inflammatory activation. The presence of the transplantation or histocompatibility antigen HLA-DR provides one convenient gauge of the degree of inflammation in cells in atheroma. Resting cells in normal arteries seldom express this transplantation antigen. However, macrophages and smooth-muscle cells at sites of human coronary artery plaque disruption do bear this inducible cell-surface marker. Therefore, the presence of HLA-DR-positive macrophages and T cells indicates an ongoing inflammatory response at sites of plaque rupture [50,51].

Inflammatory mediators may actually regulate processes that govern the integrity of the plaque's fibrous cap and hence its propensity to rupture. For example, the T cell-derived cytokine IFN-γ found atherosclerotic plaques and required to induce the HLA-DR-present muscle cells. Cytokines derived from activated macrophages such TNF-α or IL-1 in addition to T cell-derived IFN-δ can elicit the expression of genes that encode the proteinases that can degrade in extracellular matrix of the plaque's fibrous cap. Thus, inflammatory mediators can impair collagen synthesis required for maintenances

repair of the fibrous cap and trigger degradation of extracellular matrix macromolecules, processes that should weaken the plaque's fibrous cap and enhance its vulnerability to rupture. In contrast to vulnerable plaques, those with a dense extracellular matrix and relatively thick fibrous cap without substantial tissue factor-rich lipid cores seem generally resistant to rupture and unlikely to provoke thrombosis [20].

In conclusion, we now appreciate that features of the biology the atheromatous plaque in addition to its degree of luminal encroachment influence the clinical manifestations of this disease. This enhanced understanding of plaque biology provides insight into the diverse ways in which atherosclerosis can present clinically, and why the disease may remain silent or stable for prolonged periods and punctuated by acute complications at certain times. Increased under standing of atherogenesis provides new insight into the ways in which, current therapies may improve outcomes and also suggests new targets for future intervention.

DIAGNOSIS OF CORONARY ARTERY DISEASE

Diagnosis of coronary artery disease can be done by various investigations. Some are non invasive and some are invasive.

Non invasive

Resting ECG : The ECG may show evidence of previous myocardial infraction but is often normal even in patients with left main or severe three-vessel coronary artery disease. Occasionally there is T-wave flattening or inversion in some leads, providing non-specific evidence of myocardial ischemia or damage.

The most convincing ECG evidence of myocardial ischemia is obtained by demonstrating reversible ST segment depression or elevation, with or without T-wave inversion, at the time the patient is experiencing symptoms (whether spontaneous or induced by exercise testing).

Exercise (Stress) ECG : A formal exercise tolerance test (ETT) is usually performed using standard treadmill or bicycle ergometer protocol while monitoring the patient's ECG, blood pressure and general condition. Planar or down-sloping ST segment depression of 1 mm or more is indicative of ischemia; up sloping ST depression is less specific and often occurs in normal individuals.

Exercise testing can be used to confirm or refute a diagnosis of angina, and is also useful means of assessing the severity of coronary disease and identifying high-risk individuals. For example, the

amount of exercise that can be tolerated and the extent and degree of any ST segment change provide a useful guide to the likely extent of coronary disease.

Exercise testing not infallible and may produce false positive results in the presence of digoxin therapy, left ventricular hypertrophy, left bundle branch block or Wolf-Parkinson-White syndrome. The predictive accuracy of exercise testing is lower in women than men. The test should be classed as non-contributory (and not negative) if the patient cannot achieve an adequate level of exercise because of locomotor or other non-cardiac problems.

Echocardiogram

Two-dimensional echocardiography : Echocardiography is similar to other forms of ultrasound imaging and allows the structures of the heat to be visualized as a two dimensional 'slice'. Images are obtained by placing the ultrasound transducer on the chest wall, so it is a non-invasive procedure. Contraction of the ventricles can easily be seen in ' real time' and this technique is the simplest available for assessing ventricular function can be demonstrated, and vegetations may detecting intracardiac masses, such as thrombi or tumors, and can be sued to define complex structural abnormalities in congenital heart disease.

Doppler Echocardiography

This technique depends on the fundamental principle that sound wave reflected from moving objects, such as intracardiac red blood cells, undergo a frequency shift. The speed and direction of movement of the red cells, and thus of blood, can be defected in the heart chambers and great in moving. The derived information can be presented either in the heart or as a color overlay on a two-dimensional real-time echo picture. Doppler echocardiography is valuable in detecting abnormal directions blood flow, e.g. aortic or mitral reflux, and in estimating pressure gradients.

Invasive

Trans esophageal Echocardiogram : In this technique an ultrasound probe, in the shape of an endoscope, is passed into the oesophagus and positioned immediately behind the left atrium. This produces very clear images; in endocarditis, for example, it is often possible to see vegetations that are too small to be detected by ordinary echocardiography. The high-quality images that can be obtained make the technique particularly valuable for investigating patients with prosthetic valve dysfunction, patients with congenita abnormalities (e. g. atrial septal defect), and patients with systemic embolism in whom

a cardiac defect in suspected but cannot be identified by Transthoracic echocardiography.

Coronary Angiogram : This provides detailed information about the extent and nature of coronary disease and is usually performed with a view to coronary bypass grafting or percutaneous coronary intervention. In some patients, diagnostic coronary angiography may be indicated when non-invasive tests have failed to elucidate the cause of atypical chest pain. The procedure is performed under local anesthesia and requires specialized radiological equipment, cardiac monitoring and an experienced operating team.

Cardiac catheterization : [54]

History Aspects

According to Andre Cournand, cardiac catheterization was first performed in 1844 by Claude Bernand, who catheterized both the right and the left ventricle of a horse by means of a retrograde approach from the jugular vein and carotid artery. There followed an era of investigation of cardiovascular physiology in animals that resulted in the development of many important techniques and principles –including pressure manometry and the application of the

Fick principle for measuring cardiac output-subsequently applied to the study of patients with heart disease.

Although others had previously passed catheters in to great veins, Werner Forssmann, is generally credited as the first to pass a catheter into the heart of a living human being. At age 25 yrs, as a surgical resident, he exposed a vein in his own left arm, introduced a ureteral catheter in to the venous system, and advanced it under fluoroscopic control in to the right atrium.

The potential of Forssmann's technique was appreciated by other investigators. In 1930, Klein reported on catheterization of the right ventricle in 11 patients and measurement of cardiac out put using the Fick principle. The 1950s and beyond, further development came rapidly. Retrograde left-heart catheterization was first introduced by Zimmerman and Limon Lason and their respective coworkers in 1950. Percutaneous technique developed by Seldinger in 1953 was soon applied to cardiac catheterization of both left and right heart chambers.

Selective coronary arteriography was developed by Sones et al. in 1959 and was performed in the ensuring years. In this technique a specially designed catheter is inserted into a vein or artery and advanced into the heart under radiographic fluoroscopic guidance.

This allows the operator to measure intracardiac pressures, take samples from individual cardiac chambers, and obtain angiograms by injecting contrast medial into area of interest.

Right and left–heart catheterization by the way of the femoral approach usually is performed from the right groin, although the left groin may be used, if necessary.

Technique of Coronary Arteriography [55]

The Judkins femoral arterial catheterization technique permits selection of various preformed or preshaped catheters in contrast to the Sone's technique where the operator must form loop to position the catheter. The catheter can be manipulated through the skin directly or through an introducer sheath.

Improvements in catheter manufacturing technology have made large lumen preshaped coronary catheters (6 French or smaller preferred in many laboratories).

All the femoral catheters are inserted with a J –tipped guide wire. The J-tipped guidewire is advanced into the ascending thoracic aorta under fluoroscopic guidance and is followed by the catheter. Once the catheter tip has reached the desired location in the aorta, the

guidewire is removed, the catheter is aspirated (2-3 mm of blood), flushed, and connected to pressure manifold.

Coronary angiography can be completed using Judkins catheters from the femoral approach in more than 90% of patients. The Judkins catheters have special preshaped curves and tapered end–hole tips. The Judkins left coronary catheter has a double curve. The length of the segment between the primary and secondary curve determines the size of the catheter (i.e., 3.5,4.0,5.0, or 6.0 cm). The proper size of the left Judkins catheter is selected depending on the length and width of the ascending aorta

In a small person with a small aorta, a 3.5 –cm catheter is appropriate, while in a large person or in those with an enlarged or dilated ascending aorta, a 5.0 or 6.0 –cm catheter may be required.

The ingenious design of the left Judkins catheter permits cannulation of the left coronary artery without any major catheter manipulation except the slow advance of the catheter under fluoroscopic control. The catheter tips follows the ascending aorta border and falls into left main coronary ostium, often with an abrupt jump. In the words of it inventor the (Judkins) catheter knows where to go if not thwarted by the operator.

A left 4-cm Judkins catheter fits in most adult patients.

When catheter size is adequate, the catheter tips is aligned with the long axis of the left main coronary trunk. A smaller catheter in same patient will tip upward and a large catheter will tip downward in to coronary cusp. When the coronary orifice is not cannulated appropriately, the catheter should be replaced with a better –fitting catheter rather than manipulated into the coronary artery. Sometimes a slight counterclockwise rotation of the catheter may be necessary to improve alignment of the catheter tip with the left main trunk.

The Judkins right coronary catheter is sized by the length of the secondary curve and comes in 3.5, 4.0, and 5.0 cm2 sizes. The 4.0-cm catheter is adequate in majority of the cases.

The right Judkins catheter is advanced in to the ascending aorta (usually the LAO projection) with the tip directed caudally.

The right coronary artery can be entered in most cases by one of the two maneuvers.

1. The catheter is advanced in to the right coronary cusp. The catheter is rotated 45° to 90 ° clockwise while the tip is pulled back 2-3 cm at the same time. Rotation of the tip toward the right cusp

and downward motion of the catheter are seen while engaging the right coronary orifice.

2. The catheter tip is advanced to 2-4 cm above the valve. When the catheter is rotated clockwise for 45° to 90°, the tip will rotate toward the right cusp and descend approximately 1-2 cm engaging the right coronary ostium from the above.

Femoral ventriculography catheter

The pigtail catheter has a tapered tip, preshaped to make a full circle 1 cm in diameter. Five to twelve side holes are located on the straight portion of the catheter above the curve. To enter the left ventricle, the pigtail catheter is advanced to the aortic valve. The loop is positioned to the left in the RAO projection and the catheter is pushed against the valve to make a U shape that facilitates the entry into the ventricle during inspiration.

The catheter is placed in front of the mitral valve with the loop directed away from the valve (in the RAO position).

Left ventricular angiography is used to determine the size and function of the left ventricle; coronary angiography is used to detect stenoses and guide revascularization procedures such as balloon angioplasty and stenting or CABG.

The goal of Coronary angiography is to visualize the coronary arteries, its branches and anomalies with enough detail to make a precise diagnosis of and plan the treatment.

Strategy for coronary heart disease

Technique

Hand injection for coronary arteriography. Contrast media, an iodinated solution used to opacify the coronary arteries, is injected by hand through multivalve manifold.

Flow rates are usually 2-4 ml/sec with volume of 4-8 ml in the right coronary artery and 7-10 ml for the left coronary artery

Power injections for coronary arteriography. Power injection of the coronary arteries has been utilized in many laboratories and is equal in safe to hand injection.

Views

In order to obtain the optimal information from coronary ciangiography, various views unveiling overlapped vessel segments must be used. These views or projections high light specific and distinct segment of the coronary anatomy and permit a discrete view of underlying pathology.

Left coronary artery: The AP view, the LAO/cranial views, the RAO caudal view, RAO/cranial view, LAO/caudal view and lateral view are used.

Right coronary artery: The LAO/cranial view, the RAO view and lateral view are used.

Assessment of coronary stenosis

The evaluation of the degree of a stenosis relates to the percentage reduction in the diameter of the vessel. This is calculated in the projection where the greatest narrowing can be observed. Exact evaluation is almost impossible and in fact, the lesions are roughly classified. Six categories can be distinguished in this way:

0. Normal Coronary artery
1. irregularities of the vessel
2. Narrowing of less than 50% (Non significant)
3. Stenosis between 50% and 75% (Significant)
4. Stenosis between 75% and 95% (Sub occlusion)
5. Total occlusion (Occlusion)

TREATMENT

Treatment includes medical and surgical modalities of treatment. Medical treatment include non pharmacological and pharmacological regime. Non pharmacological measures include weight reduction, dietary modification, regular exercise etc. Pharmacological treatment include antiplatelet therapy and antianginal drug treatment. Surgical treatment or interventional therapy include Percutaneous Transluminal Coronary Angioplasty (PTCA) with or without stinting and Coronary Artery Bypass Grafting (CABG).

ROLE OF *H pylori* IN ATHEROSCLEROSIS

Numerous studies have reported an association of coronary atherosclerosis with certain bacterial and viral infections.

Helicobacter pylori can cause persistent infections of the gastrointestinal tract. It has been suggested that persistent infection of arteries with these bacteria can contribute to the development of atherosclerosis.

Farsak et al. studied 85 patients undergoing coronary artery bypass grafting, carotid endarterectomy, and surgery of the abdominal aorta for atherosclerotic obstructive lesions to determine the presence of *C. pneumoniae* and *H pylori* DNA in atherosclerotic plaque samples by PCR [56]. They found that *C. pneumoniae* DNA was found in 12 (26%) of 46 endarterectomy specimens and none of the healthy vascular-wall specimens, while *H pylori* DNA was found in 17 (37%) of 46 endarterectomy specimens and none of the controls. Either *C. pneumoniae* or *H pylori* DNA was positive in 23 (50%) of 46 patients and none of the controls. Six of the atherosclerotic lesions showed coexistence of both of the microorganism DNAs. The presence of *C. pneumoniae* and *H pylori* DNA in a considerable number of atherosclerotic plaques but their absence in healthy vascular wall supports the idea that they may have a role in the

development of atherosclerosis, especially in countries where infection is prevalent and where conventional risk factors fail to explain the high prevalence of atherosclerotic vascular disease.

Klein C et al. showed that IgG antibodies against *H pylori* was associated with advanced atherosclerosis (> or =2 vascular regions), adjusted for age, sex, cardiovascular risk factors, and highly sensitive C-reactive protein [57]. The mortality rate was also increased with advanced atherosclerosis with more seropositivity. But in long term follow up, if it is adjusted with risk factors like body mass index, total and high-density lipoprotein, diabetes mellitus, smoking and hypertension, the relation with IgG antibodies against *H pylori* was not associated with cardiovascular diseases [58].

The *H pylori* seropositivity reached 69.79% of CHD and it was significantly higher than that in controls without CHD (subgroup II)--40.62%, the odds ratio (OR) being 3.38 95% CI: 1.8598-6.1306 for *H pylori* in CHD. CagA IgG detection was also significantly higher (58.20%).

H pylori infection increased risk for MI with modest association and it was not evident in non-smokers or when adjusted for education [59].

Causing extent of atherosclerosis

In hypothesizing an association between infectious agents and the development of atherosclerosis, we would expect a correlation to the extent of atherosclerosis. Moreover, this effect could be multiplied by the number of pathogens to which an individual had been exposed. Elevated IgA antibodies against *C pneumoniae* and IgG antibodies against *H pylori*, cytomegalovirus, and herpes simplex virus 2 were associated with advanced atherosclerosis (> or =2 vascular regions), adjusted for age, sex, cardiovascular risk factors, and highly sensitive C-reactive protein. Infectious burden divided into 0 to 3, 4 to 5, and 6 to 8 seropositivities was significantly associated with advanced atherosclerosis. Cardiovascular mortality rate in 3.2 years follow up was 7.0% in patients with advanced atherosclerosis and seropositive for 0 to 3 pathogens compared with 20.0% in those seropositive for 6 to 8 pathogens. Their results support the hypothesis that infectious agents are involved in the development of atherosclerosis. Thus many studies have shown a significant association between infectious burden and the extent of atherosclerosis. Moreover, the risk for future death was increased by the number of infectious pathogens, especially in patients with advanced atherosclerosis [57].

Potential mechanisms whereby chronic infections may play a role in atherogenesis are myriad. In the case of *C. pneumoniae*, the effect may result from direct vessel wall colonization, which may damage the vessel directly or indirectly by initiating immunologic responses. In other cases, the effect may simply be that of enhancing the preexisting chronic inflammatory response of the body to standard risk factors, such as hyperlipidemia. Even though the infectious agent may not directly infect the vessel wall, it may perform its critical role from afar. Chronic infection might also influence preexisting plaque by enhancing T cell activation or other inflammatory responses that may participate in the destabilization of the intimal cap. Chronic infection may play a role in the initiation, progression, or destabilization of atherosclerotic plaques. The infectious agents with the most evidence to support a causative role in atherosclerosis include *C pneumoniae* and cytomegalovirus. Evidence is mounting for a variety of other potential agents, including *H pylori*, various periodontal agents, and even hepatitis A. Future studies are expected to elucidate further the pathophysiologic relationship between chronic infection and atherosclerosis and to evaluate the potential of a variety of treatment approaches, including antibiotics [60].

Exploring the causes of atherosclerosis in coronary artery diseases, the inflammatory process has been found to have major role since old days. There is now a convincing evidences that atherosclerosis has a major inflammatory component and is much more than the simple vascular accumulation of lipids. Infectious agents that have been linked to an increased risk of coronary heart disease (CHD) include *Helicobacter* pylori, *Chlamydia pneumoniae*, cytomegalovirus, and herpesviruses. The evidences supporting *Helicobacter* pylori infection has positive relation with coronary artery disease though the large multi-center studies are necessary to establish the confirmatory relationship between *H pylori* infection and coronary artery disease.

SUBJECTS AND METHODS

Study Design: A cross sectional analytical study.

Place of Study: National Institute of Cardiovascular Diseases (NICVD), Dhaka.

Subjects:

A total of 112 patients who underwent coronary angiography and consented to enter the study were included. Of them 86 patients were with angiographically documented CHD (Case group) and 26 were CHD negative (who comprised the Comparison group).

Inclusion Criteria

Subjects who underwent CAG and consented to enter the study.

Exclusion Criteria

1. Subjects with rheumatic valvular heart disease
2. Acute infection
3. Subjects taking lipid lowering agent(s)

Methods:

Sample size

The minimum sample size was calculated using the following formula [61]:

Sample size, $n = (z^2 pq)/d^2$, where Z= Standard deviate of the sample, p= prevalence of the disease, q=1-p, d= allowable error.

As there is no report of prevalence of *H pylori* infection in our country we took the prevalence to be 50%. By taking the values as follows, Z= 1.96, p= 50%, q=50%, d= 20% of p=10%,

$N=((1.96)^2 \times 50 \times 50)/10^2 = 96.$

A total of 112 subjects were included in the study, which is higher than the minimum number required.

Sampling technique

Purposive sampling technique was followed; consecutive subjects who fulfilled the criteria were included in the study. This technique was followed due to the following reasons: a) as CAG, an invasive and costly procedure, is advised only in a section of CHD patients, b) there is a limitation of total maximum number of CAG that can be done in the NICVD existing facilities, and c) moreover, for the purpose of reliability, CAG done from a single unit (under the principal investigator) was accepted.

Biochemical parameters

Fasting plasma glucose, fasting serum total cholesterol, HDL cholesterol, LDL cholesterol and total triglycerides done within a week prior to CAG were noted from the patients' records.

Blood were collected for measurement of serum Anti- *H pylori* IgG antibodies and were preserved at –20°C for later assay. The IgG antibodies to *H pylori* were measured by an enzyme linked immunosorbent assay (ELISA) method (Appendix III).

Coronary angiography

Selective coronary angiography was performed on the patients using the Judkins femoral arterial technique. Coronary artery disease was assessed by angiography by visual inspection in two or more orthogonal views.

Briefly four coronary arteries were considered for the assessment of coronary stenosis: left main coronary, left anterior descending, circumflex and right coronary. Patients with – lesion to a minimum 50% narrowing of any of these four coronary arterial segments were included in the CHD positive group.

Patients with angiographically normal coronary arteries were classified in the CHD negative group.

Statistical Analysis

Data were expressed as mean ± SD or median range as appropriate. The data were managed using the Statistical Package for Social Sciences (SPSS) for Windows program version 11.5. The mean values of the two groups (case vs comparison groups) were compared by Student's *t* test; The median values of the two groups (case vs comparison groups) were compared by Mann Whitney U test. The odds ratio (OR) for the risk measurement of CHD associated with the *H pylori* infection was estimated by the binary logistic regression model, adjusting by forward steps for age, gender, history of smoking and diabetes, BMI, serum total cholesterol, triglycerides, LDL cholesterol and HDL cholesterol.

Ethical Issues

The protocol was approved by the PhD Committee of the Faculty of the Postgraduate Medical Science and Research, Dhaka University. The study protocol was also approved by the Director, NICVD. Written informed consent was taken from all the subjects. Helsinki Declaration VI was followed through out the study.

RESULTS

Results:

Table IA. Selected characteristics of the study population by cardiovascular disease status (N=112).

Characteristics	Cardiovascular disease positive N=86	Cardiovascular disease negative N=26	p-value
Sex			
Male	82 (91%)	8 (9%)	< 0.001
Female	4 (18%)	18 (82%)	
Age			
Mean ± S.D. (yrs)	49.9 ± 9.0	48.3 ± 7.8	ns
< 50 years	40 (74%)	14 (26%)	ns
≥ 50 years	46 (79%)	12 (21%)	
Smoked			
Yes	64 (91%)	6 (9%)	< 0.001
No	22 (52%)	20 (48%)	
BMI			
Mean ± S.D.	24.7 ± 2.8	24.9 ± 3.5	ns
< 23.0	20 (83%)	4 (17%)	ns
≥ 23.0	62 (76%)	20 (24%)	
H pylori status			
Positive	58 (81%)	14 (19%)	ns
Negative	28 (70%)	12 (30%)	
Diabetes mellitus			
Yes	30 (83%)	6 (17%)	ns
No	56 (74%)	20 (26%)	

Values are presented as number (percentage) unless otherwise specified. P-values for categorical variables were calculated by $\chi 2$ test. P-values for differences for means were calculated by Student's t-test.

The Table IA shows that the relative frequency of CHD in males was higher than females (91% vs 18%, p<0.001). The relative frequency of CHD was higher in smokers than non-smokers (91% vs 52%, p<0.001). And the relative frequency of CHD did not differ in

respect of age, BMI, *H pylori* infection status (Figure 4.1), and presence/absence of diabetes mellitus.

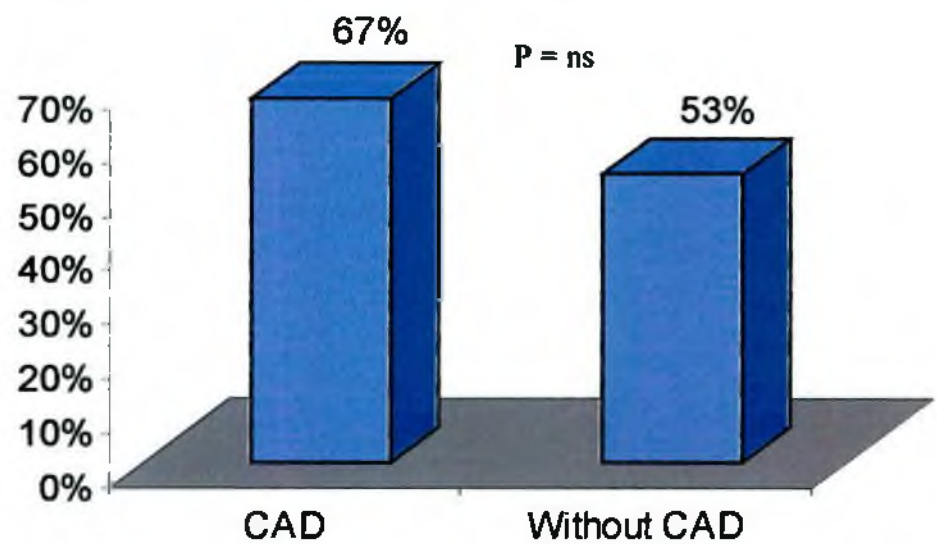

Fig 4.1 Relative frequency of *H Pylori* infection among the study subjects

Table IB. Selected characteristics of the study population by cardiovascular disease status (N=112).

Characteristics	Cardiovascular disease positive N=86	Cardiovascular disease negative N=26	p-value
Triglycerides			
Median	196 (95-689)	207 (130-534)	ns
(range)	22 (92%)	2 (8%)	0.046
< 150 mg/dl	62 (72%)	24 (28%)	
≥ 150 mg/dl			
Total Cholesterol			
Mean ± S.D.	197 ± 35	194 ± 40	ns
< 200 mg/dl	54 (77%)	16 (23%)	ns
≥ 200 mg/dl	30 (75%)	10 (25%)	
LDL Cholesterol			
Mean ± S.D.	113 ± 22	103 ± 37	ns
<130 mg/dl	62 (78%)	18 (22%)	ns
≥ 130 mg/dl	16 (80%)	4 (20%)	
HDL			
Cholesterol	36 ± 7	40 ± 9	0.046
Mean ± S.D.	34 (90%)	4 (10%)	0.019
< 35 mg/dl	50 (69%)	22 (31%)	
≥ 35 mg/dl			

Values are presented as number (percentage) unless otherwise specified. P-values for categorical variables were calculated by $\chi 2$ test. P-values for differences for means were calculated by Student's t-test and P-values for differences for medians were calculated by Mann-Whitney U-test. LDL= low density lipoprotein. HDL= high density lipoprotein

The Table IB shows that the relative frequency of CHD in subjects with high serum triglycerides (≥150 mg/dL) was higher than subjects with normal serum triglycerides (<150 mg/dL) (92% vs 72%, p=0.046).

The relative frequency of CHD in subjects with low serum HDL-C (<35 mg/dL) was higher than subjects with normal serum HDL-C

(≥35 mg/dL) (90% vs 69%, p=0.019, Figure 4.2). And the mean (±SD) serum HDL-C was lower in CHD subjects than those without CHD (36 ± 7 vs 40 ± 9 mg/dL, P =0.046). But the mean (±SD) serum total-cholesterol, LDL-C and triglycerides levels of the subjects with and without CHD were comparable.

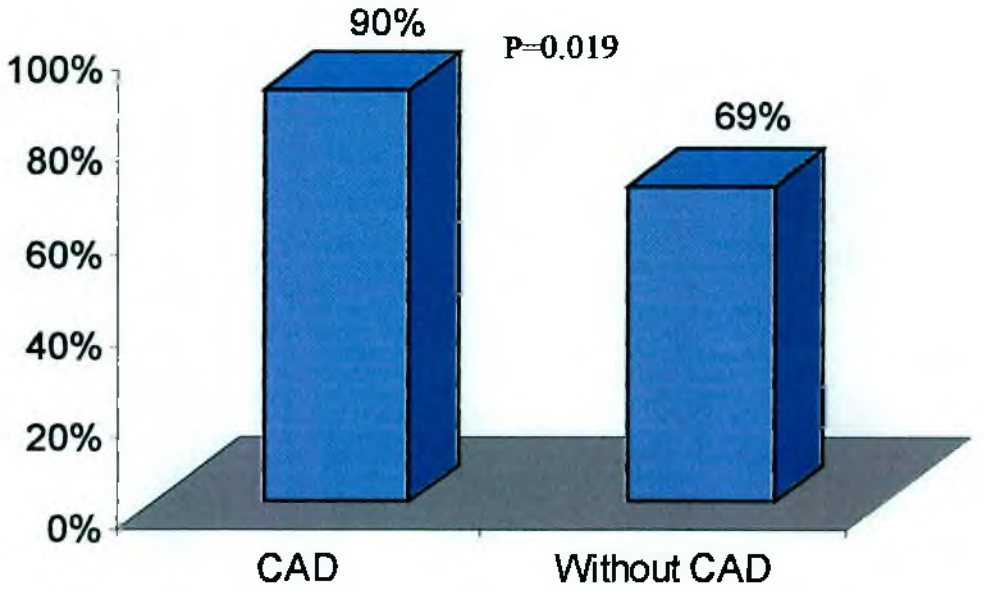

Fig 4.2 Relative frequency of low HDL-C among the study subjects

Table II. Selected characteristics of the study population in terms of cardiovascular disease controlling for *H pylori* antibody status (N=112)

Characteristics	*H pylori* positive (N=72)			*H pylori* negative (N=40)		
	Have CVD N=58	No CVD N=14	P-value	Have CVD N=28	No CVD N=12	P-value
Sex						
Male	56 (90%)	6 (10%)	< 0.001	26 (93%)	2 (7%)	< 0.001
Female	2 (20%)	8 (80%)		2 (17%)	10 (83%)	
Age						
Mean ± S.D.	51 ± 8	49 ± 7	ns	48 ± 10	48 ± 9	ns
< 50 years	28 (78%)	8 (22%)	ns	12 (67%)	6 (33%)	ns
≥ 50 years	30 (83%)	6 (17%)		16 (73%)	6 (27%)	
Smoked						
Yes	44 (92%)	4 (2%)	0.002	20 (91%)	2 (9%)	0.001
No	14 (58%)	10 (42%)		8 (44%)	10 (56%)	
BMI						
Mean ± S.D.	24.3 ± 2.7	23.5 ± 3.7	ns	25.5 ± 3.0	26.9 ± 1.9	ns
< 23.0	16 (80%)	4 (20%)	ns	4 (100%)	0 (0%)	ns
≥ 23.0	38 (79%)	10 (21%)		24 (71%)	10 (29%)	

Values are presented as number (percentage) unless otherwise specified.
P-values for categorical variables were calculated by χ^2 test.
P-values for differences for means were calculated by Student's t-test.
CVD= Cardiovascular disease, BMI=body mass index

The Table II shows that the relative frequency of CHD in males was higher than females both within the *H pylori* positive group (90% vs 20%, p<0.001) and with the *H pylori* negative group (93% vs 17%, p<0.001). The relative frequency of CHD was higher in smokers than non-smokers both within the *H pylori* positive group (92% vs 58%,

p=0.002) and with the *H pylori* negative group (91% vs 44%, p<0.001). And the relative frequency of CHD did not differ in respect of age and BMI considering *H pylori* infection status.

Table III. Differences in biochemical parameters of the study population in terms of cardiovascular disease controlling for *H pylori* status (N=112).

Characteristics	*H pylori* positive (N=72)			*H pylori* negative (N=40)		
	Have CVD N=58	No CVD N=14	P-value	Have CVD N=28	No CVD N=12	P-value
Triglycerides						
Median (range)	197 (120-529)	176(130-445)	ns	189(95-689)	244 (192-534)	ns
< 150 mg/dl	16 (89%)	2 (11%)	ns	6 (100%)	0 (0%)	ns
≥ 150 mg/dl	40 (77%)	12 (23%)		22 (65%)	12 (35%)	
Total Cholesterol						
Mean ± S.D.	197 ± 36	192 ± 38	ns	198 ± 34	195 ± 43	ns
< 200 mg/dl	38 (79%)	10 (21%)	ns	16 (73%)	6 (27%)	ns
≥ 200 mg/dl	18 (82%)	4 (18%)		12 (67%)	6 (33%)	
LDL Cholesterol						
Mean ± S.D.	197 ± 36	197 ± 36	ns	114 ± 20	100 ± 33	ns
<130 mg/dl	40 (80%)	10 (20%)	ns	22 (73%)	8 (27%)	ns
≥ 130 mg/dl	12 (86%)	2 (14%)		4 (67%)	2 (33%)	
HDL Cholesterol						
Mean ± S.D.	37 ± 7	41 ± 12	ns	36 ± 8	38 ± 2	
< 35 mg/dl	20 (83%)	4 (17%)	ns	14 (100%)	0 (0%)	0.007
≥ 35 mg/dl	36 (78%)	10 (22%)		14 (54%)	12 (46%)	

Values are presented as number (percentage) unless otherwise specified.
P-values for categorical variables were calculated by $\chi 2$ test.
P-values for differences for means were calculated by Student's t-test and for medians were by Mann-Whitney U-test.
LDL= low density lipoprotein
HDL= high density lipoprotein

The Table III shows that, within the *H pylori* negative group, there is higher relative frequency of CHD in subjects with *low* serum HDL-C (<35 mg/dL) than those with *normal* serum HDL-C (≥35 mg/dL) (100% vs 54%, p=0.007).

The mean (±SD) serum total-cholesterol, LDL-C and triglycerides levels of the subjects with CHD and those without CHD, both within the *H pylori* positive group and within the *H pylori* negative group, were comparable.

Table IV. Logistic regression model coefficients for development of cardiovascular disease

Variable	Beta	SE of beta	P value	Odds Ratio (95% CI)
Sex	-16.375	5.336	.002	0.001 (0.001-0.003)
DM	-6.067	2.453	.013	0.002 (0.001-0.284)
LDL-C	.209	.077	.007	1.233 (1.059-1.435)
Anti *H pylori* antibody status	-4.015	1.828	.028	.018 (0.001-0.649)
BMI	-.439	.229	.055	0.645 (0.411-1.010)

DM= Diabetes mellitus, LDL-C= Low density lipoprotein cholesterol, BMI=Body mass index, SE= Standard error, CI=Confidence interval

Table IV shows logistic regression analysis revealed that Sex (β = -16.375, P=0.002), LDL-C (β=0.209, P=0.007), history of diabetes (β= -6.067, P=0.013), and anti *H pylori* antibody status (β= -4.015, P= 0.028) are significant predictors of development of CHD independent of age, BMI, smoking status, serum total cholesterol, HDL-C, triglyceride levels.

Table V General Linear Model (GLM) Parameter Estimates

Dependent Variable: Total cholesterol

Parameter	Beta	SE	Sig.	95% CI	
				Lower Bound	Upper Bound
Intercept	-18.298	14.197	.202	-46.659	10.063
Girth	.710	.226	.003	.258	1.162
Age	-.250	.088	.006	-.425	-7.368E-02
LDL-C	.875	.054	.000	.768	.982
HDL-C	1.156	.126	.000	.905	1.407
TG	.185	.014	.000	.156	.213
BMI	-1.250	.630	.051	-2.508	8.297E-03
Sex	8.151	4.882	.100	-1.603	17.905
Smoking status	23.235	10.858	.036	1.545	44.926
DM	6.486	5.557	.247	-4.615	17.586
***H pylori* Ab Status**	13.940	6.625	.039	.705	27.174
HTN	-28.255	13.077	.034	-54.379	-2.132

DM= Diabetes mellitus, LDL-C= Low density lipoprotein cholesterol, HDL-C= High density lipoprotein cholesterol, BMI=Body mass index, TG= triglycerides, HTN=Hypertension, Ab= Antibody, SE= Standard error, CI=Confidence interval

Table V shows that General Linear Model analysis revealed that girth circumference, age, LDL-C, HDL-C, TG, smoking status and anti *H pylori* antibody status and hypertension are significant predictors of variance of serum total cholesterol independent of sex, history of diabetes and BMI.

Table VI General Linear Model (GLM) Parameter Estimates

Dependent Variable: Triglyceride

Parameter	B	Std. Error	Sig.	95% CI	
				Lower Bound	Upper Bound
Intercept	171.468	62.699	.008	46.213	296.723
Girth	-1.921	1.095	.084	-4.108	.266
Age	.801	.419	.060	-3.560E-02	1.637
LDL-C	-3.597	.336	.000	-4.268	-2.926
BMI	.821	2.988	.784	-5.149	6.791
TC	3.922	.302	.000	3.317	4.526
HDL-C	-4.422	.688	.000	-5.796	-3.048
Sex	-25.138	22.767	.274	-70.621	20.345
Smoking status	54.440	51.342	.293	-48.128	157.007
DM	-32.221	25.560	.212	-83.283	18.842
***H pylori* Ab status**	-55.886	30.782	.074	-117.380	5.609
HTN	-45.882	62.152	.463	-170.045	78.280

DM= Diabetes mellitus, TC=Total cholesterol, LDL-C= Low density lipoprotein cholesterol, HDL-C= High density lipoprotein cholesterol, BMI=Body mass index, HTN=Hypertension, Ab= Antibody, SE= Standard error, CI=Confidence interval

Table VI shows General Linear Model analysis revealed that LDL-C, HDL-C and serum total cholesterol are significant predictors of variance of serum triglycerides independent of age, sex, girth

circumference, history of diabetes, BMI, smoking status, hypertension and anti *H pylori* antibody status.

Table VII General Linear Model (GLM) Parameter Estimates
Dependent Variable: LDL-cholesterol

Parameter	Beta	SE	Sig.	95% CI	
				Lower Bound	Upper Bound
Intercept	8.251	14.722	.577	-21.159	37.661
Girth	-.198	.248	.428	-.694	.298
Age	.182	.093	.055	-3.836E-03	.368
BMI	.263	.665	.693	-1.065	1.592
TC	.921	.056	.000	.809	1.034
HDL-C	-.914	.160	.000	-1.234	-.595
TG	-.178	.017	.000	-.212	-.145
Sex	.271	5.119	.958	-9.955	10.497
Smoking status	-4.372	11.522	.706	-27.390	18.647
DM	-1.525	5.760	.792	-13.031	9.982
***H pylori* Ab status**	-1.500	7.028	.832	-15.540	12.539
HTN	5.117	13.887	.714	-22.625	32.859

DM= Diabetes mellitus, TC=Total cholesterol, HDL-C= High density lipoprotein cholesterol, TG=Triglycerides, BMI=Body mass index, HTN=Hypertension, Ab= Antibody, SE= Standard error, CI=Confidence interval

Table VII shows General Linear Model analysis revealed that total cholesterol, triglycerides and HDL-C are significant predictors of variance of serum LDL-C independent of age, sex, girth circumference, history of diabetes, BMI, smoking status, hypertension and anti *H pylori* antibody status.

Table VIII General Linear Model (GLM) Parameter Estimates

Dependent Variable: HDL-cholesterol

Parameter	B	SE	Sig.	95% CI	
				Lower Bound	Upper Bound
Intercept	34.0	8.933	.012	5.232	40.925
Girth	-.409	.151	.014	-.703	-.102
Age	.175	.057	.003	5.165E-02	.281
LDL-C	-.391	.065	.001	-.499	-.241
TG	-.111	.014	.001	-.116	-6.118E-02
BMI	.169	.416	.726	-.184	1.478
TC	.536	.054	.001	.386	.600
Sex	-8.551	3.050	.020	-15.205	-3.017
Smoking status	-28.1	7.125	.001	-28.244	.225
DM	-3.759	3.633	.281	-11.200	3.314
***H pylori* Ab status**	-26.5	4.367	.0001	-16.415	1.035
HTN	41.7	8.134	.001	11.497	43.997

DM= Diabetes mellitus, TC=Total cholesterol, LDL-C= Low density lipoprotein cholesterol, TG=Triglycerides, BMI=Body mass index, HTN=Hypertension, Ab= Antibody, SE= Standard error, CI=Confidence interval

Table VIII shows General Linear Model analysis revealed that age, sex, girth circumference, hypertension, total cholesterol, LDL-C, triglycerides, anti-*H pylori* antibody status and smoking status are significant predictors of variance of serum HDL-C independent of sex, BMI and history of diabetes.

DISCUSSION

Discussion

The coronary heart disease (CHD) is emerging as a huge burden for health care services for developing countries, especially in the South Asian Subcontinent. The role of the risk factors other than the conventional ones for the development of CHD, such as- low birth weight, infection and inflammation in the South Asians is under thorough investigation. The present study was undertaken to investigate the relationship between *Helicobacter pylori* infection and coronary heart disease, which in long run may create a scope for preventive and curative treatment of CHD. This study, to the best of the investigator's knowledge, is the first one in Bangladesh with the above objective.

The role of conventional risk factors

Age

The mean (+SD) age of the subjects with CHD was 49 (+9) yrs. The the subjects between 40-58 years are particularly vulnerable to develop CHD. This supports previous findings in Bangladeshi population.

Sex

Males are about 46 times more likely to develop CHD than the females of same age group. The female subjects are less vulnerable to develop CHD due to protective effect of oestrogen.

Smoking

The smokers are about 10 times more likely to suffer from CHD than nonsmokers. The association of smoking with CHD is well established. About 74% of the subjects were smokers. This may be considered very high despite active all out anti-smoking campaigns all over the country. Education and counseling regarding ill effects of smoking still remains the first major step towards prevention of CHD in this population.

Family history

Positive family history of CHD has been demonstrated again to a risk factor for CHD. Among the CHD subjects, about 44% had positive family history as opposed to 15% in the comparison group. Counseling and regular screening of subjects with positive family history may be proposed to prevent the progression of CHD in these subjects

Dyslipidemia

High LDL-C has been shown to be most the important lipid abnormality in CHD subjects in this population. This is similar to previous studies that have also reported strong association of high LDL-C and CHD. However, no association was found between the abnormalities of other serum lipid parameters (such as- high total cholesterol, low HDL-C and high triglycerides) and CHD. The findings of the study emphasizes the importance of doing lipid profile regularly and introducing measures to control LDL-C in high-risk subjects by dietary modification, regular exercise and drugs.

Diabetes mellitus

The prevalence of diabetes mellitus in CHD subjects was 83%, which was comparable to that in comparison group subjects (70%). But, the diabetic subjects are certainly at a greater risk to develop CHD considering all other risk factors. The control of diabetes and regular screening of glucose tolerance of nondiabetic/prediabetic subjects should be adopted for any preventive measure to reduce the prevalence of CHD.

The role of Helicobacter pylori infection

In this study, multivariate analysis revealed that *H pylori* antibody status is a significant predictor of development of CHD irrespective of age, sex, BMI, smoking status, history of diabetes, serum total cholesterol, LDL-C HDL-C, tryglyceride levels. The findings of the study are similar to several previous studies that have shown an association between *H pylori* infection and coronary heart disease [3,7,12]. But, the findings of the study are in contrast to several previous studies that have failed to show any association between *H pylori* infection and coronary heart disease [62].

H pylori infection and serum lipid levels

In this study, a significant association was found between *H pylori* infection and serum total cholesterol level. *H pylori* antibody positive subjects had around 20 mg/dL higher serum total cholesterol level than *H pylori* antibody negative subjects. Also, a significant association was found between *H pylori* infection and serum HDL-cholesterol level controlling for other risk factors.. It was notable that, *H pylori* positive subjects had lower level of HDL-C than *H pylori* negative subjects. This finding is similar to Niemela *et al.* who have demonstrated statistically significantly lower level of HDL-C in *H pylori* positive subjects [6]. But no significant association was found

between *H pylori* infection and other lipid parameters (serum LDL-C or fasting triglyceride levels).

Study limitations

As this was a cross sectional study the results of which cannot be compared in equal platform with the previous longitudinal studies. The small sample size of the study makes it less representative of the population. A larger sample study could not be carried out due to logistic and financial limitations. The study did not attempt to identify the most virulent strain of *H pylori* (cagA) which has been particularly shown to be involved in the pathogenesis of CHD. The Comparison group subjects of the study were not healthy subjects; although they showed normal coronary angiogram, presence of CHD due to vasospasm cannot be excluded. Moreover, as they all had chest pain as presenting complaint, many of them may have peptic ulcer disease, which is commonly associated with *H pylori* infection. The study involved chronic subjects with complaint of chest pains who have under gone elective coronary angiogram and all acutely ill patients were excluded. Thus the study cannot comment on the role of *H pylori* infection to precipitate acute myocardial infarction as reported in some previous studies.

Conclusions

The conclusions of the study are-

a) There is a significant association between *H pylori* infection and increased risk of CHD irrespective of age, sex, BMI, history of hypertension and diabetes, smoking and serum total cholesterol, HDL-C, LDL-C and triglycerides levels

b) There is a significant association between *H pylori* infection and serum total cholesterol. *H pylori* antibody positive subjects had higher serum total cholesterol level than *H pylori* antibody negative subjects. Also, a significant association was found between *H pylori* infection and serum HDL-cholesterol level. *H pylori* antibody positive subjects had lower serum total cholesterol level than *H pylori* antibody negative subjects. But no significant association was found between *H pylori* infection and other lipid parameters (serum LDL-C or fasting triglyceride levels).

c) Further large-scale, population-based and prospective studies are needed to define the role of *H pylori* infection in the pathogenesis of CHD.

SUMMARY

Summary

The coronary heart disease (CHD) is emerging as a huge burden for health care services for developing countries, especially in the South Asian Subcontinent. The role of the risk factors other than the conventional ones, such as- low birth weight, infection and inflammation in these populations is under thorough investigation. Several studies have shown the association of *Helicobacter pylori* infection with CHD. Although there are reports of very high *H pylori* infection rate in Bangladesh, its association with CHD has not yet been explored.

The present study was undertaken with the *General Objective* to investigate the relationship between *Helicobacter pylori* infection and CHD, which in long run may create a scope for curative and preventive treatment of CHD. The *Specific Objectives* of the study were- a) To find out whether *H pylori* infection is an independent risk factor for CHD in our country, and b) To find out whether *H pylori* infection modifies serum lipid concentrations in a way that may increase the risk of CHD.

This study, to the best of the investigator's knowledge, is the first one in Bangladesh with the above objectives.

Using a analytical cross-sectional (Case-Comparison) design we studied 112 subjects who underwent coronary angiography for the first time for suspected or known CHD at the Department of Cardiology, NICVD between July and December 2002. Arterial stenosis of 50% or more was taken as significant. Among the study subjects 86 (77%) had CHD (Cases) and 26 (23%) did not have CHD (Comparison group). Demographic data, history and physical examination findings were recorded. Major risks factors considered: family history of CHD, diabetes mellitus, smoking, dyslipidemia and hypertension. Anti- *H pylori* IgG antibody titers were determined by specific ELISA method.

Fifty eight (67%) of CHD patients and 14 (54%) of the Comparison group subjects were seropositive for *H pylori*.

Logistic regression analysis revealed that sex (β= -16.375, P=0.002), LDL-C (β=0.209, P=0.007), history of diabetes (β= -6.067, P=0.013), and anti-*H pylori* antibody status (β= -4.015, P= 0.028) are significant predictors of development of CHD irrespective of age, BMI, smoking status, serum total cholesterol, HDL-C and tryglyceride levels.

The findings of the study are similar to several previous studies that have shown association between *H pylori* infection and coronary heart disease [3,7,12].

The findings of the study are in contrast to several previous studies that have failed to show any association between *H pylori* infection and coronary heart disease [61].

In this study, a significant association was found between *H pylori* infection and serum total cholesterol level. *H pylori* antibody positive subjects had around 20 mg/dL higher serum total cholesterol level than *H pylori* antibody negative subjects. Also, a significant association was found between *H pylori* infection and serum HDL-cholesterol level. It was notable that, *H pylori* positive subjects had lower level of HDL-C than *H pylori* negative subjects. This finding is similar to Niemela *et al.* who have demonstrated statistically significantly lower level of HDL-C in *H pylori* positive subjects [6]. But no significant association was found between *H pylori* infection and other lipid parameters (serum LDL-C or fasting triglyceride levels).

The conclusions of the study are-

a) There is a significant association between *H pylori* infection and increased risk of CHD irrespective of age, sex, BMI, history of hypertension and diabetes, smoking and serum total cholesterol, HDL-C, LDL-C and triglycerides levels

b) There is a significant association between *H pylori* infection and serum total cholesterol. *H pylori* antibody positive subjects had higher serum total cholesterol level than *H pylori* antibody negative subjects. Also, a significant association was found between *H pylori* infection and serum HDL-cholesterol level. *H pylori* antibody positive subjects had lower serum total cholesterol level than *H pylori* antibody negative subjects. But no significant association was found between *H pylori* infection and other lipid parameters (serum LDL-C or fasting triglyceride levels).

c) Further large-scale, population-based and prospective studies are needed to define the role of *H pylori* infection in the pathogenesis of CHD.

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APPENDICES

Appendix I

Data Sheet

ID: ___ Hospital Regn: ___ CD No ___ Date: ___

Patient Name: _____ Age: ___ Sex: M/ F

Ht: Wt: Girth:

Education: Primary/SSC/HSC/Graduate/Postgraduate

FH of CAD: Y/ N SM: Y/N, If yes

Current/Past

HTN: Y/ N DM: Y/ N, If yes Type: 1 / 2 Rx:

Diet/Insulin/OHA

H/o OCP: Y/ N H/o Oophorectomy: Y/ N H/o

Menopause: Y/ N Socioeconomic Status: Low/ Middle/ Upper

Lipid Profile: TC ___ mg/dL LDL ___ mg/dL

TG ___ mg/dL HDL ___ mg/dL

Anti-*H pylori* Ab titer: ___ u/L

Positive/Negative

Clinical Diagnosis: MI/Stable Angina/UA/Post Infarction Angina/Others

Type of MI: Ant/ Inf/ Post/ Lat

CAG: LM ___ % ___ LAD ___ % ___ 1 Disc/ 2 Tub/ 3 Dif

D_1 ___ % ___ D_2 ___ % ___ D_3 ___ %

Inter: ___ % ___ LCX ___ % ___

OM_1 ___ % ___ OM_2 ___ % ___ OM_3 ___ % ___

RCA ___ % ___

Conclusion: Left main/Single vessel/Double vessel/Triple vessel

Signature: _____ Date: ___

H. pylori IgG (EIA-3057)

Enzyme Immunoassay for the Quantitative Determination of IgG Antibodies to *Helicobacter pylori* in Human Serum

FOR IN VITRO DIAGNOSTIC USE ONLY

Store at 2 to 8°C.

PROPRIETARY AND COMMON NAMES

H. pylori IgG Enzyme Immunoassay

SUMMARY OF ASSAY PROCEDURE

1. Sample dilution 1:40

5 µl / 200 µl

2. Three incubations at room temperature

Diluted Sample 100 µl	Enzyme Conjugate 100 µl	TMB Reagent (One-Step) 100 µl
30 min.	30 min.	20 min.

3. Stop with 100 µl of acid. Read O.D. at 450 nm

INTENDED USE

The *Helicobacter pylori* IgG Test Kit is intended for the quantitative determination of IgG antibodies to *Helicobacter pylori* in human serum.

INTRODUCTION

Helicobacter pylori is a spiral bacterium cultured from human gastric mucosa discovered by B.J. Marshall in 1982. Studies have indicated that the presence of *H. pylori* is associated with a variety of gastrointestinal diseases including gastritis, duodenal and gastric ulcers, non-ulcer dyspepsia, and gastric adenocarcinoma and lymphoma. The organism is present in 95-98% of patients with duodenal ulcers and 60-90% of patients with gastric ulcers. The studies have also demonstrated that removal of the organism by anti-microbial therapy is correlated with the resolution of symptoms and cure of diseases.

Patients who present clinical symptoms relating to the gastrointestinal tract can be diagnosed for *H. pylori* infection by two methods:

(1) Invasive techniques – include biopsy followed by ulture or histologic examination of biopsy specimen or direct detection of urease activity.

(2) Non-invasive techniques – include urea breath tests and serological methods.

All of the testing performed on biopsy samples is subject to errors related to sampling and interference of contaminated bacteria. An ELISA test of the presence of *H. pylori* specific IgG antibody is the technique of choice for serologic tests because of its accuracy and simplicity.

PRINCIPLE OF THE TEST

EIA-3057 H. pylori IgG

Purified *H. pylori* antigen is coated on the surface of microwells. Diluted patients serum is added to the wells, and the *H. pylori* IgG- specific antibody, if present, binds to the antigen. All unbound materials are washed away. Enzyme conjugate is added, which binds to the antibody-antigen complex. Excess enzyme conjugate is washed off and a solution of TMB Reagent is added. The enzyme conjugate catalytic reaction is stopped at a specific time. The intensity of the color generated is proportional to the amount of IgG-specific antibody in the sample. The results are read by a microwell reader compared in a parallel manner with calibrator and controls.

Reagents

Materials provided with the kit:

- Purified *H. pylori* antigen coated microtiter plate, 96 wells.
- Enzyme Conjugate Reagent (red color), 13 ml.
- Sample Diluent (green color), 22 ml.
- *H. pylori* Negative Control, < 6.25 U/ml, 150 µL.
- *H. pylori* Standards, 0, 6.25, 12.5, 25, 50, and 100 U/ml, 150 µL each.
- *H. pylori* Positive Control, > 100 U/ml, 150 µL.
- Wash Buffer (20×), 50 ml.
- TMB Reagent (One-Step), 11 ml.
- Stop Solution (1N HCl), 11 ml.

Materials required but not provided:

- Distilled water.
- Precision pipettes: 0.01, 0.10, 0.20, and 1.0 ml.
- Disposable pipette tips.
- Vortex mixer or equivalent.
- Absorbent paper or paper towel.

Specimen Collection and Preparation

1. Serum should be prepared from a whole blood specimen obtained by acceptable medical techniques. This kit is for use with serum samples without additives only.

2. Specimens may be refrigerated at 2-8°C for up to 7 days or frozen for up to 6 months. Avoid repetitive freezing and thawing of serum sample.

Storage of Test Kits and Instrumentation

Unopened test kits should be stored at 2-8°C upon receipt and the microtiter plate should be kept in a sealed bag with desiccants to minimize exposure to damp air. Opened test kits will remain stable until the expiration date shown, provided it is stored as described above. A microtiter plate reader with a bandwidth of 10nm or less and an optical density range of 0-2 OD or greater at 450 nm wavelength is acceptable for use in absorbance measurement.

Reagent Preparation

1. All reagents should be allowed to reach room temperature (18-25°C) before use.

2. Dilute 1 volume of Wash Buffer (20×) with 19 volumes of distilled water. For example, dilute 50 ml of Wash Buffer (20×) into distilled water to prepare 1000 ml of Wash Buffer (1×). Wash Buffer is stable for 1 month at 2-8°C. Mix well before use.

Assay Procedure

1. Secure the desired number of coated wells in the holder.

2. Prepare 1:40 dilution for test samples, all six *H. pylori* standards, negative control, and positive control by adding 5 µl of the sample to 200 µl of sample diluent. Mix well.
3. Dispense 100µl of diluted sera, six standards, and controls into the appropriate wells. For the reagent blank, dispense 100 µl sample diluent in 1A well position. Tap the holder to remove air bubbles from the liquid and mix well.
4. Incubate at room temperature for 30 minutes.
5. At the end of the incubation period, remove liquid from all wells. Rinse and flick the microtiter wells 4 times with diluted wash buffer (1×) and then one time with distilled water. (Please do not use tap water.)
6. Dispense 100 µl of enzyme conjugate to each well. Mix gently for 10 secoonds.
7. Incubate at room temperature for 30 minutes.
8. Remove enzyme conjugate from all wells. Rinse and flick the microtiter wells 4 times with diluted wash buffer (1×) and then one time with distilled water.
9. Add 100 µl of TMB Reagent to each well. Mix gently for 10 seconds.
10. Incubate at room temperature for 20 minutes.
11. Add 100µl of Stop Solution to each well including the 2 blanks.
12. Mix gently for 30 seconds. ***It is important to make sure that all the blue color changes to yellow color completely.***
13. Read the optical density at 450 nm ***within 15 minutes*** with a microtiter plate reader.

Important Note:
The wash procedure is critical. Insufficient washing will result in improper color development.

CALCULATION OF RESULTS

1. Calculate the mean absorbance value (A_{450}) for each set of reference standards, controls and patient samples.
2. Construct a standard curve by plotting the mean absorbance obtained from each reference standard against its concentration in U/ml on graph paper, with absorbance values on the vertical or Y axis, and concentrations on the horizontal or X axis.
3. Use the mean absorbance values for each specimen to determine the corresponding concentration of *H. pylori* IgG in U/ml from the standard curve.

EXAMPLE OF STANDARD CURVE

Results of a typical standard run with optical density readings at 450nm shown in the Y axis against *H. pylori* IgG concentrations shown in the X axis. This standard curve is for the purpose of illustration only, and should not be used to calculate unknowns. Each user should obtain his or her own data and standard curve.

H. pylori (U/ml)	Absorbance (450nm)
0	0.059
6.25	0.573
12.5	0.901
25	1.450
50	1.988
100	2.591

EXPECTED VALUES

A cut-off level is set at 20 U/ml for normal samples. Values below 20 U/ml are considered normal. Values above 20 U/ml are regarded as positive. Values above 100 U/ml should be re-assayed at a higher dilution, e.g. 1:802 (first with 1:41, and then 1:20). Results obtained from this 1:802 dilution should be multiplied by 20 to reflect the true *H. pylori* IgG concentration.

The comparison of the *H. pylori* IgG ELISA test to a commercial ELISA kit results are summarized in the following table.

		Reference ELISA			
		N	E	P	Total
H. pylori IgG ELISA	N	96(D)	1	4(B)	101
	E	2	2	1	5
	P	3(C)	0	105(A)	108
	Total	101	3	110	214

Sensitivity = A / (A+B) = 107 / 109 = 99%

Specificity = D / (C+D) = 96 / 99 = 97%

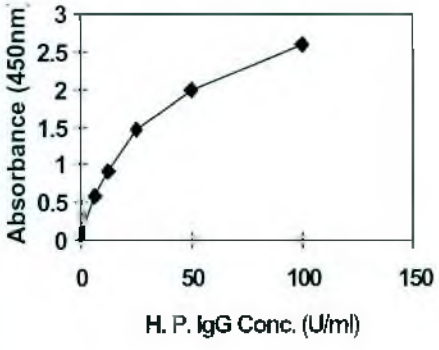
Accuracy = (A+D) / (A+B+C+D) = 201 / 208 = 97%

The precision of the assay was evaluated by testing three different sera of 20 replicates over 4 days. The intra-assay and inter-assay C.V. are summarized below.

	7.5 U/ml	22 U/ml	80 U/ml
Intra-assay	9.1%	8.5%	6.4%
Inter-assay	10.5%	8.9%	7.5%

LIMITATIONS OF THE PROCEDURE

1. The assay should be used only to evaluate patients with clinical signs and symptoms suggestive of gastrointestinal disease.
2. A positive test result does not allow one to distinguish between active infection and colonization by *H. pylori*. It



does not necessarily indicate that gastrointestinal disease is present.

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