The association of CagA-positive *Helicobacter pylori* serotype and atherosclerosis in Najran area, Saudi Arabia

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Abstract: Infections with virulent CagA-bearing *H. pylori* strains has been contributed to the pathogenesis of atherosclerosis. There is abundant circumstantial evidence that chronic *H. pylori* infections induce immune responses and trigger the development of atherosclerosis. The present study evaluated the role of CagA positive *H. pylori* as a risk factor for atherosclerosis, in atherosclerotic male patients from Najran area, Saudi Arabia. A total of 130 male patients. They were divided into 2 groups: group I (GI) included 30 apparently healthy controls without a history or presence of definite or suspected vascular diseases; and 100 male patients with atherosclerosis, group II (GII). The studied individuals were subjected to complete clinical examination, history and detection of CRP seropositivity. Detection of anti-*H. pylori* and anti-*H. pylori* CagA seropositivity were done using ELISA. In addition, biochemical measurements including glucose, total cholesterol, high density lipoprotein (HDL), low density lipoprotein (LDL) and triglycerides (TG) were measured. Results demonstrated that the prevalence of anti-*H. pylori* IgG antibodies in atherosclerotic patients were (68%) in (GII)and (56.7%) in (GI). The incidence of the anti-CagA seropositivity was significantly prevalent in GII (29%) than in GI (10%). The familial history including atherosclerosis (in one or more members of the family), obesity, hypertension, dyslipidemia, diabetes and smoking were significantly increased in GII compared to GI. The seropositivity of the inflammatory marker C-reactive protein (CRP) was significantly increased in GII (45%) compared GI (20%). In conclusion, this is the first work to show the relationship between atherosclerosis and CagA positive *H. pylori* in Najran, Saudi Arabia. However, the exact mechanisms need further study.
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1. Introduction

Cardiovascular diseases, including coronary atherosclerosis are the most prominent circulation disorders around the world and remain the leading cause of deaths in the developed and developing countries despite of declining mortality *(Jia et al., 2009 and Sun and Jia, 2012).Tiong and Brieger (2005)* reported that, coronary atherosclerosis is a multifactorial process where chronic inflammation plays a pivotal role, while other risk factors such as genetic factors, dyslipidemia, hypertension, diabetes mellitus and smoking also contribute to the pathogenesis of the disease. Moreover, it was reported that chronic inflammation plays an important role in the initiation and progression of atherosclerosis and its complications *(Ballantyne and Nambi, 2005)*. However, the underlying mechanism of the chronic inflammatory process in the pathogenesis of atherosclerosis is still unknown. As a possible trigger, different viruses and bacteria may be associated with atherosclerotic diseases *(Danesh et al., 1998; Tsai and Huang*, *2000 and Ozdogru et al., 2007)*. Reducing the adverse cardiovascular outcomes of atherosclerosis through risk factor identification and

modification has been an active area of research over the past few decades *(Al-Omran, 2012)*.

Helicobacter pylori (*H. pylori*) is a Gram-negative bacterium that colonizes the human stomach. *H. pylori* infection is now considered an epidemic, approximately 50% of the human population worldwide is infected with *H. pylori* *(Blaser and Atherton., 2004; Amieva and El-Omar, 2008 and Palframan et al.,2012)*. In the last years, several studies has linked *H. pylori* infection to different diseases outside of the stomach. Epidemiological studies based on serological findings have suggested an association between chronic *H. pylori* infection and atherosclerosis *(Yasunori et al., 2005; Pasceri et al., 2006 and Banić et al.,, 2012)*. Several epidemiologic studies have reported an association between *H. pylori* bearing the cytotoxin-associated gene-A (CagA), a strong virulence factor, and atherosclerosis leading to ischemic stroke *(Cremonini et al., 2004 and Diomedi et al., 2004)*. Also, many authors reported a strong predictive role of infection with CagA-positive strains for the incidence of first-ever stroke in patients with atherosclerosis suggested a causal relationship *(Preusch et al., 2004 and Diomedi et al., 2008)*.

There is accumulating evidence of the role of inflammation in the pathogenesis of atherosclerosis. In this regard inflammatory markers, such as highly sensitive C-reactive protein (CRP), have been associated with an increased risk of atherosclerosis. The association of *H. pylori* infection with coronary vascular disease (both coronary heart disease and cerebrovascular disease) has been suggested in many but not all studies *(Danesh et al., 1997; Murray et al., 2000; Stone et al., 2001 and Haider et al., 2002)*. *H. pylori* infection has been reported to be hyperendemic in Saudi Arabia. Reports in the 1990s have shown a prevalence of 68–82.2% *(Al-Moagel et al., 1990 and Rashed et al., 1992)*. In addition, it was found that the prevalence is markedly increased with age and ranged from 32.4% in those aged 5–10 years to more than 66.4% in those aged 20–30 years and 75% in those over 50 years *(Marie, 2008)*. *World Health Organization (2010)* reported that atherosclerotic disease is the leading cause of death in Saudi Arabia. At the same time, a high prevalence of *H. pylori* infection in Saudi Arabia has been shown in various studies *(Mohamed et al., 1994; Khan,1998; Almadi et al., 2007; Marie, 2008 and Al-Akwaa., 2010)*. The aim of the present study is to investigate the association between chronic infection with *H. pylori* bearing the cytotoxin-associated gene-A (CagA) and atherosclerosis in atherosclerotic patients from Najran area, Saudi Arabia.

2.Materials and methods

This study aimed to investigate the effect of CagA-positive *Helicobacter pylori* (*H. pylori*) infection on coronary atherosclerosis in Saudi men in Najran area, Saudi Arabia. Also, to elucidate how cytotoxin-associated gene A (CagA)-positive *H. pylori* infections were involved in coronary heart disease by examining the levels of serum lipid, and C-reactive protein (CRP). The individuals were informed in detail about the research and the protocol was approved by the Institutional Research Ethics Committee of Najran University.

A total of 130 male patients were included in the study. They were divided into 2 groups: group I (GI) included 30 apparently healthy controls without a history or presence of definite or suspected vascular diseases; and 100 male patients with atherosclerosis (GII) who were admitted to the Prince Sultan Center of Cardiology in Najran.

Clinical examination and history:

The studied individuals were subjected to complete history of familial atherosclerosis in one or more members of the family, body weight, smoking, hypertension and diabetes. Thorough physical examination, electrocardiography, echocardiography and coronary angiography were done for all individuals.

Blood samples:

Blood samples from a total of 130 male individuals were aspirated in the morning after an overnight fast (12 hours). Serum was separated as soon as possible after centrifugation of blood, divided into small aliquots and stored at -20°C until used. Their age ranged between 50-70 years.

Detection of *H. pylori* seropositivity:

Blood samples were collected from all patients and controls, and stored at −80 °C until analysis. Serologic studies were performed blindly; sera of patients and controls were analyzed at the same time. Specific anti-*H. pylori* IgG antibodies Kits from BioHit, Finland were used by Enzyme-linked immuno-sorbent assay, according to the manufacturer's instructions. It is based on sandwich enzyme immunoassay (ELISA) technique with purified *H. pylori* bacterial antigen adsorbed on a microwell plate.

Detection of *H. pylori* CagA seropositivity:

The test was performed using ELISA kit for the detection of anti-*H. pylori* CagA IgG antibodies (MyBioSource, San Diego, California, USA). Procedures were done according to the manufacturer's instructions. Samples with antibody index more than 0.9 were considered positive.

Detection of CRP seropositivity:

CRP seropositivity was detected using HumaTex CRP latex kit (HUMAN GmbH, Wiesbaden, Germany). Distinct agglutination indicates a CRP content of more than 6 mg/l in the non-diluted specimen.

Biochemical measurements:

Glucose, total cholesterol, high density lipoprotein (HDL), low density lipoprotein (LDL) and triglycerides (TG) were measured using COBAS INTEGRA automatic analysers (Roche Diagnostics GmbH, USA).

Statistical analysis:

All statistical analyses were performed using GraphPad InStat 3 (GraphPad Software, Inc.2236 Avenida de la Playa La Jolla, CA 92037 USA). Statistical analysis was done in both groups using chi square and P value. We used a two-tailed test, and a value of *P<0.05* was considered to be statistically significant.

3. Results

The demographic characteristics of all participants:

The demographic and clinical characteristics of all participants as well as the distribution of the most relevant risk factors for atherosclerosis in both atherosclerotic patients (GII) and the apparently healthy controls (GI) are summarized in **Table (1)**. The mean age of the participants was non-significantly different in the tested groups (63.45±8.73 years in GI, and 66.63±15.82 years in GII). Familial history of atherosclerosis, in one or more individuals of the family, was significantly increased in GII (39%) compared to that of GI (16.7%), ($P=0.04$). Obesity was significantly increased in GII (53%) compared to that of GI (26.7%), ($P=0.02$). History of arterial hypertension was significantly increased in GII (75%) compared to that of GI (30%), ($P<0.0001$). History of diabetes was significantly increased in GII (58%) compared to that of GI (33.3%), (P=0.03). History of smoking was significantly increased in GII (60%) compared to that of GI (26.7%), ($P=0.003$).

Regarding to the lipid profile, the present study investigated the presence of dyslipidemia in atherosclerotic patients and the apparently healthy control group. We found significant increases in the serum cholesterol, TG, HDL and LDL levels in GII compared to that of GI. The total serum cholesterol level was 205.6±76.1 mg/dl in GII, while it was 159.6 ± 46.9 mg/dl in GI. Also, serum level of TG was 169.7±66.9 mg/dl in GII, while it was 139.8 ± 38.3 mg/dl in GI. The serum HDL level was 69.82 ± 51.14 mg/dl and 39.62 ± 21.16 mg/dl in GII and GI, respectively. On the other hand, serum level of LDL was 118.54 ± 62.26 mg/dl in GII, while it was 89.51± 22.46 mg/dl in GI, **Table (1)**.

The Relation between both *H. pylori*/CagA and CRP seropositivity and atherosclerosis:

There was a relationship between both *H. pylori*/CagA and CRP seropositivity and atherosclerosis in the apparently healthy control group and atherosclerotic patients group. A trend toward higher prevalence of *H. pylori* infection was observed in GII with respect to GI, but non statistically significant difference was reached. The anti-*H. pylori* IgG antibodies were detected in 68 of 100 in GII and in 17 of 30 in GI (68% *vs.* 56.7%; *P=0.18*), **Table (2)**. The CagA-positive strains resulted to be significantly more prevalent in patients with atherosclerosis than in controls. In particular, anti-CagA antibodies were detected in 29 of 100 patients and in 3 of 30 controls (29% *vs.* 10%; *P=0.04*), **Table (2)**. The inflammatory marker C-reactive protein (CRP) was investigated in the apparently healthy control group (GI) and atherosclerotic patients group (GII). We found that CRP was positive in 20% and 45% in GI and GII, respectively. This increase in the seropositivity of CRP in GII was significantly compared to GI.

Table (1): Demographic and Clinical characteristics of the apparently healthy control group and atherosclerotic patients group.

Parameters	Apparently Healthy Control (n= 30)	Atherosclerotic Patients (n= 100)	*P value*
Age (mean±SD), years	63.45±8.73	66.63±15.82	0.29
Familial history	5(16.7%)	39 (39%)	0.04*
Obesity	8 (26.7%)	53 (53%)	0.02*
Hypertension	9 (30%)	75(75%)	<0.0001***
Diabetes	10 (33.3%)	58 (58%)	0.03*
Smoking	8 (26.7%)	60 (60%)	0.003**
Lipid profile: Cholesterol (mg/dl)	159.6 ± 46.9	205.6 ± 76.1	0.002**
Triglycerides mg/dl	139.8 ± 38.3	169.7 ± 66.9	0.02*
HDL (mg/dl)	39.62 ± 21.16	69.82 ± 51.14	0.002**
LDL (mg/dl)	89.51 ± 22.46	118.54 ± 62.26	0.01*

$P<0.05$ is considered significant

Table (2): Relationship between both *H. pylori* /CagA and CRP seropositivity and atherosclerosis in the apparently healthy control group and atherosclerotic patients group

Parameters	Apparently Healthy Control (n= 30)	Atherosclerotic Patients (n= 100)	P
H. pylori	17 (56.7%)	68 (68%)	0.18
CagA	3 (10%)	29 (29%)	0.04*
CRP	6 (20%)	45 (45%)	0.01*

$P<0.05$ is considered significant

Fig. (I): H. Pylori and CagA seropositivity in the apparently healthy control group (GI) and atherosclerotic patients group (GII)
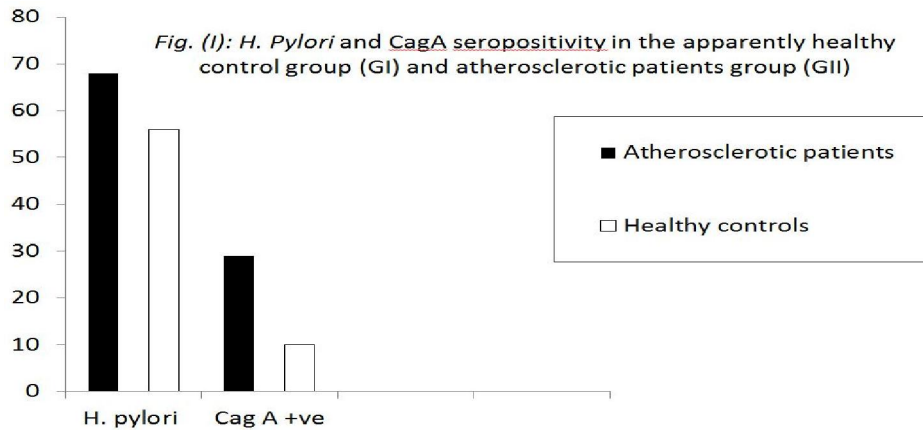
4. Discussion

Atherosclerosis of the major arteries is present universally and appears to start early in childhood *(Fong et al., 2000)*. It is a multifactorial process and no single factor could account for all the causes of cardiovascular disease. Chronic inflammation was recently introduced as a risk factor *(Lobo, 2008 and Lichtman, 2013)*. Infections, like *H. pylori*, have long been suspected to play a role in the pathogeneses of atherosclerotic diseases. There is abundant circumstantial evidence that chronic infections induce immune responses in their host and trigger the development of atherosclerosis *(Blum et al., 2003; Ott et al., 2006)*.

The present study evaluated the role of CagA positive *H. pylori* as a risk factor for atherosclerosis, in atherosclerotic male patients from Najran area, Saudi Arabia. We found a trend toward higher prevalence (68%) of anti-*H. pylori* IgG antibodies in atherosclerotic patients (GII) with respect to the apparently healthy controls (GI) (56.7%), but non statistically significant difference was reached, (*P=0.18*). While, the incidence of the CagA-positivity was significantly prevalent in patients with atherosclerosis (29%) than in controls (10%), (*P=0.04*).

Our results are consistent with many other studies which suggested a strong relationship between CagA positive *H. pylori* and atherosclerosis. Chronic infection by *H. pylori* occurs in approximately half of the world's population causing gastrointestinal and extra-gastrointestinal disorders *(Tunca et al., 2004 and Fagoonee et al., 2010)*. Virulent *H. pylori* strains have cytotoxin-associated gene-A (CagA) toxin *(Zhu et al., 2004)*. It was documented that local gastric inflammatory and immune responses against *H. pylori* can induce systematic immune response *(Riccioni and Sblendorio, 2012)*. Many studies detected *H. pylori*, especially infective CagA-seropositive strains, in atherosclerotic plaques *(Ameriso et al., 2001 and Ghirardi et al., 2006)*. Moreover, *Mayr et al., (2003)*

reported that, there was a clear dose-response relation between anti-CagA antibodies and both intima-media thickness and atherosclerosis risk.

The high incidence of anti-CagA antibodies in atherosclerotic patients in our study supports the hypothesis that the cytotoxic strains of *H. pylori* bearing the CagA have a greater potential for eliciting a systemic immune response and are associated with increased inflammation in the development of atherosclerosis *(Lindsberg and Grau, 2003)*. Regarding to the other risk factors for atherosclerosis. Our study demonstrated that familial history (atherosclerosis in one or more members of the family), obesity, history of hypertension, dyslipidemia, diabetes and smoking were significantly increased in GII compared to GI. Our findings are in agreement with a large body of earlier studies which suggested that many traditional risk factors, including hypertension, smoking, obesity, diabetes mellitus, dyslipidemia and hyperlipidemia, could be implicated in the pathogenesis of atherosclerosis *(Ashtari et al., 2008, Lobo, 2008, Mulder 2012 and Lichtman, 2013)*.

Our study demonstrated that dyslipidemia was a common factor among atherosclerotic patients. We found significant increases in the serum cholesterol, TG, HDL and LDL levels in GII compared to that of GI. Our findings are in agreement with the majority of earlier studies which reported that atherosclerosis is a chronic inflammatory reaction of the vascular wall in response to dyslipidemia and endothelial distress. It involves the inflammatory recruitment of leukocytes and the activation of resident vascular cells *(Riccioni and Sblendorio, 2012)*. Moreover, it was found that the chronic inflammation of arterial vascular wall leads to multifocal plaque development *(Davies et al., 2000 and Hansson., 2005)*. Other pathogenesis, *Sala et al., (2012)* postulated that HDL acts as reservoir for a number of biologically active substances that may impact the immune system during atherogenesis. Of

note, modifications in the lipid and lipoprotein profiles are highly correlated to lymphocyte T effector memory cells and the risk of cardiovascular events *(Keul et al., 2007)*. Therefore, the ability of HDL to promote cholesterol efflux results in the modulation of a series of responses in the immune cells involved in atherosclerosis *(Sala et al., 2012)*.

Our results demonstrated that the seropositivity of the inflammatory marker C-reactive protein (CRP) was significantly increased in the atherosclerotic patients compared to the controls. A large body of literature supports the idea that inflammation (and in particular CRP) plays a pivotal role in all phases of atherosclerosis, from the fatty streak lesion formation to the acute coronary event due to vulnerable plaque rupture. Indeed, vascular inflammation contributes to the pathogenesis of atherosclerosis, and later in the disease process *(Riccioni and Sblendorio, 2012)*.

Simanek et al., (2011) reported that CRP has been linked with cardiovascular disease outcomes and mortality and may play an important role in the occurrence of the mortality. *Mayr et al., (2003)* reported that the risk of atherosclerosis associated with CagA seropositivity was amplified by elevated C-reactive protein levels. Infections with virulent CagA-bearing *H pylori* strains may contribute to the pathogenesis of early atherosclerosis by aggravating immune-inflammatory reactions.

Conclusion:

Our study demonstrated the strong association between CagA-bearing *H. pylori* infections with the pathogenesis of atherosclerosis in southwestern, Saudi Arabia. This finding might open new perspectives for atherosclerosis prevention.

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