No detection of *Helicobacter pylori* in atherosclerotic plaques in end stage renal disease patients undergoing kidney transplantation

H. Ahmadnia, H. Vossoughinia[1], E. Mansourian, K. Gaffarzadegan[2]

Departments of Urology, [1]Gastroenterology and [2]Pathology, Mashhad University of Medical Sciences, Mashhad, Iran

ABSTRACT

Chronic infection known to be a predisposing factor for the development of atherosclerosis. Several studies have found a possible role of *Helicobacter pylori* in the pathogenesis of atherosclerosis. The aim of this study was to investigate the presence of *H. pylori* in atherosclerotic plaques in iliac arteries in 25 end stage renal disease (ESRD) patients undergoing kidney transplantation. Esophagogastroduodenoscopy was performed in all patients before transplantation. Biopsy specimens obtained from gastric antrum were sent for pathologic evaluation. Gastric *H. pylori* infection was confirmed by microscopic assessment and rapid urease test. Arterial specimens were obtained from iliac arteries during kidney transplantation. Presence of *H. pylori* DNA in atherosclerotic plaques and healthy vessel samples was evaluated by the polymerase chain reaction (PCR). The mean age of patients was 44.1 ± 22.6 years. Risk factors in patients with atherosclerosis were hypertension (68%), diabetes mellitus (20%), hyperlipidemia (20%), positive family history (16%). Atherosclerotic plaques were found in 21 (84%) patients. PCR analysis did not detect *H. pylori* in any case. There was a significant relationship of atherosclerosis with hypertension ($P = 0.006$) but not with diabetes mellitus and hyperlipidemia ($P = 0.5$). There was no significant relationship between atherosclerosis and gastric *H. pylori* infection ($P = 0.6$). This study revealed no association between the presence of *H. pylori* as a pathogen of vessel walls and atherosclerosis in ESRD.

Key words: Atherosclerosis, end stage renal disease, *helicobacter pylori*, kidney transplantation

Introduction

Atherosclerosis is a frequent cause of renovascular lesions especially in the elderly.[1,2] More than half of the end stage renal disease (ESRD) patients on dialysis are over 60 years old, the age in which atherosclerosis is more common.[3] The accelerated atherosclerotic process in chronic kidney disease and particularly those with ESRD cause a higher prevalence of coronary artery disease than the general population. High mortality rate in ESRD patients is related to cardiovascular disease, including accelerated atherosclerosis and its complications.[4]

Although diabetes mellitus, hypertension, smoking, and hyperlipidemia are the major known risk factors for the development ofatherosclerosis,[2,5,6] atherosclerosis may occur in the absence of these risk factors.Novel risk factors contributing to initiation and progression of atherosclerosis include inflammation and infections.[7-9] Some studies have found the association of elevated levels of C-reactive protein and future risk of coronary heart disease and stroke, supporting the hypothesis that inflammation plays a role in the pathogenesis of atherosclerosis.[10,11] Although the clinical association between non-traditional risk factors and atherosclerosis is not yet proved, several studies suggest the possible role of different bacterial and viral pathogens as initiators of the inflammatory processes. Numerous infections have been concerned in human atherosclerosis such as *Helicobacter pylori*, Chlamydia pneumoniae, Herpes simplex virus, and Cytomegalovirus (CMV).[12-17] Considering cardiovascular or cerebrovascular diseases, *H. pylori* infections may be associated with stroke and coronary heart disease as a result of the atherosclerotic changes in blood vessels

Address for correspondence:
Dr. Ehsan Mansourian, No. 220, South Bozorg Mehr Street, Malek Abad Blv, Postal Code: 9186953141, Mashhad, Iran.
E-mail: ehsan@live.com

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that is due to a chronic inflammatory response.[18] Low socioeconomic condition may predispose the individuals to *H. pylori* infection and cardiovascular disease.[19]

The role of chronic inflammation as a suggested cause of high morbidity and mortality in these patients by acceleration of atherosclerosis has received considerable attention in recent years. The aim of this study was to search for the presence of *H. pylori* genomic deoxyribonucleic acid (DNA) in atherosclerotic plaques using polymerase chain reaction (PCR) in ESRD patients undergoing kidney transplantation.

Materials and Methods

Study population

This study was approved by the Research Ethics Committee of Mashhad University of Medical Sciences, Iran. The study population consisted of 25 patients, who were admitted to the kidney transplantation Department of the Ghaem hospital, for renal transplantation between November 2010 and March 2011. All subjects provided informed consent.

Baseline evaluation

Baseline evaluation included the collection of demographic information, medical history, risk factors for atherosclerosis, medication use, psychosocial evaluation, physical examination, and routine biochemical and hematological studies such as sampling of blood in the fasting state for sugar, lipid; blood grouping, white blood cell cross-match, viral studies (hepatitis B, hepatitis C, CMVand human immunodeficiency virus), electrocardiogram, and chest radiography.

Specimen collection and *H. pylori* assessment

All patients underwent esophagogastroduodenoscopy using a forward-viewing endoscope (Olympus CLV-160, Tokyo, Japan). The endoscopic appearance of esophagus, stomach, and duodenum were recorded. During endoscopy, biopsy specimens of gastric antrum were sent for pathologic evaluation. For detection of *H. pylori* infection, H and E staining on biopsy specimens and rapid urease test were used.

Arterial specimens containing atherosclerotic plaqueswere obtained from iliac arteries (internal or common iliac artery) during kidney transplantation under aseptic conditions.Tissue samples were fixed in formalin and stored at–80°C for histologic studies. Presence of *H. pylori* DNA in atherosclerotic plaques and in healthy vessel samples was evaluated by the PCR using the Sbsbio tissue Kit (Beijing, China). Presence of atherosclerosis was confirmed by microscopic assay.

Statistical analysis

Statistical analysis was performed using the Fisher's exact test or Mann–Whitney's U test.Logistic regression analysis was carried out to evaluate the independent influence of each variable (sex, age, atherosclerotic factors like smoking, diabetes mellitus, hypertension, hyperlipidemia, obesity, family history and *H. pylori* infection). *P* values less than 0.05 were considered statistically significant.

Results

There were 25 patients, 9 women and 17 men. The mean age was 44.1 ± 22.6 (range 26-67) years. Upper gastrointestinal (GI) endoscopy revealed that gastric erosion and duodenal mucosal erythema were the most frequent types of mucosal lesions (16%), followed by esophagitis (8%), gastric ulcer (4%), and esophageal varices (4%). Normal upper GI endoscopy was found in 10 cases (40%). Of the 25 patients enrolled in the study, 16 (64%) were positive and 9 (36%) patients were negative for gastric *H. pylori* infection [Table 1].

Risk factors present in patients with atherosclerosis were hypertension (68%), diabetes mellitus (20%), hyperlipidemia (20%), and positive family history (16%). None of the patients were smokers or morbidly obese. Atherosclerotic plaques were found in 21 (84%) patients. There was a significant relationship between atherosclerosis and hypertension (*P* value = 0.006) but neither was any relationship between atherosclerosis and diabetes mellitus and hyperlipidemia (*P* = 0.5). There was no significant relationship between atherosclerosis and gastric *H. pylori* infection (*P* = 0.6).PCR analysis did not detect *H. pylori* in any of cases.

Discussion

Atherosclerosis is a multi-factorial disease. The importance of conventional risk factors in pathogenesis

Table 1: Clinical characteristics of individuals according to *helicobacter pylori* status

Item	*H. pylori positive* (*n*=16)	*H. pylori negative* (*n*=9)	*P* value
Age (years)	44.5±23.9	43.5±23.1	ns
Gender male/female	11/5	6/3	ns
FBS (mg/dl)	104.69±72.10	103.11±58.28	ns
SBP (mm Hg)	142.50±31.54	142.67±40.70	ns
TG (mg/dl)	208.18±75.7	201.40±152.46	ns
Chol (mg/dl)	224.00±79.02	227.78±91.01	ns
BMI (m^2/kg)	28.12±6.10	25.78±7.26	ns
Smoking	0	0	ns

FBS: Fasting blood sugar, SBP: Systolic blood pressure, TG; Triglyceride, Chol: Cholesterol, BMI: Body mass index, ns: Not significant

of atherosclerosis is well known. To refine newer risk factors in an individual, many studies have been done. Many studies have found a possible association between different microbial infections and atherogenesis. Although a positive association between *H. pylori* infection and atherosclerosis in carotid and coronary atherosclerotic diseases has been found,[9,15-17,20-22] some studies could not confirm this association.[23-26]

This study was not designated to investigate the causative effect of *H. pylori* on atherosclerosis nor to prove atherosclerosis as a certain etiology of ESRD. Our study showed no significant relationship between atherosclerosis and *H. pylori*infection. In this study, we could not detect *H. pylori* in atherosclerotic plaques of iliac artery (internal or common) in ESRD patients undergoing kidney transplantation whereas *H. pylori* was detected in 64% of patients in their gastric specimens.

We found *H. pylori* infection in 64% our patients. Sixty percent of evaluated patients had endoscopic abnormalities which are similar to other reports, occurring in 25-64% ESRD patients.[27] These findings can support the possibility of occurrence of post-transplantation peptic ulceralthough future prospective studies with larger study population are needed to confirm this hypothesis.

PCR is a sensitive, accurate diagnostic tool for *H. pylori* infection. In comparison to other methods for diagnosing *H. pylori*, PCR yields high sensitivity and specificity for *H. pylori*. PCR methods would increase the probability of *H. pylori* detection.[28,29] Careful attention must be paid to ensure no cross-contamination, while gathering thebiopsies and laboratory phases.[28,30] The false-positive result may possibly be due to the presence of cross-reacting DNAs such as yet-uncultured human micro-organisms. Another possible explanation for false positive is that there were no viable bacteria in the plaques biopsy samples but only dead organisms or chromosomal DNA remained in the cells after *H. pylori* infection.[31] One important disadvantage of conventional PCR in detection of *H. pylori* is inhibition of PCR amplification by a specific inhibitor, causing false negative by preventing positive finding.[28-30]

Studies investigating the presence of *H. pylori* in iliac atherosclerotic plaque are few in the literature and this may be due to the difference in epidemiology of atherosclerosisin coronary, carotid, and iliac arteries andaccording to our knowledge there is no previous study in ESRD patients undergoing kidney transplantation to investigate the relationship between *H. pylori* and atherosclerosis.

The exact way by which inflammation associates with atherosclerosis is not known. The possible mechanisms contributing to the pathogenesis of atherosclerosis are induction of vascular inflammation, endothelial dysfunction and proinflammatory cytokines that may cause instability and rupture of pre-existing atherosclerotic plaques.[15,26,32]

There are conflicting data about relationship of *H. pylori* in atherosclerosis. Although some studies found *H. pylori* in atherosclerotic plaques,[16,17,20,21] some other researchers found no evidence of *H. pylori* in the endarterectomy plaques of patients who were seropositive for *H. pylori*.[33-39] Thus, the issue of an association between *H. pylori* and atherosclerosis remains controversial.[40]

Blasi *et al*., studied 51 patients who underwent abdominal aortic aneurysm surgery for presence of *H. pylori*. In all cases PCR showed no evidence of *H. pylori* presence in atherosclerotic plaque specimensalthough 92.1% of patients were seropositive for *H. pylori*.[33,34] Kaklikkaya *et al*., reported 21 patients undergoing surgery for aortoiliac occlusive disease, searching for *H. pylori* in aorta-iliac atherectomy specimens by PCR. They did not find *H. pylori* in any of specimens.[35] Dore *et al*., found no association between *H. pylori* and atherosclerosis in any of the 32 patients with cardiovascular disease.[36]

H. pylori infection can cause vascular disease directly, as a pathogen of vessel walls or indirectly through the inflammatory process. Activation of inflammatory pathways by infiltration of inflammatory cells that secrete the inflammatory cytokines induces adipocyte dysfunction, the main pathway to atherosclerosis; destabilize the fibrous cap tissue, endothelial desquamation, and plaques rupture which occur in atherosclerosis due to action of various inflammatory markers.[5,7,8,18] In addition, *H. pylori* may have a role in promoting atherosclerosis by modifying lipid metabolism by elevating lowdensitylipoproteinand decreasing high density lipoprotein (HDL).[19,27,41-43] *H. pylori* positive subjects have severe endothelial dysfunction which predisposes them to atherosclerosis.[19,26]

The present study has several limitations. Atherosclerosis is a multi-factorial disease, and different risk factors should be considered. Since this was a descriptive study, we could not elucidate upon the mechanism by which *H. pylori* infection causes atherosclerosis. To discover this relationship, prospective cohort model studies should be performed. The relatively small population studied is a limiting factor and to elucidate the role of *H. pylori* in atherosclerosis, other studies should be carried out with larger number of patients.

Conclusion

This study revealed no association between the presence of *H. pylori*, asa pathogen of vessel walls, and atherosclerosis in ESRD. Although *H. pylori* may have a role in atherosclerosis by secretion of different types of cytokines which can promote a proinflammatory, procoagulant, and proatherogenic environment which does not necessarily need to be the result of the presence of *H. pylori* antigens on the walls of vessels in ESRD. Thus, these results suggest that the *H. pylori* infection is unlikely to be a direct cause of atherosclerosis but do not preclude an indirect role in the process.

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