Evaluation of Anti-*Helicobacter pylori* Antibodies in A group of Iraqi Patients with Atherosclerosis and Coronary Artery Disease

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Abstract

Coronary artery disease (CAD) is the end result of the accumulation of atheromatous plaque within the walls of the coronary arteries resulting in decrease of oxygen and ischemic heart disease (IHD). It was consider as one of the most common diseases and major causes of morbidity and mortality worldwide. The present study evaluated the anti- *Helicobacter pylori* IgG and the role of virulence factor of *H. pylori* cytotoxin associated gene (Cag A) as a risk factors for CAD. Also the detection of proinflammatory markers such as C-reactive protein. The level of serum IgG was done by indirect immunofluorescent (IIF) whereas Cag A and high sensitive C- reactive protein (hs-CRP) measured by enzyme linked immunosorbent assay (ELISA). Seventy Iraqi patients with CAD were included in this study Their mean ages were 58.80 ± 5.13 years ranged between 41-85 years ; and 20 individuals as a control group which was divided into 2 subgroups: 10 apparently healthy volunteers (negative control) and the other subgroup contained 10 with normal coronary artery but had other heart disease except CAD (positive control). All blood samples were investigated biochemically such as glucose test, urea test , creatinine test and lipid profile test(cholesterol, triglyceride ,LDL and HDL) and all of them showed normal values. The result showed, there were a high significant differences (P 0.01) of anti- *H. pylori* IgG between CAD patients and both positive and negative controls. 78.57% (55/70), 100% (10/10) and 0% (0/10) respectively. Also there were a significant differences (P 0.05) in the mean value of Cag A antigen when compared to the positive and negative controls groups (2.74±0.19), (2.72±0.31) and (1.64± 0.16) respectively. Furthermore; the present study revealed significant differences of concentration levels of hs-CRP in patient group (4.95 ± 0.38 µg/ml) as compared to positive and negative control groups (0.77 ± 0.06 µg/ml, 3.96 ± 0.96 µg/ml). The results suggest there was a relationship between *Helicobacter pylori* infection and coronary artery disease.

Keywords: *Helicobacter pylori*, Antibodies, Atherosclerosis, Coronary artery disease

تقييم اضداد البكتريا الملتوية البوابية في مرضى تصلب الشرايين وامراض القلب التاجية العراقيين

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الخلاصة:

مرض تصلب الشرايين التاجية هو النتيجة النهائية لتراكم اللويحات العصيدية ضمن جدران الشرايين التاجية مسببة نقص بالاوكسجين وبالتالي حدوث مرض القلب الاقفاري. يعد من الامراض الشائعة وأحد الاسباب الرئيسية لحالات الوفاة في العالم. في هذه الدراسة تم تقييم اضداد IgG لبكتريا الملتوية البوابية وكذلك يعد عامل الضراوة السام لخلايا بكتريا الملتوية البوابية (Cag A) ودوره في تطور المرض وكونها عامل خطورة لمرض تصلب الشرايين التاجية. فضلا" عن التكهن بدور بعض العوامل الالتهابية في الامراضية مثل بروتين الطور الحاد (البروتين التفاعلي-C). تم استخدام تقنية التآلق المناعي غير المباشر Indirect immunofluorescent لتقييم مستوى اضداد IgG لبكتريا الملتوية البوابية في حين استخدمت تقنية الامتزاز المناعي المرتبط بالانظيم Enzyme linked immunosorbent assay (ELISA) لقياس مستوى كلا" من Cag A وhs-CRP في مصول المرضى ومجاميع السيطرة. شملت الدراسة 70 مريضا مصابا بمرض تصلب الشرايين التاجية. فضلا عن 20 شخص اعتبروا كمجموعة السيطرة اذ قسموا الى مجموعتين: المجموعة الاولى تشمل الاشخاص الاصحاء المتطوعين والمجموعة الثانية هم الاشخاص المصابين بآمراض قلب اخرى عدا مرض تصلب الشرايين التاجية. خضعت جميع نماذج الدم الى الفحوصات الكيميوحيوية كفحص السكر واليوريا والكرياتنين والدهون الثلاثية والكوليسترول وبروتين الدهني منخفض الكثافة والبروتين الدهنيلالاتفع الكثافة. تبين من خلال هذه الدراسة ان نسبة التعبير المصلي الايجابي لل IgG قد ارتفعت بشكل عالي المعنوية (P 0.01) في مرضى تصلب الشرايين التاجية بنسبة 78.57% مقارنة مع مجاميع السيطرة (الموجبة والسالبة) 100% و 0% على التوالي. من جانب اخر فقد اظهرت نتائج الدراسة الحالية وجود فروق معنوية (P 0.05)في قيمة المتوسط الحسابي لعامل الضراوة Cag A بين مجموعة مرضى تصلب الشرايين التاجية (2.74±0.19) ومجموعتي السيطرة الموجبة والسالبة (2.72±0.31) و (1.64±0.16)على التوالي. علاوة على ذلك، فقد بينت نتائج هذه الدراسة ان هناك فرقا" معنويا" (P<0.05) في معدل تركيز البروتين التفاعلي-C (CRP) بين مجموعة مرضى تصلب الشرايين التاجية (4.95±0.38 µg/ml) ومجموعتي السيطرة الموجبة والسالبة(3.96±0.96 µg/ml) و(0.77±0.06 µg/ml) على التوالي. وعلى ضوء نتائج هذه الدراسة تبين هناك علاقة بين الاصابة ببكتريا الملتوية البوابية ومرض الشرايين التاجية.

Introduction:

Coronary artery disease (CAD) is the end result of the accumulation of atheromatous plaque within the walls of the coronary arteries resulting in decrease of oxygen and ischemic heart disease (IHD). When the artery becomes partially blocked, chest pain (angina) occurs, if the artery becomes completely blocked, cells in the heart began to die and heart attack may occur [1]. Coronary artery disease is the leading cause of morbidity and mortality in the world. Every year, more than 19 million people worldwide die of an acute coronary event [2]. Inflammation play important role in the pathophysiology of acute coronary syndrome (ACS) for example level of the inflammatory marker C - reactive protein associated with an increases risk of cardiovascular events. Moreover inflammatory markers such as Interleukin-6(IL-6), Interleukin-8(IL-8) or Monocyte Chemoattractant Protein-1(MCP-1) are elevated in acute myocardial infarction (AMI) and are predictive for recurrent plaque instability [3]. Infectious agents may cause a spectrum of systemic effects and induce coronary atherosclerosis in several different ways. For instance, by increasing the production of circulating cytokines, through the generation of acute phase reactants (white blood cells and C- reactive protein) and the stimulation of immune-mediated responses, such as the production of antibodies targeted to the invading pathogens, *etc* [4]. Several authors have also reported that infections might stimulate smooth muscle cell proliferation, migration, and lipid accumulation; apoptosis of endothelial cells can be inhibited and many procoagulant effects could be produced [5, 6]. *H. pylori* infection is one of the most widely spread infectious diseases in human. This microorganism infects half the world population and causes chronic gastritis [7]. One of *H. pylori* strains which included highly virulent strains, which creates *cytotoxin-associated gene-A* (*CagA*) toxin and VacA toxin, which is strongly related to *CagA*, one of the *Cag* genes [8].Recent epidemiological surveys have indicated that *H.*

pylori infection may be associated with atherosclerotic vascular diseases [9]. They revealed the presence of *H. pylori*, especially infective *CagA*-seropositive strains, in atherosclerotic plaques and showed *H. pylori*'s association with atherosclerotic diseases [10]. There is now clear evidence 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 atherothrombosis [11]. The aim of the present study is to evaluate the seroprevalence of anti-*Helicobacter pylori* antibodies IgG, explore the seroprevalence of Helicobacter pylori Cag A and estimate the level of a sensitive marker of systemic inflammation high sensitive C-reactive protein.

Materials and methods:

Seventy Iraqi patients with coronary artery disease (CAD) aged 41-85 years, Mean 58.80 ± 5.13 years, were included in this study. They were admitted to Ibn Al-Bitar specialist center for cardiac surgery in Baghdad between October 2013 and January 2014**.** All patient and control groups from Baghdad. They were selected from non-smoker, non-diabetic, non-hypertention patients and none of them had a history of any underlying autoimmune or chronic infectious disease. This prospective study was carried out after obtaining a permission from requisite ethics committee and informed consents from patients. Control group was divided into 2 subgroups: 10 apparently healthy volunteers(with normal coronary angiography and without other heart diseases) which called negative control, the other subgroup contained 10 positive control with normal coronary artery but had other heart disease. Negative and positive control included in this study as a control group, with ages mean 54.85 ± 5.12 years ranged between 37-70 years. Tubes containing CAD and control sera were labeled and carried out to measure anti-*Helicobacter pylori* IgG and high sensitive C-reactive protein (hs-CRP) by using indirect immunofluorescent (IIF) where as measured and *H. pylori* cytotoxin associated gene (Cag A) by using enzyme linked immunosorbent assay (ELISA), according to manufacturer´s instruction (Euroimmune, Germany for anti-*H. pylori* IgG, Cusabio, China for Cag A and Demeditec, Germany for hs-CRP). All immunological studies achieved in Baghdad Teaching Hospital Laboratories- Ministry of Health- Baghdad.

Statistical analysis:

The Statistical Analysis System used included T-test (or Least significant difference–LSD) and Chi-square test. P value 0.05 was considered statistically significant, and P value 0.01 was considered statistically highly significant.

Results and discussion:

A total of 70 cases with coronary artery disease and 20 (negative and positive control) subjects were enrolled in this study. Their ages mean were 58.80 ± 5.13 years ranged between 41-85 years and ages mean 54.85 ± 5.12 years ranged between 37-70 years for patients and healthy control respectively. The male: female ratio was 9:1(M:F= 63:7). The subjects were selected from non-smoker, non-diabetic, non-hypertention patients (without any risk factors). Many of biochemical tests were done on patient´s blood such as glucose test, urea test, creatinine test and lipid profile test(cholestrol, triglyceride, LDL and HDL), all results showed normal value. Normal values for glucose test, urea test and creatinine test were (3.6-6.1 mmol/L, 3.3-7.5mmol/L, 62-124 mmol/L) respectively. While normal value for lipid profile (cholesterol, triglyceride, HDL and LDL) was (5.2mmol/L, 2.**6** mmol/L, 1.17 mmol/L and 3.4 mmol/L respectively [12].

Figure 1 and 2 showed the seroprevalence of anti-*H. pylori* IgG. It showed seroprevalence of anti -*H. pylori* IgG. The overall positive of anti- *H. pylori* IgG in CAD patients was 78.57% (55/70) while 100% (10/10) and among positive control group and there were no seropositive result in negative control group 0%(0/10). These results showed, a highly significant differences between CAD patients group and both control groups, when analyzed by Chi-square test(P 0.01).

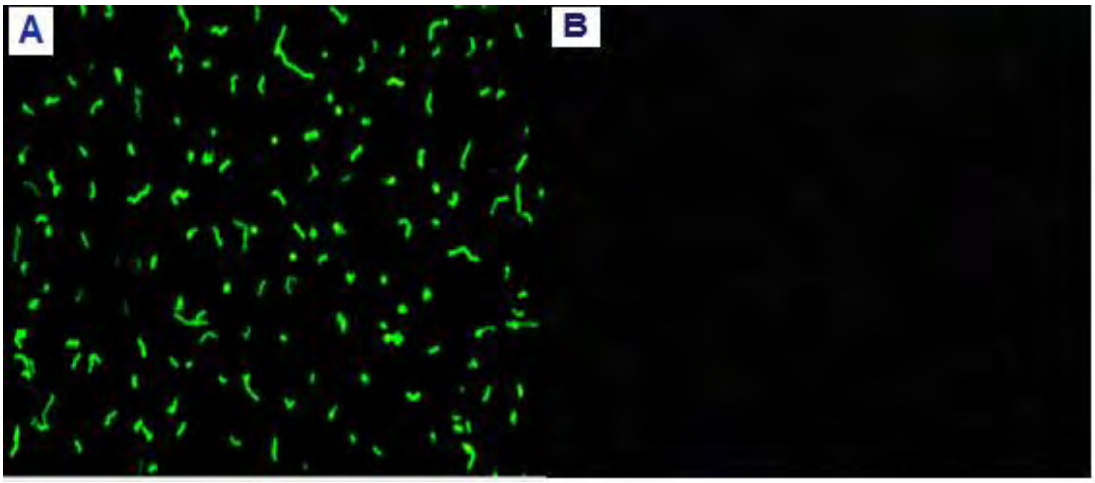

Figure 1-IIF staining of *H. pylori* IgG (40X) showing: A: Seropositive of anti- *H. pylori* IgG in sera of CAD patients. B: Seronegative of anti- *H. pylori* IgG in sera of control groups.

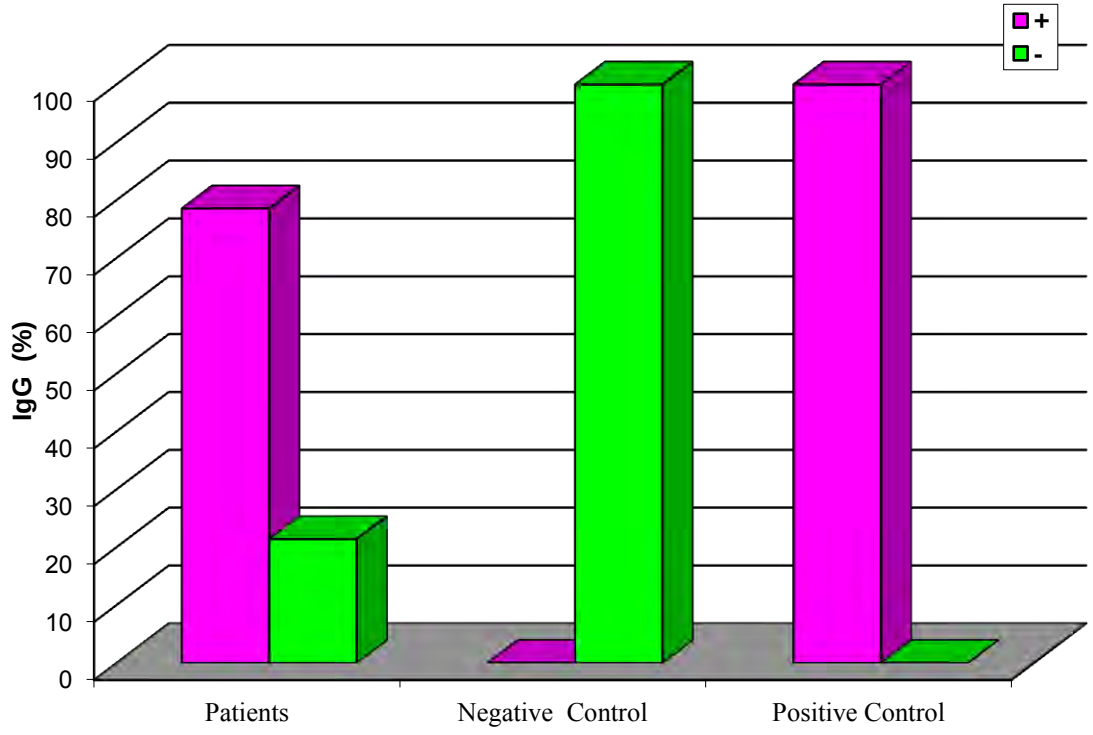

Figure 2-Seroprevalence of anti- *H. pylori* IgG among patients and control groups

The figure 3 showed the mean value of Cag A antigens between patients and control groups (negative and positive control). The result showed, a significant differences in the mean value of Cag A antigen of CAD patients when compared to the positive and negative controls groups (2.74±0.19), (2.72±0.31) and (1.64± 0.16) respectively (P 0.05).

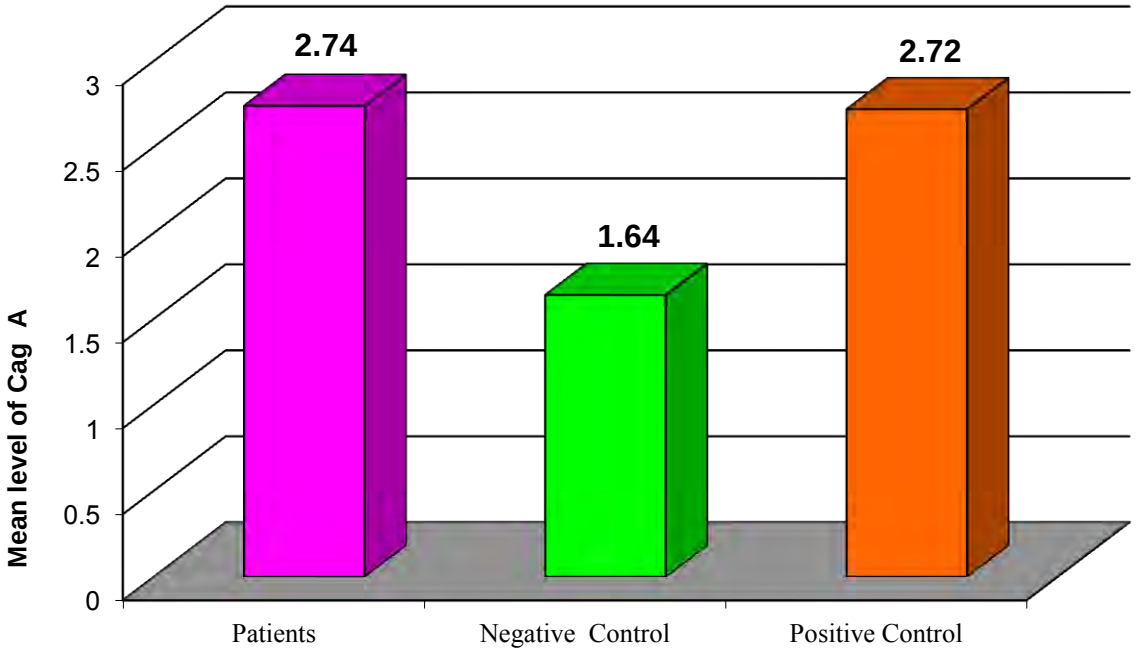

Figure 3-Mean values of Cag A among studied groups

In figure 4; the serum sample of patients and controls were tested for evaluation the levels of C-reactive protein. A significant difference was observed when the concentration levels of CRP of both patients and controls groups were analyzed based on LSD value test of significance. The results showed the mean value of patients was (4.95 ± 0.38 µg/ml and 0.77 ± 0.06 µg/ml, 3.96 ± 0.96 µg/ml) of negative and positive control respectively.

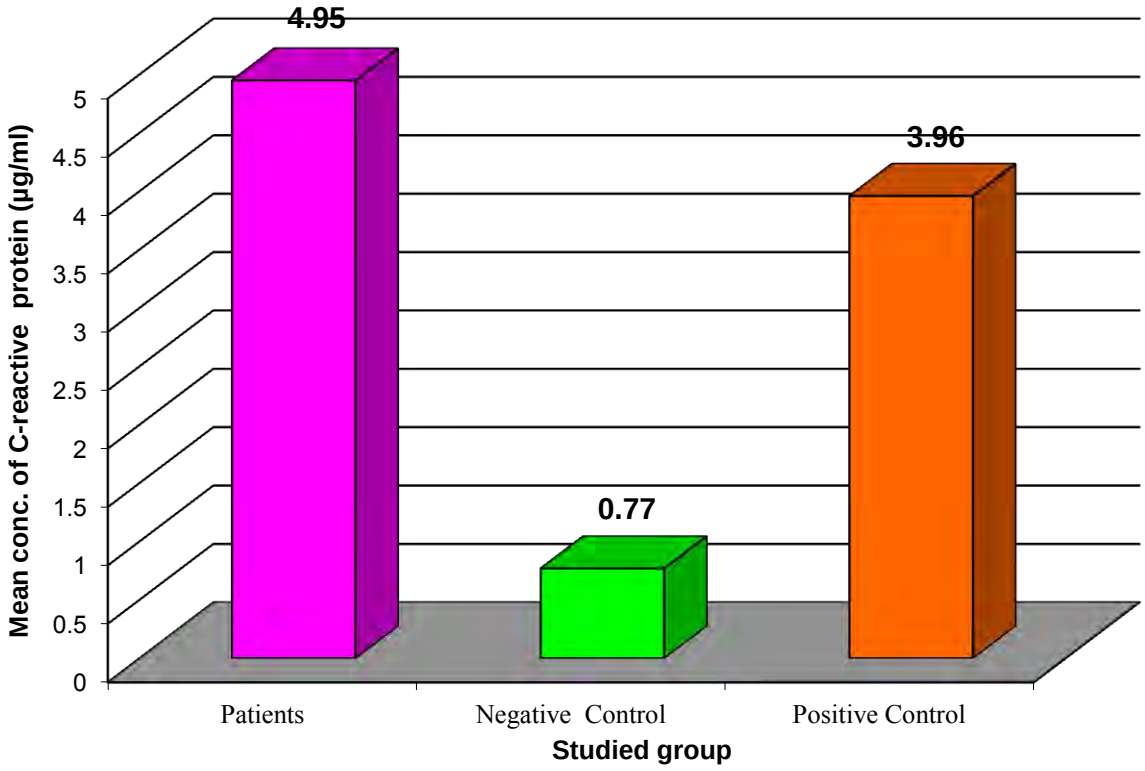

Figure 4-Mean concentration of hs- CRP(µg/ml) among studied groups

This result agrees with local study, which revealed that 80% of CAD patients showed seropositive anti-*H. Pylori* IgGs [13]. Also, the results of current study agree with several recent studies. Vcev ***et. al.,*** showed that the prevalence of seropositivity for *H. pylori* was higher in patients compared to controls [14]. Also, Vafaeimanesh *et al*., showed seropositivity for anti-*H. pylori* immunoglobulin G IgG [9, 15].This study and other studies suggest this hypothesis that *H. pylori* can be associated with CAD or even consider it as a risk factor that plays a role in atherosclerosis plaque formation [15]. *Helicobacter pylori* infection has been suggested to influence the development of atherosclerotic changes in coronary arteries indicating a damaging effect of this bacterium or its products (e.g. cytokines, endotoxins, cytotoxins, and other virulence factors) on the coronary endothelium [9]. Some of recent studies revealed that no relation between lipid profile and CAD [9, 16]. Similarly; the current study showed the same results, as all studied group, revealed normal lipid profile. The present study evaluated the role of Cag A positive *H. pylori* as a risk factor for atherosclerosis, in atherosclerotic patients. The incidence of the CagA-positivity was significantly prevalent in patients with atherosclerosis than in controls. These results agree with several recent studies, Al-Qurashi and his colleagues showed that, there was significantly prevalent of Cag A in atherosclerotic patient. The results are consistent with many other studies, which suggested a strong relationship between CagA positive *H. pylori* and atherosclerosis [10]. Chronic infection by *H. pylori* occurs in approximately half of the world's population causing gastrointestinal and extra-gastrointestinal disorders [17]. Virulent *H. pylori* strains have cytotoxin-associated gene-A (Cag A) toxin [8]. It was documented that local gastric inflammatory and immune responses against *H. pylori* can induce systematic immune response [18].

Many studies detected *H. pylori*, especially infective Cag A-seropositive strains, in atherosclerotic plaques [19]. Other study, Zhang et *al*., found statistical significance between the Cag A-seropositive strains and coronary heart disease in all subgroups and total population [11]. Establishing a causal link between this infection and CAD would be of a major public health importance, since the eradication of the bacterium is easy and much less expensive than long-term treatment of the other risk factors [20].

C-reactive protein is a sensitive and important biomarker of inflammatory reactions, which is produced by liver and may be a causal agent promoting atherosclerotic initiation and progression [21]. Increasing level of CRP may be the earliest event in vascular inflammatory process, inducing endothelial dysfunction, the first step in atherosclerosis [22]. The results agreed with Al-Qurashi and Hodhod, which demonstrated that the seropositivity of the inflammatory marker CRP was significantly increased in the atherosclerotic patients compared to the controls [10]. C-reactive protein serves as a pattern-recognition molecule in innate immunity. It may directly contribute to a proinflammatory state in atheroma by inducing adhesion molecule expression on endothelial cells, stimulating cytokine release of monocytes, and activating the complement cascade [23]. Ishikawa and his colleagues suggested CRP localization in atherosclerotic plaque. They also suggested that CRP plays an important role on plaque vulnerability and in the pathogenesis of unstable angina, as well as restenosis after coronary intervention [24]. The following conclusions can be extracted from the results obtained outs of this study: There was a significant association between *H. pylori* IgG with CAD among Iraqi patients. Infection with *H. pylori* can be one of the risk factor for the development of CAD in Iraqi patients. A significant increase was observed in Cag A positive H. *pylori* seroprevalence among Iraqi patients with CAD. Hs-CRP concentration has been elevated in sera of CAD patients versus healthy control. So, inflammatory changes in the vessel wall may play an important role in pathogenesis of CAD.

The recommendations can be made from this study: sample size should be taken large enough to achieve a suitable and convincing result. The detection of *H. pylori* specific DNA in atheromatous plaque material from coronary arteries and its association to the positivity of *H. pylori* and clinical symptoms could be interpreted as an evidence for the involvement of a *H. pylori* infection in the progression of CAD. Further studies are needed to assess the relationship between early life exposure to *H. pylori* and subsequent risk of coronary heart disease. Because *H. pylori* infection may be easily eradicated by specific treatments, the accurate definition of this new risk factor may lead to new strategies for the prevention of coronary heart disease.

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