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Original Research

Serum levels of high-sensitivity C-reactive protein (hs-CRP) in *Helicobacter pylori* infected patients

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Abstract

Objective: C-reactive protein (CRP) is a protein found in the blood, the levels of which rise in response to inflammation. Its physiological role is to bind to phosphocholine expressed on the surface of dead or dying cells. In this study we aimed to examine the association between *Helicobacter pylori* (*H.pylori*) infection and serum levels of high sensitivity C-reactive protein (hs-CRP).

Background: Recently, many studies have suggested that there is an association between *H.pylori* infection and serum high levels of hs-CRP.

Subjects: 200 subjects with *H.pylori* infection (128 male, 72 female) aged 40-68 years (mean 49 years) and 50 healthy control subjects (36 male, 14 female) aged 35-70 years (mean 52.5 years) were included in this study. Blood samples were collected to determine serum hs-CRP.

Results: The mean serum level of hs-CRP in patients with *H.pylori* infection subjects (0.048±0.005 mg/dl) was significantly higher than in healthy control subjects (0.037±0.003 mg/dl). Serum level of hs-CRP was high in 24% of patients group, while it was high in 12% of healthy group. No significant differences were shown among *H.pylori* infected subjects and healthy subjects regarding to age, sex, smoking, body mass index, and socio-economic status.

Conclusion: Serum hs-CPR levels were higher in *H.pylori* infected patients, based on these results serum hs-CRP could be affected by *H.pylori* infection.

INTRODUCTION

Helicobacter pylori(H.pylori) is a spiral-shape, gram negative bacterium that one of the most common chronic bacterial infections in humans and is recognized as a major etiologic agent for several gastro duodenal diseases including gastric ulcer, duodenal ulcer, gastric MALT, lymphoma, and distal gastric cancer [1]. In 1994 *H.pylori* was categorized in group I of carcinogens by World Health Organization [2]. *H.pylori* can elicit lifelong inflammatory and immune responses with release of various bacterial and the host-dependent cytotoxic substances [3]. Recently, several number of studies have been reported that *H.pylori* infection consider as a factor that increasing the coronary heart disease [4-6].

C-reactive protein (CRP) is an acute-phase protein originates from the liver. CRP has been identified as a marker of inflammation that can be used for diagnosis and follow-up of some inflammatory diseases [7]. CRP has been considered as independent risk factor for cardiovascular diseases and can also be consider as a valuable tool for estimation of at risk persons [8].The measurements of serum levels of CRP using high-sensitivity assay (hs-CRP) can detect subclinical inflammatory status which may reflect vascular inflammation [7, 8].

Examination of the association between *H.pylori* infection and serum hs-CRP levels seemed to be important to explain the relationship of *H.pylori* infection and coronary diseases [9]. In the present study

we aimed to evaluate serum hs-CRP levels in *H.pylori* infected patients and to compare it with serum hs-CRP levels in healthy uninfected subjects to examine the association between *H.pylori* infection and serum hs-CRP levels in Jordanian groups.

MATERIAL AND METHOD

Subject

The subjects of this study were selected from patients attending the outpatient specialty clinics in King Hussein Medical Center during the period from 15 May 2012 to 30 October 2012. 250 subjects were selected, 200 of them *H.pylori* infected (128 male, 72 female) aged 40-68 years (mean 49 years) while 50 are healthy (36 male, 14 female) aged 35-70 years (mean 52.5 years). After interview for 3 minutes all subjects completed a written informed agreement for participation in this study. Blood samples were collected from these subjects for *H.pylori* IgG antibodies and C-reactive protein. All tests were done in Princess Iman Center for Research and Laboratory Sciences in King Hussein Medical Center in Amman-Jordan.

Exclusion criteria

In our study the following cases were excluded: (i) previous myocardial infarction, (ii) previous percutaneous transluminal coronary angioplasty, (iii) history of *H.pylori* eradication or antacids drug take, (iv) liver disease, (v) chronic alcoholism, (vi) inflammatory bowel disease, (vii) previous gastrointestinal surgery, (viii) those receiving anti-ulcer treatment in the last four months, (ix) those receiving proton-pump inhibitors.

Determination of *H.pylori* IgG

Serum levels of anti-*H.pylori* IgG were measured by the use of commercial ELISA kits (DIA, PRO. Diagnostic Bioprobes, Via.G.Carduccu n 27, 20099 San Giovanni (Milano-Italy) based on a monoclonal antibody against 64-kD *H.pylori* antigen.

Briefly, 100 µl of undiluted serum sample and calibrators were added to micro wells coated with an *H.pylori* solicited preparation. Then 100 µl of peroxidase conjugated HpN45 were added to all wells. After 1 hour of incubation, the plate was washed, color was developed with a 3,3',5,5' tetramethylbenzidine (TMB) solution and absorbance read at 450 nm. Antibody concentration was expressed as units/ml. Negative values are considered < 450 U/ml. Sensitivity was 100% and specificity was 90%.

Determination of serum hs-CRP levels

Serum hs-CRP levels we measured in duplicate by the use of commercial ELISA kits (Ara. Gen Biotech. Amman–Jordan. JPM Europe Gmbh Rothenbaumehaussee 95, 20148, Hamburg, Germany). Serum levels of hs-CRP were quantitied by the use of standard samples containing known concentration of CRP. Serum hs-CRP levels are expressed as mg/dl. Subjects with hs-CRP more than 0.1 mg/dl were considered high serum hs-CRP individual.

Statistical Analysis

All statistical analysis were performed using SPSS for windows 15.0 (SPSS Inc. Headquarters, Chicago, III., USA) software program and Microsoft Excel 2007 program. The difference in mean of serum hs-CRP between *H.pylori* positive subjects and negative subjects was statistically tested with a t-test. The proportion of hs-CRP subjects was compared between the two groups with Fisher exact test. $P < 0.05$ was considered to be statistically significant.

RESULTS

250 subjects were participated in this study, 200 subjects with *H.pylori* infection (128 male, 72 female) aged 40-68 years (mean 49 years) and 50 healthy control subjects (36 male, 14 female) aged 35-70 years (mean 52.5years). Blood samples were collected to determine serum levels of hs-CRP. *H.pylori* infection status was determined by IgG antibody test.

hs-CRP values

Mean serum levels of hs-CRP in *H.pylori* positive subjects and healthy group are shown in Table 1. The mean serum level of hs-CRP in *H.pylori* seropositive group (0.048±0.005 mg/dl) was significantly higher than that in healthy group (0.037±0.003 mg/dl).

Subject characteristics

The characteristics of our study subjects are shown in Table 2. There were no significant differences shown among *H.pylori* positive and healthy subjects regarding to age, sex, smoking, body mass index (BMI), and socio-economic status. Males were dominant (66%) and 45% were older than 50 years.

High serum hs-CRP levels were more predominant in females in *H.pylori* positive subjects and also in healthy group. There was no significant difference in the serum hs-CRP level across age group. The serum hs-CRP geometric mean was significantly higher in *H.pylori* positive subjects than in healthy subjects 0.048and 0.037 respectively ($p<0.001$ by t-test).

High serum hs-CRP level was found in 24% of *H.pylori* infected subjects and in 12% of healthy group. (Those with serum hs-CRP level more than 0.1 mg/dl defined as high hs-CRP individuals).

Table 1. Serum hs-CRP levels in *H.pylori* infected and control subjects.

	Gender	n= 250 %	Mean of(hs-CRP)	P values
***H.p*-positive**	Male 128(64%)			
	Female 72(36%)	200(80%)	0.048±0.005 mg/dl	p<0.001
***H.p*-negative**	Male 36(72%)	50(20%)	0.037±0.003 mg/dl	p<0.001
	Female14 (28%)			

Table 2. Characteristics of study groups.

	Group A ***Hp*-positive** **n=200**	**Group B** ***Hp*-negative** **n=50**	**P-value**
Sex			
Female Male	128 72	36 14	0.233
Age	50±9.6	49±8.7	0.276
BMI			
Normal Overweight obese	136 39 25	33 17 2	0.345
Smoking			
Never Former current	67 55 78	23 13 14	0.324
Socio economic			
Lower class Middle class Upper class	35 139 26	5 39 6	0.113
Hs-CRP			
<0.1 >0.1	153(76%) 47(24%)	44(88%) 6(12%)	0.04

DISCUSSION

In the present study we examined the association between *H.pylori* infection and serum hs-CRP levels. Two groups were selected from Jordanian population for this aim, 200 subject with *H.pylori* infection and 50 healthy as control group.

H.pylori infection is associated with coronary artery disease, the pathway to elevate the disease is not clear. There are various possibilities for the mechanism underlying a causal role of *H.pylori* infection in endothelial dysfunction [9-11]. The first possibility *H.pylori* has the direct effect on the structure and function of vascular endothelial cells [9,10]. The second possibility is the nutritional effect of *H.pylori* [9,11]. *H.pylori* infection may cause malabsorption of vitamin B12, vitamin B6 and folic acid. This defect could lead to failure of methylation of 5-methyletetrafolic acid and posterior hyperhomocysteinemia, which is toxic to endothelial cells [9]. In previous study we found that the serum levels of vitamin B12 and folate are lower in *H.pylori* infected subjects in comparison with healthy subjects, resulting that there a strong association between

H.pylori infection and serum vitamin B12 deficiency.

CRP is a sensitive and important biomarker of inflammatory reactions which is produced by liver and may also be a causal agent promoting atherosclerotic initiation and progression [7,12,13]. Increasing level of CRP may be the earliest event in vascular inflammatory process, inducing endothelial dysfunction, the first step in atherosclerosis [14]. There are several mechanisms by which CRP can promote a proatherogenic environment in endothelial cells including the following [13], (i) decreasing prostacyclin and nitric acid oxide synthesis, (ii) increasing endoththelin-1 concentration and cell adhesion molecules such as monocyte chemoattractant protein-1. Moreover, In vascular smooth muscle cells CRP has been show to increases nuclear factor kappa B (NF-κB) and upregulate angiotensin type-1 receptor leading to increased reactive oxygen species and smooth muscle cell proliferation. Taken the previous facts together with observational studies, CRP levels > 3 mg/dl predict future risk for cardiovascular diseases in evidently healthy subjects [15].

In the present study we found that the mean serum level of hs-CRP was significantly higher in *H.pylori* infected subjects (0.048±0.005) in comparison with the mean serum level of hs-CRP in healthy subjects (0.037±0.003 mg/dl) ($p<0.001$ by t-test). These results are similar to that recently reported by Ishida et al [9] and Jafarzadeh et al [16].

Previous study in Turkey reported that serum CRP level was significantly reduced among 57 patients after successful *H.pylori* eradication [17]. Another study report that aging, smoking, *H.pylori*, Chlamydia pneumonia and BMI were all raised the concentration of serum hs-CRP levels [18]. Current study elucidate that *H.pylori* infection elevates serum hs-CRP levels which might increase systemic risks.

The production of proinflammatory cytokines such as tumor necrosis factor (TNF), interleukin (IL)1-6 and IL-8 stimulates by *H.pylori* infection [19]. Elevated serum levels of TNF, IL-1, IL-6 and IL-8 was found in *H.pylori*-infected individuals [20]. Production of CRP is regulated by cytokines, principally interleukins, as well as hormones such as cortisol and insulin [21]. Therefore the association between CRP concentration and *H.pylori* infection could be explain by the action of cytokines, hormones or both of them.

This study has different limitations. First, *H.pylori* infection was determined by serum antibody, not by other methods which more specific and sensitive such as urea breath test or histological examination. Second, serum hs-CRP levels may affected by the other factors like chronic infections.

In conclusion, the results in the present study indicated that serum hs-CRP levels in *H.pylori* infected subjects were significantly higher than that in healthy controls, supporting that infection with *H.pylori* may increase serum hs-CRP levels.

REFERENCES

1. Siavoshi F, Malekzadah R, Daneshmand M, Ashktorab H. *Helicobacter pylori* endemic and gastric disease. Dig Dis Sci. 2005;50:2075-80.
2. International Agency for Research on Cancer Working Group on the Evaluation of Carcinogenic Risks to Human. Schistosomes, Liver flukes and *Helicobacter pylori*: infection with *Helicobacter pylori*. IARC Monogr Eval Carcinog Risks Hum.1994; 61:177-240.
3. Rouses A, Philippou N, Gourgoliannis KI. *Helicobacter pylori* infection and respiratory disease: a review. World J Gastooenterol. 2003;9:5-8.
4. Laurila A, Bioligu A, Nayha S. Association of *Helicobacter pylori* infection elevated serum lipids. Atherosclerosis. 1999;142:207-10.
5. Kinjio K, Sato H. Sato H. Prevalence of *Helicobacter pylori* infection and its link to coronary risk factors in Japanese patients with acute myocardial infarction. Circ J. 2002;66:805-10.
6. Niemela S, Karrunen T, Korhonen T. Could *Helicobacter pylori* infection increase the risk of coronary heart disease by modifying serum lipid concentration? Heart. 1996;75:573-5.
7. Singh SK, Suresh MV. Voleti B, Agrawal A. The connection between C-reactive protein and the atherosclerosis. Am Med 2008;40:110-20.
8. Wilson AM, Ryan MC, Boyle AJ. The novel role of C-reactive protein in cardiovascular diseases; risk marker of pathogen. Int J Cardiol .2006;106:291-7.
9. Ishida Y, Suzuki K,Taki K, Niwa T, Kurotsuchi S, Ando H, Iwase A, Nishio K, Wakal K, Ito Y, Hamajima N. Significant association between *Helicobacter pylori* infection and serum C-reactive protein. Int J. Med Sci 2008;5:224-9.
10. Kalia N, Jones C, Bardhan KD. Effects of genotypically different strains of Helicobacter pylori on human microvascular endothelial cells in vitro. Dig Dis Sci. 2001;46:54-61.
11. Sung JJ, Sanderson JE. Hyperhomocysteinaemia, *Helicobacter pylori*, and coronary heart disease. Heart. 1996;76:305-7.
12. De Maat MP, Trion A. C-reactive protein as a risk factor versus risk marker. Curr Opin Lipidol. 2004;15:651-7.
13. Jialal I, Devaraj S, Venugopal SK. C-reactive protein: risk marker or mediator in atherothrombosis? Hypertension. 2004;44:6-11.

14. Oshima T, Ozono R, Yano Y, Oishi Y, Teragawa H, Hagashi Y, Yoshizuma M, Kambe M. Association of *Helicobacter pylori* infection with systemic inflammation and endothelial dysfunction in healthy male subject. J Am Coll Cardiol. 2005;45:1219-22.

15. Ridker PM. Clinical application of C-reactive protein for cardiovascular disease detection and prevention. Circulation. 2003;107:363-9.

16. Jafaezadeh A, Hassanshahi GH, Nemati M. Serum levels of high-sensitivity C-reactive protein (hs-CRP) in *Helicobacter pylori*-infected peptic ulcer patients and its association with bacterial CagA virulence factor. Dig Dis Sci. 2009;54:2612-6.

17. Suat S, Bekir K, Mustafa A. Do procalcitonin and C-reactive protein levels have a place in the diagnosis and follow up of *Helicobacter pylori* infection? J Med Microbiol. 2004;53:639-44.

18. Mendall MA, Praful P, Lydia B. C-Reactive Protein and its relation to cardiovascular risk factors: a population based cross sectional study. BMJ. 1996;312:1061-5.

19. Mehmet N, Refik M, Harputluoglu M, Ersoy Y, Aydin NE, Yildirim B. Serum and gastric fluid levels of cytokines and nitrates in gastric disease infected with *Helicobacter pylori*. New Microbiol. 2004;27:139-48.

20. Nari A, Moore MA, Kono S. Impact of C-reactive protein on disease risk and its relation to dietary factors: literature review. Asian Pac J Cancer Prev. 2007;8:167-77.

21. Vermeire S, Van Assche G, Rutgeetrs P. C-reactive protein as a marker for inflammatory bowel disease. Inflamm Bowel Dis. 2004;10:661-5.