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Original Research

Helicobacter pylori infection and iron deficiency in patients with coronary artery disease

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Abstract: The aim of this study was to investigate whether impact of the seropositivity to *Helicobacter pylori* (*H pylori*) infection on ferritin and iron levels is an independent risk factor for atherosclerosis in patients with cardiovascular disease. The anti *H pylori* IgG, IgA levels, serum ferritin and iron concentration of 86 patients with cardiovascular disease and 64 participants free of cardiovascular disease as control subjects were determined by ELISA assay. The results of present study showed that seropositivity to *H pylori* IgG and IgA levels of coronary artery disease (CAD) patients was higher than controls and CAD patients with negative anti *H pylori* IgG and IgA significantly. A significant negative correlation was found between seropositivity to *H pylori* IgG and IgA, ferritin and iron levels of CAD patients with seronegativity and seronegativity to *H pylori* IgG and IgA in comparison with controls. The achieved results from present study suggest that the involvement of *H pylori* infection in atherosclerosis process is based on the chronic inflammation which might facilitate the CAD-related pathologies. Moreover, impact of the presence of *H pylori* infection on reduction of the ferritin and iron levels of CAD patients as a risk factor independent of other classic factors including lipid profiles and inflammatory factors was remarkable.

Key words: Ferritin, Iron, helicobacter pylori, cardiovascular disease.

Introduction

Deaths attributed to Cardiac heart disease have increased in the last two decades**.** The first report about a decrease in blood hemoglobin content with increasing severity of congestive heart failure (CHF) was published (1). Since then, several studies have documented both a greater mortality associated with anemia and a high prevalence of anemia in patients with advanced CHF. Several evidence show that cardiac function, functional capacity, and quality of life of patients with advanced CHF were improved significantly by anemia elimination (2-5).

The high prevalence of iron deficiency anemia in coronary artery disease (CAD) patients, which probably plays a causative role in the progression of CHF, seems to be multifactorial, and at least partially the result of a defective release of iron from cells (1). Prevalent classic risk factors such as hyperlipidaemia, hypertension, and cigarette smoking always are not the main etiological agents for all cases of coronary artery disease (2). Since classic risk factors do not explain all cases of coronary heart disease (CHD), the concept that atherogenesis may have infectious background should be considered as factors implicated in the development of CHD. Since the majority of CHD symptoms are induced by both local and systemic inflammatory responses, recently the attention is focused on the role of inflammation in the development of atherosclerosis (3). Chronic infections may influence the course of CHD via different mechanisms such as chronic inflammatory reactions, an autoimmune processes and modification of classic CHD risk factors (3). The indirect association between the prevalence of helicobacter pylori (*H pylori)* and the occurrence of CHD is confirmed by many research studies. According to majority of findings the involvement of *H pylori* in this process is based on the chronic inflammation which might facilitate the CHD-related pathologies (3). *H pylori* is a gram-negative bacterium with perfect adaptation to the acidic environment of the stomach and high affinity to gastric epithelial cells. *H pylori* is one of the most common infections in the world, with an estimated 50% of the world's population being carriers of the bacterium (4). Based on many evidence infection-related chronic inflammation from *H pylori* is introduced as one of the CAD risk factor, because the CAD risk factors plasma fibrinogen, C-reactive protein, and blood leukocyte count have been elevated in seropositive subjects (3). *H pylori* infection is the most common infection worldwide especially in developing countries (4). According to many research reports, 70-90% of apparently healthy people of developing countries are estimated to be infected with *H Pylori*. An indirect association between the prevalence of *H pylori* and the occurrence of CHD is demonstrated by many research studies (5-8). A causal association between *H pylori* gastritis and iron deficiency anemia (IDA) was published in a patient with hardy unexplained IDA for first time and the anemia was resolved following treatment of *H pylori*-related gastritis (6). One of the serious complications of iron deficiency is anemia, which can lead to serious damage of heart function. Theoretically, severe anemia leads

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to inadequate oxygen delivery to tissues, which in the heart could cause myocyte dysfunction (7)**.** The results of a study on patients with decompensated advanced CHF suggest that the majority of anemic patients with advanced CHF are iron deficient anemia and, an imperative trial of iron-replacement therapy for them was recommended (1). The harmful effects of anemia on the heart have long been recognized. Numerous evidences show that up to 48 percent of individual who have had heart failure are anemic and 43 percent of subjects who hospitalized for a heart attack, were found to have anemia. It is found that anemic subjects are at a 41-percent greater risk of heart attack or needing approaches to treat heart disease as compared to those without anemia (8). Clinical and endoscopic evaluation of more than one-third patients with iron deficiency do not show a lesion to attribute to their iron deficiency. Many evidences suggest that *H pylori* gastritis, without peptic ulcer, can be associated with low iron stores and anemia (8). Epidemiologic studies also support an association between *H pylori* infection and low iron stores. Several reports have shown resolution of hardy cases of anemia after *H pylori* treatment. The results of a study showed that during a follow up period of 12-24 months, all patients with *H pylori* infection and iron deficiency anemia who were cured of *H pylori* infection had resolution of anemia and iron deficiency without further iron supplementation (1). It is believed that H pylori colonisation (possibly) reduces iron uptake and increase iron loss or depletion. In addition, epidemiologic studies have shown that subjects with seropositive for *H pylori* infection(12-16).

Serum ferritin assay has become the standard test for the assessment of iron stores (1, 17). The results of many studies on serum ferritin concentration of *H pylori*- seropositive population from different country showed that infected population with *H pylori* were at 40% increased risk of having significant reduced ferritin level (<30 μg/L) compared to seronegative individuals (after adjustment for age, gender, menopausal status, socioeconomic status, blood donation, and alcohol consumption (12-15). Therefore, knowing that inflammation as a cardiovascular risk factor in the one hand and *H Pylori* involvement in extra digestive disorders, ferritin levels decrease and iron deficiency anemia in CAD incidence on the other hand made authors to evaluate the impact of the seropositivity to *H pylori* role in atherosclerosis processes. Thus, the authors investigated the association between levels of total iron-binding capacity (TIBC), ferritin, iron and seropositive *H Pylori* IgG and IgA levels in patients with CAD in comparison with controls.

Materials and Methods

Sampling and coronary angiography

This cross-sectional study was performed in Rasool Akram Hospital of Tehran. 96 consecutive CAD patients (100 men and 65 women; mean age 52. 95± 1.25 and 51.32±1.61 years old respectively) and 64 controls were enrolled into the study and candidate for coronary angiography and informed consent and after adjustment for age, gender, menopausal status, socioeconomic status, blood donation, and alcohol consumption were selected. Before catheterization, all subjects completed a semi-structured questionnaire regarding their past medical and drug history. The diagnosis was based on the decision of an experienced clinician. Coronary angiography was carried out by left-heart catheterization and arteriography using Judkins method, and then a cardiologist separately reviewed the angiography films. According to angiography reports, the clinical and laboratory evaluated patients with ≥50% coronary stenosis were considered as CAD positive group and participants with <50% coronary stenosis considered as CAD negative group or controls. Accordingly, patients with hepatic dysfunction, autoimmune disease, thyroid dysfunction and/or adrenal dysfunction as well as patients who consumed any kinds of glucocorticoids were excluded from study. This study was approved by the Ethical Committee of Iran University of Medical Sciences.

Biochemical Measurements

Fasting blood sample of catheterization participants were taken to measure lipid profiles, immunoglobulins G and A (anti *H pylori* IgG and IgA), ferritin and iron (TIBC) levels. ELISA kit (Diagnostic kit, PISHTAZ TEB Company, Teharan, Iran) was used to measure the ferritin and iron levels. Anti *H pylori* antibody status was determined by measuring IgG and IgA antibody by ELISA assay (Diagnostic kit, PISHTAZ TEB Company). Spectrophotometric assay was used for lipid profiles assay (Zist chimi kit; Zist chimi CCompany).

Statistical data analysis

Statistical analyses were carried out using SPSS software (version 16.0, Chicago, IL, USA). Unpaired student t-tests and ANOVA test were used for comparing continuous variable. Chi-square test was used for discrete variables. To compare the association of *H pylori* infection with ferritin and iron (TIBC) and thereby CAD, logistic regression tests were used by adjusting sex and age plus history of diabetes, dyslipidemia, and/or Hypertension.

Results

Demographic characteristics of four study groups were presented in tables 1 and 2. No significant differences were found in terms of demographic characteristics between CAD patients and controls with seropositivity and seronegativity to *H pylori* IgG and IgA levels**.** As it was shown in Table 1, the seropositivity to *H pylori* IgG (73.59 ± 3.94U/ml) and IgA (47.62 ± 4.04 U/ml) levels of CAD patient with seropositive to *H pylori* IgG were significantly more than those were found in CAD patients with *sero*negative *to H pylori* IgG (8.84 ± 1.93 and 11.97 ± 1.98 U/ml, respectively). 7.95 ± 0.38 and 14.68 ± 3.9 8 U/ml were achieved for anti *H pylori* IgG and anti *H pylori* IgA of controls with seronegative to *H pylori* IgG, respectively, which are lower significantly than those found for CAD patient with seropositive and seronegative to *H pylori* IgG respectively. The anti *H pylori* IgG (68.29 ± 4.10 U/ml) and anti *H pylori* IgA (40.01 ± 3.58 U/ml) levels of the control group with seropositive to *H pylori* IgG were lower than CAD patient with seropositive to *H pylori*

Table 1. Iron, ferritin and Anti *H. Pylori* IgG and IgA levels and other demographic characteristics of CAD patients with positive and negative Anti *H. Pylori* IgG and the control subjects with positive and negative Anti-H. *Pylori* IgG.

			Control	Non-CAD + Anti-H.P. IgG Positive	CAD + Anti-H.P. IgG Negative	CAD + Anti-H.P. IgG Positive	P value
Gender		Male	14	18	10	58	0.011
		Female	9	23	9	19	
Smoking		Yes	7	7	7	23	0.349
		No	16	34	12	54	
Diabetes History		Yes	2	4	6	9	0.342
		No	21	37	13	68	
Medication	Aspirin	Yes	12	34	15	67	0.139
		No	7	11	4	10	
	Statin	Yes	9	18	11	57	<0.001
		No	14	23	8	20	
	Losartan	Yes	7	9	7	21	0.507
		No	16	32	12	57	
Ferritin (ng/ml)			181.76±14.06	141.38±8.79	123.74±19.43	129.31±6.32	0.005
Iron (µg/dl)			103.37±12.28	77.43±6.32	70.15±11.85	69.25±3.48	0.012
TIBC (µg/dl)			198.76±1.07	220.03±2.76	208.1.34± 0.001	210.01± 1.02	
Anti H.P. IgG (U/ml)			7.95 ± 0.38	68.29 ± 4.10	8.84 ± 1.93	73.59 ± 3.94	<0.001
Anti H.P. IgA (U/ml)			14.68 ± 3.98	40.01 ± 3.58	11.97 ± 1.98	47.62 ± 4.04	<0.001
LDL-C (mg/dl)			104.63 ± 6.64	94.07 ± 3.57	100.07 ± 5.63	97.98 ± 3.42	0.621
HDL-C (mg/dl)			40.34 ± 2.52	39.16 ± 2.16	34.97 ± 1.59	38.66 ± 1.03	0.610
Cholesterol (mg/dl)			190.16 ± 12.08	166.78 ± 5.97	169.01 ± 8.05	170.22 ± 4.47	0.165
TG (mg/dl)			153.02 ± 21.23	143.01 ± 17.30	173.12 ± 24.62	127.62 ± 6.96	0.143
FBS (mg/dl)			101.94 ± 5.06	102.98 ± 7.04	107.06 ± 6.87	103. 18 ± 2.33	0.912
Age (Years)			58.06 ± 2.15	56.39 ± 1.75	55.45 ± 2.67	58.59 ± 1.63	0.237
SBP (mmHg)			127.64 ± 2.53	130.79 ± 3.01	131.92 ± 1.63	130.94 ± 16.31	0.531
DBP (mmHg)			79.41 ± 1.99	79.92 ± 2.01	82.07 ± 1.94	84.21 ± 1.60	0.323
BMI (Kg/m^2)			27.89 ± 0.62	27.53 ± 0.42	27.34 ± 0.86	27.32 ± 0.32	0.831

Table 2. Iron, ferritin and Anti *H. Pylori* IgG and IgA levels and other demographic characteristics of CAD patients with positive and negative Anti *H. Pylori* IgA and the control subjects with positive and negative Anti-H. *Pylori* IgA.

			Control	Non-CAD + Anti-H.P. IgA Positive	CAD + Anti-H.P. IgA Negative	CAD + Anti-H.P. IgA Positive	P value
Gender		Male	8	24	8	60	0.093
		Female	9	23	5	23	
Smoking		Yes	6	7	7	24	0.431
		No	11	40	6	60	
Diabetes History		Yes	1	4	3	10	0.082
		No	11	48	7	76	
Medication	Aspirin	Yes	11	37	8	76	0.089
		No	6	10	3	9	
	Statin	Yes	6	20	7	65	<0.001
		No	10	28	3	23	
	Losartan	Yes	5	9	4	22	0.458
		No	11	41	7	63	
Ferritin (ng/ml)			181.76±14.06	141.38±8.79	123.74±19.43	129.31±6.32	0.008
Iron (µg/dl)			103.37±12.28	77.43±6.32	70.15±11.85	69.25±3.48	0.012
TIBC (µg/dl)			242.01 ± 6.11	251.98 ± 5.56	225.94± 5.61	263.95 ± 4.12	0.008
Anti H.P. IgG (U/ml)			15.78 ± 5.42	57.14 ± 4.83	20.98 ± 11.42	65.02 ± 3.02	<0.001
Anti H.P. IgA (U/ml)			7.01 ± 0.73	37.94 ± 4.14	6.97 ± 0.51	43.56 ± 2.99	<0.001
LDL-C (mg/dl)			101.42 ± 8.09	98.04 ± 4.87	104.32 ± 9.21	98.24 ± 8.21	0.818
HDL-C (mg/dl)			39.73 ± 1.98	38.41 ± 1.89	39.32 ± 3.32	37.33 ± 0.76	0.431
Cholesterol (mg/dl)			177.32 ± 10.86	174.73 ± 8.12	177.76 ± 12.78	167.42 ± 4.21	0.632
TG (mg/dl)			143.45 ± 19.42	140.62 ± 13.34	193.04 ± 34.36	129.61 ± 7.69	0.071
FBS (mg/fl)			100.23 ± 5.64	102.12 ± 7.36	111.23 ± 8.91	102.65 ± 2.43	0.753
Age (years)			56.82 ± 3.12	56.78 ± 1.76	58.22 ± 2.32	58.48 ± 1.43	0.732
SBP (mm Hg)			129.32 ± 2.97	131.59 ± 2.82	135.01 ± 5.01	130.12 ± 1.92	0.642
DBP (mmHg)			79.02 ± 2.98	80.02 ± 1.87	82.34 ± 3.02	83.02 ± 1.52	0.329
BMI (Kg/m^2)			28.03 ± 0.81	27.65 ± 0.53	26.79 ± 1.84	26.84 ± 0.23	0.453

IgG but significantly higher than CAD patients with negative anti *H pylori* IgG. According to Table 2, 65.o2 ± 3.02 and 43.56 ± 2.99 U/ml of anti *H pylori* IgG and IgA respectively of CAD patient with seropositive to *H*

pylori IgA were significantly (P<0.01) higher than those found for CAD patients with seronegative *to H pylori* IgA (20.98 ± 11.42 and 6..97 ± 0.51 U/ml), controls with seronegative to H *pylori* IgA (15.78 ± 5.42 and 7.01± 0.73U/ml) and the control subjects with seropositive to H *pylori* IgA (57.14 ± 4.83 and 37.94 ±4.14 U/ml) respectively. As it was shown in Table1 and 2, the difference between serum triglyceride (TG) and very low density lipoprotein (VLDL) levels of the control group and patient subjects was not significant. The data obtained for high-density lipoprotein (HDL), low-density lipoprotein (LDL), and cholesterol concentrations of the patient group was not different significantly from controls. As it was shown in Table 1 and Figure 1 (A), the serum ferritin concentration of CAD patients with positive anti *H pylori* IgA (129.31 ± 6.32 µg/dl) was not significantly different from those of CAD patients with negative anti *H pylori* IgA (123.74 ± 19.43µg/dl). The difference between ferritin levels of CAD patients with positive anti *H pylori* IgA (129.31 ± 6.32 µg/d) and the control group with positive anti H pylori IgG (141.38±8.79 µg/dl) was not significant (P=0.145). The ferritin levels of CAD patients with positive anti *H pylori* IgA (129.31 ± 6.32 µg/dl) was significantly (P<0.001) lower than control subject with negative anti *H pylori* IgA (181.76 ± 14.06µg/dl). The difference between serum ferritin concentration of the control subjects with negative anti *H pylori* IgA (181.76±14.06 µg/dl) and the control group with positive anti *H pylori* IgG (141.38±8.79) was significant (P= 0.002).

A significant negative correlation with r= -0.207, P=0.042 was identified between anti *H pylori* IgA and ferritin levels of CAD patients with positive anti *H pylori* IgG (Figure 1. B). The correlation between anti *H pylori* IgA and ferritin levels of CAD patients with negative anti *H pylori* IgA was also significant (r= 0.004, P=0.289). The correlation between anti *H pylori* IgA and ferritin levels of control group with positive was significant (r= -0.345, P=0.037), while for control group with negative anti *H pylori* IgA was not significant (r= -0.113, P= 0.242). It is worth to note that correlation between anti *H pylori* IgA and ferritin levels of all subjects was significant (r= -0.176, P= 0.013).

As it was shown in Figure 2 (A), the serum ferritin concentration of CAD patients with positive anti *H pylori* IgG (131.49 ± 5.11 µg/dl) was not significantly different

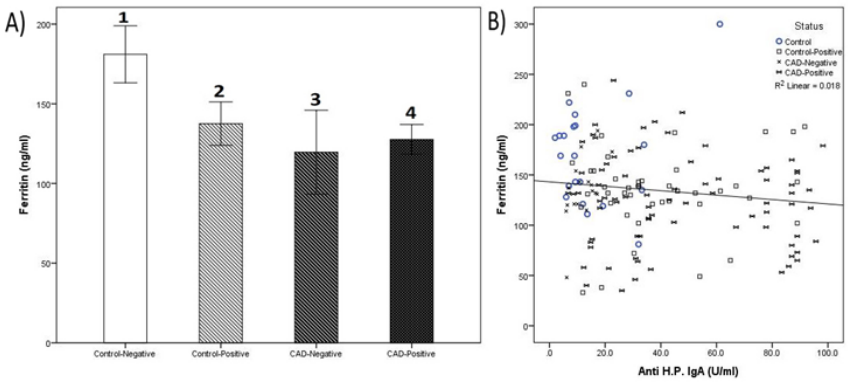

Figure 1. (A): Ferritin levels of CAD patients and controls with seropositive and seronegative to *H pylori* IgA. 4, P<0.001 (in comparison with1); 4, P=0.145 (in comparison with 2); 4, P=0.733 (in comparison with 3); 3, P=0.042 (in comparison with 2); 2, P=0.002 (in comparison with 1); 3, P<0. 001(in comparison with 1). **(B).** Correlation between ferritin and anti *H pylori* IgA of CAD patients and controls with seropositive and seronegative to *H pylori* IgA. Negative correlation between all subjects, r=- 0.176, P=0.013. ○) Negative correlation for controls with negative anti *H pylori* IgA, r= -0.113, P= 0.242 □) correlation for controls with positive anti *H pylori* IgA, r= - 0.345, P=0.037; ♦) Negative correlation for CAD patients with negative anti *H pylori* IgA, r=-0.289, P=0.004; ♦♦) Negative correlation for CAD patients with positive anti *H pylori* IgA, r=-0.207, P=0.042.

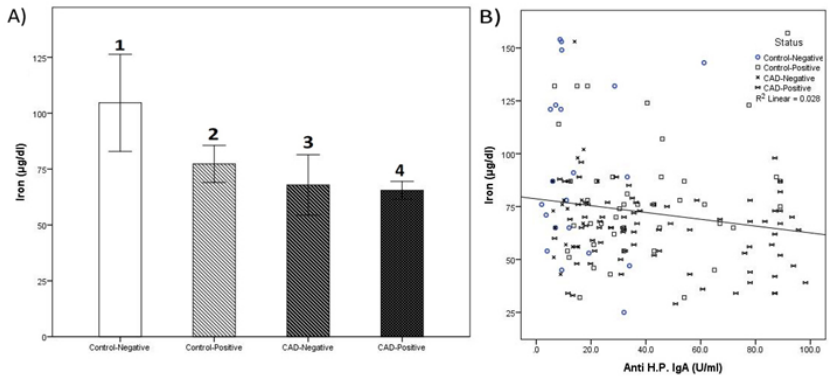

Figure 2. (A): Iron levels of CAD patients and controls with seropositive and seronegative to *H pylori* IgA. 4, P<0.001 (in comparison with1); 4, P=0.009 (in comparison with 2); 4, P=0.840 (in comparison with 3); 3, P=0.241 (in comparison with 2); 2, P=0.004 (in comparison with 1); 3, P<0. 001(in comparison with 1). **(B):** Correlation between ferritin and anti *H pylori* IgA of CAD patients and controls with seropositive and seronegative to *H pylori* IgA. Negative correlation between all subjects, r=-0.199, P=0.002. ○) Negative correlation for controls with negative anti *H pylori* IgA, r= -0.243, P= 0.125; □) correlation for controls with positive anti *H pylori* IgA, r= 0.090, P=0.285; ♦) Negative correlation for CAD patients with negative anti *H pylori* IgA, r=-0.360, P=0.078; ♦♦) Negative correlation for CAD patients with positive anti *H pylori* IgA, r=-0.205, P=0.013.

from that is in CAD patients with negative anti *H pylori* IgA (123.74 ± 19 .34µg/dl). The difference between ferritin levels of CAD patients with positive anti *H pylori* IgA (129.31 ± 6.32 µg/dl (and the control group with positive anti *H pylori* IgG (141.38±8.79 µg/dl) was not significant (P=0.145). The ferritin levels of CAD patients with positive anti *H pylori* IgA (129.31 ± 6.32 µg/dl) was significantly (P<0.001) lower than control subjects with negative anti *H pylori* IgA (141.38±8.79µg/dl). The serum ferritin concentration of the control subjects with negative anti *H pylori* IgA (181.76±14.06 µg/dl) and control group with positive anti *H pylori* IgG (141.38±8.79) was different significantly (P= 0.002). A significant negative correlation with r= -0.207, P=0.042 was identified between anti *H pylori* IgA and ferritin levels of CAD patients with positive anti *H pylori* IgG (Figure 2. B). The correlation between anti *H pylori* IgA and ferritin levels of CAD patients with negative anti *H pylori* IgA was also significant (r= 0.004, P=0.289). The correlation between anti *H pylori* IgA and Ferritin levels of control group with positive was significant (r= - 0.345, P=0.037) while for control group with negative anti *H pylori* IgA was not significant (r= -0.113, P= 0.242). It is worth to note that correlation between anti *H pylori* IgA and ferritin levels of all subjects was significant (r= -0.176, P= 0.013). As it was shown in Tables 2 and Figure 2 (A), there was not a significant (P=1) difference between the homocysteine levels of CAD patients with positive anti *H pylori* IgA (24.70 ± 0.80 µmol/L(as comparison with CAD patients with negative anti *H pylori* IgA (26.50± 4.49 µmol/L). Serum homocysteine concentration of CAD patients with positive anti *H pylori* IgA (24.70 ± 0.80 µmol/L µmol/L) was not significantly (P= 0.1) higher than control subjects with positive anti *H pylori* IgA (22.79± 1.12 µmol /L) but was higher than controls with negative anti *H pylori* IgA positive (17.85± 1.07 µmol/L) significantly (P=0.01). The difference between homocysteine levels of the control subjects with positive anti *H pylori* IgA (22.79 ± 1.12 µmol/L) and controls with negative anti *H pylori* IgA (17.85± 1.07 µmol/L) was not different significantly (P=0.34). Serum homocysteine concentration of CAD patients with negative anti *H pylori* IgA (26.50± 4.49 µmol/L µmol/L) was not significantly (P= 0.75) higher than control subjects with positive anti *H pylori* IgA (26.50 ± 4.49 µmol /L). A significant correlation (P<0.001, r=0.691) was found between anti *H pylori*. IgA and anti *H pylori* IgG of CAD patients in comparison with non CAD patients (Figure 3).

Discussion

Present study showed that patients with cardiac heart disease had a significant iron deficiency. Iron deficiency, in our patients, was associated with the expected decrease in ferritin concentration. It is suggested that this relative decrease in iron and ferritin concentrations might be the result of the inflammation that accompanies the heart disease. As it was shown in results, seropositivity to *H pylori* IgG and IgA levels of patient with eropositive to *H pylori* IgG were significantly more than those were found in CAD patients with *sero*negative *to H pylori* IgG. These results suggest that *H pylori* infection could be a frequent cause of iron deficiency and ferritin decrease in patients with heart disease, which might accelerate the CHD incidence. According to many evidence, the consequence of iron deficiency in roughly one fourth of individual is anemia. Echocardiography survey has demonstrated that some of the hemodynamic changes and cardiovascular failure are accompanied with iron-deficiency anemia**.** In a study of iron-deficient children, 24% of those with hemoglobin levels of less than 5 g/dL manifested CHF (8). It has been demonstrated that rats fed iron-deficient diets developed dilated cardiomyopathy that was histologically associated with atrophic rather than hypertrophic cardiac myocytes. Myocardial iron concentrates were dramatically reduced. It has been revealed that both children and rats without anemia show a skeletal muscle dysfunction, when fed short-term iron-deficient diets (7).

Based on many reports between one third to two thirds of patients with severe anemia have shown cardiomegaly on chest radiography, and the cardiac silhouette reportedly returns to normal within a few weeks of the resolution of anemia (7)**.** Available evidence showed that anemia is prevalent in chronic heart failure patients and is associated with an impaired prognosis (10). It has been identified that permeability of microcyic erythro-

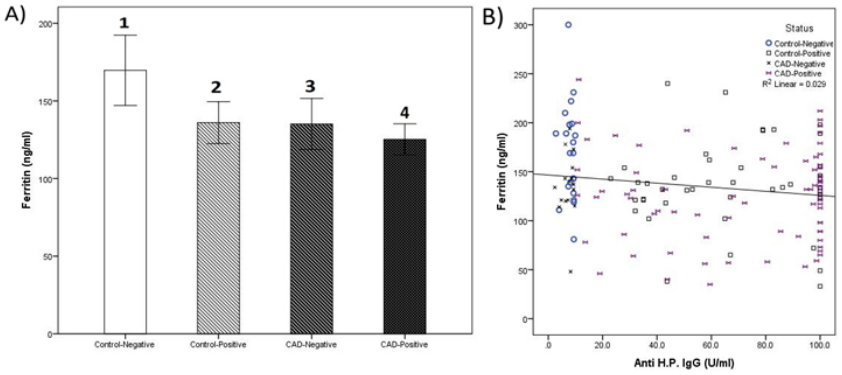

Figure 3. (A): Ferritin levels of CAD patients and controls with seropositive and seronegative to *H pylori* IgG. P<0.001, 4 in comparison with1; P=0.458, 4 in comparison with 2; P=0.559, 4 in comparison with 3; P=0.978, 3 in comparison with 2; P=0.004, 2 in comparison with 1; P=0. 003, 3 in comparison with 1. **(B):** Correlation between ferritin and anti *H pylori* IgG of CAD patients and controls with seropositive and seronegative to *H pylori* IgG. Negative correlation between all subject, r=- 0.181, P=0.002. ○**)** Negative correlation for controls with negative anti *H pylori* IgG, r= -0.092, P= 0.283; □**)** correlation for controls with positive anti *H pylori* IgG, r= -0.053, P=0.371; ♦) Negative correlation for CAD patients with negative anti *H pylori* IgG, r=-0.230, P=0.187; ♦♦) Negative correlation for CAD patients with positive anti *H pylori* IgG, r=-0.283, P=0.009.

cytes decreases in anemic patients especially those with microcytic iron deficiency anemia in comparison to normocytic cells. Therefore, thromboemblolic and cardiovascular events are encountered more commonly in this patients. It was emphasized that more than one third of the patients with chronic cardiac heart disease had iron deficiency anemia (11). It has been identified that *H pylori* colonization in gastric mucosa may impair iron uptake and increase iron loss, potentially leading to iron deficiency anemia (IDA). It is suggested that *H pylori* colonization in the gastric mucosa may disturb some functions of the mucosa and it leads to a decrease in iron absorption and increases iron loss. The impact of the *H pylori* eradication and ferrous supplement consumption on IDA improvement was demonstrated by four meta-analyses (11, 20-22). However inconsistent with our results, some studies could not document the association of *H pylori* infection with CAD (12). On the contrary, the results of a study showed that *H pylori* is not associated with iron deficiency anemia in male patients with normal gastrointestinal tract endoscopy results unless for patients with abnormal gastrointestinal tract endoscopy (13). A significant correlation between lower serum ferritin concentration and iron deficiency and *H pylori* infection was reported. It was shown that after *H pylori* eradication, the serum ferritin concentration was increased and iron deficiency of approximately half of seropositive persons was resolved (14). Several mechanisms have been proposed by which *H pylori* is causing anemia iron deficiency, including reduced iron absorption from hypo or achlorhydria resulting from chronic gastritis is a more likely mechanism. Ascorbic acid facilitates iron absorption by reducing iron to the ferrous form. Decreased concentration of ascorbic acid in gastric juice is another important effect of *H pylori* gastritis. Increased, hepcidin production from hepatocytes in response to IL-6 production associated with *H pylori* gastritis is another hypothesized mechanism. *H pylori* needs iron as a growth factor like many bacteria. Iron up take of *H pylori* is facilitated by an iron-binding protein like ferritin (Pfr), which may play a role in storage of excessive iron. Fur is product of ferric uptake regulator gene. Fur controls the iron uptake and storage *in H pylori*. Mutant *fur* could cause an increased *H pylori* whole cell iron content and probably iron deficiency. Separation of iron in lactoferrin in the gastric mucosa is the another mechanism. Iron take up by *H pylori* is mediated via a receptor and lactoferrin secretion in the gastric mucosa is influenced by the *H pylori* organism (15). Lactoferrin is a glycoprotein which shows high affinity for iron even at low pH and sites of infection and inflammation, which is caused by the metabolic activity of bacteria. In such circumstances, lactoferrin also binds to the released iron from transferrin, which prevents its further usage for bacterial proliferation. It is worth to note that due to an increase of lactoferrin concentration during the most inflammatory reactions and some viral infections, several studies classify lactoferrin as an acute-phase protein (15). Lactoferrin may play an important role in IDA. It has been shown that gastric mucosa lactoferrin levels of *H pylori* positive IDA subjects is higher significantly than persons who are non-anemic *H pylori* -negative, non- anemic *H pylori -p*ositive and *H pylori* –negative with IDA (15). There are several evidences that show the heart failure patients improvement with treatment of iron. Many evidences show that there were small increases in the hemoglobin concentration after treatment. Most of the experimental evidence suggests that iron improves muscle function. In severely iron-deficient rats with a hemoglobin concentration of 4.1 to 5.2 g/dL, walking duration was increased 6- to 10-fold for 15 to 18 hours after iron dextran therapy. This rapid improvement in exercise capacity without change in hemoglobin concentration suggests that iron is a cofactor needed for exercise. It has been demonstrated that in mitochondrial preparations of skeletal muscle, the rate of glycerophosphate phosphorylation as substrate was associated with increase in work performance with treatment of the iron-deficient rats(25, 26).

Considering this fact that difference between TG, VLDL, HDL-C, LDL-C, and cholesterol levels as classic CAD risk factors of the control group and patients with seropositive to IgG and IgA were not significant (Tables 1 and 2), therefore the significant decrease in iron, TIBC and ferritin levels of CAD patients with seropositive to IgG and IgA as comparison with controls could be attributed to chronic *H Pylori* infection as a risk factor independent of other classic factors including lipid profiles for CAD*,* which could induce or accelerate the heart dysfunction processing. Therefore, testing for *H pylori* infection and subsequent treatment is suggested to be considered in persons with unexplained iron deficiency. Thus, the possible incidence of heart dysfunction will be prevented.

In conclusion, the present study demonstrated an inverse relationship between iron and ferritin levels and *H pylori* seropositivity especially IgG and IgA and atherosclerosis occurrence in patients with CAD. Since classic risk factors are not able to explain all cases of CAD, the results of present study suggest that chronic *H pylori* infection affect the development or maintenance of CAD, since it induces chronic long term infection within gastric epithelium which leads to not only local but systemic inflammation. According to our findings the involvement of *H pylori* in this process is based on the chronic inflammation which might facilitate the CAD-related pathologies. Moreover, impact of the presence of *H pylori* was found on iron and ferritin levels in such patients.

Study limitations

In this study, small sample size was investigated and these observations should be confirmed in a larger sample of patients with more analysis works. We analyzed only two independent variables, it should be worthwhile to consider other probable variables involving in CAD disease in future studies.

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