Impact of iron status and inflammatory indices on atherosclerotic burden of patients with Helicobacter Pylori infection and coronary arterial disease

Baris Sensoy¹, Nur Ozer Sensoy²

1 Health Sciences University Bursa Yuksek Ihtisas Training and Research Hospital, Cardiology Clinic, Bursa, TR
2 Bursa Mudanya State Hospital, Internal Medicine Clinic, Bursa, TR

* Corresponding Author: Baris Sensoy E-mail: gs2000bs@yahoo.com

ABSTRACT

Objective: Both inflammation and iron deficiency are suggested to be associated with coronary arterial diseases (CAD) and H. Pylori infection. The explanatory interaction depending on serum iron status and inflammatory biomarkers for the extent of atherosclerosis in H. Pylori infection is obscure. Therefore, we aimed to analyze the impact of iron Status and inflammatory indices on atherosclerotic burden of seropositive CAD patients with CagA (cytotoxin-associated gene A) strains of H. Pylori.

Materials and Methods: This was an observational study of patients' undergone elective and urgent coronary angiography due to CAD. Serologic H. pylori infection status and iron status was determined in all of 293 subjects. Further seropositive patients were divided into groups to evaluate the extent of coronary atherosclerosis according to Syntax scoring system. Propensity score matching and covariate- adjusted multivariate logistic regression were used to adjust for baseline differences between study groups.

Results: The odds ratio of positive serology for the presence of iron deficiency and acute coronary syndromes were 2.5 (95% CI (1.1-5.4); p = 0.02) and 3.0 (95% CI (1.3-7.0); p = 0.007) respectively. After controlling for diabetes mellitus, smoking, MPV, RDW and haemoglobin levels; Tsat ≤ 24.5 remained negatively associated with advanced atherosclerosis (OR:9.9, 95% CI (4.1-24.3); p < 0.0001). In our matched sample, multivariable linear regression analysis showed that association of syntax score with Tsat was independent of hs-CRP (p=0.001).

Conclusions: Irrespective of inflammatory status, transferrin saturation can be the decisive mirror indicator of advanced atherosclerosis in seropositive CAD patients with CagA strains of H. Pylori.

Keywords: H. Pylori, CagA, Coronary arterial disease, , Iron status, Inflammation

INTRODUCTION

Helicobacter pylori (H. Pylori) infection is one of the most common infectious diseases in the world, and it affects more than 50% of the world’s population (1). H.Pylori infection is well known as responsible for an immune-inflammatory response, and CagA (cytotoxin-associated gene A) positive H. pylori strains are recognized as a marker of greater potential for inducing such a response (2-5). The occurrence and progression of atherogenesis is suggested to be linked to H. pylori infection through a low-grade, persistent inflammatory stimulation (6, 7). However, the relation between H. Pylori infection and stable or unstable forms of cardiac syndromes is controversial (8, 9). Recently, a meta-analysis showed a significant, positive association between CagA IgG seropositivity and the occurrence of acute coronary syndromes (ACS) (4). Also, prolonged exposure to CagA bearing strains of H. Pylori infection is suggested to be associated with H. Pylori related advanced disease manifestations such as more severe gastric injury, unexplained iron-deficiency anaemia, progression of gastric preneoplastic lesions and development of gastric adenocarcinoma (10, 11). In addition to the close link between H. Pylori infection and iron deficiency, it was defined that under conditions of iron depletion, H. pylori virulence had increased (10, 12, 13).
Furthermore, low iron status was claimed as the eventual late sign of ischemic heart disease and Transferrin saturation (Tsat) was emphasized to be a good screening test for iron deficiency in patients with the coronary arterial disease (CAD) (14, 15). Lower iron levels may promote free-radical induced lipid peroxidation that leads to vascular inflammation and atherosclerosis subsequently (16). In addition to circulating iron status biomarkers and high-sensitive C-reactive protein (hs-CRP), routinely reported, quickly obtained hematologic parameters are able to reflect systemic ongoing inflammation and may be helpful in cardiovascular risk stratification (17).

In addition to persistent inflammatory stimulation, the explanatory mechanisms for the progression of atherogenesis in H. pylori infection are uncertain. Therefore, the impact of simple hematologic and circulating iron status parameters on the extent of coronary atherosclerosis would be investigated in patients with positive CagA serology.

**MATERIAL and METHODS**

**Study population**

A total of 293 patients undergoing elective and urgent coronary angiography in our cardiology department with various manifestations of ischemic heart disease were included. In this observational study, all of the patients were suspected of having CAD due to clinical symptoms or the results of clinical tests including electrocardiography, blood test, treadmill exercise test and exercise 201-Tl myocardial scintigraphy. Subjects with angiographically normal coronary arteries were excluded. Patients with an identified risk for iron deficiency and a red cell disorder including heavy menstrual periods, known bleeding from a gastrointestinal site, known elevated levels of lead, thalassemia, sickle cell disease, sideroblastic anaemia, aplastic anaemia, vegan diet or a disorder associated with acute or chronic infection/inflammation and those taking iron supplements were excluded from the study. None of the subjects included in the study had clinical evidence of connective tissue disease, liver dysfunction, hypothyroidism, severe chronic heart failure (NYHA class III-IV), moderate or severe renal dysfunction (eGFR < 60 mL/min/1.73 m²) and malignant diseases. Additionally, patients with any surgery within the previous four weeks, prior upper gastrointestinal tract and coronary arterial bypass surgery, use of nonsteroid anti-inflammatory drugs, blood transfusion during the last three months and incomplete data were excluded. Also none of the 293 individuals recruited had a history of eradication therapy for H. Pylori infection or had received any antibiotic treatment during the study.

All subjects were screened with a questionnaire. Demographic data and risk factors for CAD were recorded in all participants. Participants were asked about medical history, including specific questions related to physician-diagnosed hypertension, diabetes, heart failure and hyperlipidemia. Furthermore, current medication, socio-demographic data, and lifestyle habits, including smoking, were recorded. Individuals whose income was lower than at least two times of the minimum wage in our country were defined as lower socioeconomic status. The education level was divided into <10 years and ≥10 years. Among the main cardiovascular risk factors, the presence of family history of CAD (in a first-degree relative <55 years of age), hypertension (systolic or diastolic blood pressure >140 and 90 mm Hg, respectively, or pharmacological therapy with antihypertensive drugs), diabetes mellitus (fasting glucose plasma concentrations >126 mg/dL or pharmacological therapy with antidiabetic drugs or insulin), hyperlipidemia (low-density lipoprotein (LDL) cholesterol levels ≥130 mg/dL or being treated with lipid-lowering medication) were considered definitions. Current smoking status was defined as at least 20 cigarettes per month for more than 6 months of usage. Exercise angina was determined by the presence of chest pain on walking that was relieved within 10 minutes after stopping or by ST-segment of ECG down-sloping in a standard 12-lead electrocardiogram during chest pain or by positive stress testing. The diagnosis of AMI was established by using American College of Cardiology/European Society of Cardiology criteria (18). Iron deficiency was defined as a serum iron < 40 μg/dL or Tsat < 20 % according to the definition previously used (19).

Participation was voluntary, and written-informed consent form was obtained from each subject. The study protocol was approved by the ethics committee of our Hospital. The inclusion period was six months.

**Laboratory Methods**

Indices of iron status and other laboratory measurements assessed in peripheral blood

All blood samples were drawn before the procedure after an overnight fasting under standardized conditions. Haematological parameters were measured using a Beckman Coulter LH780 Hematology Analyzer (Beckman Coulter, Inc).

Within 30 minutes, the remaining blood was centrifuged at 3000g for 10 minutes, immediately divided into aliquots, and frozen at -70°C until analysis. Serum samples were separated at the same time by centrifugation at room temperature for measurement of the following laboratory parameters: Iron, ferritin, transferrin saturation (Tsat) (the ratio of serum iron and total iron binding capacity) and hsCRP.

Iron status was assessed by serum iron (Beckman Coulter AU 5800 analyzer), ferritin (Beckman Coulter DXI 800 analyzer) and transferrin saturation (Beckman Coulter AU 5800 analyzer). HsCRP (Siemens BN-II kinetic nephelometry analyzer) was used as a marker of inflammation. Hs-CRP values were classified by American Heart Association (AHA) standards for risk for cardiovascular disease: Low (<1mg/L), Intermediate (1–3 mg/L), or High (>3mg/L) (20).

Specific H pylori anti-CagA IgG antibodies were measured by use of a commercial Enzyme-linked immune-sorbent assay (ELISA) (Radim Diagnostics, Rome, Italy) according to manufacturer's instructions. Titers were defined as positive or negative according to a cutoff value of 30 UR/mL. The sensitivity and specificity of the tests of Radim-TM was 88% and 93.8%, respectively (21). Patients were divided into 2 groups according to CagA IgG serostatus. All measurements were processed according to standard laboratory practice in a blinded fashion.
Determinant of Coronary Arterial Disease

Coronary angiography was performed by a femoral approach using the standard Judkins technique (Axiom Artis zee 2011; Siemens, Munich, Germany). Coronary arteries were demonstrated in the left and right oblique planes with cranial and caudal angulations. Left ventricular and aortic pressures were recorded. Coronary arteries were opacified with manual injections of Iohexol (Omnipaque, Nycomed Ireland, Cork, Ireland) at each position. Coronary artery disease has been defined as stenosis of at least one major epicardial coronary vessel at any degree. The evaluation of the degree of a stenosis relates to the percentage reduction in the diameter of the vessel. Abnormal angiograms were classified as one, two or three-vessel diseases according to the amount of stenosis higher than 50% in a major epicardial vessel. Furthermore, each angiographic lesion identified was scored according to the Syntax score (SXScore) system (22, 23). All lesions causing ≥50% stenosis in a coronary artery with a diameter ≥1.5 mm were included in SXScore calculation. For the analysis, the software on the Web site (http://www.syntaxscore.com) was used. The SXScore was evaluated separately by two interventional cardiologists blinded to the study protocol and patient characteristics. In the presence of a controversy between the two results, the opinion of a senior interventional cardiologist was applied, and a common consensus was obtained.

Further, in order to increase the statistical analytic reliability, patients with CAD and positive CagA IgG serology who had SXScore of 0 were excluded and remaining seropositive 183 patients were divided into two groups to evaluate the severity of CAD according to Syntax scoring system; mild for 0-22 scores and moderate-severe for > 22 scores.

Statistical Analysis

We used the Kolmogorov-Smirnov test to assess the normality of numeric variables and analyzed homogeneity of numeric variables using the Levene test. Continuous variables with a normal distribution were expressed as means with standard deviations. Continuous variables with a skewed distribution (Neutrophils, Lymphocytes, MPV, RDW, iron, ferritin, Tsat) were expressed as medians with lower and upper quartiles. The categorical variables were expressed as numbers with percentages.

The Student t test, the Mann–Whitney U test and the chi-square (x2) tests (or Fisher’s exact test if any expected cell count was <5) were used to compare baseline characteristics according to H. Pylori serology, atherosclerotic severity and CAD type. Comparisons of parameters among the hs-CRP risk groups were performed by the Kruskal-Wallis test due to the lack of parametric test assumptions. Bonferroni adjustment Mann-Whitney U test was used as a post hoc test for multiple comparisons between the groups.

Our study groups exhibited significant demographic and atherosclerotic risk factor differences (Table 1 and 2). To minimize the confounding effect of these factors and to obtain the best balance among groups, we performed a multivariate logistic regression model based on the significant variables (Propensity score-matched analysis) (24).

To evaluate the correlations of hematologic and iron status parameters with each other and between the SXScore, we used the Spearman's ρ correlation analysis. Furthermore, in order to estimate the ability of circulating blood markers to predict atherosclerosis severity, the receiver–operating characteristic (ROC) curve analysis was done to estimate area under curve (AUC). For the most accurate cut-off values of subsequent iron biomarkers, sensitivity (true positive/true positive + false negative) and specificity (true negative/false negative + false positive) were calculated (and expressed in %).

Univariate logistic regression was used to investigate the relation between coronary disease severity and confounding parameters in our entire sample. After performing univariate analysis, significantly obtained variables (diabetes mellitus, smoking, Tsat ≤ 24%, MPV, RDW and haemoglobin levels) were used in multivariate logistic regression analysis.

To assess the effect of hs-CRP on the association between possible confounding laboratory parameters and the severity of coronary atherosclerosis, multivariable linear regression analysis was performed with and without including hs-CRP in the model in our matched sample.

Continuous variables with a skewed distribution were logarithmically transformed and results were expressed as odds ratio (OR) with 95% confidence intervals (CI). A P-value ≤0.05 was considered statistically significant. Statistical tests were two-sided. All analyses were performed with IBM SPSS 14 (SPSS Statistics version 14, IBM Corp).

RESULTS

Clinical variables, H. pylori infection, iron deficiency and coronary atherosclerotic disease.

The general features of patients according to specified groups for both entire and matched samples are summarized in Tables 1, 2, 3 and Supplementary Table 1. Tests for CagA-positive strains of H. pylori infection were performed in all subjects with positive results in 81.2%. Prevalence of diabetes mellitus, family history of CAD and current smoking was higher in seropositive patients than the seronegative ones (p < 0.05).

Also SXScores, presence of acute coronary events and prevalence of iron deficiency were higher in seropositive patients (p < 0.05). The odds ratio of positive serology for the presence of iron deficiency and acute coronary syndromes were 2.5 (95% CI (1.1-5.4); p = 0.02) and 3.0 (95% CI (1.3-7.0); p = 0.007) respectively.

After balancing the groups for significant confounding CAD risk factors; higher lymphocyte counts, higher rates of iron deficiency and presence of acute coronary syndrome were observed in seropositive subjects compared to seronegative ones (p < 0.05).

In the matched sample, while the difference of median SXScores was no longer significant between the groups; H. pylori infection was still increasing the likelihood of iron deficiency and acute coronary syndromes (OR:2.7, 95% CI (1.04-7.0); p = 0.03) and (OR:3.5, 95% CI (1.3-9.3); p = 0.02) respectively (Table 2).

As we found significant associations between iron deficiency, CAD risk factors, acute coronary syndromes and CagA-positive strains of H. pylori infection, a second analysis was...
performed in seropositive CAD patients (n=183) according to the extent of coronary atherosclerosis. Overall, 75 (41%) subjects had more severe coronary atherosclerosis. Prevalence of diabetes mellitus and current smoking was higher in high SXScore group than the low SXScore group (p < 0.05). Also, levels of MCV, MPV, RDW, Hb, iron and Tsat differed significantly between the groups (p < 0.05) (Supplementary Table 1).

The ROC curve analysis showed that the Tsat at a cut point of 24.5% had 81% sensitivity and 61% specificity to determine severe coronary atherosclerosis (AUC = 0.84, p < 0.0001). For iron, RDW and MPV; the values were 68.5 (AUC = 0.65, p = 0.001; 72% sensitivity, 64% specificity), 13.7 (AUC = 0.61, p = 0.009; 73% sensitivity, 47% specificity) and 8.5 (AUC = 0.60, p = 0.02; 77% sensitivity, 48% specificity) respectively. After balancing the groups for significant confounding CAD risk factors; levels of MPV, iron, Tsat and frequency of iron deficiency remained significantly different between the groups (Table-3).

### CAD type

When the analyses was done according to CAD type, while none of the iron status or hematologic indices were different between the groups in our entire sample (p > 0.05); only median Tsat value for ACS patients was lower compared to stable CAD patients in our matched sample (n= 76) (21 (17-24) vs. 27 (18-29); p = 0.04). Iron deficiency rates tended to be higher in patients with ACS according to stable CAD patients (85% vs.74%; p = 0.09). SXScore, anti-CagA IgG titer were higher and were independently associated with acute coronary events in entire and matched sample groups (p < 0.05) data not shown.

### Hs-CRP

When the indices of haematology and iron status were analyzed according to classified hs-CRP levels; only Tsat values were significantly and gradually decreasing from low risk to high risk groups (p<0.001) (Table-4).

### Medical treatments

Acetylsalicylic acid (ASA) treatment rate was higher in patients with iron deficiency compared to non-iron deficiency patients in both of our entire and matched samples (63% vs. 47% and 84% vs. 57%; p < 0.05 respectively). Positive CagA serology remained significant for the risk of iron deficiency after adjustment for ASA medication (OR:2.9, 95% CI (1.1-7.7); p = 0.03). Treatment rates with ACEI, beta-blocker, calcium channel blocker and statin were similar between the groups according to serostatus (p > 0.05). Also, medical treatment rates were identical in our patients according to the extent of coronary atherosclerosis for both entire and matched samples (p < 0.05; n=183 and n=76).

### Correlations of variables with each other and the SXScore

Correlations of hematologic and iron status parameters with each other and between the SXScore for entire and matched group of patients (n= 293 and n=100) are presented in Supplementary Table 2. Tsat correlated negatively with SXScore (r = -0.22, p < 0.05), number of diseased vessels (r = - 0.26, p < 0.05), white blood cell count (r = - 0.45, p < 0.01) and hs-CRP (r = -0.33, p < 0.01) in both of our entire and matched samples. Hs-CRP levels correlated positively with SXScore (r = 0.26, p < 0.05) number of diseased vessels (r = 0.66, p < 0.01) and RDW (r = 0.42, p < 0.01) in both of our entire and matched samples. Also positive correlation between anti-CagA IgG titer and lymphocyte count was determined (r = 0.55, p < 0.01). Correlation between CagA titer and SXScore lost its significance after matching groups (r = 0.22, p < 0.0001 vs. r = -0.01, p > 0.05).

### Regression Analysis

The association of severe coronary atherosclerosis with confounding parameters was investigated by univariate and multivariate analyses. After controlling for diabetes mellitus, smoking, MPV, RDW and haemoglobin levels; Tsat ≤ 24.5 remained negatively associated with advanced atherosclerosis (OR=9.9, 95% CI (4.1-24.3); p < 0.0001). (Table-5). In our matched sample, multivariable linear regression analysis showed that association of SXScore with Tsat was independent of hs-CRP (p=0.001) (Supplementary Table 3).

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**Table 1.** Demographic, clinical and laboratory characteristics of our study group according to CagA serostatus.

|                           | All  n=293 | Entire sample (n=293) | CagA IgG-seronegative n=55 | CagA IgG-seropositive n=238 | p-value |
|---------------------------|-----------|-----------------------|---------------------------|--------------------------------|--------|
| Age, years                | 60±14     | 60±16                 | 60±14                     | <0.0001                        |        |
| Female, n (%)             | 169 (58)  | 44 (88)               | 125 (51)                  |                                | <0.0001|
| Family history of CAD, n (%) | 174 (59)  | 22 (44)               | 152 (63)                  |                                | <0.0001|
| Socioeconomic status, n (%) |          |                      |                           |                                |        |
| Low                       |           |                       |                           |                                |        |
| Middle-High               | 112 (38)  | 16 (32)               | 96 (39)                   |                                | 0.32   |
| Education <10 years, n (%) | 140 (48)  | 27 (54)               | 113 (46)                  |                                | 0.33   |
| Diabetes, n (%)           | 129 (44)  | 10 (20)               | 119 (49)                  |                                | <0.0001|
| Hypertension, n (%)       | 188 (64)  | 33 (66)               | 155 (64)                  |                                | 0.77   |
| Dyslipidemia, n (%)       | 153 (52)  | 26 (52)               | 127 (52)                  |                                | 0.97   |
| Smoking, n (%)            | 130 (44)  | 10 (18)               | 120 (50)                  |                                | <0.0001|
| WBC (10³ /μL)             | 9.4±3.1   | 10.2±3.8              | 9.3±2.9                   | 0.07                           |        |
| Platelets (10³ /μL)       | 228±61    | 211±56                | 231±62                    | 0.03                           |        |
| Neutrophils (10³ /μL)     | 6.3 (4.8-7.9) | 7.4 (5.0-8.1) | 5.6 (4.8-7.8)             | 0.04                           |        |
| Lymphocytes (10³ /μL)     | 1.8 (1.3-2.4) | 1.4 (1.2-1.8) | 1.9 (1.4-2.7)             | <0.0001                        |        |
| MCV, fL                   | 89.6±5.9  | 88.7±6.5              | 89.8±5.6                  | 0.21                           |        |
| MPV, fL                   | 8.8 (7.8-9.4) | 9.1 (8.2-9.3) | 8.6 (7.7-9.5)             | 0.20                           |        |
| RDW, %                    | 14.0 (13.4-15.1) | 14.1 (13.2-15.1) | 13.9 (13.4-15.0) | 0.83                           |        |
| Creatinine, mg/dL         | 1.0±0.3   | 1.1±0.3               | 1.0±0.3                   | 0.23                           |        |

CAD, coronary arterial disease; Cag A, cytotoxin-associated gene product; IgG, immunoglobulin G; MCV, mean corpuscular volume; MPV, mean platelet volume; RDW, red cell distribution width; WBC, white blood cell.

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Comparison of laboratory parameters by American Heart Association (AHA) risk for cardiovascular disease group.

| Parameters | Low (hs-crp < 1 mg/L) n=10 | Intermediate (hs-crp 1-3 mg/L) n=40 | High (hs-crp > 3 mg/L) n=133 | p-value* | p-value** |
|------------|---------------------------|-----------------------------------|-----------------------------|---------|---------|
| RDW, %     | 13.9 (13.4-13.9)          | 13.4 (12.8-15.0)                  | 14.3 (13.6-15.1)            | 0.005 * |         |
| MPV, fL    | 8.0 (8.0-9.4)             | 8.6 (7.5-8.7)                     | 8.9 (7.7-9.7)               | 0.016 **|         |
| Tsat, %    | 41 (0.05-0.46)            | 28 (0.06-0.58)                    | 21 (0.07-1.00)              | <0.001 **|         |
| Iron, µg/dL| 142 (113-142)             | 80 (62-91)                        | 65 (47-84)                  | <0.001 **|         |
| Syntax score | 5 (5-7)                   | 17 (14-28)                        | 19 (12-33)                  | 0.008 **|         |

*Comparison of groups with each other by using Mann-Whitney test; Bonferroni-adjusted P value is 0.016.
**p-value* denotes the difference between the low group and high group is statistically significant (p<0.016).
**p-value** denotes the difference between the low group and intermediate group is statistically significant (p<0.016).
***p-value*** denotes the difference between the intermediate group and high group is statistically significant (p<0.016).
DISCUSSION

This propensity score matched observational study has confirmed that CagA positive H. pylori infection prevalence is high and is associated with higher prevalence of iron deficiency, CAD risk factors and acute coronary events in CAD patients. The major and novel finding of this study is the independently association of Tsat with advanced coronary atherosclerosis in CagA positive H. pylori infection.

Nearly all of the samples collected from East Asian countries comprise CagA strains of H. pylori, contrary to western countries, in where it decreases to a half (25). In Turkey, a developing country, seroprevalence of H. Pylori IgG is between 41% to 83% (25). In a study regarding CAD patients from our country, seroprevalence of H. Pylori IgG was 80.2% (26). In another study from United States of America, 45% of CAD patients were seropositive for H. Pylori (27). However, the importance of anti-CagA seropositivity wasn't analyzed in these studies. Different prevalence rates of infected populations between developing and developed countries are explained partly by higher socioeconomic levels in developed countries (1, 25). In line with the literature, we found the prevalence of virulent H. pylori infection 81.2% in patients with CAD. The socioeconomic class was also recognized as a risk factor for atherosclerosis (1). Also, other factors predisposing to both of these diseases had been suggested to affect the association between CAD and H. Pylori infection (10, 28). Major factors responsible for the increasing rate of CAD occurrence like diabetes mellitus and smoking, were linked with higher prevalence of H. Pylori infection (28). This was supported by the findings in our study as male gender, family history of CAD, history of diabetes mellitus, and current smoking were the covariates associated with positive CagA IgG serology.

The relation between H. Pylori infection and stable or unstable forms of cardiac syndromes is controversial (2-5, 8, 9). Population-based cohort studies have not shown a significant association of H. pylori infection and CAD (3, 8). However, such a significant, positive association between CagA IgG seropositivity and the occurrence of ACS was concluded in a meta-analysis and a prospective, case-control study with a 12-year follow-up period (4, 9). Moreover, anti-CagA antibody titer was the only independent predictor of the extent of coronary atherosclerosis in a cross-sectional study with 60 patients by Niccoli et al. (5). Different from their research, that compared dissimilar groups for CAD risk factors; our study was carried out at a larger population by adjusting confounding factors. The predictive ability of anti-CagA antibody titer on the extent of coronary atherosclerosis lost its significance in our matched sample. In the literature, propensity score match analysis hasn’t been used in this regard and discrepancy between the investigations may be attributed to cohort heterogeneity and differences in exposure time to the infection. Besides, most of these studies were based mainly on Enzyme-linked immune-sorbent assay (ELISA) that failed to confirm the existence of ongoing infection and the likelihood of different active H. Pylori infection rates might have affected the outcomes of studies. After ACS, ongoing infection by H. pylori may be responsible for platelet aggregation and local inflammation within the vascular wall that diminish along with plaque stabilization during subsequent weeks (6, 7). The importance of acute infection had been claimed in unstable forms of cardiac syndromes by determining the significant association of seropositivity for H. Pylori with the risk of only short-term outcomes, but not with the risk of long-term outcomes (6, 7). Although the rate of acute infection was obscured due to the design of our study, the association of the presence of acute coronary syndromes with CagA titer independent from the SXScore strengthened this suggestion.

In addition to CAD; the prevalence of other H. Pylori related advanced disease manifestation rates such as more severe gastric injury, unexplained iron-deficiency anemia, progression of gastric pre-neoplastic lesions and development of gastric adenocarcinoma are suggested to be higher with CagA bearing strains (10, 11). Strain-specific virulence constituents may act in concert with environmental factors to influence pathogenic outcomes (11). Iron deficiency is also shown to be associated with an increased risk for neoplasms that arise within the gastrointestinal tract, including the stomach (12). H. pylori infection contributes to iron deficiency (12), and bacterial eradication results in reversal of this disorder (29). Virulent strains of H. Pylori translocate CagA into host cells by a bacterial type IV secretion system (T4SS) in order to affect and alter host cell morphology, signaling, and inflammatory responses. It was defined that under conditions of iron depletion H. pylori virulence had increased by enhancing assembly and function of the cag T4SS (13). H. pylori infection-related chronic mucosal inflammation, especially the presence of lymphoid follicles was shown to be reflected in the amount of higher peripheral blood lymphocytes and lower blood MPV levels (30). By determining 2.5 times higher iron deficiency risk and higher lymphocyte counts in our anti-CagA positive patients compared to seronegative ones, we emphasize the importance of interaction between inflammation, iron status and advanced disease manifestation.

As iron status, in conjunction with the presence of CagA positive H. pylori strains was considered as a risk factor for more advanced associated diseases; we investigated the association of iron deficiency with advanced coronary arterial diseases in terms of acute coronary events and advanced coronary atherosclerosis in seropositive patients. In the Hunt study, a large prospective-cohort study with a 11.4 year follow-up period, low iron status was demonstrated to be the eventual late sign of ischemic heart disease (14). In another study, the diagnostic accuracy of iron status parameters for determining iron deficiency in CAD patients was investigated. When iron stores in bone marrow aspirates were taken as the diagnostic gold standard test; it was observed that irrespective of concomitant anaemia many stable CAD patients had bone marrow iron deficiency. Tsat, as a more widely available screening tool, was emphasized to be a good alternative to serum soluble transferrin receptor for iron deficiency in patients with CAD (15). This evidence was confirmed by a systematic review and meta-analysis that examined the association between body iron status biomarkers and CAD by determining the only significant association between serum Tsat and CAD (16). Our results concerning the associations between advanced coronary arterial diseases and iron deficiency was in line with the current knowledge and also suggested the probable role of
CagA positive *H. Pylori* infection in the progression of both disorders.

Although DNA of *H. Pylori*, especially CagA positive, can be identified in atherosclerotic plaques of patients with severe CAD, low grade general inflammatory response is suggested to be the primary mechanisms linking *H. Pylori* infection to the development of atherosclerosis and ACS occurrence (2, 6, 7). Therefore, we evaluated the explanatory interactions depending on serum iron status and inflammatory biomarkers for the extent of atherosclerosis. Risk stratification of CAD by circulating blood biomarkers including direct or indirect inflammatory markers such as hs-CRP, Tsat, iron and more simple, inexpensive and widely available markers like RDW and MPV was asserted (16, 17, 20). However, the most critical limitation which could not be avoided in these studies was the inadequate adjustment of other cardiovascular risk factor effects. Although, in keeping with previous observations, we had found individual relationships between RDW, MPV, iron, Tsat and advanced coronary atherosclerosis; our study tried to solve most of this limitation. Therefore, these relationships were investigated after balancing the groups for significant confounding CAD risk factors. When hs-CRP was added in the model MPV lost its significance, while Tsat was still a significant variable for determining SXXScore.

Strengths of our study include the large sample size relative to similar studies, fully matched compared samples for probable confounders and a detailed description of the quantitative coronary angiography method used. It is a limitation of our study that *H. pylori* diagnosis based on serology, which may reflect not only present but also recent or past *H. pylori* infection. So, the association of acute *H. Pylori* infection with the presence of acute coronary syndromes is uncertain. No history of eradication therapy for *H. Pylori* infection in none of the recruited 293 individuals points out this limitation at least partly. On the other hand, *H. pylori* status was determined among all cases with the same method reducing internal variability, and, although the test showed good correlation with previous *H. pylori* tests used in former studies, no specific local validation was performed. Our measurements of subsequent markers were based on a single determination, and the time-course relationship with vascular events cannot be extrapolated from the study. Observational studies are always open to residual confounding factors that cannot always be completely controlled. Here, we reported estimates of OR adjusted by the most widely recognized independent risk factors. In addition, although the study is not based on a random sample from the general population, in our country, CagA positive *H. Pylori* infection was also frequent in the general population as previously reported (25). When the higher rate of coexistent CAD risk factors with the infection is taken into account, our findings may have clinical importance for the general population.

**CONCLUSIONS**

In summary, these results may have clear implications for clinical practice in line with the previously reported studies (10, 16, 26, 28). The high seroprevalence of *H. Pylori* infection and the frequent coexistence of positive CagA IgG serology with covariates responsible for the occurrence of coronary atherosclerosis highlight the necessity of investigation for CAD in CagA positive *H. Pylori* infection. Besides, those more pathogenic strains can be responsible for higher rates of both iron deficiency and more severe coronary disease. Also, we have shown that, Tsat might be the decisive mirror indicator of *H. Pylori* related advanced disease manifestations irrespective of inflammatory status. To better clarify that issue, larger prospective studies, including time-course relationship of Tsat with prognostic and progressive disease outcomes are needed.

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Supplementary Table 1. Demographic, clinical, laboratory and angiographic characteristics of our entire study group according to severity and complexity of coronary atherosclerosis

| Characteristics                      | Entire sample | Syntax score ≤22 | Syntax score >22 | p-value |
|--------------------------------------|--------------|------------------|------------------|---------|
| Age, (years)                         | 61±14        | 61±13            | 62±16            | 0.57    |
| Male, n (%)                          | 76 (42)      | 39 (36)          | 37 (49)          | 0.09    |
| Female, n (%)                        | 123 (67)     | 70 (65)          | 53 (71)          | 0.41    |
| Socioeconomic status, n (%)          | Low          |                  |                  |         |
|                                      | Middle-High  |                  |                  |         |
| Education ≤10 years, n (%)           | 90 (49)      | 50 (46)          | 40 (53)          | 0.35    |
| Diabetes, n (%)                      | 103 (56)     | 54 (50)          | 49 (65)          | 0.04    |
| Hypertension, n (%)                  | 133 (70)     | 76 (70)          | 57 (76)          | 0.40    |
| Dyslipidemia, n (%)                  | 108 (59)     | 58 (54)          | 50 (67)          | 0.08    |
| Smoking, n (%)                       | 76 (42)      | 30 (28)          | 46 (61)          | <0.0001 |
| WBC (10³ /μL)                        | 9.8±2.7      | 9.9±3.2          | 9.6±1.9          | 0.26    |
| Platelets (10³ /μL)                  | 238±65       | 239±66           | 236±63           | 0.76    |
| Neutrophils (10³ /μL)                | 7.1 (5.1-8.2)| 7.1 (4.8-8.7)    | 6.8 (5.3-7.8)    | 0.80    |
| Lymphocytes (10³ /μL)                | 1.9 (1.3-2.7)| 1.9 (1.2-2.9)    | 2.2 (1.7-2.7)    | 0.48    |
| MCV, fL                              | 90.5±4.8     | 91.6±4.3         | 88.9±5.1         | <0.0001 |
| MPV, fL                              | 8.6 (7.7-9.4)| 8.6 (7.7-9.2)    | 8.7 (8.6-9.7)    | 0.02    |
| RDW, %                               | 14.0 (13.4-15.0) | 13.9 (13.4-15.0) | 14.3 (13.6-16.0) | 0.009   |
| HsCRP, mg/dL                         | 6.2±4.0      | 5.8±4.3          | 6.7±4.3          | 0.24    |
| Hemoglobin, g/dL                     | 13.9±1.9     | 14.3±1.5         | 13.5±2.2         | 0.011   |
| Iron µg/dL                           | 70 (48-91)   | 84 (57-113)      | 61 (37-79)       | 0.001   |
| Iron deficiency, n (%)               | 59 (32)      | 25 (23)          | 34 (45)          | 0.002   |
| Ferritin µg/L                        | 98 (50-124)  | 112 (40-124)     | 63 (55-100)      | 0.29    |
| Tsat, %                              | 22 (18-29)   | 28 (19-31)       | 21 (12-23)       | <0.0001 |
| Tsat ≤24.5, n (%)                    | 84 (46)      | 34 (32)          | 50 (67)          | <0.0001 |
| Creatinine, mg/dL                    | 1.1±0.3      | 1.1±0.3          | 1.0±0.2          | 0.25    |
| LDL, mg/dL                           | 112±33       | 111±29           | 112±37           | 0.83    |
| CagA IgG titer UR/mL                  | 143±81       | 144±77           | 142±88           | 0.90    |
| CAD type, n (%)                      | Stable CAD   |                  |                  |         |
|                                      | ACS          |                  |                  | 0.23    |
| Multivessel disease, n (%)           | 62 (37)      | 31 (29)          | 51 (68)          | <0.0001 |
| Syntax score                         | 10 (5.2)     | 11 (5.8)         | 6 (5.0)          |         |

ACS, acute coronary syndrome; CAD, coronary arterial disease; CagA, cytotoxin-associated gene product; HsCRP, high sensitive C-reactive protein; IgG, immunoglobulin G; LDL-C, low-density lipoprotein cholesterol; MCV, mean corpuscular volume; MPV, mean platelet volume; RDW, red cell distribution width; Tsat, transferrin saturation; WBC, white blood cell.

Supplementary Table 2. Correlations of angiographic and laboratory parameters in our study group

| Correlation coefficient | Matched Sample n=100 |
|-------------------------|----------------------|
| Age, (years)            | 0.14**               |
| WBC (10³ /μL)           | 0.38**               |
| Platelets (10³ /μL)     | -0.03                |
| Neutrophils (10³ /μL)   | 0.35**               |
| Lymphocytes (10³ /μL)   | 0.02                 |
| MCV, fL                 | 0.005                |
| MPV, fL                 | 0.22**               |
| RDW, %                  | 0.32**               |
| Iron µg/dL              | -0.18**              |
| Ferritin µg/L           | 0.21**               |
| Tsat, %                 | -0.23**              |
| CagA IgG titer UR/mL    | 0.07                 |

CagA, cytotoxin-associated gene product; HsCRP, high sensitive C-reactive protein; IgG, immunoglobulin G; MCV, mean corpuscular volume; MPV, mean platelet volume; RDW, red cell distribution width; Tsat, transferrin saturation; WBC, white blood cell.

Significance * p<0.05, ** p<0.01. Parameters significant in both samples are shown in bold.
Supplementary Table 3. Univariable and multivariable linear regression analysis of Syntax score and potential confounding variables in our matched sample group.

| Variables | Univariable linear regression analysis | Multivariable linear regression analysis | Standardized β | Collinearity statistics |
|-----------|--------------------------------------|----------------------------------------|----------------|------------------------|
|           | β (95% CI) | p-value | β (95% CI) | p-value |                      |
| **Analysis with hs-crp** | | | | | |
| MPV | 84 (24; 143) | 0.006 | 53 (-7; 113) | 0.08 | 0.21 | 1.18 |
| RDW | 93 (11; 177) | 0.03 | -13 (-96; 70) | 0.75 | -0.04 | 1.32 |
| hs-CRP | -0.7 (0.2; 0.02) | 0.14 | - | - | - | |
| LDL | -38 (-55; -21) | <0.0001 | -33 (-51; -14) | 0.001 | -0.43 | 1.24 |
| Iron | -4 (-18; 11) | 0.63 | - | - | - | |
| **Analysis without hs-crp** | | | | | |
| MPV | - | - | 64 (7; 121) | 0.03 | 0.26 | 1.20 |
| RDW | - | - | 2 (-76; 81) | 0.95 | 0.007 | 1.32 |
| Tsat | - | - | -36 (-55; -19) | <0.0001 | -0.46 | 1.10 |

β, Regression coefficient; CI, confidence interval; hs-CRP, high sensitive C-reactive protein; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; LVEF, left ventricular ejection fraction; MPV, mean platelet volume; NLR, neutrophil to lymphocyte ratio; RDW, red cell distribution width; Tsat, transferrin saturation; VIF, variance inflation factor.