Serum hsCRP and procalcitonin levels in dyspeptic patients infected with CagA-positive *Helicobacter pylori*

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INTRODUCTION

Helicobacter pylori (HP) is a Gram-negative bacteria that infects approximately half of the world's population (1). These bacteria colonize the mucosa of the stomach antrum and cause chronic gastritis. Although the prevalence of this infection is high, most individuals remain asymptomatic. Factors that cause HP-associated disease are called virulence factors. CagA is one of the important virulence factors that trigger inflammation. CagA is associated with peptic ulcers, gastric adenocarcinoma, and mucosa-associated lymphoid tissue (MALT) lymphoma (2).

Coronary heart disease, iron-deficiency anemia, pernicious anemia, and idiopathic thrombocytopenic purpura have been linked to HP infection (3). These extragastric manifestations of HP infection are thought that they are derived from HP-triggered systemic inflammation.

C-reactive protein (CRP) is an acute phase reactant that is sensitive to systemic inflammation. Because of its sensitivity, it is used as a clinical marker of infection and tissue damage. CRP is not only specific for infection, serum CRP levels usually increase to high levels after infections, myocardial infarction, stress, trauma, neoplastic proliferation, and surgery (4, 5). High-sensitivity CRP (hsCRP) assays allow measuring very low levels of circulating CRP. Levels of hsCRP are affected by diverse states other than systemic infection, including body mass index, alcohol, and tobacco consumption, and diseases such as diabetes mellitus and coronary heart disease (6). In the past few decades, hsCRP has emerged as a predictor of coronary heart disease (7).

Procalcitonin (PCT) is a polypeptide precursor of calcitonin that is produced by thyroid gland (8). In the early

1990s, it was observed that PCT levels increase after bacterial infections. Because PCT does not increase during neoplastic and autoimmune processes, or viral infections, it is used as a marker for sepsis and severe bacterial and fungal infections (9). PCT levels are increased independently from calcitonin in bacterial infections. Its major production sites are not exactly known but, it has become apparent that hepatocytes, neutrophils, and mononuclear cells can produce PCT (10).

CagA is an important virulence factor for HP infection. We hypothesized that CagA-positive HP infection would cause more serious systemic responses vs. CagA-negative HP infection. Thus we investigated the effect of CagA positivity to serum levels of CRP and PCT in patients with HP infection.

MATERIALS AND METHODS

This study was conducted between June 2010 and June 2012 in the Department of Gastroenterology of Kecioren Research and Training Hospital, Ankara, Turkey after approval by the local ethics committee. Informed consent was obtained from all patients who were enrolled into the study. Patients who presented with dyspeptic complaints and on whom were performed upper gastrointestinal endoscopy were evaluated for study inclusion. Individuals without organic lesions such as duodenal ulcers,

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gastric ulcers, or adenocarcinoma, and who were diagnosed with HP infection by histopathology were enrolled. Exclusion criteria included alcohol and tobacco consumption, pregnancy, antioxidant consumption, HIV infection, diabetes mellitus, hypertension, coronary artery disease, renal failure, heart failure, liver failure, acute bacterial infections, rheumatoid arthritis, malignant disease, and cerebrovascular disease. Patients who had received anti-HP therapy were also excluded. All patients with HP infection received a quadruple anti-HP therapy that consists of bismuth salisilate.

At least two biopsies from antrum and corpus were taken from patients who admitted with dyspepsia and who had no organic lesions discernable by endoscopy. Tissue sections were stained with Giemsa and hematoxylin-eosin for HP infection and histopathological assessment, respectively. The Sydney Classification (11), which includes HP density, atrophy, intestinal metaplasia, and inflammation, was used for histopathological assessment. Patients with HP infection were evaluated for study enrollment. Blood samples were collected from all patients who fulfilled the criteria. CagA status was detected using a commercial kit (DIA.PRO Diagnostic Bioprobes Srl®, Milano, Italy), hsCRP was detected by a kit (Image Immunochemistry Systems; Beckman Coulter®, Brea, USA), and serum PCT levels were analyzed by a kit (BRAHMS PCT®, Henningsdorf, Germany), according to the manufacturer's instructions.

Statistical analyses were made with SPSS version 15.0 statistical software (SPSS Inc./IBM, Chicago, IL). Continuous variables are expressed as mean $\pm$ standard deviation and categorical variables are reported as percentages. Results were analyzed for normal distribution using the Kolmogorov–Smirnov test. Student's *t*-test or Mann–Whitney *U* test were used where appropriate. Chi-square test was used to compare categorical data. Correlation between serum levels of hsCRP, PCT, and parameters of Sydney classification was determined by Spearman's correlation test. *P*-values < 0.05 were accepted as statistically significant.

RESULTS

A total of 82 patients were enrolled, including 27 males and 55 females. Mean patient age was 45.5 ± 8.7 years (range 29 to 67 years). Forty-four patients (54%) of the patients were positive for CagA, and 38 patients (46%) were negative for CagA. There was no significant difference between the CagA positive and CagA negative patients in the mean of age. Percentage of gender between CagA positive and negative patients was not different (Table 1).

Serum CRP levels were similar in CagA-positive patients (0.377 ± 0.050 mg/dl) and CagA-negative patients (0.379 ± 0.053 mg/dl) (Fig. 1; $P > 0.05$). PCT

TABLE 1. Comparing Baseline Characteristics of Patients

	CagA positive	CagA negative	<i>P</i> -value
Male/Female (<i>N</i> ,%)	18/26 (53.65%)	9/29 (46.35%)	0.10
Mean age (age $\pm$ SD)	46.45 ± 9.37	44.47 ± 7.78	0.28

levels were significantly elevated by $\approx 24\%$ in CagA patients (0.210 ± 0.008 ng/ml) vs. CagA-negative patients (0.170 ± 0.011 ng/ml) (Fig. 2; $P = 0.01$). Normal range of PCT was 0–0.05 ng/ml according to the manufacturer's instructions.

There was no correlation between hsCRP, gastric mucosal damage, and *H. pylori* intensity. (r : -0.092 , $P > 0.05$; r : 0.135 , $P > 0.05$, respectively). Also PCT levels did not correlate with gastric mucosal damage and *H. pylori* intensity. (r : 0.054 , $P > 0.05$; r : -0.079 , $P > 0.05$, respectively).

DISCUSSION

The current study revealed that CagA positivity, an important virulence factor for HP infection, did not affect serum hsCRP levels. However PCT serum level significantly elevated in CagA-positive vs. CagA-negative HP-infected patients.

HP leads to chronic infection of the antrum epithelium, and HP also triggers systemic inflammation thus contributing to extra gastrointestinal manifestations (12). Localized infection can cause systemic responses via direct effects on vascular wall or indirectly by regulating inflammatory mediator expression (13). Inflammatory cytokines such as IL-18, IFN- α , and IL-1 β play crucial roles in the inflammation induced by HP infection. Chronic HP infection can last for decades and this situation can induce systemic effects.

The associations between HP infection and chronic diseases are interesting. Some studies revealed associations between HP infection and membranous glomerulopathy, Behcet's Disease, and systemic sclerosis (14, 15). But, atherosclerosis can be considered the most important HP-associated disease. Atherosclerosis is a multistep process that involves vascular wall inflammation involved in all of steps; all primary risk factors for atherosclerosis involve contribution to the underlying inflammatory state (16). Various microbiologic pathogens have roles in atherosclerosis pathogenesis (17), and HP is an established risk factor for coronary artery disease. The association between HP and coronary artery disease was first investigated by Mendall et al. in 1994 (18). Since then, Gunn et al. found a relationship between HP infection and atherosclerosis, and identified HP IgG seropositivity as a risk factor for coronary artery disease (19). Individuals with acute myocardial infarction have higher levels of HP IgG than controls (20).

Fig. 1. Serum hsCRP level of CagA positive and negative patients.

Elevated CRP contributes to chronic diseases such as the chronic inflammation associated with atherosclerosis, and circulating CRP is an independent risk factor for predicting cardiovascular disease (21). Clinical studies revealed that higher levels of CRP not only predict myocardial infarction risk but also predict the morbidity and morbidity during the postinfarction period (22). CagA is an important virulence factor for HP infection. It is known that CagA causes serious damage to the gastric mucosa and also increases inflammation by inducing cytokines such as IL-8 (23).

Because CRP is an inflammation marker, some authors conducted studies that aimed the relationship between hsCRP values and HP infection. A population-based epidemiologic study revealed that HP-infected individuals have higher serum CRP levels than individuals without HP infection (24). Another study on healthy patients during routine checkups reported that HP-seropositive patients have higher hsCRP levels than HP-seronegative individuals (13). One study investigated the relationship between CagA status and hsCRP revealed that HP-infected patients with peptic ulcers higher CRP levels than asymptomatic HP-infected patients or HP-negative individuals, but that CagA status did not affect hsCRP levels (25). Our results confirm these earlier findings regarding

the lack of association between CagA status and CRP levels.

There are few studies that investigated the relationship between hsCRP, CagA status, and coronary artery disease. One study of 159 coronary artery disease patients showed that HP-positive/CagA-positive patients have higher levels of hsCRP and oxidized low-density protein than the HP-positive/CagA-negative and HP-negative group (26). After excluding all patients with coronary artery disease and other chronic diseases in our study, we did not observed increased hsCRP in CagA-positive HP-infected patients.

PCT is an inflammation marker that is used for diagnosing, grading, and monitoring septic shock. Also PCT can be used to assess the activation of systemic inflammatory response, to predict the prognosis and treatment success (27, 28). In recent decades PCT, has been used to diagnosis and assess the severity of malaria and bacterial meningitis (29, 30). PCT is also associated with atherosclerosis severity and has prognostic value in acute myocardial infarction (31, 32).

Because hsCRP is a nonspecific marker of inflammation and PCT is sensitive for bacterial infection, some authors conducted studies that evaluated the clinical utility of both markers together in assessing bacterial

Fig. 2. Serum procalcitonin level of CagA positive and negative patients.

infection. A meta-analysis of 12 studies concluded that PCT is more sensitive and specific than hsCRP for differentiating bacterial infection (33). To the best of our knowledge there are few studies that have evaluated the relationship between hsCRP, PCT, and HP infection. One study compared the levels of hsCRP and PCT between HP-infected patients, HP-negative patients with dyspepsia, healthy blood donors, and patients with bacteraemia. In that study, HP-positive patients had higher PCT levels than both HP-negative patients and healthy donors but there was no significant difference in the mean of hsCRP levels between these groups. After HP eradication, PCT levels were lower than the levels on admission but hsCRP levels did not differ from HP treatment (34).

In our study we did not include HP-negative patients, patients with chronic diseases, or those diagnosed with peptic ulcers. To date, ours is one of few studies to evaluate the effect of CagA on serum levels of PCT and hsCRP in otherwise healthy HP-infected patients. Because CagA has a relationship with atherosclerosis (35) and hsCRP and PCT can affect coronary artery disease, we hypothesized that CagA-positive patients have higher levels of PCT and hsCRP than CagA-negative individuals. PCT levels were higher in CagA-positive patients but hsCRP

levels were not. This observation may be due to the fact that PCT modulation is more sensitive for bacterial infection and hsCRP can increase in noninfectious inflammation, so CagA-positivity in HP infection only affected PCT levels. HP infection can contribute to atherogenesis and CagA-HP strains enhance coronary artery disease severity by elevating hsCRP and oxidized LDL. Our patients did not have coronary artery disease, so CagA did not increase hsCRP levels.

Our study had some limitations. First of all relatively low number of patients enrolled into the study. Also lack of HP negative group can be considered as limitation. We believe that more studies are needed to explain the association between HP infection, virulence factors, and chronic extragastrointestinal diseases. Also novel biomarkers are needed to predict the prognosis and treatment success of HP infection.

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