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The assessment of carotid intima-media thickness, lipid profiles and oxidative stress markers in *Helicobacter pylori*-positive subjects

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Background/aims: *Helicobacter pylori infection has been suggested to be associated with atherosclerosis. The issue is still controversial. It is well known that abnormal lipid profile and oxidative stress are related to atherosclerosis and the measurement of carotid intima-media thickness. The aim of this study was to investigate carotid intima-media thickness and oxidative stress along with lipid parameters in Helicobacter pylori-positive and -negative subjects.* ***Materials and Methods:*** *Thirty Helicobacter pylori-positive subjects and 31 Helicobacter pylori-negative subjects were enrolled. Helicobacter pylori infection was diagnosed by noninvasive tests. Serum total oxidant status and total antioxidant capacity levels were measured. Oxidative stress index was calculated based on total oxidant status/total antioxidant capacity ratio. Traditional cardiovascular risk factors were recorded, and laboratory analysis included measurement of serum triglycerides, high-density lipoprotein cholesterol and low-density lipoprotein cholesterol. carotid intima-media thickness was assessed by high-resolution ultrasound.* ***Results:*** *We found that the mean and maximum values of right and overall carotid intima-media thickness in Helicobacter pylori-positive subjects were significantly thicker than in Helicobacter pylori-negatives ($p<0.05$). Serum triglycerides levels of Helicobacter pylori-positive subjects were significantly higher than in Helicobacter pylori-negatives ($p<0.05$). Total oxidant status, total antioxidant capacity and oxidative stress index values were significantly higher in Helicobacter pylori-positive subjects compared with negatives ($p<0.05$). No significant correlation was observed between oxidative stress markers and carotid intima-media thickness values.* ***Conclusions:*** *Carotid intima-media thickness, total oxidant status, total antioxidant capacity, oxidative stress index, and triglycerides values are increased in Helicobacter pylori-positive subjects compared to Helicobacter pylori-negatives. These data indicated that Helicobacter pylori infection may have a role in atherosclerotic processes.*

Key words: Helicobacter infections, atherosclerosis, oxidative stress, hypertriglyceridemia

Helikobakter pilori pozitif bireylerde karotis intima media kalınlığı, lipid profilleri ve oksidatif stres belirteçlerinin çalışılması

Giriş ve Amaç: *Helikobakter pilori infeksiyonunun ateroskleroz ile ilişkili olduğu ileri sürülmüştür. Bu konu halen çelişkilidir. Anormal lipid profili ve oksidatif stresin ateroskleroz ve karotis intima media kalınlığı ile ilişkili olduğu iyi bilinmektedir. Bu çalışmanın amacı Helikobakter pilori pozitif ve negatif bireylerde karotis intima media kalınlığı ve oksidatif stresin belirteçlerini lipid parametreleri doğrultusunda araştırmaktır.* ***Gereç ve Yöntem:*** *Çalışmaya 30 Helikobakter pilori pozitif birey ve 31 Helikobakter pilori negatif birey katıldı. Helikobakter pilori infeksiyonu noninvaziv testlerle teşhis edildi. Serum total oksidatif status ve total antioksidatif kapasite düzeyleri ölçüldü. Oksidatif stres indeksi total oksidatif status/antioksidatif kapasite oranına dayanarak hesaplandı. Geleneksel kardiyovasküler risk faktörleri kaydedildi ve serum trigliserit, yüksek yoğunluklu lipoprotein kolesterol ve düşük yoğunluklu lipoprotein kolesterol dahil laboratuvar analizleri çalışıldı. Yüksek çözünürlüklü ultrason ile karotis intima media kalınlığı ölçüldü.* ***Bulgular:*** *Helikobakter pilori pozitif bireylerde ortalama ve maksimum sağ ve genel karotis intima media kalınlığı değerlerini Helikobakter pilori negatiflere göre anlamlı olarak daha kalın bulduk ($p<0.05$). Helikobakter pilori pozitif bireylerin serum trigliserit seviyeleri Helikobakter pilori negatiflerden anlamlı olarak daha yüksekti ($p<0.05$). Total oksidatif status, total antioksidatif kapasite, oksidatif stres indeksi değerleri Helikobakter pilori pozitif bireylerde negatiflere kıyasla anlamlı olarak daha yüksekti ($p<0.05$). Oksidatif stresin belirteçleri ve karotis intima media kalınlığı değerleri arasında anlamlı korelasyon gözlenmedi.* ***Sonuç:*** *Helikobakter pilori pozitif bireylerde negatiflere kıyasla karotis intima media kalınlığı, total oksidatif status, total antioksidatif kapasite, oksidatif stres indeksi ve trigliserit değerlerinde anlamlı yükselme vardır. Bu bilgiler Helikobakter pilori infeksiyonunun ateroskleroz mekanizmasında bir rolü olabileceğini işaret eder.*

Anahtar kelimeler: Helikobakter infeksiyonları, ateroskleroz, oksidatif stres, hipertrigliseridemi

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INTRODUCTION

Helicobacter pylori (Hp) is a gram-negative curved bacillus that causes severe gastric pathologies, including chronic active gastritis, peptic ulcer, gastric adenocarcinoma, and type B low-grade mucosa-associated lymphoid tissue lymphoma (1,2). Several studies have demonstrated that *Hp* infection is also associated with the development of coronary atherosclerosis (3). Pathogenesis of atherosclerosis includes abnormal lipid metabolism, endothelial dysfunction, inflammatory and immunological factors, plaque rupture, and smoking (4).

There is increasing evidence that microbial pathogens induce oxidative stress (OS) in infected host cells, and this may present an important mechanism leading to epithelial injury (5). It has been suggested that exposure to sustained high levels of endotoxin constitute a risk factor for atherosclerosis in animal models, and likely in humans (6,7).

Hyperlipidemia is a well-known risk factor for coronary heart disease (6), and oxidative modification of low-density lipoproteins (LDL) in the arterial wall by reactive oxygen species (ROS) plays an important role in atherosclerosis (8).

Existing cardiovascular diseases (CVDs) are found to be correlated with carotid artery intima-media thickness (CIMT) measured by ultrasound and are predictive of CVD in individuals without clinically evident disease. CIMT is now widely used as an early marker for atherosclerotic disease (9).

The aim of the present study was to evaluate the association between OS parameters and CIMT values in patients with *Hp*. For this purpose, we measured the CIMT and compared the serum total antioxidant capacity (TAC) and total oxidant status (TOS) levels and blood lipids between *Hp*-positive and -negative subjects.

MATERIALS AND METHODS

This study was approved by the institutional ethical committee of Akdeniz University, Faculty of Medicine. The subjects were selected among adults who visited the Department of Gastroenterology because of dyspeptic symptoms and were tested with noninvasive methods for *Hp* infection in Akdeniz University Hospital. *Hp* infection was identified by the presence of positivity of *Hp* stool antigen test or carbon 14-labeled urea breath test. We excluded patients who had acute infectious, rheumatologic or cardiovascular disease. For the study, we included 30 *Hp*-infected individuals (14 male, 16 female). Thirty-one healthy noninfected (15 male, 16 female), age- and sex-matched individuals were enrolled in this study as controls. All subjects were informed about the study and their written consent was obtained. We obtained detailed medical history about smoking habits, the presence of diabetes mellitus, hypertension, hyperlipidemia, or family history of CVD, and medications including antihypertensive and antihyperlipidemic drugs. Blood pressure was measured with manual sphygmomanometer. Body mass index (BMI; kg/m^2) was calculated by dividing the body weight (kg) by height squared (m^2). Their routine laboratory tests, which included complete blood count, serum glucose, creatinine, and alanine aminotransferase (ALT) levels, were recorded.

Measurement of Serum Lipid and Lipoprotein Levels

Venous blood samples were obtained following an overnight fasting state. Serum samples were separated and stored at -80°C until the analysis. Serum high-density lipoprotein cholesterol (HDL-C) and triglyceride (TG) levels were measured with the enzymatic colorimetric method by using commercial kits on a Modular PPP auto-analyzer (Roche Diagnostics). Serum LDL-C levels were calculated using Friedewald formula (10).

Measurement of Serum Total Antioxidant Capacity (TAC)

Total antioxidant capacity (TAC) of serum was determined using a novel automated measurement method as previously described (12). In brief, hydroxyl radical, which is the most potent biological radical, was produced. In the assay, ferrous ion solution, which is present in reagent 1, was mixed with hydrogen peroxide, which is present in reagent 2. The sequentially produced radicals, such as brown-colored dianisidinyl radical cation produced by the hydroxyl radical, are also potent radicals. Using this method, the antioxidative effect of the sample on the potent free radical reactions initiated by the produced hydroxyl radical was determined. The assay achieved excellent precision values lower than 3%. The results were expressed as mmol Trolox equivalent/L.

Measurement of Total Oxidant Status (TOS)

Total oxidant status (TOS) of serum was determined using a novel automated measurement method as previously described (13). Oxidants present in the sample oxidized the ferrous ion-o-dianisidine complex to ferric ion. The oxidation reaction

was enhanced by glycerol molecules abundantly present in the reaction medium. The ferric ion produced a colored complex with xylenol orange in an acidic medium. The color intensity, which could be measured spectrophotometrically, was related to the total amount of oxidant molecules present in the sample. The assay was calibrated with hydrogen peroxide, and the results were expressed in terms of micromolar hydrogen peroxide equivalent per liter (μmol H_2O_2 equivalent/L).

Determination of Oxidative Stress Index (OSI)

The ratio of TOS to TAC was accepted as the oxidative stress index (OSI). For calculation, the resulting unit of TAC was changed to mmol/L, and the OSI value was calculated according to the following formula (14): OSI (arbitrary unit) = TOS (μmol H_2O_2 equivalent/L)/TAC (mmol Trolox equivalent/L).

Ultrasound Scanning Procedure

Subjects were evaluated for CIMT and plaque occurrence using high-resolution grey-scale Doppler ultrasonography. In a semidark room, all subjects lay supine with their necks slightly hyperextended and rotated away from the imaging transducer. Both carotid arteries were scanned. CIMT was defined as the distance between the leading edge of the lumen intimal interface and the leading edge of the media adventitia interface of the far wall (9).

Statistical Analysis

Calculations were performed with a statistical software package (SPSS for Windows, Version 16.0, SPSS Inc, Chicago, IL). Quantitative data were expressed as mean (±SD) or as medians. The comparisons of parameters were performed using Student's t-test and Mann-Whitney U test. Correlation analyses were performed using Spearman's correlation test. A p value of <0.05 was considered as significant.

RESULTS

We included 30 subjects (14 male, 16 female) infected with *Hp* and 31 subjects (15 male, 16 female) without *Hp* infection. The demographic and clinical characteristics of the study population are shown in Table 1. There were no statistically significant differences between the two groups with regard to demographic and clinical characteristics (age, gender, BMI, smoking habits, history of diabetes mellitus, hypertension, family history for CVD).

As shown in Table 2, serum TG levels were significantly higher in *Hp*-positive subjects than *Hp*-negative subjects (161±70 mg/dl vs 117±64 mg/dl, p<0.05). There were no statistically significant differences in HDL-C and LDL-C levels between the two groups. Serum TOS and TAC levels and OSI ratio were significantly higher in the *Hp*-positive group than in the control group (17.5±6.2 vs 12.7±7.7, p<0.05; 2.22±0.24 vs 1.95±0.23, p<0.05; 0.78±0.24 vs 0.65±0.31, p<0.05, respectively).

Structural measurements of vessels in *Hp*-positive and -negative subjects at enrollment are shown in Table 3. Atherosclerotic plaques in the common carotid artery were shown in 1% (3 of 30) of *Hp*-positive patients and only 0.3% (1 of 30) of control subjects. The mean and maximum values of the

Table 1. Demographic and clinical characteristics of *Helicobacter pylori*-positive and -negative subjects

Parameter	*Hp*-positive group (n=30)	Control group (n=31)	P value
Mean age (year)	40.9±10.3	42.3±9.4	NS
Male/Female	14/16	15/16	NS
DM	1	2	NS
HT	4	4	NS
HPL	8	10	NS
Smoking	12	15	NS
Family history of CVD	3	4	NS
SBP (mmHg)	119±3	121±8	NS
DBP (mmHg)	78.5±8.8	79±6.6	NS
Mean BMI (kg/m²)	27.1±3.7	26.2±3.8	NS

DM: Diabetes mellitus. HT: Hypertension. HPL: Hyperlipidemia. CVD: Cardiovascular disease. SBP: Systolic blood pressure. DBP: Diastolic blood pressure. BMI: Body mass index
Results are expressed as mean±SD or number of patients.

Table 2. Biochemical variables in *Helicobacter pylori*-positive and -negative subjects

Parameter	*Hp*-positive group (n=30)	Control group (n=31)	P value
Hb (g/dl)	13.6±1.8*	13.8±1.5	NS
WBC (x10³/mm³)	7380±1450	7566±1980	NS
Glu (mg/dl)	99±39	90±12.2	NS
ALT (U/L)	28.4±21	25.6±23	NS
LDL-C (mg/dl)	106±31	110±30	NS
HDL-C (mg/dl)	44.7±11.7	47±15	NS
TG (mg/dl)	161±70	117±64	0.014
Cr (mg/dl)	0.72±0.15	0.77±0.11	NS
TOS (μmol H_2O_2 Equiv./L)	17.5±6.2	12.7±7.7	0.002
TAC (mmol Trolox Equiv./L)	2.22±0.24	1.95±0.23	0.000
OSI	0.78±0.24	0.65±0.31	0.027

Hb: Hemoglobin. WBC: White blood cell. Glu: Glucose. ALT: Alanine aminotranferase. LDL-C: Low-density lipoprotein-cholesterol. HDL-C: High-density lipoprotein-cholesterol. TG: Triglyceride. Cr: Creatinine. TOS: Total oxidant stress. TAC: Total antioxidant capacity. OSI: Oxidative stress index.
* Results are expressed as mean ± SD

Table 3. Structural and functional parameters of vessels for *Helicobacter pylori*-positive and -negative subjects

Parameter	*Hp*-positive group (n=30)	Control group (n=31)	P value
Mean right CIMT (mm)	0.70±0.09*	0.64±0.06	0.007
Max right CIMT (mm)	0.81±0.10	0.74±0.07	0.012
Mean left CIMT (mm)	0.72±0.14	0.67±0.08	NS
Max left CIMT max (mm)	0.83±0.15	0.79±0.1	NS
Mean general CIMT (mm)	0.71±0.10	0.65±0.06	0.024
Max general CIMT (mm)	0.82±0.11	0.77±0.01	0.040
Plaque	3	1	

CIMT: Carotid intima-media thickness.
* Results are expressed as mean ± SD or number of patients.

right CIMT were significantly increased in *Hp*-positive subjects compared with -negative subjects ($p<0.05$). The mean and maximum values of left CIMT tended to be higher in subjects with *Hp*, but the differences were not statistically significant between the two groups. We calculated mean and maximum overall CIMT by using left and right CIMT measurements. The mean and maximum values of overall CIMT were significantly higher in *Hp*-positive subjects than negative subjects ($p<0.05$) (Table 3).

Serum TG levels were significantly correlated with maximum right CIMT value ($r=0.293$, $p<0.05$), serum TAC levels ($r: 0.478$, $p<0.05$), serum TOS levels ($r: 0.381$, $p<0.05$), and OSI ratio ($r: 0.281$, $p<0.05$). No significant correlation was found between TOS, TAC, OSI values and CIMT values.

DISCUSSION

A relation between atherosclerosis and chronic *Hp* infection was found in epidemiological studies (15-17). Pellicano et al. (18) reported significantly higher prevalence of *Hp* infection in patients with coronary artery disease than in controls (77% vs 59%). Although these studies have suggested a relationship between *Hp* infection and coronary heart disease, some of the underlying mechanisms still need to be discovered. It has been reported that chronic *Hp* infection results in these lipid alterations that could partially contribute to the initiation and development of coronary atherosclerosis (6,19,20). Infection and inflammation are associated with dyslipidemia. Induction of changes in lipoproteins by cytokines indirectly predisposes patients to atherosclerosis (21). In the present study, we did not find any significant difference in

HDL-C and LDL-C levels between *Hp*-positive and -negative subjects. However, *Hp*-positive subjects had significantly higher plasma TG levels than negative subjects. Laurila et al. (22) found significantly increased TG (1.17 vs 1.00 mmol/L) and total cholesterol (6.34 vs 5.87 mmol/L) levels in 460 *Hp*-positive subjects compared with 269 *Hp*-negative subjects, but HDL-C levels were found to be similar in the two groups.

Major risk factors of atherosclerosis may explain only 50% of its etiology. Therefore, a search for new risk factors for atherosclerosis is necessary. Several studies have determined the potential role of OS in atherosclerosis. OS can lead to modification of LDLs in the arterial wall and endothelial injury. Microbial pathogens can induce OS in infected host cells (5). *Hp* causes inflammation of the gastric mucosa by inducing infiltration of neutrophils, macrophages and lymphocytes, which leads to tissue damage (23).

Since a large variety of oxidant system and antioxidant system members are produced in an organism, we used TOS and TAC measurements to reflect all oxidant and antioxidant systems. We also used OSI, the ratio of the total plasma TOS level to TAC, as an indicator of OS. OSI reflects the redox balance between oxidation and anti-oxidation (12,13). Only a few studies have investigated serum TOS and TAC levels in *Hp*-infected subjects. Similar to previous studies, we found a significant increase in serum levels of TOS and OSI in the *Hp*-positive group compared with controls (23-25).

We found a significant increase in TAC levels in the *Hp*-positive group compared with the controls. According to our knowledge, our study is the first showing that *Hp*-positive subjects had higher serum TAC levels than controls. These increased TAC levels might be considered as a response to increased OS in these patients.

There is considerable evidence suggesting that ultrasonic measurements of early atherosclerosis are clinically significant. In prospective studies, increased IMT has been related to an increased risk of CVDs (26,27). There are conflicting data regarding CIMT and *Hp* infection. Some researchers have reported no relationship between *Hp* and CIMT (21,28,29). However, Hamed et al. (30) reported a significant association between the two in diabetic patients. In our study, the mean and maximum values of right and overall CIMT were significantly increased in *Hp*-positive subjects compared with -negative subjects. The mean and maximum values of left CIMT tended to be higher in *Hp*-infected subjects, but the differences were not significant between the two groups.

Although serum TOS and TAC levels and cardiovascular risk factors have been examined in *Hp*-infected subjects previously (16,19,20,24,25), to our knowledge, this study is the first to investigate the association between serum TOS and TAC levels and lipid profile with CIMT in *Hp*-infected subjects. We determined the correlations between TOS, TAC, OSI, lipid profile, and CIMT. While max right CIMT was positively correlated with TG, it showed no correlation with TOS, TAC and OSI.

The current study has certain limitations. Our study group was limited. Because of using noninvasive methods, we could not determine the grade of gastric inflammation or virulence factors of *Hp*.

In conclusion, right and overall CIMT were significantly thicker with increased levels of TOS, TAC, and OSI in our *Hp*-positive patients. Although we found no correlation between CIMT and TOS, TAC and OSI values, these results implied that increased OS may have an important role in vascular structural changes induced by *Hp* infection. This study suggests that high TG levels in *Hp*-positive subjects may trigger OS and atherosclerotic changes. Further studies with larger populations are needed to explore whether there is a strong relationship between OS and atherosclerosis in *Hp* infection.

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