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Relationship between Helicobacter pylori and thiol-disulfide homeostasis: A prospective observational study

Helicobacter pylori ve tiyol-disülfid homeostazı arasındaki ilişki: Prospektif, gözlemsel bir çalışma

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Abstract

Aim: Helicobacter pylori (HP) infection causes inflammation and oxidative stress at a cellular level. In the present study, we aimed to evaluate the possible relationship between HP and thiol-disulfide homeostasis (TDH), a novel indicator of oxidative stress.

Methods: Medical data of a total of 53 patients admitted with persistent dyspepsia and undergoing gastroscopy were evaluated prospectively. The patients were divided into two groups, based on the result of gastric biopsy, as HP-positive (+) and HP-negative (-). Demographic data, ferric-reducing ability of plasma (FRAP), ischemia-modified albumin (IMA), native thiol, total thiol, disulfide, and malondialdehyde (MDA) levels of the patients were recorded and compared between the two groups.

Results: The native thiol (451.03 mmol/L vs. 407.03mmol/L, p=0.005) and total thiol (493.20 mmol/L vs. 456.40 mmol/L, p=0.027) levels were significantly higher in the HP (+) group than in the HP (-) group. The disulfide levels and disulfide/native thiol, disulfide/total thiol and native thiol/total thiol ratios were similar between the HP (+) and HP (-) groups. Although the FRAP was lower in the HP (+) group than in the HP (-) group, this difference was not statistically significant (0.94 mmol/L vs. 1.10 mmol/L). No statistically significant difference was found between the groups in the IMA and MDA levels..

Conclusion: In this study, oxidative status of HP patients was evaluated in several different methods. Among them, only elevated native thiol and total thiol levels were found in HP-induced gastritis. There is a need for further studies involving a larger number of patients and a subgroup analysis to examine whether elevated serum thiol-disulfide levels in HP infection suggest an antioxidant or pro-oxidant status.

Key words: Helicobacter pylori, homeostasis, thiol, disulfide, oxidative stress

Öz

Amaç: Helicobacter pylori (HP) enfeksiyonu, hücresel düzeyde inflamasyona ve oksidatif strese neden olur. Bu çalışmada, oksidatif stresin bir göstergesi olan tiyol-disülfid homeostazı (TDH) ve HP arasındaki olası ilişkiyi değerlendirmeyi amaçladık.

Yöntemler: Dispepsi yakınması ile başvuran ve gastroskopi uygulanan toplam 53 hastanın tıbbi verileri prospektif olarak değerlendirildi. Hastalar gastrik biyopsi sonucuna göre HP-pozitif (+) ve HP-negatif (-) olarak iki gruba ayrıldı. Hastaların demografik verileri, plazma ferik indirgeme kabiliyeti (FRAP), iskemi modifiye albümin (IMA), native tiyol, total tiyol, disülfid ve malondialdehit (MDA) düzeyleri kaydedildi ve iki grup arasında karşılaştırıldı.

Bulgular: Doğal tiyol (451,03 mmol/L ve 407.03 mmol/L, p=0.005) ve toplam tiyol (493.20 mmol/L ve 456.40 mmol/L, p=0,027) seviyeleri HP (+) grubunda HP (-) grubuna göre anlamlı olarak daha yüksekti. Disülfid düzeyleri ve disülfid / native thiol, disülfid/total tiyol ve native tiyol / total tiyol oranları HP (+) ve HP (-) grupları arasında benzerdi. FRAP, HP (+) grubunda HP (-) grubuna göre daha düşük olmasına rağmen, bu fark istatistiksel olarak anlamlı değildi (0,94 mmol/L ve 1,10 mmol/L). IMA ve MDA düzeylerinde gruplar arasında istatistiksel olarak anlamlı bir fark saptanmadı.

Sonuç: HP kaynaklı gastrit gibi oksidatif stresin arttığı koşullar altında serum tiyol-disülfid düzeylerinin yükselmesi görülebilir. HP enfeksiyonunda yüksek serum tiyol-disülfid düzeylerinin antioksidan veya pro-oksidan durumu gösterip göstermediğini incelemek için daha fazla sayıda hasta ve alt grup analizi içeren çalışmalara ihtiyaç vardır.

Anahtar Kelimeler: Helicobacter pylori, homeostaz, tiyol, disülfid, oksidatif stres

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Introduction

Helicobacter Pylori (HP) is a spiral gram-negative, microaerophilic bacterium, highly adapted, that selectively colonizes the human stomach [1]. It is transmitted by fecal-oral, gastro-oral and oral-oral routes, assuming that the infection is mainly in childhood. According to statistics, it affects around 3-4 billion people worldwide, with the rate of infection in developing countries very high, reaching up to 80% [2]. During the host colonization process, HP induces a strong inflammatory response, characterized histologically by superficial epithelial degeneration and infiltration of the gastric mucosa by inflammatory cells [3].

HP results in reactive oxygen species (ROS) and reactive nitrogen species (RNS) production from the gastric mucosa. Low and moderate amounts of ROS have a beneficial effect on several physiological processes including the killing of invading pathogens, wound healing and tissue regeneration processes [4]. Although immune system is capable of creating an immune response to the infection, it usually fails to clear HP. The inability of the host to clear HP results in a chronic inflammation with continued oxidative stress within the gastric tissue. Determination of oxidative stress in gastric inflammation may be necessary for a better understanding of its pathophysiology [5].

Oxidative stress plays an important role in the pathogenesis of inflammation, the level of which is measured based on such parameters as FRAP, IMA, MDA, and thiol-disulfide

[6-9]. Moreover, a relationship between oxidative stress and the development of HP infection has been demonstrated in many studies [5, 10-12]. Elevated serum thiol-disulfide levels can be seen under conditions with increased oxidative stress such as HP-induced gastritis. In the present study, we aimed to evaluate the possible relationship between HP and thiol-disulfide homeostasis, a novel indicator of oxidative stress.

Material and methods

Medical data of a total of 53 patients who were admitted to hospital between April 2018 and June 2018 with persistent dyspepsia, and had undergone gastroscopy, were evaluated prospectively. Detailed information about the procedure and study was supplied to all patients and written informed consent was obtained. The study protocol was approved by the local Ethics Committee. The study was conducted in accordance with the principles of the Declaration of Helsinki. Ethical approval was obtained from the hospital's ethical committee (2011-KAEK-25 2018/06-06).

Inclusion and Exclusion Criteria

This study included 53 literate patients with chronic dyspeptic complaints (lasting more than three months), aged between 18 and 70 years, who agreed to participate in the study. Patients with varicose, inflammatory or active ulcerous lesions in the upper gastrointestinal tract that could cause hemorrhage and those with a previous history of gastric surgery were excluded.

Interventions

Gastroscopic interventions were performed under sedation by a single endoscopist in the endoscopy unit. A biopsy was obtained from four different quadrants in the antral mucosa. difference between the groups (p=0.388). FRAP was also lower in the HP (+) group, although the difference between the two groups was not statistically significant (p=0.059). The mean A 5-mL blood sample was withdrawn into two separate tubes before the gastroscopic intervention, and the samples were centrifuged at 4,000 rpm for 10 min. The sera were stored in the Eppendorf tubes at -80°C.

After the measurement of weight and height according to the standard protocol, body mass index (BMI) was calculated using the following formula: BMI (kg/m^2) = weight (kg) / height^2 (m^2). Blood pressure measurements were performed at the out-patient clinics of the general surgery department according to the standard protocol.

Grouping

The patients were divided into two groups, as 27 patients with HP infection (HP-positive) or 26 patients without HP infection (HP-negative), based on the results of a pathological examination of gastric biopsies. Participants who never smoked were recorded as smoking (-) patients and active smokers were the smoking (+) group.

Outcomes

Demographic data, the ferric reducing ability of plasma (FRAP), ischemia-modified albumin (IMA), native thiol, total thiol, disulfide, and malondialdehyde (MDA) levels of all patients were recorded. IMA levels were analyzed spectrophotometrically according to the method described by Bar Or et al. [10]. Serum TDH was analyzed by a novel automated method [13]. Total antioxidant capacity (TAC) was measured with the FRAP method which measures all available antioxidants aside from thiols [7].

Statistical Analysis

Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS) version 21.0 software (IBM Corp., Armonk, NY, USA). The Shapiro-Wilk test was used to evaluate whether the variables were normally distributed. The data were expressed in mean ± standard deviation (SD) or median (min-max) and number and frequency. Based on the results of the normality test, either an independent samples t-test or Mann-Whitney U-test was used to compare the groups. The categorical variables were compared using a chi-square test. A binary logistic regression analysis was carried out to examine the independent risk factors affecting the occurrence of HP. A p value of <0.05 was considered statistically significant.

Results

Of the study patients, 28.3% were males and 71.7% were females with a mean age of 46.28 ± 9.57 years and a mean body mass index of (BMI) of 28.03±5.6 kg/m^2. Comparisons of HP groups with baseline demographic and clinical characteristics of patients are shown in Table 1. There was no significant difference between the two groups in terms of age, sex, BMI, systolic and diastolic blood pressure, and smoking status.

The laboratory data of the patients in the HP groups are presented in Table 2. The native thiol and total thiol levels were significantly higher in the HP (+) group than in the HP (-) group (p=0.005 and p=0.027, respectively) (Table2 and Figure 1 and Figure2).

There was no significant difference between the groups in terms of the disulfide levels, disulfide/native thiol, disulfide/total thiol, and native thiol/total thiol ratios. The mean IMA value was 0.81-absorbance unit (au) in the HP (+) group and 0.84 au in the HP (-) group, indicating no statistically significant

MDA level was 3.9 mol/L in the HP (+) group and a slightly lower 3.8 mol/L in the HP (-) group, although not statistically significant.

Table 1: Comparisons of general characteristics in Helicobacter Pylori groups.

Variable	HP (+)	HP (-)	p
Age (years) †	43.85±8.80	48.81±9.85	0.059
Sex (F/M) ‡	20 (74.1)/7(25.9)	18 (69.2)/8(30.8)	0.696
BMI (kg/m^2) ¶	27.34 (19.53-46.07)	26.72 (17.58-52.79)	0.838
Systolic BP (mmHg) ¶	126.50 (102-210)	138 (110-187)	0.332
Diastolic BP (mmHg) ¶	82.50 (65-122)	86 (70-137)	0.248
Smokers ‡	4 (14.8)	7 (26.9)	0.277

†: mean ± standard deviation, ‡: n (%), ¶: median (min-max).
HP: Helicobacter pylori; BMI: body mass index, BP: blood pressure.

Table 2. Comparisons of the laboratory results in Helicobacter Pylori groups.

Variable	HP (+) (n=27)	HP (-) (n=26)	P
Albumin (g/dL) ¶	4.30 (2.80-5.20)	4.10 (1.70-5.30)	0.161
IMA (AU) ¶	0.81 (0-0.97)	0.84 (0.61-0.96)	0.388
IMA/Albumin ¶	0.19 (0-0.32)	0.20 (0.11-0.53)	0.168
Native thiol (mmol/lt) †	451.03±55.95	407.03±52.19	0.005
Total thiol (mmol/lt) ¶	493.20 (411.80-675.20)	456.40 (344.90-548)	0.027
Disulfide (mmol/lt) ¶	21.95 (5.30-37.25)	22.63 (14.40-41.25)	0.612
SS/native thiol (%) ¶	4.95 (0.90-7.33)	5.59 (3.45-10.72)	0.068
SS/total thiol(%) ¶	4.50 (0.90-7.33)	5.03 (3.23-8.83)	0.070
Native/total thiol (%) ¶	90.99 (85.35-98.21)	89.95 (82.34-93.54)	0.070
FRAP (µmol/L) ¶	0.94 (0.64-2.07)	1.10 (0.61-1.87)	0.059
Malondialdehyde (µmol/L) †	3.9±1.0	3.8±1.0	0.610

†: mean ± standard deviation, ¶: median (min-max).
HP: Helicobacter pylori, IMA: ischemia-modified albumin, FRAP: ferric reducing ability of plasma, AU: absorbance unit, SS: disulfide.

Discussion

In this study, the possible relationship between HP and thiol-disulfide homeostasis was evaluated. The native thiol and total thiol levels were significantly higher in the HP (+) group than in the HP (-) group. No significant differences between the groups were observed in FRAP, IMA, MDA and disulphide levels. The FRAP levels was lower in the HP (+) group, but the difference between the two groups was not statistically significant.

A study conducted in humans showed higher MDA levels in the gastric mucosa of HP-infected patients, compared to healthy tissues, and that MDA levels returned to normal after HP eradication [8]. Remarkably increased serum MDA, catalase, and superoxide dismutase levels were demonstrated in patients with HP infection [14], although, in the present study, we found no statistically significant difference between the MDA levels of the two groups. IMA increases in oxidative stress conditions associated with ischemia and is considered a biomarker of oxidative stress, which is, in turn, associated with chronic kidney disease, hypercholesterolemia, and type2 diabetes mellitus [15-17]. In the present study, we found no statistically significant difference in IMA and albumin levels or IMA/albumin ratios.

FRAP is one of the most widely used technique for the measurement of total antioxidant capacity [7]. Several studies have reported total oxidant status, total antioxidant capacity, and oxidative stress index to be significantly higher in HP-infected patients than in non-HP infected patients [18]. However, in our study, we found no statistically significant difference between the FRAP levels of the two groups.

Interestingly, contrary to expectations, a comparison of the native thiol and total thiol parameters of the HP (+) and HP (-) groups revealed significantly higher levels in the HP (+) group in our study. Thiols are considered to be the main antioxidant buffer, and play an important role in protecting against the detrimental effects of ROS [19]. Proteins containing thiol groups act as an antioxidant buffer, but are also involved in the regulation of the redox system [20]. Thiols are both antioxidant and pro-oxidant molecules, and although thiols are mostly considered antioxidant molecules, they may act as pro-oxidant molecules, depending on the physical status of the organism [21, 22]. The level of oxidative stress in an organism is the determinant of the behavior of thiols, and this is maintained with a dynamic balance in the body. Naja et al. has found no significant difference in the plasma thiol level in HP infected patients compared with controls [23]. Baykan et al. reported that there was no difference between the total thiol, native thiol, disulphide/native thiol and dissulphide/total thiol ratios of the HP (+) patients and control group [24]. They claimed severity of inflammation affected their results. In the present study, disulfide levels, disulfide/native thiol ratio, and disulfide/total thiol ratio were found to be similar between the two groups, suggesting that TDH is not impaired in the HP (+) group.

Nonetheless, there are some limitations to the present study. First, our sample size was relatively small. Second, it was conducted in a single center. Therefore, the results cannot be generalized to other populations or settings. Smokers are another limitation of the study since smoking may interfere with the results. We recommend further large-scale, multi-center, prospective studies to establish a definitive conclusion.

In conclusion, we evaluated oxidative status of HP patients in several different methods. Among them, only elevated native thiol and total thiol levels were found in HP-induced gastritis. There is a need for further studies involving a larger number of patients and a subgroup analysis to examine whether elevated serum thiol-disulfide levels in HP infection suggest an antioxidant or pro-oxidant status.

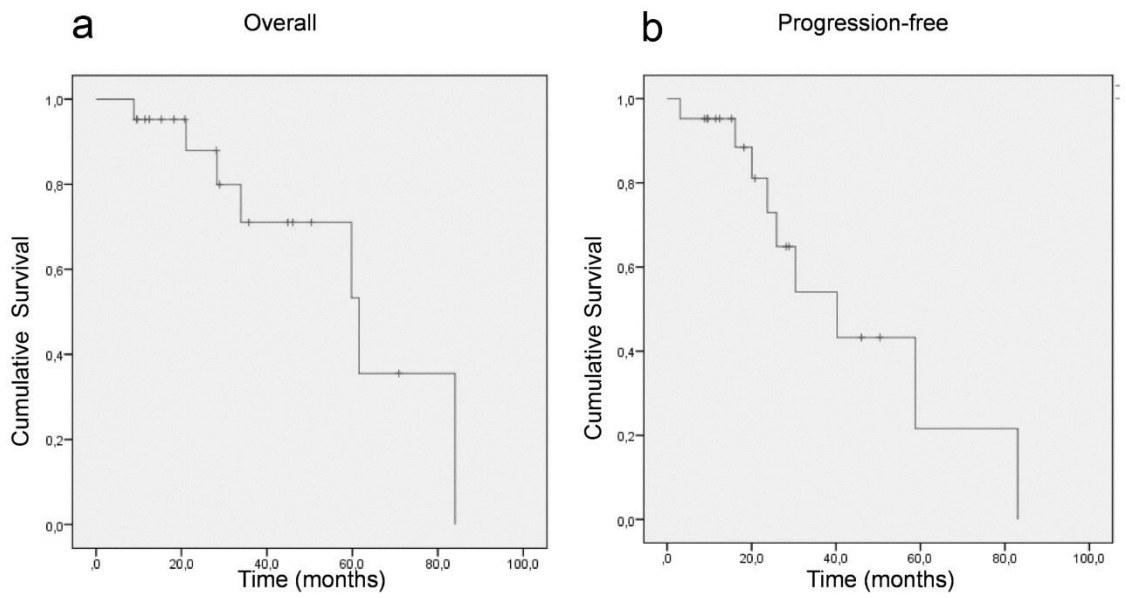

Figure1. Relationship between native thiol (mmol/lt) and Helicobacter pylori.
HP: Helicobacter pylori.

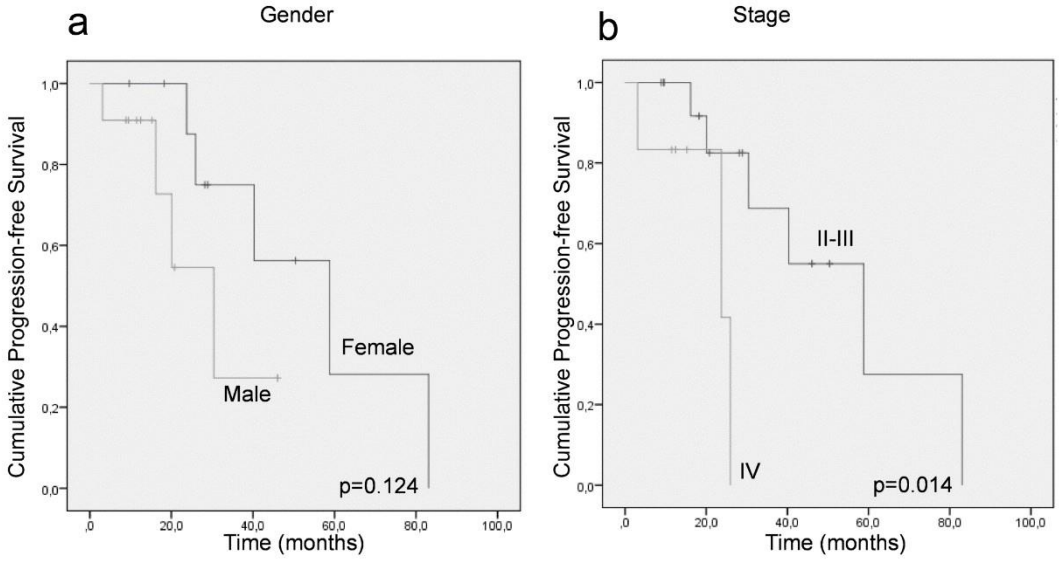

Figure2. Relationship between total thiol (mmol/lt) and Helicobacter pylori.
HP: Helicobacter pylori.

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