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The effect of decrease in serum nitric oxide concentration on portal hemodynamics after *Helicobacter pylori* treatment: An open-label pilot study

Helikobakter pilori tedavisinden sonra serum nitrik oksit konsantrasyonundaki azalmanın portal hemodinamikler üzerine etkisi: Açık etiketli pilot çalışma

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Background/aims: *Helicobacter pylori-induced gastritis increases serum nitrate and nitrite concentrations. The relationship between splanchnic hemodynamics and nitrate and nitrite levels has been demonstrated. We aimed to determine the effect of Helicobacter pylori eradication treatment on portal hemodynamics.* ***Methods:*** *Nineteen patients with liver cirrhosis and Helicobacter pylori gastritis were included. Nine patients had esophageal varices indicating over portal hypertension. After histopathologic examination of gastric mucosa, Doppler ultrasonographic measurement of the portal veins and serum sampling to determine nitrate and nitrite concentration, treatment for Helicobacter pylori eradication was initiated for each patient. Evaluation of histopathology, Doppler measurements and sampling for serum nitrate and nitrite levels were repeated six weeks after the end of therapy.* ***Results:*** *The rate of eradication of Helicobacter pylori was 73.7%. The median inflammation score, Helicobacter pylori density, and the median serum concentration of nitrate and nitrite decreased significantly after therapy (p values were 0.021, 0.001, and 0.018, respectively). After treatment, the patients with varices showed significant decreases in serum nitrate and nitrite levels whereas those without varices did not. Considering portal measurements, alteration in the congestion index approached statistical significance (0.15 versus 0.1; p=0 .066) in the patient group with varices.* ***Conclusions:*** *Reducing effect of Helicobacter pylori eradication treatment on serum nitrate and nitrite concentration seems to have some beneficial influence on portal hemodynamics.*

Key words: Helicobacter pylori, nitric oxide, portal hemodynamics

Amaç: *Helikobakter pylori gastriti serum nitrat ve nitrit konsantrasyonunu artırmaktadır. Splanknik hemodinamiklerle serum nitrat ve nitrit seviyesi arasında ilişki olduğu gösterilmiştir. Helikobakter pilori eradikasyon tedavisinin portal hemodinamikler üzerindeki etkilerini incelemeyi amaçladık.* ***Metod:*** *Karaciğer sirozu ve Helikobakter pilori gastriti olan 19 hasta çalışmaya alındı. Dokuz hastada belirgin portal hipertansiyonu gösteren özofagus varisleri vardı. Gastrik mukozanın histopatolojik değerlendirmesi, portal venlerin Doppler ultrasonografik ölçümü ve nitrat ve nitrit konsantrasyonu için serum örneklemesi yapıldıktan sonra hastalara Helikobakter pilori eradikasyon tedavisi başlandı. Histopatoloji, Doppler ölçümleri ve serum nitrat ve nitrit seviyeleri tedavi bitiminden 6 hafta sonra tekrar değerlendirildi.* ***Bulgular:*** *Helikobakter pilori eradikasyon oranı %73,7 idi. Mukozanın inflamasyon skoru, Helikobakter pilori yoğunluğu ve serum nitrat ve nitrit konsantrasyonu tedavi sonrasında anlamlı biçimde azaldı (p değerleri sırasıyla 0,021; 0,001 ve 0,018 idi). Tedaviden sonra serum nitrat ve nitrit seviyesi varisli olan hastalarda anlamlı azalma gösterirken varisi olmayanlarda anlamlı değişiklik olmadı. Portal ölçümler açısından konjesyon indeksindeki değişiklik varisli hasta grubunda istatistiki anlama yaklaştı (0,15'e karşılık 0,1; p=0,066).* ***Sonuç:*** *Helikobakter pilori eradikasyon tedavisinin serum nitrat ve nitrit konsantrasyonu üzerine azaltıcı etkisinin portal hemodinamikler üzerine faydalı etkileri olabilir.*

Anahtar kelimeler: Helikobakter pilori, nitrik oksit, portal hemodinamikler

INTRODUCTION

Inflammation of gastric mucosa by *Helicobacter pylori (H. pylori)* is associated with increased mucosal inducible nitric oxide synthase (iNOS) enzymatic activity and has been demonstrated in both *in vivo* and in vitro studies (1, 2). Nitric oxide (NO) and its products (nitrates and nitrites, or NOx) increase not only in gastric mucosa but also in the serum of patients with *H. pylori* infection (3).

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Endothelial NOS (eNOS) is upregulated in the splanchnic circulation in patients with chronic liver disease (4), while its function is downregulated in the liver (5). NO appears to be a key mediator of hyperdynamic circulation in cirrhosis and has been shown to play a prominent role in mediating splanchnic vasodilation. The results of many studies strongly support the hypothesis that increased NO production is a major factor in the peripheral arterial vasodilation of cirrhosis. An association between portal hypertension and hyperdynamic circulation was first described by Abelmann in 1953 (6). Peripheral vasodilation seems to produce a hyperdynamic circulatory state by an increase in cardiac output due to afterload reduction (7). Splanchnic vasodilation as a part of peripheral vasodilation is a key process not only for portal hypertension but also for some severe complications of cirrhosis such as hepatorenal syndrome.

We designed this study to examine whether or not the expected decrease in the serum level of NOx after *H. pylori* treatment had any effect on portal hemodynamics in cirrhotic patients.

MATERIALS AND METHODS

Patients

We included 19 non-creatinemic cirrhotic patients (median age: 58 years; range: 41-76 years; 9 women, 10 men) with *H. pylori* gastritis diagnosed by histopathologic examination of mucosal specimens taken during endoscopies performed for esophageal varices evaluation. The diagnosis of cirrhosis was made on clinical, radiological or histological basis. One patient had undergone band ligation therapy of esophageal varices three times two years before. The patients were requested to avoid drugs such as proton pump inhibitors, antibiotics and β-blockers that may influence the results of tests used in this study in the four weeks prior to the study and during the study period. Blood samples were collected after overnight fasting. After serum samples had been collected for NOx measurement and after Doppler ultrasonography (USG) had been performed to evaluate portal venous homodynamics (mean blood flow velocity, blood flow volume, and congestion index [CI]), patients received *H. pylori* eradication regimen, mostly classical triple therapy (lansoprazole, 20 mg twice daily; clarithromycin, 500 mg twice daily; and amoxicillin, 1000 mg twice daily). Serum was stored at -80°C until the measurement of NOx. Endoscopic biopsy and Doppler USG were repeated six weeks after the end of therapy.

Informed consent was obtained from each subject before examination.

Histopathologic Evaluation

Two biopsy specimens, each taken from the antrum and the corpus, were assessed using the Sydney classification for *H. pylori* density and inflammation. The tissues were prepared with hematoxylin and eosin and Giemsa staining.

Serum NOx Measurement

Nitrite and nitrate levels were determined using a photometric endpoint determination (Roche Diagnostic GmbH, Mannheim, catalog No: 1 756 281). The principle depends on reduction of nitrate to nitrite by nicotinamide adenine dinucleotide phosphate in the presence of the enzyme nitrate reductase. The nitrite reacts with sulfanilamide and N-(1-naphthyl)-ethylenediamine dihydrochloride to give a red-violet diazo dye. The diazo dye is measured on the basis of its absorbance in the visible range (550 nm).

Doppler USG for Portal Vein Measurement

USG examinations were performed using a high-definition imaging 5000 sonography machine (Philips Medical Systems, Bothell, WA, USA) with a 3.5-5 MHz convex transducer. All ultrasound examinations were performed by the same radiologist (MAP). Before volume flow measurements were performed, routine gray-scale examinations of the liver and spleen were made. The portal vein was assessed with color Doppler imaging first to exclude thrombosis or stenosis, and then by duplex Doppler imaging for flow velocity and volume measurements. The standard protocol of Doppler USG examination of the portal vein included diameter, area, mean blood flow velocity, blood flow volume, and congestion index. Portal blood velocity was measured at the site of the main portal vein in the supine position after more than eight hours of fasting. The Doppler insonation angle was adjusted below 60°. The portal blood volume measurements were automatically calculated using the software of the sonography machine. The CI of the portal vein was calculated according to Moriyasu's formula (8): [cross-sectional area/mean portal flow velocity (PFV)]/10. The cross-sectional area was derived from the diameter, assuming a circular shape of the vessel, and mean PFV was the time-averaged maximum velocity x 0.57.

Statistical Analyses

Results are presented as the median and range. We compared paired data before and after eradication treatment using the nonparametric Wilcoxon test. A *P* value less than. 05 was considered significant.

RESULTS

Most of our patients had viral etiology and early stage of cirrhosis. All patients except two had only gastritis without duodenal ulcer. Nine patients had some degree of esophageal varices indicating the presence of portal hypertension (Table 1). We did not measure portal pressure in patients with or without varices. In this study, the eradication rate was 73.7% (89% in the group with and 60% in the group without varices; p>0.05). The serum NOx (nitrate and nitrite) concentration decreased significantly after H. pylori eradication therapy [126 µM (range: 47-248 µM) vs 79 µM (range: 30-277 µM); p=0.018]. *H. pylori* eradication clearly reduced serum NOx concentration from 99 µM (range: 46-216 µM) to 61µM (range: 30-277 µM) (p=0.03) in the patient group in which *H. pylori* was eradicated, while this decrease was not significant in the group in which *H. pylori* was not eradicated (p=0.34) (Table 2). Although the level of NOx seemed to be higher in patients whose *H. pylori* was not eradicated than in those with successful treatment, the difference was not significant. Eradication of *H. pylori* also significantly reduced mucosal inflammation (p values for the eradication and noneradication groups were 0.005 and 0.2, respectively).

The decrease in serum NOx concentration was evident in the patient group with varices [from 126 µM (range: 82-172 µM) before treatment to 61 µM (range: 31-146 µM) after treatment; p=0.02] compared with the group without varices [from 121 µM (range: 47-248 µM) before treatment to 112 µM (range: 30-277 µM) after treatment; p=0.6] (Table 3). All the patients without varices were Child A cirrhotic, whereas 4 of 9 and 1 of 9 patients with varices were Child B and Child C cirrhotic, respectively. Child B and C patients showed statistically similar serum NOx levels to those of Child A patients before treatment.

Before treatment, PFV was significantly higher in the patient group without varices than in the group with varices (p=0.041), while the CI was higher (numerically) in the group with varices when compared to the group without varices (p=0.072) (Table 4). We also observed some nonsignificant changes in the parameters of the portal vein after treatment. In a sub-analysis based on the presence of varices, the decrease in CI value approached statistical significance (p=0.066). A nonsignificant increase in flow velocity (p=0.1) in the group with

Table 1. Patient characteristics

Patients	n = 19
Age, years, median (range)	58 (41-76)
Male: Female (n)	10:9
Etiology of cirrhosis (n)	
HBV	6
HCV	10
Alcoholic	2
Cryptogenic	1
Esophageal varices (n)	
Absent	10
1st degree	5
2nd degree	2
3rd degree	2
Stage of cirrhosis (n)	
Child-Pugh A	16
Child-Pugh B	2
Child-Pugh C	1
Portal gastropathy (n)	
Absent	11
Mild	6
Severe	2
Duodenal ulcer (n)	2

Table 2. Comparison of pre- and post-treatment serum levels of NOx based on *H. pylori* eradication status

	Median levels (range) of serum NOx (µM)		***P value***
	Before treatment	**After treatment**	
Pts with *H. pylori* eradication	99 (47 – 216)	61 (30 – 277)	0.03
Pts without *H. pylori* eradication	190 (63 – 248)	146 (37 – 261)	0.34

Table 3. Comparison of pre- and post-treatment serum levels of NOx based on the presence of varices

	Median levels (range) of serum NOx (µM)		***P value***
	Before treatment	**After treatment**	
Pts with varices	126 (82 – 172)	61 (31 – 146)	0.01
Pts without varices	121 (46 – 248)	112 (30 -277)	0.6

varices was observed (Table 5), while there was no response to treatment in terms of flow velocity in patients without varices (Table 6).

DISCUSSION

Nitric oxide and its reaction products (nitrates and nitrites) have various roles in many biologic processes. After studies reporting induced production of gastric mucosal NOx during *H. pylori* gastritis (1), serum levels of NOx have been shown to be elevated during this infection (3). One of the subjects in gastroenterology related to NO metabolism is portal hemodynamics in cirrhosis. In rats, plasma NOx concentrations are increased with experimentally induced cirrhosis (9). Furthermore, studies designed with NOS inhibitors suggest that NO is the key mediator of hyperdynamic circulation in cirrhosis (10). Given this, we wondered whether any possible change in serum NOx concentration after treatment of *H. pylori* gastritis would affect portal hemodynamics in cirrhotic patients.

To our knowledge, this is the first report in the literature to show a decrease in serum NOx concentration after treatment for *H. pylori* gastritis. This is in accordance with the study by Kodama and coworkers (3), who showed higher serum NOx concentrations in serologically *H. pylori*-positive patients than in *H. pylori*-negative patients. If NOx has some beneficial or adverse effects on tissues or systems other than sites where it is produced, we would expect some functional alterations in systems related to NOx after its serum level decreased following treatment for *H. pylori* gastritis.

Our patients with esophageal varices had significantly lower PFVs and numerically higher CI values than did patients without varices. Recent studies (11,12) have reported lower PFVs and CI values in cirrhotic patients compared with healthy controls. Our data are consistent with these studies since patients without varices may have normal or at least lower portal venous pressures compared with patients with varices. After eradication therapy, when we analyzed cirrhotic patients irrespective of the presence of varices, CI and portal vein diameter showed no significant alterations. In the subgroup analysis, the decrease in CI and increase in PFV became more evident in patients with varices than in those without varices; we attribute this to the near normal values of basal portal measurements in patients without varices and/or lower level of decrease in serum NOx concentration probably due to the lower (but statistically nonsignificant) eradication rate in patients without varices. Higher cure rate of infection in

Table 4. Basal hemodynamics in patient groups based on the presence of esophageal varices

Parameter	Patients with varices median (range)	Patients without varices median (range)	*P value*
PFV (cm/s)	7.7 (7.1-9.7)	10.0 (4.7-15.6)	0.041
CI	0.15 (0.08-0.3)	0.12 (0.05-0.16)	0.072
PVF (ml/minute)	626 (300-933)	720 (217-1032)	0.78
PVD (mm)	13 (9-16)	11 (9-14)	0.35

PFV: Portal flow velocity; CI: Congestive index; PVF: Portal venous flow (flow volume); PVD: Portal vein diameter.

Table 5. Hemodynamic changes after the treatment of *H. pylori* in patients with varices

Parameter	Before treatment median (range)	After treatment median (range)	*P value*
PFV (cm/s)	7.7 (7.1-9.7)	10.5 (6.7-13.2)	0.1
CI	0.15 (0.08-0.3)	0.1 (0.05-0.21)	0.066
PVF (ml/minute)	626 (300-933)	657 (324-1172)	0.67
PVD (mm)	13 (9-16)	12 (8-15)	0.168

PFV: Portal flow velocity; CI: Congestive index; PVF: Portal venous flow (flow volume); PVD: Portal vein diameter.

Table 6. Hemodynamic changes after the treatment of *H. pylori* in patients without varices

Parameter	Before treatment median (range)	After treatment median (range)	*P value*
PFV (cm/s)	10.0 (4.7-15.6)	8.1 (5.9-16.8)	0.31
CI	0.12 (0.05-0.16)	0.11 (0.05-0.19)	0.72
PVF (ml/minute)	720 (217-1032)	502 (280-958)	0.17
PVD (mm)	11 (9-14)	12 (8-15)	0.391

PFV: Portal flow velocity; CI: Congestive index; PVF: Portal venous flow (flow volume); PVD: Portal vein diameter.

the patient group with varices may be attributed to the change in drug metabolism in cirrhotic patients having advanced liver disease (13, 14). In this study, there were advanced cirrhotics (Child B or C categories) among the patients with varices who showed high cure rate.

One might ask if the decrease in serum NOx concentration will improve the portal hemodynamics or, conversely, if it will increase portal pressure, since nitrates given as medications for primary (15) or secondary prophylaxis (16) of variceal bleeding are expected to reduce portal pressure. In any event, the effects of nitrates in this indication and in reducing portal pressure are not evident and not commonly accepted as that of propranolol (17). In the present study, approach of CI and PFV values of cirrhotic patients with varices to those of healthy controls after treatment of *H. pylori* suggested an improvement in portal hemodynamics.

Our limitation was lack of a control group (*H. pylori* cases without cirrhosis or uninfected cirrhosis). However, our data suggest a beneficial effect of *H. pylori* eradication treatment on portal venous hypertension or splanchnic congestion. We can say that this treatment has no detrimental effect on portal hemodynamics in cirrhotic patients when eradication of *H. pylori* is indicated. A possible but not absolute effect of *H. pylori* eradication demonstrated with this small-scale study should be evaluated with large-scale studies to determine short- and long-term benefits on portal hypertensive bleeding and survival.

REFERENCES

1. Fu S, Ramanujam KS, Wong A, et al. Increased expression and cellular localization of inducible nitric oxide synthase and cyclooxygenase 2 in *Helicobacter pylori* gastritis. Gastroenterology 1999; 116: 1319-29.
2. Wilson KT, Ramanujam KS, Mobley HL, et al. *Helicobacter pylori* stimulates inducible nitric oxide synthase expression and activity in a murine macrophage cell line. Gastroenterology 1996; 111: 1524-33.
3. Kodama K, Sumii K, Kawano M, et al. *Helicobacter pylori* infection increases serum nitrate and nitrite more prominently than serum pepsinogens. Helicobacter 2002; 7: 9-13.
4. Wiest R, Groszmann RJ. The paradox of nitric oxide in cirrhosis and portal hypertension: too much, not enough. Hepatology 2002; 35: 478-91.
5. Rockey DC. Vascular mediators in the injured liver. Hepatology 2003; 37: 4-12.
6. Abelmann WH. Hyperdynamic circulation in cirrhosis: a historical perspective. Hepatology 1994; 20: 1356-8.
7. Groszmann RJ. Hyperdynamic circulation of liver disease 40 years later: pathophysiology and clinical consequences. Hepatology 1994; 20: 1359-63.
8. Moriyasu F, Nishida O, Ban N, et al. "Congestion index" of the portal vein. Am J Roentgenol 1986; 146: 735-9.
9. Bomzon A, Blendis LM. The nitric oxide hypothesis and the hyperdynamic circulation in cirrhosis. Hepatology 1994; 20: 1343-50.
10. Campillo B, Chabrier PE, Pelle G, et al. Inhibition of nitric oxide synthesis in the forearm arterial bed of patients with advanced cirrhosis. Hepatology 1995; 22: 1423-9.
11. Siringo S, Piscaglia F, Zironi G, et al. Influence of esophageal varices and spontaneous portal-systemic shunts on postprandial splanchnic hemodynamics. Am J Gastroenterol 2001; 96: 550-6.
12. Yin XY, Lu MD, Huang JF, et al. Color Doppler velocity profile assessment of portal hemodynamics in cirrhotic patients with portal hypertension: correlation with esophageal variceal bleeding. J Clin Ultrasound 2001; 29: 7-13.
13. Giannini E, Fasoli A, Borro P, et al. Impairment of cytochrome P-450-dependent liver activity in cirrhotic patients with *Helicobacter pylori* infection. Aliment Pharmacol Ther 2001; 15: 1967–73.
14. Andersson T, Olsson R, Regardh CG, Skanberg I. Pharmacokinetics of [^{14} C] omeprazole in patients with liver cirrhosis. Clin Pharmacokinet 1993; 24: 71–8.
15. Angelico M, Carli L, Piat C, et al. Isosorbide-5-mononitrate versus propranolol in the prevention of first bleeding in cirrhosis. Gastroenterology 1993; 104: 1460-5.
16. Merkel C, Sacerdoti D, Bolognesi M, et al. Hemodynamic evaluation of the addition of isosorbide-5-mononitrate to nadolol in cirrhotic patients with insufficient response to the beta-blocker alone. Hepatology 1997; 26: 34-9.
17. D'Amico G, Pagliaro L, Bosch J. Pharmacological treatment of portal hypertension: an evidence-based approach. Semin Liver Dis 1999; 19: 475-505.