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The effect of duodenogastric reflux on *Helicobacter pylori* presence and gastric histopathologic changes

Duodenogastrik reflünün *Helicobacter pylori* varlığı ve gastrik histopatolojik değişikliklere etkileri

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BAckground/aims: *In this study, we investigated the presence of Helicobacter pylori infection and gastric histological changes in unoperated patients with and without duodenogastric reflux.* ***Methods:*** *Twenty-two women and 15 men, mean age of 51.17 years, who had duodenogastric reflux during endoscopy were included. Thirty-seven age-and gender-matched patients without duodenogastric reflux served as controls. The Helicobacter pylori infection was detected by histology. The Helicobacter pylori density and gastric histology according to the modified Sydney system were evaluated with specimens from antrum, corpus and fundus.* ***Results:*** *The presence of Helicobacter pylori, Helicobacter pylori density, chronic inflammation score, lymphoid follicles, atrophy, and intestinal metaplasia were similar in patients with and without duodenogastric reflux ($p>0.05$). Acute inflammation was found to be lower in the antrum ($p<0.05$) in the duodenogastric reflux-positive group.* ***Conclusion:*** *Duodenogastric reflux does not affect the presence of Helicobacter pylori and gastric histopathologic changes.*

Keywords: Duodenogastric reflux, Helicobacter pylori, gastric histopathology

Amaç: *Bu çalışmada duodenogastrik reflünün Helicobacter pylori varlığı ve gastrik histopatolojik değişikler üzerine etkileri araştırıldı.* ***Yöntem:*** *Endoskopide duodenogastrik reflü saptanan 22'sı kadın ve 15'i erkek, yaş ortalaması 51.17 yıl olan 37 olgunun sonuçları, endoskopide duodenogastrik reflü saptanmayan yaş ve cins özellikleri benzer olan 37 olgunun sonuçları ile karşılaştırıldı. Helicobacter pylori varlığı histolojik olarak saptandı. Antrum, korpus ve fundustan alınan biopsilerde Helicobacter pylori yoğunluğu ve gastrik histopatolojik değişiklikler modifiye Sydney sistemi ile değerlendirildi.* ***Bulgular:*** *Duodenogastrik reflülü olgularda antrumda akut inflamasyon skoru istatiksel açıdan anlamlı bir farklılık gösterirken Helicobacter pylori varlığı, Helicobacter pylori yoğunluğu, kronik inflamasyon skoru, lenfoid follikul, atrofi, intestinal metaplazi varlığı açısından her iki grup arasında farklılık saptanmadı.* ***Sonuç:*** *Duodenogastrik reflü Helicobacter pylori varlığı ve gastrik histopatolojik değişiklikleri etkilememektedir.*

Keywords: Duodenogastrik reflü, *H. pylori,* gastrik histopatoloji

INTRODUCTION

Duodenogastric reflux (DGR) is commonly defined as the transport of duodenal contents from the duodenum to the stomach (1). The pathogenic importance of DGR is accepted for postoperative bile vomiting and reflux esophagitis following gastrectomy (2, 3). In an unoperated stomach, the pathogenesis of DGR and its role in digestion is unknown. Chernov et al concluded that DGR is involved in the formation of the internal gastric environment, plays a role in gastric digestion and that its regulation is affected by the coordinated motor and evacuatory performance of the gastroduodenal junction and duodenum (4). Castedal et al have shown that late phase **III** contractions of the duodenum act as a retroperistaltic pump, retropelling duodenal contents to the stomach (5). Experimental studies have demonstrated the destructive effects of bile and pancreatic juice on the gastric mucosa (6).

Although it has been shown that bile acids possess antibacterial activity against *H. pylori* (7, 8), there are few reports on the presence of *H. pylori* and the severity of gastritis in patients with DGR, which are also conflicting. In this study, we investigated the presence of *H. pylori* infection and gastric histological changes in unoperated patients with and without DGR.

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MATERIALS AND METHODS

Twenty-two women and 15 men, with a mean age of 51.17 years, who had gastric yellow-green fluid during endoscopy were included in the study. All included patients had increased bihrubin and/or amylase in their gastric fluid. Thirty-seven age- and gender-matched patients who had normal gastric fluid with normal amylase and bihrubin levels (without duodenogastric reflux) served as controls. None of the patients had received previous antibiotic treatment for *H. pylori* eradication or were presently taking nonsteroidal anti-inflammatory drugs. Patients with previous gastric operation and recent treatment with proton pump inhibitors (<4 weeks) were excluded from the study. Age, sex, and medications taken before endoscopy were recorded at baseline. The upper endoscopic examination was performed by an experienced endoscopist, and all endoscopic findings, including the signs of gastritis, gastric-duodenal ulcers, erosions, masses, and the presence of blood, and/or of duodenogastric reflux in the lumen were noted. During upper endoscopy, we aspirated the residual gastric fluid and measured the bihrubin and/or pancreatic amylase content within it. The presence of yellow-green fluid with increased bihrubin and/or amylase content was accepted as the sign of duodenogastric reflux. Alpha-amylase was assessed by means of the CNPG3 test (2-chloro-4-nitrophenyl-oc-D 25 maltotrioside substrate, Abbott Park, IL). A value above 130 U/L was considered borderline. Total bihrubin was assessed by using a method which oxidized to biliverdin by sodium nitrite resulting in a decrease in absorbance at 444 nm on Abbott Aeroset Autoanalyzer (Abbott Park, IL). A value above 1.2 mg/dl was considered borderline. The *H. pylori* status was evaluated by means of histology. In each patient, a total of six samples divided equally from antrum, corpus and fundus for histopathological examination were obtained. The biopsy specimens were fixed in 10% buffered formalin, embedded in paraffin, and stained with Giemsa and hematoxylin-eosin. Each biopsy specimen was examined by the same pathologist who was unaware of the clinical background and the endoscopic findings of the patients. The *H. pylori* density and gastric histologic changes were stratified similar to the criteria-modified Sydney system cited by Yang et al (9).

Statistical analysis

Data were analyzed using the SPSS 11.0 Statistical package program. Univariate analysis, using either independent-samples t test or chi-square test, was performed between patient groups with and without DGR. The comparison of the histologic parameters among subgroups with different bacterial densities was done by chi-square test or a one-way ANOVA model as appropriate. A p value <0.05 indicated a statistical significance.

RESULTS

A total of 74 patients (33 male and 41 female) with a mean age (±SD) of 49.8± 15.6 years were enrolled into the study. No significant differences in gender ratio (0.68 vs 0.94) or age were observed between groups A and B (51.17±16 vs 48.5±14.5 years, p>0.05). In patients with DGR (group A), the median value of amylase and total bihrubin was 9433 U/L (range 0 to 54230 U/L) and 3.2 mg/dl (range 0 to 18 mg/dl), respectively. The presence of *H. pylori* infection was similar in patients with and without DGR (group B) (antrum: 27, corpus: 24, and fundus: 27 vs antrum: 28, corpus: 27, and fundus: 24, respectively; p>0.05). As seen in Tables 1, 2 and 3, histological parameters were corn-

Table 1. Comparison of the antral histology in Hp (+) patients with and without duodenogastric reflux (DGR)

Histologic parameters	DGR negative	DGR positive	P
H. pylori density	2.00±0.17	1.83±0.15	NS
Acute activity	1.11±0.16	0.54±0.14	<0.05
Chronic inflammation	2.15±0.09	1.91+0.08	NS
Intestinal metaplasia (%)	7.1	3.7	NS
Lymphoid follicle (%)	46.15	45.83	NS
Atrophy (%)	19.23	12.5	NS

Table 2. Comparison of the corpus histology in Hp (+) patients with and without duodenogastric reflux (DGR)

Histologic parameters	DGR negative	DGR positive	P
H. pylori density	1.38±0.16	1.52±0.20	NS
Acute activity	0.65±0.17	0.43±0.15	NS
Chronic inflammation	1.88±0.08	1.69±0.09	NS
Intestinal metaplasia (%)	0	4.34	NS
Lymphoid follicle (%)	34.6	39.1	NS
Atrophy (%)	3.8	4.34	NS

Table 3. Comparison of the fundus histology in Hp (+) patients with and without duodenogastric reflux (DGR)

Histologic parameters	DGR negative	DGR positive	P
H. pylori density	1.07±0.14	1.22±0.52	NS
Acute activity	0.61±0.14	0.36±0.14	NS
Chronic inflammation	1.57±0.09	1.50±0.12	NS
Intestinal metaplasia (%)	0	0	NS
Lymphoid follicle (%)	19.23	27.27	NS
Atrophy (%)	3.84	0	NS

pared between cases with and without DGR in antrum, corpus and fundus. Acute inflammation in antrum of DGR (+) cases was found to be statistically different. Acute inflammation was found to be lower in antrum in DGR (+) group. There were no differences of chronic inflammation scoring, intestinal metaplasia, atrophy, or lymphoid follicle among all anatomic locations.

DISCUSSION

Bile acids and *H. pylori* interaction is complex. Bile acids (either cheno or ursodeoxycholic acid) (8) and fresh human bile have been shown to be detrimental on *H. pylori* viability and existence (10). However, some studies have reported *H. pylori* in bile or bile stones retrieved during biliary surgery (11). Reports on *H. pylori* infection in patients with **DGR** are also conflicting. The relation of **DGR** with *H. pylori* infection is important for two reasons. First, the sensitivity of the CLO test that has been widely used in the diagnosis of *H. pylori* is related to *H. pylori* density and the severity of gastritis (12). Second, recent studies have shown a close link between *H. pylori* infection, atrophic gastritis, and intestinal metaplasia (IM) (13, 14). The reflux of duodenal contents into the stomach has also been reported as a causative factor in the development of atrophy and IM (15, 16). To evaluate the risk in cases with DGR and *H. pylori*, we compared the above cases with matched controls without DGR. However, other than the differences in acute inflammation, there were no differences in the other parameters examined between the **DGR** (+). and (-) groups. Furthermore, since *H. pylori* density and severity of gastritis were not altered in the presence of duodenogastric reflux, we may speculate that sensitivity of the CLO test would not be changed in patients with DGR. Thus, its use can be justified.

Most reports could not establish a relationship between **DGR** and *H. pylori* infection (17, 18), while the O'Connor (19) and Offerhaus (20) teams reported that bile reflux had eradicated *H. pylori*. We observed no difference in presence of *H. pylori* or in its density between groups. The differences between the studies may be due to the limited number of cases, history of gastric surgery, or the fact that human bile inhibited *H. pylori* reproduction in a dose-dependent manner, as reported by Graham et al (10). Furthermore, the amount of bile reflux, bile acid composition and concentration changes and gastric acidity should have affected the results. It has been demonstrated that chenodeoxycholic acid was a more effective inhibitor of adherence, in that the number inhibited and percentage of inhibition were greater than with ursodeoxycholic acid (21). For some technical reasons, we did not measure the amount of bile acid composition or concentration. DGR has no uniform description nor any specific gold standard diagnostic tool. Even though bile is often used as a marker, DGR consists of other components such as pancreatic juice and duodenal secretions. Fuchs et al (22) evaluated the subjects for signs of DGR based on the combination of elevated intragastric pH, **bili**rubin exposure, and intragastric evidence of pancreatic enzymes. In this trial we utilized endoscopy and measurement of amylase/bilirubin levels in gastric juice.

Since *H. pylori* was reported to be carginogenic, we tried to observe whether *H. pylori* existence and DGR increased the risk of precancerous conditions. However, in our trial, there was no statistically significant difference between the two groups regarding existence of lymphoid follicle, atrophy, and intestinal metaplasia. Sobala et al reported increased atrophy and intestinal metaplasia in DGR (+) patients with previous gastric surgery (16). In contrast, Karttunen et al attributed the atrophy to *H. pylori* rather than bile reflux in patients with gastric ulcer (23). Nakamura et al (15) demonstrated that DGR is associated with marked IM, supported by results showing statistically significant increases in gastric pH and bile acid concentration in the IM group compared with corresponding values in the control group. In contrast to our study, Nakamura et al used endoscopic white mucoid-like patches as the criteria instead of microscopic IM. The exact relationship between atrophy and/ or intestinal metaplasia and DGR should be investigated thoroughly to reveal a possible role of bile acids and/or duodenogastric reflux in gastric carcinogenesis.

In conclusion, DGR does not appear to affect *H. pylori* presence or gastric histopathologic changes in the unoperated stomach. However, further studies with larger sample sizes may emphasize the correlation between amylase and total bilirubin levels.

REFERENCES

1. Fein M, Fuchs KH, Bohrer T, et al. Fiberoptic technique for 14-hour bile reflux monitoring. Dig Dis Sci 1996; 41: 216-25.
2. Robles-Campos R, Lujan-Mompean JA, Parrilla-Paricio P, et al. Role of *Helicobacter pylori* infection and duodenogastric reflux in the pathogenesis of alkaline reflux gastritis after gastric operations. Surg Gynecol Obstet 1993; 176: 594-8.
3. Muller-Lissner SA. Is a duodenogastric reflux of pathogenic significance? Z Gastroenterol 1988; 26: 637-42.
4. Chernov VF, Kutnetsov AP, Danilova AV, et al. [Duodenogastric reflux in humans.] Vestn Ross Akad Med Nauk 2000; 3: 37-41.
5. Castedal M, Bjornsson E, Gretarsdottir J, et al. Scintigraphic assessment of interdigestive duodenogastric reflux in humans: distinguishing between duodenal and biliary reflux material. Scand J Gastroenterol 2000; 35: 590-8.
6. Wickbom G, Bushking FL, Linares C, Dragstedt LR. On the corrosive properties of bile and pancreatic juice on living tissue. Arch Surg 1974; 108: 680-4.
7. Itoh M, Wada K, Tan S, et al. Antibacterial action of the bile acids against *Helicobacter pylori* and changes in its ultrastructural morphology: effect of unconjugated dihydroxy bile acid. J Gastroenterol 1999; 34: 571-6.
8. Bode G, Malfertheiner P, Strohle A, et al. Polymorphism in *Helicobacter pylori-a* key function in recurrence of infection? Med Klin 1992; 87: 179-84.
9. Yang HB, Sheu BS, Su LJ, et al. Clinical application of gastric histology to monitor treatment of dual therapy in *H. pylori* eradication. Dig Dis Sci 1997; 42: 1835-40.
10. Graham DY, Osato MS. *H. pylori* in the pathogenesis of duodenal ulcer: interaction between duodenal acid load, bile and H pylori. Am J Gastroenterol 2000; 95: 87-91.
11. Monti J, Fay M, Banchio C, et al. Detection of *Helicobacter pylori* by polymerase reaction in bile samples from gallbladder and bile stones. Acta Gastroenterol Latinoam 1999, 29: 251-3.
12. Madani S, Rabah R, Tolia V. Diagnosis of *Helicobacter pylori* infection from antral biopsies in pediatric patients is urease test that reliable? Dig Dis Sci 2000; 45: 1233-7.
13. Sozzi M, Valentini M, Figura N, et al. Atrophic gastritis and intestinal metaplasia in *Helicobacter pylori* infection: the role of CagA status. Am J Gastroenterol 1998; 93: 375-9.
14. Guarner J, Herrera-Goepfert R, Mohar A, et al. Gastric atrophy and extent of intestinal metaplasia in a cohort of *Helicobacter pylori-infected* patients. Hum Pathol 2001; 32: 31-5.
15. Nakamura M, Haruma K, Kamada T, et al. Duodenogastric reflux is associated with antral metaplastic gastritis. Gatrointest Endosc 2001; 53: 53-9.
16. Sobala GM, O'Connor HJ, Dewar EP, et al. Bile reflux and intestinal metaplasia in gastric mucosa. J Clin Pathol 1993; 46: 235-40.
17. Niemela S, Karttunen T, Heikkla J, et al. Relationship of Campylobacter pylori and duodenogastric reflux. Dig Dis Sci 1989; 34: 1021-4.
18. Kellosalo J, Alavaikko M, Laitinen S. Effect of biliary tract procedures on duodenogastric reflux and the gastric mucosa. Scand J Gastroenterol 1991; 26: 1272-8.
19. O'Connor HJ, Wyatt JI, Dixon MF, Axon ATR. Campylobacter like organisms and reflux gastritis. J Clin Pathol 1986; 39: 531-4.
20. Offerhaus GJA, Rieu PNMA, Jansen BJM, et al. Prospective comparative study of the influence of postoperative bile reflux on gastric mucosal histology and Campylobacter pylori infection. Gut 1989; 30: 1552-7.
21. Mathai E, Arora A, Cafferkey M, et al. The effect of bile acid on the growth and adherence of *Helicobacter pylori*. Aliment Pharmacol Ther 1991;5: 653-8.
22. Fuchs K-H, Maroske J, Fein M, et al. Variability in the composition of physiologic duodenogastric reflux. J Gastrointest Surg 1999; 3: 386-96.
23. Karttunen T, Niemela S. Campylobacter pylori and duodenogastric reflux in peptic ulcer disease and gastritis. Lancet 1998: 1; 118.