The role of gastric juice ammonia and gastric juice urea nitrogen/ammonia in the diagnosis of *Helicobacter pylori* infection

Helicobacter pylori enfeksiyonu tanısında mide suyu amonyak ve üre nitrojen/amonyak düzeylerinin önemi

Orhan ÖZGÜR MD, Gürden GÜR MD, Mete ÖZDOĞAN MD, Sedat BOYACIOĞLU MD

Başkent University, School of Medicine, Department of Gastroenterology, Ankara

SUMMARY: *Urease activity of Helicobacter pylori (H.pylori) may result in increased concentrations of gastric juice ammonia. In this study, we aimed to assess the importance of gastric juice urea nitrogen and ammonia concentrations in the diagnosis of H.pylori infection. The diagnosis of* H.pylori *infection in 80 dyspeptic patients was made by concomitant positivity of urease test and histological detection of* H.pylori. *We measured the gastric juice urea nitrogen and ammonia concentrations obtained during upper endoscopy. We also calculated the gastric juice urea nitrogen to ammonia ratios (R) in both* H.pylori *positive and* H.pylori *negative patients. The mean gastric juice urea nitrogen concentrations were not different between* H.pylori *positive and negative groups. (5.28±1.92 mmol/L vs. 4.60±0.90) (p>0.05). The mean gastric juice ammonia concentration in* H.pylori *positive and negative groups were 3.08±1.87 vs. 0.41±0.43 mmol/L respectively (p (0.001). The mean R value was significantly lower in the* H.pylori *positive group than that of the* H.pylori *negative group. (2.91±2.80 vs. 29.29±33.40) (p<0.001). In conclusion, we believe that measurement of gastric juice ammonia concentrations and the calculation of gastric juice urea/ammonia ratios may be of value in the diagnosis of* H.pylori *infection.*

Key words: *Helicobacter pylori,* gastric juice ammonia

ÖZET: *Helicobacter pylori'nin (H.pylori) üreaz aktivitesi mide suyu amonyak düzeyinde artışa neden olabilir. Bu çalışmada, mide suyu amonyak ve üre nitrojen düzeylerinin* H.pylori *infeksiyonu teşhisindeki öneminin ortaya konması amaçlanmıştır.* H.pylori *infeksiyonu varlığı 80 dispeptik hastada araştırıldı. Üreaz testi ve histolojik incelemenin* H.pylori *yönünden birlikte pozitifliği durumunda* H.pylori *infeksiyonu varlığı kabul edildi. Üst endoskopi sırasında elde olunan mide suyu örneklerinde üre nitrojen ve amonyak düzeyleri ölçüldü. Üre nitrojen/amonyak (R) oranları* H.pylori *pozitif ve negatif hastalarda saptandı. Ortalama mide suyu üre nitrojeni* H.pylori *pozitif ve negatif gruplarda farklılık göstermemekteydi. (5.28 ± 1.92 mmol/L, 4.60 ±0.90) (p>0.05). Ortalama mide suyu amonyak konsantrasyonu* H.pylori *pozitif ve negatif gruplarda sırasıyla 3.08 ± 1.87 ve 0.41 ± 0.43 mmol/L olarak bulundu (p>0.001). Ortalama R değeri* H.pylori *pozitif grupta negatif gruba göre anlamlı olarak düşüktü. (2.91 ± 2.80, 29.29±33.40) (p<0.001).*

Bu sonuçların ışığı altında mide suyu amonyak ölçümü ve üre nitrojen/amonyak oranının saptanmasının H.pylori *infeksiyonu tanısında değerli olabileceğini düşünmekteyiz*

Anahtar sözcükler: *Helicobacter pylori,* mide suyu amonyağı

There is a close relationship between antral mucosal Helicobacter pylori (*H.pylori*) infection and gastroduodenal diseases (1, 2). Eradication of the microorganism reduces the relapse rate of duodenal ulcer and may have beneficial effect on non-ulcer dyspepsia (3). Therefore, the primary detection of this organism and the confirmation of its eradication has become increasingly important in the management of gastroduodenal diseases.

H.pylori is known by its high urease activity which is widely used to detect the presence of infection (4). Gastric juice urea concentration decreases in the presence of *H.pylori* infection (5-7). On the other hand, Kim et al found no difference in gastric juice urea concentrations between *H.pylori* positive and negative patients (8).

The high urease activity of the microorganism might theoretically also result in raised gastric juice ammonia concentration. Previous studies showed that gastric juice ammonia and ammonium concentrations increase in the presence of *H.pylori*infection (5-9). The urea to ammonium (or

Geliş tarihi: 9.12.1997 Kabul tarihi: 28.4.1998

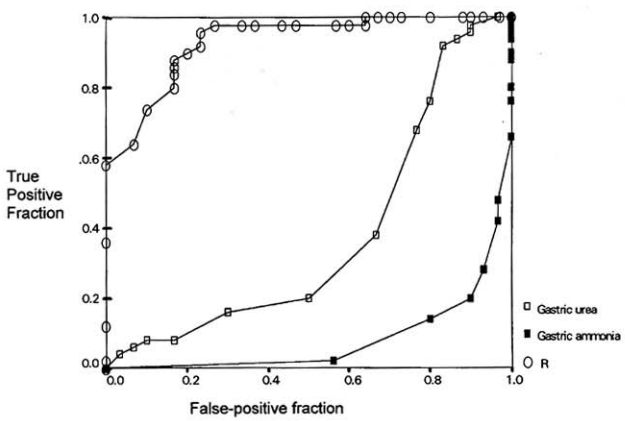

Figure 1. Receiver-operating-characteristic curves show the accuracy of R value in the best diagnostical return

Table 1. Mean blood urea nitrogen, ammonia, gastric juice urea nitrogen and ammonia levels in Helicobacter pylori positive and negative patients.

	Hp-positive	*Hp-negative*	*p*
BUN (mg/dL)	17.87±3.03	17.48±1.48	>0.05
Blood ammonia (μmol/L)	19.55±5.30	20.19±5.16	>0.05
Gastric juice urea nitrogen (mmol/L)	5.28±1.92	4.60 (0.90	>0.05
Gastric juice ammonia(mmol/L)	3.08±1.87	0.41±0.43	<0.001
R	2.91±2.80	29.29±33.40	<0.001

Hp: *Helicobacter pylori*, R: Gastric juice urea nitrogen/gastric juice ammonia

ammonia) ratio has been shown to have better agreement with *H.pylori* infection (6, 10). We therefore assessed the value of measuring gastric juice urea nitrogen and ammonia concentrations and calculated the urea nitrogen to ammonium ratios in patients undergoing diagnostic endoscopy, as this might provide a rapid and safe detection of *H.pylori* infection.

MATERIALS AND METHODS

Eighty patients (30 men and 50 women) with chronic dyspepsia were included in the study. None had taken antibiotics and/or anti secretory therapy within 4 weeks prior to the study. Patients who had chronic liver disease and/or chronic renal failure were excluded from the study as urea and ammonia metabolism is altered in these groups of patients.

Following 12 hours of fasting, upper gastrointestinal endoscopies were performed on each patient. During endoscopy, just after entering the stomach, about 2 ml of gastric juice was aspirated from the lumen by a cannula, which was passed through the biopsy channel. Routine examination of the upper gastrointestinal tract was then performed and antral mucosal biopsies were obtained for urease test and histology. Sterile biopsy forceps were used for each biopsy. For the urease test, antral mucosal specimens were placed in 1 mL of 3.9% urea solution (Harnstoff-Boulcion urea broth) and incubated for up to 24 hours at 37ºC. The specimens for histological examination were fixed in standard formalin solution before staining with hematoxylen and eosin. The histological examination was performed by the same pathologist. The gastric juice samples were sent to the laboratory immediately after endoscopy and microcentrifugated at 3000 cycles per minute for 12 minutes to remove the mucus. Then, urea nitrogen concentrations of supernatant fluid were determined by Cobas-Mira equipment with the UV kinetic method (human). We used Ektachem DT 60 analyzer for the measurement of supernatant fluid ammonia concentrations. The gastric juice urea nitrogen / gastric ammonia values were calculated and defined as R values.

Patients with histologically shown *H.pylori* infection of the antral mucosa and with a positive urease test were considered *H.pylori* positive. Patients who had no histologically demonstrable microorganism and urease test negativity were considered *H.pylori* negative. Patients who were positive for *H.pylori* only by histology or urease test were also excluded from the study. Serum urea nitrogen and plasma ammonia concentrations were also measured in all patients.

Results were expressed as mean±standard deviation (SD). Blood urea nitrogen and ammonia data were compared by the Student's t test whereas gastric urea nitrogen, ammonia and R values were compared by the Mann-Whitney test. Differences with two-tailed P values lower than 0.05 were considered to be statistically significant. Receiver-Operating-Characteristic (ROC) curves were

Table 2. Sensitivity and specificity of the R values

R	*Sensitivity*	*Specificity*
≤ 3.0	74%	96%
≤ 3.5	80%	93%
≤ 4.0	84%	93%
≤ 4.5	86%	93%
≤ 5.0	88%	93%
≤ 5.5	90%	90%
≤ 6.0	92%	86%
≤ 6.5	96%	83%

R: Gastric Juice urea nitrogen / gastric juice ammonia

Table 3. Sensitivity and specificity of the gastric juice ammonia concentrations

Ammonia concentration	*Sensitivity*	*Specificity*
≥ 0.80	92%	77%
≥ 1.00	93%	74%
≥ 1.50	94%	65%

made for illustrative purposes only and were not used for statistical analysis. The ROC curve of gastric urea nitrogen, ammonia and R values were presented and, on this basis, defined what parameter and which cut-off values gave the best diagnostical return.

RESULTS

Fifty-three patients were *H.pylori* positive and 27 patients were *H.pylori* negative. Mean ages of *H.pylori* positive and *H.pylori* negative patients were 40.33±11.34 and 39.59±9.41 years respectively (p>0.05). Serum urea nitrogen and plasma ammonia concentrations were similar in the two groups (Table 1). The mean gastric juice urea nitrogen concentrations in *H.pylori* positive patients was 5.28±1.92 mmol/L and in *H.pylori* negative patients was 4.60 (0.90 mmol/L. The difference between these groups was not significant (p>0.05).

The mean gastric juice ammonia concentration in *H.pylori* positive patients was 3.08±1.87 mmol/L and in *H.pylori* negative patients was 0.41±0.43 mmol/L. The difference between the two groups was significant (Table 1, p<0.001). Some cut off values for the diagnosis of *H.pylori* are shown in Table III. The mean R value for *H.pylori* positive patients was 2.91±2.80 and for *H.pylori* negative patients was 29.29±33.40. The difference between the two groups was significant (Table 1, p<0.001). The favorable cut-off R values for the best diagnostical return are shown in Table II.

The ROC curve of the gastric juice urea nitrogen, ammonia and R values are shown in figure I. On the ROC curve base, the R values seemed to be superior in the diagnosis of *H.pylori* infection compared with ammonia values..

DISCUSSION

After the discovery of the role of Helicobacter pylori infection in various gastrointestinal disorders, attempts were made to establish a quick, safe and inexpensive diagnosis (11-17). *H.pylori* infection is most frequently diagnosed clinically from its urease activity. Two diagnostic tests are available using this urease activity. Firstly, the 14C-urea breath test and secondly the antral mucosal urease test. The 14C urea breath test is a highly sensitive and reproducable test, but requires special equipment. The analysis of breath samples is also time consuming. Another disadvantage is that it involves the administration of a small dose of radioactivity that makes it unsuitable for children and pregnant women (13). The urea breath test using 13C eliminates the risk of radioactivity but a mass spectrometry is needed, therefore it is not suitable for mass screening (12). Antral mucosal urease tests are also highly specific and moderately sensitive but require endoscopy and sometimes 24 hours are needed for the diagnosis. Serodiagnosis of *H.pylori* infection is a suitable screening test, but it is not always possible to differentiate active disease from passive immunization (16, 19).

In theory, urease activity of *H.pylori* may result in higher than normal gastric juice ammonia and lower than normal gastric juice urea nitrogen by converting urea to ammonia and CO2. There are some controversial studies concerning gastric juice urea nitrogen in *H.pylori* infected patients. Marshal and Longtan have found a significant

decrease in gastric juice urea nitrogen content in *H.pylori* infected subjects compared with *H.pylori* negative patients (7). On the other hand, Kim et al and Pedriali et al found no difference in gastric juice urea nitrogen between *H.pylori* positive and negative patients (8-18). Regarding gastric juice ammonia concentration, Kim et al found this value to be 5.48 mmol/L and 1.26 mmol/L in *H.pylori* positive and *H.pylori* negative patients to be respectively (8). Differences between these values were significant. Weber et al also observed that gastric juice ammonia concentration was elevated in *H.pylori* infected patients. There are also some other studies which show that gastric juice ammonia concentration in *H.pylori* positive patients is higher than *H.pylori* negative patients (5, 6, 9, 17).

In our study, gastric juice urea nitrogen concentrations were not different in *H.pylori* positive and *H.pylori* negative patient groups, but the gastric juice ammonia concentration was significantly elevated in the *H.pylori* positive group compared with the *H.pylori* negative group (3.08 ± 1.87 mmol/L vs. 0.41 ± 0.43 mmol/L, $p<0.001$). This can be explained by the high urease activity of *H.pylori*. The ROC curve illustration of our study also shows that R values are superior to gastric juice ammonia concentrations in diagnosing *H.pylori* infection. Neithercut et al and Pedriali et al also found that R value is more accurate than gastric juice ammonia measurement in diagnosing *H.pylori* infection (6, 18).The mean R value for *H.pylori* positive patients was 2.91 ± 2.80 whereas it was 29.29 ± 33.40 for *H.pylori* negative patients. The calculated R value for the diagnosis of *H.pylori* infection was 93% specific and 88% sensitive for values (5.00. The sensitivity decreased and the specificity increased as the R values decreased (Table II).

With respect to gastric juice ammonia, values (1.50 mmol/L were 94 % sensitive but 65 % specific for the diagnosis of *H.pylori* infection. On the other hand, values (0.80 mmol/L were more specific (77%) but less sensitive (92%).

The measurement of gastric juice ammonia and urea nitrogen could be completed within 30 minutes which is faster than urea breath tests or histology, and compatible with urease tests. Endoscopy is not obligatory and a naso or orogastric tube could be used for gastric juice aspiration in clinics where endoscopy is not available.

In conclusion, we believe that measurement of gastric juice ammonia concentrations and also the calculation of gastric juice urea nitrogen to ammonia concentrations provide a simple, quick, sensitive method for the detection of *H.pylori* infection.

REFERENCES

1. Graham DY, Go MF. *Helicobacter pylori*: Current status. Gastroenterology 1993; 105: 279-82.
2. Tytgat GNJ, Rausus EAJ. Campylobacter pylori and its role in peptic disease. Gastroenterol Clin N Am 1990; 19: 183-96.
3. Mc Carthy C, Patchett S, Collins R, et al. Long term effect of *Helicobacter pylori* eradication in non-ulcer dyspepsia. Gastroenterology 1991; 100.A121.
4. El Nujumi AM, Rowe PA, Dahil S, et al. Role of ammonia in the pathogenesis of the gastritis, hypergastrinemia and hyperpepsinogenemia caused by *Helicobacter pylori* infection. Gut 1991; 32: 21-24.
5. Chittajallu RS, Neithercut WD, McDonald AMI, McColl KEL. Effect of increasing *Helicobacter pylori* ammonia production by urea infusion on plasma gastrin concentrations. Gut 1991; 32: 21-4.
6. Neithercut WD, Milne A, Chittajallu RS, et al. Detection of *Helicobacter pylori* infection of the gastric mucosa by measurement of gastric aspirate ammonium and urea concentrations. Gut 1991; 32: 973-6.
7. Marshall BJ, Langton SR. Urea hydrolysis in patients with *Campylobacter pyloridis* infection. Lancet 1988; 1: 965-6.
8. Kim H, Park C, Jang WI, et al. The gastric urea and ammonia levels in patients with *Campylobacter pylori*. Am J Clin Pathol 1990; 94: 187-91.
9. Yang DH, Bom HS, Joo YE, et al. Gastric juice ammonia v.s CLO test for the diagnosis of *Helicobacter pylori* infection. Dig Dis Sci 1995, 40 (5): 1083-6.
10. Neithercut WD, Rowe PA, Nujumi AME, et al. Effect of *Helicobacter pylori* on intragastric urea and ammonium concentrations in patients with chronic renal failure. J Clin Pathol 1993; 46: 544-7.
11. Morris A, Sose T, Wicholson G. Rapid diagnosis of *Campylobacter pylori* infection. Lancet 1986; i: 704.
12. Graham DY, Klein PD, Ewans DJ, et al. *Campylobacter pylori* detected noninvasively by the 13C urea breath test. Lancet 1987; 1: 1174-7.
13. Rauws EA, Royen EA, Langerburg W, et al. 14C urea breath test in *Campylobacter pylori* gastritis. Gut 1989; 30: 798-803.
14. Weinstein WM. Gastritis and gastropathies. In: Sleisenger MH, Fordtran JS (eds). Gastrointestinal disease, pathophysiology, diagnosis and management. Philadelphia, WB Saunders Company, 1993: 545-71.
15. Dooley CP, Cohen H, Fitzgibbons PL, et al. Prevalence of *Helicobacter pylori* infection and histologic gastritis in asymptomatic persons. N Engl J Med 1989; 321: 1562-6.
16. Talley NJ, Newell OG, Ormand JE, et al. Serodiagnosis of *Helicobacter pylori*. Am J Clin Pathol 1988; 90: 450-3.

17. Hazell SL, Borody TJ, Gol A, Lee A. *Campylobacter pyloridis* gastritis I: Detection of urease as a marker of bacterial colonisation and gastritis. Am J Gastroenterol 1987; 82: 292-6.
18. Pedriali R, Cantarini D, Gullini S. The importance of determining both urea and ammonium levels in gastric juice for rapid diagnosis of *Helicobacter pylori* infection. Endoscopy 1992; 24 : 292-6.
19. Veendaal RA, Pena AJ, Meijer JL, et al. Long term serological surveillance after treatment of *Helicobacter pylori* infection.Gut 1991; 32: 1291-4.
20. Weber J, Astheimer W, Riemann JF. Detection of *Campylobacter pylori* by measuring gastric ammonia concentration. (Abstract). Gastroenterology 1988; 94: 490.