Gastric juice ammonia and urea concentrations and their relation to gastric mucosa injury in patients maintained on chronic hemodialysis

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Abstract

Purpose: **This study was undertaken to test the hypothesis that high concentrations of urea in gastric juice would have an influence on *Helicobacter pylori* infection in patients maintained on chronic hemodialysis (HD).**

Material and methods: **We investigated 30 patients (17 males, 13 females; mean age 50.8 ± 2.9 years) with end-stage renal disease (ESRD) undergoing hemodialysis treatment (HD) for at least 6 months, who were compared to 31 patients (16 males, 15 females; mean age 61.3 ± 2.2 years) with dyspeptic symptoms. Biopsies from the gastric antrum and body were taken for histological investigation. Urea and ammonia were measured in gastric juice, and the severity of gastritis was evaluated according to Sydney criteria.**

Results: ***H. pylori*** **infection was found in 19 (63%) HD patients and in 22 (71%) control subjects. Gastric juice urea concentration was significantly higher in HD patients than in controls and *H. pylori* infection caused a significant decrease in urea concentration in both groups. There was an inverse correlation between urea and ammonia concentration in gastric juice in both groups. Ammonia concentration in both groups was higher in *H. pylori* infected patients. In *H. pylori* negative subjects ammonia/urea ratio was lower in HD patients in comparison to controls. Ammonia/urea ratio was raised by *H. pylori* infection in both groups, and the difference between HD and control groups persisted. *H. pylori* infection was associated with polymorphonuclear infiltration of gastric mucosa. There was a significant correlation between gastric ammonia and mucosal polymorphonuclear leukocytes infiltration and gastritis score.**

Conclusions: **Higher urea levels in the gastric juice of chronically hemodialyzed patients do not seem to be a risk factor for infection with *Helicobacter pylori*.**

Key words: *Helicobacter pylori*, gastritis, urea, ammonia, hemodialysis.

Introduction

Dyspeptic complaints are present in up to 80% of patients with uremia [1-3]. The reasons for this are not entirely clear. *Helicobacter pylori* is an important causative factor of peptic ulcer disease and chronic gastritis. It is estimated, that in Poland about 70% of population may be infected [4,5]. Data concerning frequency of *H. pylori* infection in chronic hemodialysis patients are conflicting [3,6-11]. *H. pylori* has several adaptations for an acid milieu of the stomach. One of them is urease, which converts urea into ammonia and bicarbonate [12]. The ammonium hydroxide formed raises pH of gastric juice and enables *H. pylori* colonization of gastric mucosa. It has been argued that urease is essential for gastric colonization [13], although, urease negative strains have been isolated from patients [14].

In patients with chronic renal failure high gastric juice urea concentrations, by providing substrate, might lead to elevated concentrations of ammonia, and ammonia has been incriminated as a main factor injuring gastric mucosa by several groups [15-20]. It has also been found that in patients maintained on hemodialysis there is a lack of correlation between endoscopic findings and histopathology of gastric mucosa [2,3].

Therefore this study was undertaken to test the hypothesis that high concentrations of urea in the gastric juice would have an influence on *Helicobacter pylori* infection by investigating relationship between concentrations of urea and ammonia in gastric juice and histopathologic changes of gastric mucosa in

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hemodialyzed patients in comparison to subjects with normal kidney function.

Material and methods

Patients

30 patients (17 males, 13 females; mean age 50.8±2.9 years) with end-stage renal disease (ESRD) undergoing hemodialysis treatment (HD) for at least 6 months, were compared to 31 patients (16 males, 15 females; mean age 61.3±2.2 years) with dyspeptic symptoms and normal levels of blood urea nitrogen and creatinine. ESRD patients were hemodialyzed 3 times a week for 3.5-5.5 hours with Fresenius 4008B machines. Bicarbonate dialysate was used and polysulphone dialysis membranes (Fresenius F5 and F6 dialyzers).

Study protocol

Patients from both groups were recruited for the study based on the presence of dyspeptic complaints and abstinence from alcohol. Exclusion criteria from both groups were as follows: administration of histamine$_2$-receptor antagonists, proton-pump inhibitors, bismuth, sucralfate, non-steroidal anti--inflammatory drugs, or antibiotics within 4 weeks before the study, prior gastrectomy or eradication therapy for *H. pylori* and abnormal liver function tests. The study protocol was approved by Ethics Committee of the Medical University of Białystok.

All the endoscopic procedures were performed by experienced endoscopist (KB). Patients underwent endoscopy before scheduled hemodialysis, after an overnight fast using Olympus GIF E10 endoscope. First 5 ml of gastric juice was withdrawn using sterile cannula and syringe and immediately transferred to a sterile tightly capped tube, and after cooling to 4°C centrifugated at 3000 g for 15 minutes. Five mucosa specimens were obtained using sterile biopsy forceps from the standard sites in antral region and stomach body for urease test and histopathology.

Histopathology

The formalin-fixed, paraffin-embedded tissue sections were stained with hematoxylin and eosin, as well as modified Giemsa stain, to detect *H. pylori*. All sections were assessed "blindly" by the same histopathologist according to the Sydney System [21]. *H. pylori* colonization, polymorphonuclear infiltration, mononuclear cells number, atrophy and intestinal metaplasia were scored as absent, mild, moderate or severe.

The scores for each evaluated factor from both antrum and body were added to give a total "gastritis score" for each patient. We also calculated polymorphonuclear score, mononuclear score, atrophy score, and intestinal metaplasia score by adding individual scores for antrum and corpus.

Patients were considered to be infected with *H. pylori* if either histological examination or urease test were positive.

Laboratory methods

Urease test was performed on a biopsy specimen using commercial kit (Institute of Food and Nutrition, Warsaw, Poland).

Urea concentration in gastric juice was measured with urease colorimetric method, based on hydrolysis of urea to ammonia and carbon dioxide in the presence of urease. Ammonia then reacts with oxoglutarate in the presence of GLDH and NADH, which is oxidised and measured at 340 nm, using reagents from Abbot (Urea Nitrogen List No 8D34-01) and Alcyon TM 300/300i analyzer (Abbot Laboratories). Correction was made for ammonia present in gastric juice according to Lieber [22]. Ammonia was determined with a commercial reagents (Randox Laboratories) using enzymatic UV method and Cobas Bio automatic analyzer (Roche Diagnostics). Urease activity index was calculated as ammonia/urea ratio in gastric juice.

Table 1. **Endoscopic findings in each investigated group. HD (hemodialyzed) patients**

Mucosa	HD – n (%)	Control – n (%)	P (Fisher's exact test)
Normal	10 (33)	10 (33)	NS
Chronic gastritis	10 (33)	12 (39)	NS
Chronic erosive gastritis	6 (20)	6 (19)	NS
Chronic atrophic gastritis	2 (7)	3 (10)	NS
Duodenitis	3 (10)	2 (6)	NS
Gastric ulcer	0	2 (6)	NS
Duodenal ulcer	1 (3)	4 (13)	NS

Statistical analysis

Results are expressed as mean ±SD. Chi-square test, Student's t-test, Mann-Whitney test, MANOVA, Spearman and Pearson correlations were used as appropriate to test the statistical significance of the differences between groups. A P value less than 0.2 was introduced into a backward stepwise logistic regression model. $P<0.05$ was considered statistically significant. The STATA software, version 8.2 (Stata Corporation, College Station, TX, USA) was used for statistical computations.

Results

Endoscopic findings in each investigated group are shown in *Tab. 1*.

Positive urease test was found in 19 (63%) HD patients and in 22 (71%) control subjects ($P=0.525$; NS). The presence of *H. pylori* in tissue sections was found in 7 (23%) HD patients and in 15 (48%) control subjects ($P=0.042$).

Serum urea concentration in HD patients infected with *H. pylori* was similar to that in HD subjects without *H. pylori* infection ($P=0.322$). There was a strong correlation between serum and gastric juice correlations of urea ($r=0.795$; $P<0.0001$).

As seen from *Fig. 1* and *Tab. 2* gastric juice urea concentration was significantly higher in HD patients than in controls (*Tab. 2*) and *H. pylori* infection caused statistically significant decrease in urea concentration in both groups (*Tab. 2*).

The mean gastric juice ammonia concentration in the *H. pylori* infected HD patients was 8.9±7.6 mmol/L compared with 7.4±3.1 mmol/L in the *H. pylori* positive patients with nor-

Figure 1. **Gastric juice urea concentration in *H. pylori* negative and positive control subjects and HD (hemodialyzed) patients** (for statistical significances see *Tab. 2*)

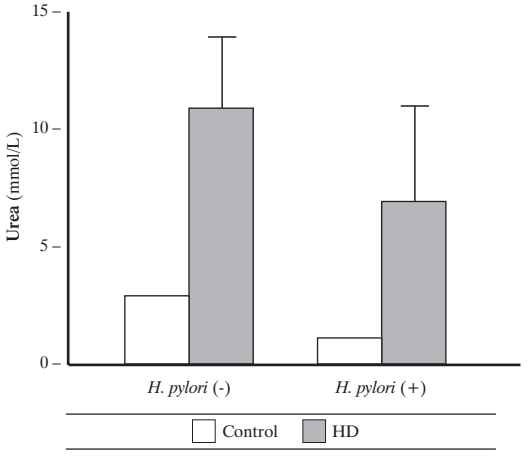
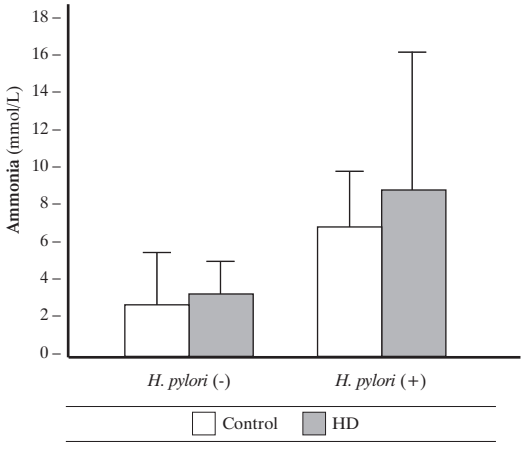


Table 2. **Influence of renal failure and *H. pylori* infection on ammonia and urea concentrations and urease index in gastric juice (MANOVA)**

	P value for		
Factor	**Ammonia**	**Urea**	**Urease index**
Renal failure	NS	<0.00001	<0.00001
H. pylori infection	<0.00001	=0.0004	<0.00001
Interaction between factors	NS	NS	0.0075

Figure 2. **Gastric juice ammonia concentration in *H. pylori* negative and positive control subjects and HD (hemodialyzed) patients** (for statistical significances see *Tab. 2*)



mal renal function (*Fig. 2*). In the *H. pylori* negative patients the values were 3.1±2.0 mmol/L and 1.5±1.0 mmol/L respectively in the HD patients and control subjects and this difference was statistically significant (*Tab. 2*).

There was an overlap of the urea and ammonia concentrations in gastric juice from both *H. pylori* positive and negative subjects (*Fig. 3*).

In *H. pylori* negative subjects ammonia/urea ratio was lower in HD patients in comparison to controls (*Fig. 4* and *Tab. 2*).

Figure 3. **Individual ammonia and urea concentrations in gastric juice from both *H. pylori* positive and negative subjects. HD (hemodialyzed) patients**

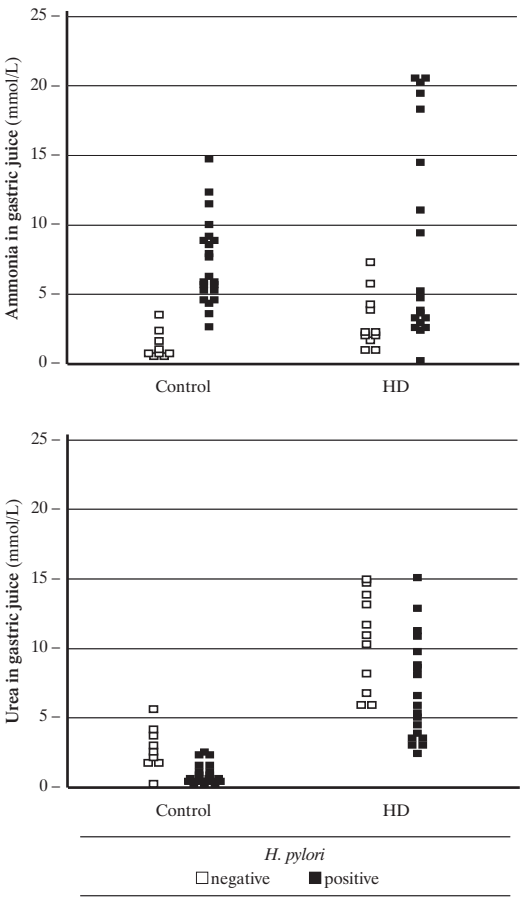
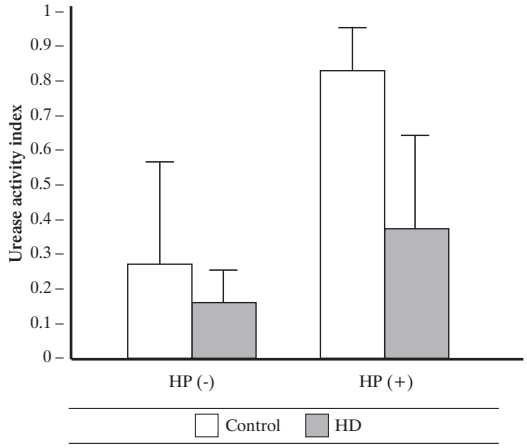


Figure 4. **Ammonia/urea ratio in gastric juice in *Helicobcter pylori* (HP) negative and positive control subjects and HD (hemodialyzed) patients** (for statistical significances see *Tab. 2*)



Ammonia/urea ratio was augmented in the presence of *H. pylori* infection in both groups, and the difference between HD and control groups persisted. Increase, however, was more pronounced in controls (*Tab. 2*).

There was an inverse correlation between urea and ammonia concentration in gastric juice in both groups (*Fig. 5*).

Figure 5. **Correlation between urea and ammonia in gastric juice. Regression for HD (hemodialyzed) patients (solid line) and controls (dashed line)**

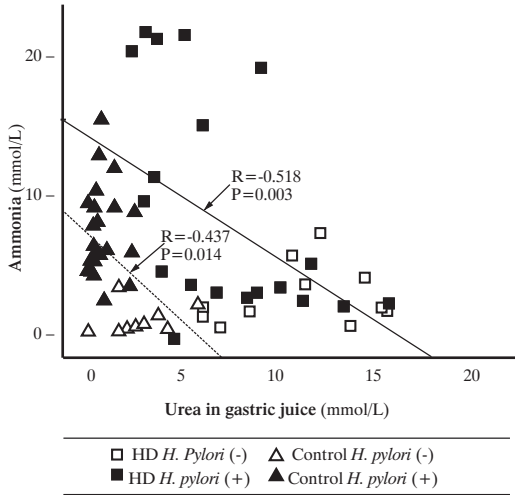


Table 3. **Results of backward stepwise logistic-regression analysis with *H. pylori* infection status as an outcome variable**

Variable	Odds Ratio	95% CI	P value
Polymorphonuclear infiltration score for antrum	4.656	1.852-11.707	0.001
Polymorphonuclear infiltration score for corpus	3.814	1.592-9.136	0.003

* Other variables that were examined but they did not have statistically significant associations with a *H. pylori* infection included patients being on HD treatment (lymphoplasmocytic infiltration score, mucosal atrophy score and intestinal metaplasia score)

The number of polymorphonuclears in antral and stomach corpus mucosa was proportional to ammonia concentration in gastric juice (Spearman's rho =0.339; P=0.008 and Spearman's rho =0.344; P=0.007 respectively).

When all the subjects were analyzed the total gastritis score was directly correlated with ammonia concentration (Spearman's rho =0.295; P=0.022), urease activity index (Spearman's rho =0.302; P=0.019) and *H. pylori* positive status (Spearman's rho =0.446; P=0.004), and inversely to urea in gastric juice (Spearman's rho =-0.284; P=0.028).

We also calculated polymorphonuclear score, mononuclear score, atrophy score, and intestinal metaplasia score by adding individual scores for antrum and corpus. Only polymorphonuclear score was significantly higher in HD in comparison to control group (P=0.045; Mann-Whitney test).

The results of backward stepwise logistic-regression analysis have shown that only polymorphonuclear infiltration in both corpus and antrum were predictive for *H. pylori* infection (*Tab. 3*).

Discussion

Since its discovery in 1982 [23], *Helicobacter pylori* has been linked to many gastroduodenal diseases, including gastritis, peptic ulcer and gastric malignancies. However, its role in the pathogenesis of gastrointestinal complications of renal failure has not been definitely proved. Uremic patients have very often dyspeptic symptoms, but their etiology is multifactorial, and most probably not related to *H. pylori* infection [2,3].

It has been argued that high gastric juice urea concentrations might create favourable environement for *H. pylori*, and therefore a higher frequency of infection in uremic patients should be expected. That has not been proved to be the case [24]. To the contrary, several studies reported lower prevalence of *H. pylori* infection in renal failure patients [3,6-8]. Frequent use of antibiotics in patients maintained on chronic HD might possibly be one of the reasons for the lower prevalence of *H. pylori* infection. We observed somewhat higher prevalence of *H. pylori* than in other studies, although not different from control subjects – fact perhaps related to the selection of patients presenting with dyspeptic complaints, as well as to rather high prevalence of *H. pylori* infection in Poland. In unselected populations it was reported to be about 70% [4,5].

Similar serum urea concentrations in both *H. pylori* positive and negative subjects indicate that differences in gastric juice urea concentration between these groups were in fact caused by infection with microorganism. This is in agreement with Neithercut et al. [25] and opposite to Ala-Kaila et al. [2]. Inverse relationship between urea and ammonia in gastric juice concentrations suggests that uremic state is an important factor causing raised ammonia concentration in gastric juice. Such effect can be mimicked by an infusion of urea into the stomach [26], and should accentuate any ammonia induced effects. Ammonia is transformed to $NH4^+$ ion (ammonium). The relative concentration of these two forms is pH dependent. Ammonia concentration raises with an increase of pH. Ammonia has been incriminated as a factor contributing to gastric mucosal injury [16]. It has been shown that ammonia accelerates apoptosis in gastric mucosa [18], inhibits proliferation and cell cycle progression at S-phase [19], cell migration and proliferation of gastric mucosa [20].

There was an overlap between urea and ammonia concentrations in gastric juice from *H. pylori* positive and negative subjects in both groups which precludes possible use of these parameters as indicators of *H. pylori* infection. It is interesting to note that ammonia/urea ratio was a good predictor of *H. pylori* infection in both groups of patients.

Total gastritis score was directly correlated with ammonia concentration and urease activity index and inversely to urea in gastric juice. It does not necessarily prove a direct causal link. It may also be an indirect marker of *H. pylori* presence which harms mucosa, as suggested by correlation with positive infection status.

Both in HD patients and control group polymorphonuclear infiltration in both corpus and antrum were predictive for *H. pylori* infection. It is well known that *Helicobacter pylori* infection causes gastric mucosa inflammation which is associated with neutrophilic activation [27,28]. Leukocytes are located

in the lamina propria of the mucosa, within the epithelium. The intensity of neutrophilic infiltration within the epithelium is correlated with the extent of mucosa damage and the intensity of *Helicobacter pylori* infection.

In conclusion higher urea levels in blood and gastric juice of chronically hemodialyzed patients do not seem to be a risk factor for *Helicobacter pylori* infection.

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