



# Asian Journal of **Biochemistry**

ISSN 1815-9923



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## Measurement of Ammonium Concentration in Gastric Juice as a Diagnostic Test for *Helicobacter pylori* Infection and the Relationship Between Ammonium Concentration and the Severity of Gastritis

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**Abstract:** In this study, we assessed whether measurement of gastric juice ammonium, would provide comparable to histopathological results. Also, in this study, we sought to examine the relationship between gastric juice ammonium concentration and severity of gastritis. All cases were selected from a database of patients who underwent gastric endoscopic biopsies from January 2001 to May 2004. Thirty-two subjects were *H. pylori* positive and 21 were *H. pylori* negative compared with non-infected subjects. Their *H. pylori* status was confirmed by histologically and serology test. One antral biopsy was obtained in each a total 53 patients and histological assessment of antral biopsy was fixed in 10% neutralized formaldehyde, embedded in paraffin and then stained with hematoxylin - eosin and Giemsa stains were performed in all cases. On the day of the biopsy had gastric juice and a blood sample drawn for the measurement of biochemical markers. The mean gastric juice urea concentration in infected subjects was  $0.6 \pm 0.1$  mmol L<sup>-1</sup>, which was lower than that in the uninfected subjects ( $1.7 \pm 0.2$  mmol L<sup>-1</sup>), (p less than 0.05). The mean gastric juice ammonium concentration in infected subjects was  $3.2 \pm 0.3$  mmol L<sup>-1</sup>, which was higher than that in the uninfected subjects ( $1.3 \pm 0.2$  mmol L<sup>-1</sup>, (p<0.05). This biochemical marker accurately predicts significant *H. pylori* infection.

**Key words:** *H. pylori*, gastric juice, ammonium, gastritis, infection

### Introduction

*Helicobacter pylori* (*H. pylori*) is a spiral bacterium that chronically infects the human stomach and causes gastritis and peptic ulcer (Blaser, 1992). It has been established that *H. pylori* is one of the most important pathogens of several gastroduodenal disease (Kawamura *et al.*, 2000). *H. pylori* is an organism involved in the pathogenesis of human active chronic gastritis, peptic and duodenal ulcer disease. The bacterium was originally isolated in 1982 from endoscopic biopsy specimens of human gastric mucosa (Velazquez and Feirtag, 1999). Progression of *H. pylori* associated gastritis is a major pathway for gastric cancer development (Graham, 2000). It has been shown to be a cause of gastritis and peptic ulcer disease (Malik *et al.*, 2002). *H. pylori* is the first bacterium that is involved in the

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development of gastric mucosa-associated lymphoid tissue lymphoma (Lehours *et al.*, 2003). *H. pylori* possesses unusually high urease activity that lowers the urea concentration and raises the ammonium concentration of the gastric juice in infected people (Neithercut *et al.*, 1991). *H. pylori* is considered as the major pathogen in pH-associated gastritis but the mechanism of its action has not been fully explained. ammonia at higher concentrations damages the gastric mucosa, while ammonium ion exerts the protective activity; the ammonia-induced gastric damage may involve the formation of reactive oxidants (Pedrial *et al.*, 1992; Konturek *et al.*, 1996). Survival of *H. pylori* is dependent upon urease in the cytoplasm and at the bacterial surface. Urease is essential to the growth and motility of *H. pylori* in the mucus layer in the acid-secreting stomach (Sidebotham *et al.*, 2003). The enzymatic method of ammonium measurement proved suitable when the effect of low gastric juice pH was controlled (Neithercut *et al.*, 1993). Effects of ammonia, tumor necrosis factor and anti-Lewis autoantibodies induced after *H. pylori* infection on the development of gastric diseases were investigated (Goto, 2003). The ammonia concentration in the gastric juice from *H. pylori* positive cases was significantly higher than that from negative cases and there was positive correlation between number of *H. pylori*, gastritis score and ammonia concentration in gastric juice. It is suggested that ammonia in gastric juice, which is produced by powerful urease activity of *H. pylori*, is one of the pathogenic factors in gastritis (Takahashi *et al.*, 1993). *Helicobacter pylori* urease activity is a potential source of ammonia in the stomach of patients with cirrhosis (Chakrabarti *et al.*, 2002). In recent years, Gastroenterology specialists have increasingly turned to an alternative hypothesis for the noninvasive prediction of *H. pylori* infection. The means available for diagnosis of the *H. pylori* infection have significant limitations. So a non-invasive test, such as gastric juice indexes of early gastritis would be of value in identifying patients at high risk for developing severe *H. pylori* infection indexes as a viable alternative to histopathology. Principle of the rapid urease test and urea-breath test is based upon urease activity of *H. pylori*. These are gold standard tests for assessment of *H. pylori* infection. However, antral biopsy is invasive and has the potential for complications. Tests requiring endoscopy are expensive and invasive also, the biopsy is difficult to perform. Culture and histology are laborious and require several days. The diagnosis of *H. pylori* infection may be accomplished by noninvasive techniques. Each of these techniques requires several hours to days before the test result is known. Therefore development of new diagnostic tests that may be performed at the time of endoscopy and rapidly interpreted may allow treatment of patients for *H. pylori* infection immediately after endoscopic examination. An alternative approach for the noninvasive prediction of *H. pylori* infection is the use of biochemical markers. In this study, we sought to determine whether direct measurement of gastric juice ammonium level by a biochemical method allows accurate diagnosis of *H. pylori* infection. Also, we sought to examine the relationship of *H. pylori* infection to gastric juice ammonium level. Many methods have been developed for urease determination. Several procedures for the quantitative estimation of ammonium in biological fluid have been described (Bertocchi *et al.*, 1996). They are based on spectrophotometric procedures or on potentiometric or enzyme electrode (Abass *et al.*, 1998). The goals of the present study were to determine the test characteristic of gastric juice ammonium level as measured by an enzymatic method for the diagnosis of *H. pylori* infection and to assess the correlation between gastric-juice ammonium level and the severity of gastritis. It has been hypothesized that the ammonium produced by bacterial urease activity may play a role in the pathogenesis of gastritis.

## **Materials and Methods**

### *Apparatus and Reagents*

#### *Apparatus*

A Ceecil, CE 1020 spectrophotometer, Clement 2000 centrifugal, Olympus, CLV-U40, STB 25 R Endoscopy were used.

#### *Chemicals*

All chemicals were of reagent grade and all solutions were prepared in deionnised water. Glutamate dehydrogenase (bovine liver) obtained as a solution in 50% glycerol containing sodium phosphate buffer (pH 7.3), NADH, 2-oxoglutarate acid  $\beta$ -nicotinamide adenine dinucleotide reduced form,  $\alpha$ -ketoglutaric acid and Glutamate dehydrogenase (EC.1.4.1.3) type III from bovine liver lyophilized powder and were obtained from Sigma Chemical Co., St. Louis, MO, USA. All other chemicals were purchased from Merck, Germany.

#### *Reagents*

A 0.005 M NADH solution was prepared by dissolving 35 mg of this compound in 10 mL of ice cold phosphate buffer, pH 7.2. A 0.15 solution of 2-oxoglutarate was prepared by dissolving 220 mg of this compound in 10 mL of water and then adjusting to pH 7.2 with 4 M NaOH. GLDH solutions of the desired concentration were prepared by diluting the stock with ice cold phosphate buffer of pH 7.2. In this paper one unit of enzyme activity is defined as the amount which causes the transformation of one micromole of 2-oxoglutarate per minute in the presence of  $\text{NH}_4$  and NADH at 25°C and pH 7.2. Standard solutions of  $\text{NH}_4$  were prepared from solid ammonium chloride dissolved in deionised water. All the reagents except ammonium standard solution were ammonium free.

#### *Patients*

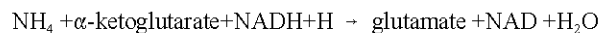
The study cohort includes consecutive outpatientss attending the Gastroenterology Clinic of Babol University of Medical Sciences Hospital from January 2001 to May 2004. The cases were mainly from the Shahid Behshti Hospital of Babol, Iran. All patients had given prior informed consent for use of data and serum for research purpose. We assessed 98 patients from a database of patients under went gastric endoscopic biopsies. Of these 45 were excluded because of use of drugs affecting gastric juice ammonium and, they had undergone previous treatment for *H. pylori*. The remaining 53 patients comprise the sample of this study. Of the patient's, 52.8% were male and 47.2% female. Of the patients were divided into two groups according to the presence ( $n = 32$ ) or absence ( $n = 21$ ) of *H. pylori* infection. Fifty-three patients (28 men, 25 women, The median ages at biopsy were  $49.2 \pm 16.5$  year) underwent upper endoscopy and testing for *H. pylori*. Indications for endoscopy included dyspepsia, gastroesophageal reflux disease, bleeding, abnormal x-ray study, anemia, dysphagia. Their *H. pylori* status was confirmed by antral biopsy and serology test. Patients scheduled for elective endoscopy at the Shahid Behshti Hospital were eligible for inclusion in the study. After informed consent, patients were interviewed, and a data collection form was completed that recorded clinical information. Each patient underwent venepuncture and collection of approximately 5 mL of blood for IgG antibody testing for *H. pylori*. All patients also had a urease test performed. Patients were included in this study who consented to undergo endoscopy with biopsy specimens taken from the antrum of gastric. Patients classified as *H. pylori* positive based on strongly positive quantitative serology, the status of *H. pylori* infection was based on antral biopsy.

In order to correctly evaluate the gastritis pattern at least 5 specimens were required (2 from the antrum, 1 from the incisura angg.

Five specimens (2 from the antrum, 1 from the incisura angularis and 2 from the gastric body) were obtained in a total 53 patients and histological assessment of antral biopsy was fixed in 10% neutralized formaldehyde, embedded in paraffin and then stained with hematoxylin-eosin and Giemsa stains were performed in all cases. Histological grading and staging histotological featur of antral biopsy specimens were analyzed according to the calssification and grading of Sydeny system (Price, 1962) by a single pathologist, who was unaware of patient characteristics. Histological interpretation of gastric biopsies was performed by a pathologist blinded to other *H. pylori* testing results. Patients undergoing upper endoscopy had collection of gastric juice that was tested for ammonium using an enzymatic method. Severity of gastritis was graded using the Sydney classification and correlated to gastric-juice ammonium level. The presence of *H. pylori*, neutrophils and mononuclear cells was graded as normal, mild, moderate or marked for each patient. The degree of the 32 cases was classified as mild in 12, moderate in 15 and severe in 5. Patients also underwent *H. pylori* testing by IgG serology, urease testing. Patients with endoscopic evidence of gastritis were not included. *H. pylori* status was evaluated in all patients by urea test and histological examination of tissue obtained at endoscopy, including serology test. A patient was considered to be infected with *H. pylori* if two of the three tests were positive and was considered not infected when all three tests were negative.

At the time of endoscopy, approximately 5-10 mL of gastric juice was aspirated from the gastric fundus and collected in a trap. The gastric juice was then frozen at -70 °C until the time of analysis. After thawing, the gastric juice specimen was centrifuged at 2500 rpm for 15 min to separate mucus and debris. The centrifuged samples were diluted 1:2 (v/v) in Tris buffer, pH = 7.2 and equilibrated to 30 °C.

Determination of gastric juice ammonium was analyzed by enzymatic conversion to glutamate catalyzed by glutamate dehydrogenase (GDH, l-glutamate:NAD(P) oxidoreductase, EC 1.4.1.3) according to the following, respective reaction (Abass *et al.*, 1998).



The disappearance of NADH has been followed spectrophotometrically and the decrease in absorbance is related to the  $\text{NH}_4$  concentration. In the present investigation, the concentration of ammonium ion was also determined indirectly by monitoring the disappearance of NADH.

#### *Assessment of H. pylori Infection*

##### *Urease Assay*

Urea was assayed of earlier described methods (Cheny and Marbach, 1962). Urease activity was assayed using a coupled enzyme assay (Dunn *et al.*, 1990). Briefly, enzyme preparations of the appropriate dilution (10  $\mu\text{L}$ ) were added to cuvette containing 2 mL of reaction mixture with Tris-HCl (pH 8.2), 1 mM 2-oxoglutarate, 250  $\mu\text{M}$  NADH, 20 units/mL glutamate dehydrogenase, 15 mM urea and 1 mM sodium sulfide to inhibit nonspecific NADH oxidase activity. Absorbance at 340 nm was read every 30 sec using a spectrophotometer. Reactions were performed at 25 °C and rates were calculated from linear portions of the curves (between 1 and 5 min). Blanks containing no urea were

run to measure NADH oxidase activity. One unit of urease activity was defined as that amount capable of hydrolyzing 1  $\mu\text{mol}$  of urea/min. Specific activity of urease was calculated as  $\mu\text{mol}$  of urea hydrolyzed per min/mg of protein. The sensitivity and specificity of the gastric juice ammonium techniques were calculated using two different standardized diagnostic techniques such as histology, serology and urease test.

#### Statistical Methods

Data are expressed as Mean $\pm$ SD. The relationship between gastric juice ammonium level and stage of histological results was analysed by the spearman rank correlation test. For all analyses,  $p < 0.05$  were considered to be statistically significant.

### Results

In this study 53 patients underwent endoscopy and collection of gastric juice. Their *H. pylori* status was confirmed by antral histopathological, urease and serology test. A cutoff value was used to define the qualitative result of serological testing for *H. pylori* ( $< 1.8$ , negative;  $1.8-2.2$ , intermediate;  $2.2$ , positive). The gastric juice ammonium concentration ranged from  $0.2$  to  $3.5 \text{ mmol L}^{-1}$ . Table 1 shows gastric juice ammonium levels were significantly higher in *H. pylori* infected persons than in those without *H. pylori* infection ( $3.2 \pm 0.3$  vs.  $1.3 \pm 0.2$ ,  $\text{mmo L}^{-1}$ ), when compared using a combined reference standards, ( $p < 0.05$ ). As shown in Table 2 the gastric juice urea level were  $0.6 \pm 0.1 \text{ mmol L}^{-1}$  significantly lower in the *H. pylori* -positive patients than in negative group  $1.7 \pm 0.2 \text{ mmol L}^{-1}$ , ( $p < 0.05$ ). No significant difference were found between sera urea in *H. pylori* infected persons and in those without *H. pylori* infection. The severity of gastritis was graded according to the updated Sydney classification system and the relationship to gastric juice ammonium concentration and classification and grading of gastritis was assessed (Table 3). Among 32 patients, gastric juice ammonium level had a significant correlation with the severity of gastritis grade and stage of histological results. Also there significant correlation between gastric juice ammonium level and *H. pylori* -related gastritis scores (e.g., neutrophil, lymphocyte and *H. pylori* density) was the coefficient correlation of the results of histopathological examination to gastric juice ammonium level was  $0.84$ .

Table 1: The mean gastric juice ammonium levels in *H. pylori* infected persons and in those without *H. pylori* infection

Without <i>H. pylori</i> infection	With <i>H. pylori</i> infection
$1.3 \pm 0.2 \text{ mmol L}^{-1}$	$3.2 \pm 0.3 \text{ mmol L}^{-1}$

Data represent mean value $\pm$ SD of at least three separate experiments. ( $p < 0.05$ )

Table 2: The mean gastric juice urea levels in *H. pylori* infected persons and in those without *H. pylori* infection

Without <i>H. pylori</i> infection	With <i>H. pylori</i> infection
$1.7 \pm 0.2 \text{ mmol L}^{-1}$	$0.6 \pm 0.1 \text{ mmol L}^{-1}$

Data represent mean value  $\pm$  SD of at least three separate experiments. ( $p < 0.05$ )

Table 3: Relationship between gastric juice ammonium level and classification and grading of gastritis

Classification and grading of gastritis	Gastric Juice ammonium level
Control	$1.25 \pm 0.25$
Mild	$1.7 \pm 0.28$
Moderate	$1.8 \pm 0.30$
Severe	$3.1 \pm 0.35$

Data represent mean value  $\pm$  SD of at least three separate experiments. ( $p < 0.05$ )

## Discussion

In the present study, gastric juice levels of ammonium were measured in patients with *H. pylori* infection and compared the results to histopathological features to assess the correlation between gastric-juice ammonium level and the severity of gastritis. In this work, we found measurement of gastric juice ammonium level is a sensitive and specific means of *H. pylori* infection diagnosis. The sensitivity of gastric juice ammonium test for the diagnosis of *H. pylori* infection a cut- off value of  $3.2 \text{ mmole L}^{-1}$  was 91.3% and its specificity was 89.5%. Present study supports a possible role of gastric juice ammonium in the severity of gastritis. As shown in Table 1 and 2 patients with *H. pylori* infection had lower levels of gastric juice urea ( $0.6 \pm 0.1$  vs.  $1.7 \pm 0.2 \text{ mmol L}^{-1}$ ,  $p < 0.05$ ) as well as higher levels of gastric juice ammonium ( $3.2 \pm 0.3$  vs.  $1.3 \pm 0.2$ ,  $\text{mmo L}^{-1}$ ,  $p < 0.05$ ), than did patients without *H. pylori* infection. We found that *H. pylori* - infected patients had significantly lower urea levels, higher ammonium concentrations and higher gastric urease activity as compared with uninfected controls. Present results were in good agreement with those reported previously (Konturek *et al.*, 1996; Sidebotham *et al.*, 2003; Neithercut *et al.*, 1993). Elevated gastric-juice ammonium concentration may be correlated to more severe gastritis by a grater density of *H. pylori* organisms colonizing the gastric pits. As shown in Table3, we conclude that the severity of gastritis increase directly with increasing gastric-juice ammonium concentration. Concerning all gastric juice data from our series, ammonium level correlated well with both *H. pylori* stage and gastritis Grade of patients. Patients with more severe neutrophilic infiltration were found to have significantly higher levels of gastric juice ammonium ( $p < 0.05$ ). This study confirmed previous observation showing that measurement of the urea and ammonium levels in gastric juice obtained during routine upper gastrointestinal endoscopy might provide a simple and rapid method of detecting *H. pylori* infection (Neithercut *et al.*, 1991; Konturek *et al.*, 1996).

### Limitation of the Study

This study has several limitations. First, the study population was relatively small, second our exclusive use of patients with diagnostic *H. pylori* infection might have led to a selection bias and third we did not investigate our cases and controls for organic disease using endoscopy. However, each subjects detailed medical record was examined and, thus, organic conditions likely to explain symptoms could be largely excluded. The physician interview assessed for any alarm symptoms and the examination helped to exclude major pathology. To compensate, further studies will be required to re-evaluate present results.

In conclusion, the clinical value of gastric juice ammonium level, is useful index to diagnose *H. pylori* infection. We conclude that gastric juice ammonium level has practical value in evaluation of the *H. pylori* infection.

## Acknowledgements

This reserch was supported by the grant No. 13815 from the Research Council of Babol University of Medical Sciences. Also, we gratefully acknowledge the assistance of the following colleague in our department, Dr. Shafigh, Dr. Siadati, Dr. Ali mohhammadnejad, Mr. H. Saruneh, Mr. M. Aghajani, Miss M. Mosavi and Miss M. Hasani.

## References

- Abass, A.K., J.P. Hart, D.C. Cowell and A. Chappell, 1998. Development of an amperometric assay for NH<sub>4</sub> based on chemically modified screen-printed NADH sensor. *Analytica Chimica Acta*, 373: 1-8.
- Bertocchi, P., D. Compagnone and G. Palleschi, 1996. Amperometric ammonium ion and urea determination with enzyme based probes. *Biosensors and Bioelectronics*, 11: 1-10.
- Blaser, M.J., 1992. *Helicobacter pylori*: Its role in disease. *Clin. Infect. Dis.*, 15: 386-93.
- Chakrabarti, P., A. Zullo, C. Hassan, A. Pandit, A. Chowdhury, A. Santra, B. Hazra, S. Morini and T. Roy, 2002. *Helicobacter pylori*, gastric juice and arterial ammonia levels in patients with cirrhosis. *J. Clin. Gastroenterol.*, 34: 578-581.
- Chen, A.L. and E.P. Marbach, 1962. Modified reagents for determination of urea and ammonia. *Clin. Chem.*, 8: 130-132.
- Dunn, B.E., G.P. Campbell, G.I. Perez-Perez and M.J. Blaser, 1990. Purification and characterization of urease from *Helicobacter pylori*. *J. Biol. Chem.*, 265: 94669-94673.
- Goto, H., 2003. *Helicobacter pylori* and gastric diseases. *Nagoya J. Med. Sci.*, 66: 77-85.
- Graham, D.Y., 2000. *Helicobacter pylori* infection is the primary cause of gastric cancer. *J. Gastroenterol.*, 35: 90-97.
- Kawamura, A., K. Adachi, T. Takashima, T. Katsube, M. Yuki and Y. Kinoshita, 2000. *Helicobacter pylori* infection and family history of gastroduodenal disease in a Japanese population. *Am. J. Gastroenterol.*, 95: 2987-2988.
- Konturek, S.J., P.C. Konturek, T. Brzozowski, J. Stachura and M. Zembala, 1996. Gastric mucosal damage and adaptive protection by ammonia and ammonium ion in rats. *Digestion*, 57: 433-445.
- Lehours, P., A.R. Fourmestraux, A. Lavergne, F. Cantet and F. Megraud, 2003. Which test to use detect *Helicobacter pylori* infection in patients with low-grade gastric mucosa-associated lymphoid tissue lymphoma. *Am. J. Gastroenterol.*, 98: 291-294.
- Malik, A.A., A.K. Ganti, A. Potti, R. Levitt and J.F. Hanley, 2002. Role of *Helicobacter pylori* infection in the incidence and clinical course of monoclonal gammopathy of undetermined significance. *Am. J. Gastroenterol.*, 97: 1371-1374.
- Neithercut, W.D., A. Milne, R.S. Chittajallu, A.M. El Nujumi and K.E. McColl, 1991. Detection of *Helicobacter pylori* infection of the gastric mucosa by measurement of gastric aspirate ammonium and urea concentrations. *Gut*, 32: 973-976.
- Neithercut, W.D., A.M. El Nujumi and K.E. McColl, 1993. Measurement of urea and ammonium concentrations in gastric juice. *J. Clin. Pathol.*, 46: 462-464.
- Pedriali, R., D. Cantarini and S. Gullini, 1992. The importance of determining both urea and ammonium levels in gastric juice for rapid diagnosis of *Helicobacter pylori* infection. *Endoscopy*, 24: 292-295.
- Price, A.B., 1991. The Sydney system. Histological division. *J. Gastroenterol Hepatol.*, 6: 209-222.
- Sidebotham, R.L., M.L. Worku, Q.N. Karim, N.K. Dhira and J.H. Baron, 2003. How *Helicobacter pylori* urease may affect external pH and influence growth and motility in the mucus environment: evidence from in-vitro studies. *Eur. J. Gastroenterol. Hepatol.*, 15: 395-401.
- Takahashi, S., H. Igarashi, K. Nakamura, N. Masubuchi, S. Saito, T. Aoyagi, T. Itoh and I. Hirata, 1993. *Helicobacter pylori* and urease activity-comparative study between urease positive and negative mutant strains. *Nippon Rinsho*, 51: 3149-3153.
- Velazquez, M. and J.M. Feirtag, 1999. *Helicobacter pylori*: characteristics, pathogenicity, detection methods and mode of transmission implicating foods and water. *Intl. J. Food Microbiol.*, 53: 95-104.