The role of *Helicobacter pylori* in minimal hepatic encephalopathy

Seham S. El-seid[a], Fatma A. Attia[a], Mona Abd El-Raouf[a], Ghada S. Abd Al-Azeem[b], Nagwa Abd EL-Ghaffar Mohammed[c], Heba Anwar[a]

[a]Departments of Internal Medicine
[b]Neuropsychiatry, Al-Azhar University
[c]Department of Clinical and Chemical Pathology, National Research Center, Cairo, Egypt

Correspondence to Seham S. El-seid, Department of Internal Medicine, Al-Azhar University, Cairo, Egypt
Tel: +20 101 548 6658
e-mail: drseham4@yahoo.com

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Background
One of the causes of death in patients with liver cirrhosis is hepatic encephalopathy (HE). Hyperammonemia is the most important cause of HE.

Aim
The aim of this work was to determine the relation between the *Helicobacter pylori* infection and minimal hepatic encephalopathy (MHE) in cirrhotic patients and to assess the outcome after treatment of *H. pylori*.

Patients and methods
This study was carried out on 50 Egyptian cirrhotic patients. The patients were divided into two groups: group A1 (32 positive *H. pylori*) and group A2 (18 negative *H. pylori*). Both groups were compared with 20 (age and sex matched) healthy individuals (group B). Patients and controls were subjected to an assessment of history, clinical examination, upper gastrointestinal endoscopy with gastric biopsy for histopathological examination of *H. pylori*, abdominal ultrasound, neuropsychiatric assessment using the figure connection test (FCT), complete blood count, liver and kidney function tests, and determination of plasma ammonia level. Plasma ammonia level and FCT were measured before and after treatment of *H. pylori* among patients with positive *H. pylori*.

Results
Plasma ammonia levels and FCT were highly significantly increased in all cirrhotic patients (group A) compared with the controls (group B) ($P < 0.01$) and in the positive *H. pylori* patients (group A1) compared with the negative *H. pylori* patients (group A2) ($P < 0.01$) and in group A1 before treatment compared with after treatment ($P < 0.01$).

Conclusions
There is a highly significant association between *H. pylori* infection and MHE in cirrhotic patients. The treatment of *H. pylori* infection reduces the mean plasma ammonia levels and improves FCT results among the infected patients. Therefore, *H. pylori* infection is an effective treatable risk factor for the clinical management of MHE.

Keywords:
figure connection test, liver cirrhosis, minimal hepatic encephalopathy, plasma ammonia

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Introduction

Hepatic encephalopathy (HE) has a broad spectrum of neurological symptoms varying from minimal hepatic encephalopathy (MHE) to deep coma and death [1]. Patients with MHE appear clinically well and lack overt encephalopathy; subtle cognitive defects may be present [2]. The diagnosis of MHE is difficult and is made on the basis of neuropsychometric tests [3].

Helicobacter pylori bacteria are rich in urease enzyme producing ammonia from gastric leumen into circulation, causing HE have observed that eradication of *H. pylori* may reduce the concentration of ammonia in cirrhotic patients [5–7].

This study was carried out to determine the relation between *H. pylori* infection and MHE in cirrhotic patients and to assess the outcome after the treatment of *H. pylori*.

Patients and methods

Study population

The study was carried out on 50 Egyptian patients with liver cirrhosis (group A) and 20 age-matched and sex-matched healthy participants as controls (group B). Patients were recruited from the outpatient clinic and from among the inpatients of the internal medicine department, Al-Zahraa University Hospital, Cairo, Egypt, from January 2013 to June 2013. All patients and controls provided their informed consent before inclusion in the study. Also, approval of ethical committee of faculty of medicine, AL-Azhar University, was obtained. Patients were subdivided into group A1 (positive *H. pylori*), which included 32 patients, 17 women and 15 men, age range 43–61 years, mean ± SD (53.63 ± 6.89 years), and group A2 (negative *H. pylori*), which included 18 patients,

11 women and seven men, age range 46–61 years, mean ± SD (50.50 ± 8.8 years). The presence or absence of *H. pylori* was diagnosed by upper gastrointestinal endoscopy and gastric biopsies for histopathological examination for *H. pylori* infection. Exclusion criteria: patients with overt HE, hematemesis and melena, a history of *H. pylori* eradication within the previous 3 months, psychological disorders other than MHE, and severe cardiac, pulmonary, cerebral, and renal disorders were excluded. All patients and controls were subjected to a full assessment of medical history and a thorough clinical examination. Patients with diseases and conditions that increase ammonia level such as overt HE, hematemesis and melena, psychological disorders other than MHE, severe cardiac, pulmonary, cerebral, and renal disorders, and a history of *H. pylori* eradication within the previous 3 months were excluded. Group A1 was subjected to anti *H. pylori* treatment in the form of Clarithromycin 500 mg twice daily plus Amoxicillin 1 g twice daily and Pantoprazol 80 mg daily for 2 weeks, followed by Pantoprazol 40 mg daily for the next 2 weeks [8]. We reassessed the effect of anti-*H. pylori* treatment on hyperammonemia and MHE by determination of plasma ammonia level and figure connection test (FCT).

Laboratory studies

Seven milliliter of fasting venous blood samples were taken from each participant and divided into parts: the first part (1 ml of blood) was placed in a tube containing EDTA for the determination of complete blood count. The second part (1 ml of blood) was placed in a tube containing heparin, the tube was filled completely, and kept tightly closed at all times. It was place immediately on ice and centrifuged, preferably at 4°C, for the determination of ammonia. The third part (3 ml of blood) was left to clot and centrifuged at 1000*g* for 15 min for routine investigations. The fourth part (1.8 ml of blood) was placed in a tube containing 0.2 ml citrate for the determination of prothrombin time.

Complete blood count was determined using a Coulter counter T890 (Coulter Counter, Harpenden, UK). Prothrombin time and prothrombin concentration (PC) were performed using the standard thromboplastin method. Liver function tests including assessment of serum AST, serum ALT, serum albumin, and serum bilirubin and kidney function tests including determination of blood urea and serum creatinine were carried out on a Hitachi auto analyzer, Hitachi 912 (Roche Diagnostics GmbH, Mannheim, Germany) using colorimetric techniques. Electrolytes including serum sodium and potassium were determined using the ion selective electrode on a Hitachi auto analyzer 912.

Plasma ammonia was determined using the enzymatic ultraviolet method on a Cobas Mira SW (8735) analyzer (Roche Diagnostics Corporation, Indianapolis, Indiana, USA) with glutamate dehydrogenase supplied from Randox Laboratories Ltd (Ardmore, Crumlin Co., Antrim, UK) [9]. Plasma ammonia was determined in all the groups studied and in the patients of group A1 after treatment of *H. pylori* infection.

Abdominal ultrasonography was performed to assess liver echogenicity, size, surface, any focal lesions, portal vein diameter, hepatic vein, intrahepatic bile radicle, size of spleen, and presence or absence of ascetic fluid.

Upper gastrointestinal endoscopy was performed using Pentax EPKI 5000 endoscopy (Pentax medical EPKI 5000, EG29K, Japan). Gastric biopsies were taken from the stomach of all patients for histopathological examination and detection of *H. pylori* infection.

Neuropsychiatric assessment was performed using the FCT [10].

We determined FCT in all normal healthy participants and this was considered the standard for FCT represented by minutes (2:35 ± 1:10) and compared it with that of the patients. Then, we determined FCT in group A1 after *H. pylori* eradication. This test is a derivative of the trail-making test and measures cognitive motor abilities. The test score is the time that a patient needs to perform the test, including the time needed to correct the errors. A low score indicates good performance.

Statistical analysis

Data were analyzed and computed using Microsoft Office 2007 (excel, SPSS Inc., Chicago, Illinois, USA) and statistical package for social science version 16. Parametric data were expressed as mean ± SD and nonparametric data were expressed as number and percentage of the total. Comparison of the mean ± SD of two groups was carried out using a paired and an unpaired Student's *t*-test. Measurement of the mutual correspondence between two values was performed using the Spearman correlation coefficient. *P* more than 0.05 was considered nonsignificant, *P* less than 0.05 was considered significant, and *P* less than 0.01 was considered highly significant.

Results

The current study enrolled 50 Egyptian cirrhotic patients who were divided into two groups, (group A1)

positive *H. pylori* and (group A2) negative *H. pylori*, together with 20 apparently healthy individuals as a normal control group (group B).

The demographic, laboratory data, and FCT of the cirrhotic patients studied are shown in Table 1. Group A1: positive *H. pylori* patients: this group included 32 Egyptian cirrhotic patients with positive *H. pylori*, 17 women (53.2%) and 15 men (46.8%), age range 43–61 years, mean age 53.63 ± 6.89 years. Group A2: negative *H. pylori* patients: this group included 18 Egyptian *H. pylori*-negative cirrhotic patients, 11 women (61.2%) and seven men (38.8%), age range 46–61 years, mean age 50.50 ± 8.8 years. Group B: this group included 20 apparently healthy individuals as a normal control group, 10 women (50%) and 10 men (50%), age range 40–62 years, mean age 52.7 ± 4.17 years.

The plasma ammonia level and FCT in all the groups studied are shown in Tables 2 and 3 and Figs 1 and 2. There was a highly significant increase in the plasma ammonia level and FCT in group A1 (positive *H. pylori*) compared with group A2 (negative *H. pylori*) ($P < 0.01$), in group A1 (positive *H. pylori*) compared with group B (control) ($P < 0.01$), and in group A1 before treatment compared with group A1 after treatment ($P < 0.01$).

In the positive *H. pylori* group (group A1), there was a significant positive correlation between ammonia level and the number of colonies of *H. pylori* in the gastric biopsy and also between FCT and the number of colonies *H. pylori* ($r = 0.761$, 0.628, $P < 0.01$, and $P < 0.01$), respectively. There was a significant positive correlation between FCT and ammonia ($r = 0.928$, $P < 0.01$) (Table 4, Figs 3 and 4).

There was a highly significant increase in the serum ammonia level and FCT in group A1 before treatment in comparison with after treatment of *H. pylori* infection.

Discussion

The results of the current study showed that there was a statistically highly significant increase in the mean plasma ammonia levels in MHE patients who tested positive for *H. pylori* infection compared with those with MHE and negative *H. pylori* infection. This finding is in agreement with the result of Agrawal *et al.* [11], who showed that ammonia levels were higher in patients with liver cirrhosis who had *H. pylori* infection in comparison with patients with negative *H. pylori*.

Agrawal A *et al.* [4] showed that there was a significant association between *H. pylori* infection and MHE

Table 1 Laboratory data and figure connection test of patients in groups A1 and A2 (mean ± SD)

Parameters	Groups		
	Group A1 (*N* = 32)	Group A2 (*N* = 18)	*P*
Age (years)	53.63 ± 6.89	50.50 ± 8.89	>0.05
Male sex (%)	46.87	38.89	>0.05
Female sex (%)	53.13	61.11	>0.05
ALT (IU/l)	38.56 ± 1.6	33 ± 2.3	>0.05
AST (IU/l)	50 ± 0.6	50.3 ± 0.4	>0.05
Serum albumin (g/dl)	2.9 ± 0.53	3.07 ± 0.48	>0.05
Total bilirubin (mg/dl)	1.8 ± 1.1	2.03 ± 1.6	>0.05
Total proteins (g/dl)	6.6 ± 0.9	6.5 ± 1.5	>0.05
PT (s)	15.97 ± 2.24	15.63 ± 2.30	>0.05
PC (%)	63.84 ± 9.54	57.67 ± 9	>0.05
INR	1.3 ± 0.26	1.4 ± 0.29	>0.05
Urea (mg/dl)	48.34 ± 35.37	51.11 ± 26.84	>0.05
Creatinine (mg/dl)	1.02 ± 0.97	0.69 ± 0.25	>0.05
Serum K (mEq/l)	3.91 ± 0.49	4.04 ± 0.70	>0.05
Serum Na (mEq/l)	133.22 ± 7.49	132.72 ± 7.89	>0.05
WBC ($10^3/cm^2$)	4.90 ± 1.75	5.73 ± 2.59	>0.05
RBC ($10^6/cm^2$)	3.96 ± 0.62	3.95 ± 0.81	>0.05
PLT ($10^3/cm^2$)	10.83 ± 1.50	98.2 ± 39.27	>0.05
Ammonia (μmol/l)	113.97 ± 32.79	54.15 ± 20.52	<0.01
FCT (min)	11:11 ± 2:26	6:12 ± 02:26	<0.01

ALT, alanine aminotransferase; AST, aspartate aminotransferase; FCT, figure connection test; INR, international normalization ratio; PLT, platelet; PT, prothrombine time; RBC, red blood corpuscle; WBC, white blood corpuscle.

Table 2 Plasma ammonia levels in all the groups studied (mean ± SD)

Groups	Parameters	
	Ammonia (μmol/l)	*P*
Group A (*N* = 50)	92.43 ± 60.79	<0.01
Group B (*N* = 20)	23.16 ± 9.52	
Group A1 (*N* = 32)	113.97 ± 32.79	<0.01
Group A2 (*N* = 18)	54.15 ± 20.52	
Group A1 before treatment	113.97 ± 32.79	<0.01
Group A1 after treatment	108.42 ± 33.54	

Table 3 Figure connection test in all the groups studied (mean ± SD)

Groups	Parameters	
	FCT (min)	*P*
Group A (*N* = 50)	9:23 ± 5:31	<0.01
Group B (*N* = 20)	2:35 ± 1:10	
Group A1 (*N* = 32)	11:11 ± 2:26	<0.01
Group A2 (*N* = 18)	6:12 ± 2:43	
Group A1 before treatment	11:11 ± 2:26	<0.01
Group A1 after treatment	7:27 ± 2:20	

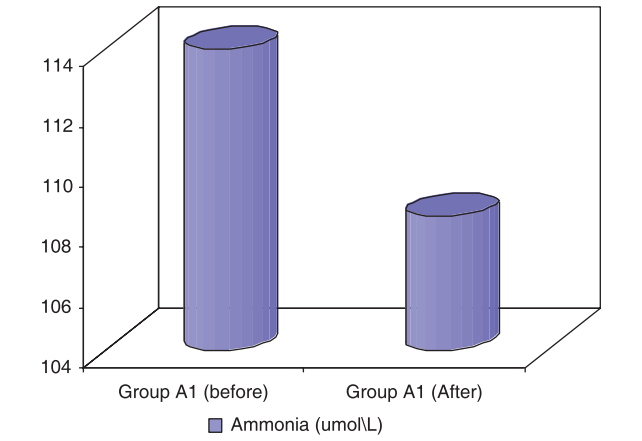
FCT, figure connection test.

Table 4 Correlation between plasma ammonia and figure connection test and number of *Helicobacter pylori* colonies

Parameters	Ammonia		FCT	
	R	*P*	*R*	*P*
Helicobacter pylori colonies	0.761	<0.01	0.628	<0.01
FCT	0.928	<0.01		

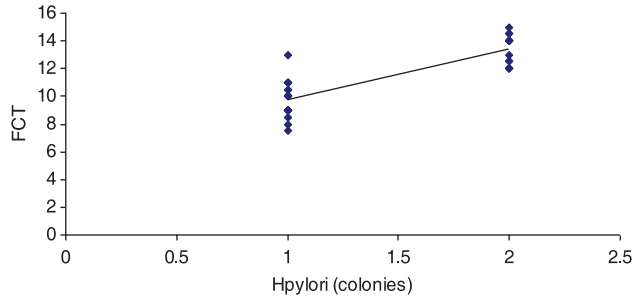
FCT, figure connection test; *R*, correlation.

Figure 1



Plasma ammonia level in group A1 before and after treatment of *Helicobacter pylori* infection.

Figure 3



Positive correlation between figure connection test (FCT) and the number of *Helicobacter pylori* colonies.

Figure 2

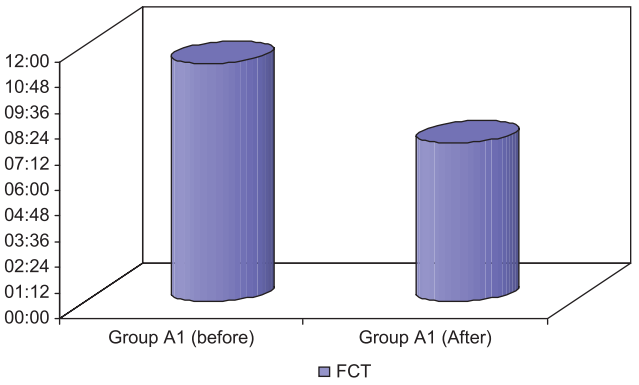
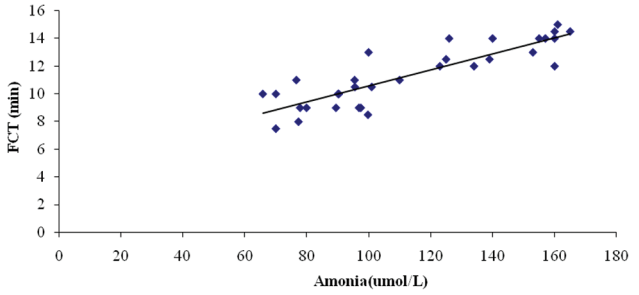


Figure connection test (FCT) in group A1 before and after treatment of *Helicobacter pylori* infection.

Figure 4



Positive correlation between figure connection test (FCT) and plasma ammonia.

in patients with liver cirrhosis. Also, another study by Si *et al.* [12] reported that the ammonia level in the portal vein of cirrhotic patients with *H. pylori* infection was significantly higher than that in patients without infection. In a study carried out by Jiang *et al.* [13], a higher blood ammonia level was detected in *H. pylori*-positive patients with MHE compared with those negative for *H. pylori*, and this result has been confirmed by Chen *et al.* [14]. Muzaffar *et al.* [7] observed that *H. pylori* eradication reduced the ammonia concentration in cirrhotic patients. However, in several studies, *H. pylori* infection generated ammonia in the stomach, but the amount appeared to be too small to affect ammonia levels in patients with cirrhosis [15,16].

The Shanid *et al.*'s study [17] showed that patients with liver cirrhosis had 35.7% seroprevalence, comparable with the data reported by Batmonabane *et al.* [18]. A significant association was found between *H. pylori* infection and portal hypertensive gastropathy (PHG) in cirrhotic patients; also, this infection is correlated to the severity of PHG. PHG provides a favorable environment for the colonization of *H. pylori*, leading to a high prevalence of this bacterium in cirrhotic patients with PHG. They suggested that edema of the gastric mucosa in cirrhosis might provide a favorable environment for the colonization of *H. pylori*, especially when there is severe hemorrhagic congestion [17].

Zullo *et al.* [19] found no significant difference between fasting venous blood ammonia concentrations in patients with *H. pylori* infection and those without *H. pylori* infection. This controversy could be explained by the fact that the amount of ammonia produced by *H. pylori* may depend on the number of bacteria and their distribution in the stomach, gastric pH, and gastric membrane permeability to ammonia, severity of liver impairment, and portal vein branch circulation [14].

Our study showed a highly statistically significant correlation in plasma ammonia level with the severity of *H. pylori* infection (colonies) and this is in agreement with Chen *et al.* [14], who observed that *H. pylori* may increase the blood ammonia concentration and induce HE when the bacterium is widely distributed in the stomach. We found a highly

significant reduction in plasma ammonia levels in *H. pylori*-positive patients with MHE after triple-drug anti-*H. pylori* treatment. This finding indicates that *H. pylori* may contribute toward the development of hyperammonemia in patients with liver disease and MHE. This result is in agreement with the result of Agrawal A *et al.* [4], who showed that anti *H. pylori* therapy results in a reduction in blood ammonia levels and improvement in MHE, and also in agreement with Schulz *et al*. [20], who observed that eradication therapy in *H. pylori*-positive cirrhotic patients may have a beneficial influence on hyperammonemia and MHE.

The results of the present study showed that treatment of *H. pylori* infection resulted in a reduction in blood ammonia levels and an improvement in MHE, and this in contrast with previous studies that showed no association between eradication of *H. pylori* infection and improvement in HE [15,21].

Rekha *et al.* [22] suggested that *H. pylori* infection may be a risk factor for HE only in individuals with advanced cirrhosis, but not in early liver disease, and their results do not rule out completely the role of *H. pylori* infection in the pathogenesis of HE in patients with more advanced liver disease. Therefore, eradication of *H. pylori* to reduce bacterial ammonia production in the stomach may be effective in patients with hyperammonemia with diffuse *H. pylori* infection in the stomach.

This current study showed a highly significant increase in the time of FCT in *H. pylori*-positive groups in comparison with *H. pylori*-negative groups, and in *H. pylori*-positive patients before treatment than after treatment. These results are in agreement with the results of Miquel *et al.* [23] and Shavakhi *et al.* [24], who reported that FCT showed a significant difference between *H. pylori*-positive and *H. pylori*-negative patients and an improvement after eradication of *H. pylori*. In contrast, Zullo *et al.* [19] showed that FCT does not improve after eradication of *H. pylori*.

The study concluded that eradication of *H. pylori* infection reduces the mean plasma ammonia levels and improves FCT results among the infected patients. Therefore, *H. pylori* infection is an effective treatable risk factor for the clinical management of MHE. On the basis of our findings, we recommend testing for *H. pylori* infection in those patients; also, patients with chronic liver disease should be screened for MHE using neuropsychiatric tests for early detection of different cognitive deficits that cannot be detected during a standard neurological examination, but adversely affect daily functioning. We recommended also before giving license of deriving especially the hepatic drivers must be mandatory screened for MHE by neuropsychiatric tests to minimize attacks of road accidents.

Acknowledgements

Conflicts of interest

There are no conflicts of interest.

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