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Helicobacter Pylori Infection and its Associated Genes in Egyptian Patients with Liver Cirrhosis

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Abstract

Background: Genes of helicobacter pylori produce copious amounts of ammonia which is among the leading precipitating factors of hepatic encephalopathy.

Aim of Study: To study the impact of H. pylori infection and its associated genes on the occurrence and severity of hepatic encephalopathy.

Patients and Methods: This study included 100 patients with liver cirrhosis and H. pylori infection in addition to 20 H. pylori infected subjects without liver cirrhosis serving as a control group. All of them were subjected to: Rapid urease test, measuring serum ammonia level, and gene expression (vacA, ureA/ureB, ureI, amiE/amiF, rocFand nixA) by RNA extraction.

Results: Serum ammonia level was higher when UreI, Ami F and Roc F genes were expressed while it was lower when Ami E, Nix A and Vac A genes were expressed in some of the studied groups. Serum ammonia level was significantly higher when Nix A gene was expressed among controls (*p*-value=0.017) and when Ure I gene was expressed among HE group (*p*-value=0.003).

Conclusions: Our data showed that despite serum ammonia was significantly higher among the cirrhotic patients with hepatic encephalopathy did not show a significant relation with different grades of hepatic encephalopathy. Also no statistical significant relation was found between serum ammonia and H. pylori genes expression.

Key Words: *Liver cirrhosis – Hepatic encephalopathy – H. Pylori gene expression.*

Introduction

THERE are various genes that encode proteins to regulate the production of ammonia **[1,2]**. Urease is composed of two types of subunit, UreA and UreB, encoded by the ureAB genes that constitutes up to 10% of the total cell protein in the bacteria **[1]**.

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The ureI gene is activated under acidic conditions, thus increasing the diffusion of urea to the cytoplasm at least 300-fold **[3,4]**.

Other genes of H. pylori, amiE and amiF are two paralogous genes that encode aliphatic amidase (AmiE) and for mamidase (AmiF), which hydrolyze short-chain amides, converting them to ammonia and an organic acid **[5]**.

An arginase gene (rocF) responsible for the hydrolysis of L-arginine to L-ornithine and urea to acid adaptation **[6]**. More acid is controlled by two important proteins encoded by the nixA and nikR genes. The urease requires Ni2+ ions in the bacterial cytoplasm for proper activity. NixA is a specific Ni2+ transporter located on the bacterial inner membrane, and NikR is a transcription regulator responsible for the regulation of various genes, including the ureAB operon, as well as nixA in the presence of Ni2+ ions **[7,8]**. We aimed to study the relationship between H pylori, vacA, ureA/ureB, ureI, amiE/amiF, rocF and nixA genes expression and hepatic encephalopathy (HE) as well as blood amonia level.

Patients and Methods

The present study included 100 cirrhotic patients infected with H. pylori, aged more than 18 years of both sexes, prospectively selected from Hepatology Department and Outpatient Clinic of Ahmed Maher Hospital, Cairo, Egypt over a period of one year from March 2017 to March 2018.

A diagnosis of cirrhosis was made on the basis of clinical & laboratory findings and abdominal ultrasonography. In addition to 20 H. pylori infected

subjects without liver cirrhosis serving as a control group who were attended the GI endoscopy unit for variable gastric symptoms not related to liver disease. The patients were further subdivided into: the non encephalopathy group included 20 patients, and the hepatic encephalopathic group (HE) included 80 patients. The diagnosis of hepatic encephalopathy was based on clinical findings mostly in the form of slurred speech, mood changes and tremors. Patients with GI bleeding, active infection, antibiotic therapy during last month, hepatocellular carcinoma (HCC), electrolyte imbalance, renal failure (Cr >2mg/dl), benzodiazepines, opium and/or alcohol consumption in last two weeks, PPI and antacids in previous days, encephalopathy of other causes such as: Encephalitis, hyperglycemia or hypoglycemia and acute fulminant hepatic failure, metabolic causes of liver cirrhosis were excluded from the study. A written consent was obtained before enrollment in the study.

The selected patients were subjected to the following: (1) Rapid urease test (CLO Test) for diagnosis of H. pylori (Clotest 2028143, Avanos Medical - 60480, USA). Gastric mucosal biopsy samples were used for rapid urease test. The decision was made (positive vs. negative) within 24 hours. Most will turn positive within 120 to 180 minutes but it is best to hold those that appear negative for 24 hours **[9]**. (2) Fasting venous samples obtained from each patient to measure ammonia concentration by ELISA ammonia assay kit. (Abcam, USA, Catalogue No. ab83360). (3) Quantitative gene expression of vacA, ureA/ureB, ureI, amiE/amiF, rocF & nixA was conducted on gastric biopsy samples by real-time PCR.

The studied genes were: Vacuolating cytotoxin (vacA), urease (UreA), urease (UreB), urease (UreaI), amidase (AmiE), Ni+2 transporter (nixA), arginase (rocF).

PCR primers were designed from GenBank RNA sequences cited at http://www.ncbi.nlm.nih.gov/tools/primer-blast. The ideal primer pair was selected with optimal factors including melting temperature (Tm: 60 to 65°C) and applicant length of about 90 to 200bp.

All cDNAs were prepared for all gene markers, glyceraldehyde 3-phosphate dehydrogenase (GAPDH), and non-template negative control. The relative abundance of mRNA species was evaluated using the SYBR® Green method (Applied Biosystems, CA, USA).

PCR primer sequences.

Gene Name	Forward and Reverse primers
VacA	Forward 5' - ATGGAAATACAACAAACACAC- 3' Reverse 5' - CTGCTTGAATGCGCCAAAC - 3'
UreA	Forward 5' - CATTCTTCCCGCTTCCACCA- 3' Reverse 5' - GCGGAAGTTGTCGTTATCGC - 3'
UreB	Forward 5' - ACATTCTTCCCGCTTCCACC- 3' Reverse 5' - CCGCTTGGGACACAAAAGTG- 3'
UreI	Forward 5' - AGGACACCCTAGAGGCGATT- 3' Reverse 5' - TGGTGGAAGCGGGAAGAATG- 3'
AmiE	Forward 5' - AAAAAGTTAAAACTCGCCCACAA- 3' Reverse 5' - AGAGTTCCTACAAACCCTCTGT- 3'
AmiF	Forward 5' - CGGGATCCAATAGTATTCTCTCGCAA TAATTG- 3' Reverse 5' - GGAATTCCCTTAAAAACGGGTTC TAGC- 3'
nixA	Forward 5' - CGCGCGTAATACGACTCAC - 3' Reverse 5' - CCGGGGGATCCACTAGTTCT- 3'
rocF	Forward 5' - TAAGCTCAGAGCATGCGAACAT- 3' Reverse 5' - CTCTTATCGCACCCACGCTA - 3'

Statistical analysis:

Analysis of data was performed using Statistical Package for Scientific Studies 17 (SPSS) for Windows. The quantitative variables were expressed by mean and SD (Standard deviation), compared using unpaired *t*-student test and ANOVA. p value is considered to be significant if <0.05 and highly significant if <0.001.

Results

Among the studied patients, age ranged from 39 to 69 years, the mean age was 54.6±7.58 SD with female predominance (52%). While, among the control group, age ranged from 35 to 65, the mean age was 52±9.04 with male predominance (60%). Table (1).

The mean of serum ammonia level was significantly higher in the studied cirrhotic groups than in the control group and was significantly higher in cirrhotic patients with hepatic encephalopathy than in those without encephalopathy with *p*-value <0.001. Table (2).

The mean of serum ammonia level was higher in grade II hepatic encephalopathy compared to grade I HE among patients with HE however did not show a statistically significant difference. Table (3).

Table (1): Demographic characteristics of the studied groups.

Gender	Control		Non encephal-opathy		HE		Chi-square	
	N	%	N	%	N	%	X^2	*p*
Male	12	60.00	10	50.00	38	47.5	1.200	0.85
Female	8	40.0	10	50.0	42	52.5		
Total	20	100	20	100	80	100		
Age							Anova	
Range	35-65		39-69		38-68			
Mean ± SD	52±9.04		54.6±7.58				F	*p*

Table (2): Serum ammonia level among the studied groups.

	S. Ammonia (µmol/L)		Anova	
	Range	Mean ± SD	F	*p*
Control	18-51	29.645±11.216	58.08	<0.001
Non encephalopathy	85-197	133.250±43.43		
HE	29.3-280	191.43±48.02		

Table (3): Serum ammonia level in relation to grading of encephalopathy.

S. Ammonia in hepatic encephalopathy group	Grading of encephalopathy		
	Grade I (n=57)	Grade II (n=23)	
HE	194.67±45.80	238.87±30.23	0.4

As regards expression of different genes. UreA/B genes were expressed among all groups.

Ami E and Ami F genes were expressed in all controls and HE patients (100%), while Roc F and Vac A genes were only expressed in all encephalopathy patients (100%).

Serum ammonia level was higher when UreI, Ami F and Roc F genes were expressed which showed a statistically significant difference (*p*-value <0.001) among HE group in relation to Ure I gene.

As regards Nix A gene, serum ammonia level was higher when the gene expressed among control group which showed a statistically significant difference (*p*-value=0.017). While serum ammonia level was higher when the gene not expressed among the studied patients which showed a statistically significant difference (*p*-value=0.026) among the non encephalopathy group.

As regards Vac A gene, serum ammonia level was higher when the gene not expressed among controls and non encephalopathy group without a statistical significant difference at *p*-value=0.79 and 0.27 respectively. Table (4).

Table (4): Relation between serum ammonia in different groups and gene expression.

		Serum ammonia		
		Control group Mean ± SD	Non encephalopathy Mean ± SD	HE Mean ± SD
UreA	Expressed	29.65±11.22	133.250±43.43	191.433±48.027
	Not expressed	0.00	0.00	0.00
UreB	Expressed	29.65±11.22	133.250±43.43	191.995±53.76
	Not expressed	0.00	0.00	0.00
UreI	Expressed	32.992±12.94	135.429±45.78	196.342±40.987
	Not expressed	24.625±5.50	128.167±40.917	98.150±97.369
	p-value	0.103	0.742	0.003
AmiE	Expressed	29.645±11.22	127.111±41.24	191.433±48.027
	Not expressed	0.00	188.500±12.02	0.00
	p-value	–	0.055	–
AmiF	Expressed	29.645±11.216	134.316±44.35	191.995±53.76
	Not expressed	0.00	113,00±0.00	0.00
	p-value	–	0.645	–
RocF	Expressed	30.056±12.325	139.417±44.189	191.433±48.03
	Not expressed	28.000±5.715	124.000±43.464	0.00
	p-value	0.753	0.452	–
NixA	Expressed	36.000±13.820	119.500±39.32	188.342±47.090
	Not expressed	24.445±4.488	165.333±37.206	219.250±54.49
	p-value	0.017	0.026	0.227
VacA	Expressed	28.714±11.011	128.647±43.418	191.433±48.027
	Not expressed	30.146±11.739	159.333±40.501	0.00
	p-value	0.794	0.271	–

Discussion

Helicobacter pylori infection with its high urease content, has been suggested as a contributor to intestinal ammonia production, which is an essential factor linked to the pathogenesis of hepatic encephalopathy (HE) **[1,6]**.

In the current study we aimed to investigate the impact of H. pylori infection and its associated genes on the occurrence and severity of hepatic encephalopathy.

In the present study, the mean value of serum ammonia level was statistically significantly higher in the cirrhotic groups than in the control group (p<0.001) as expected as liver remains crucial in ammonia disposal in patients with cirrhosis.

Moreover, we found that the mean of serum ammonia was statistically significantly higher in cirrhotic patients with hepatic encephalopathy than in those without hepatic encephalopathy. This was in agreement with Tarantino et al., through a study conducted on 201 patients with liver cirrhosis, which concluded that the serum ammonia level was increased with the level of the Child score progression **[10]**. Whereas Weissenborn, 1992 denied the role of ammonia in the pathogenesis of HE based on the observations that arterial blood ammonia levels are normal in about 10% of patients with clinically overt HE whereas several patients with cirrhosis and hyperammonemia do not manifest signs of HE **[11]**.

Regarding the relation of ammonia concentration and degree of HE, we found no statistical significant relation between serum ammonia and the severity of hepatic encephalopathy (p-value >0.05). This was in agreement with Ong et al., who concluded that there was a substantial overlap in ammonia levels for hepatic encephalopathy of grades 0 to 2 and, to some degree, grade 3 **[12]**. While Qureshi et al., had found that ammonia levels correlated with the severity of hepatic encephalopathy **[13]**.

Regarding H. pylori gene expression among the studied groups, UreA/UreB genes were expressed in 100% of all patients groups in the present study. This was in line with previous studies that demonstrated the importance of urease structural genes **[14,15]**.

When Ure I gene was expressed it showed a statistically significant difference (p-value <0.001) as among HE group in relation to serum ammonia level. Scott et al., have postulated that ureI gene product is probably a pH activated urea transporter. These observations suggest selective pressure and induction of ureI gene expression with the presence of encephalopathy **[16]**.

Concerning Nix A gene, serum ammonia level was higher when the gene expressed among control group which showed a statistically significant difference (p-value=0.017). While serum ammonia level was higher when the gene not expressed among the studied patients which showed a statistically significant difference (p-value=0.026) among non encephalopathy group.

Similarly Vac A gene, serum ammonia level was higher when the gene not expressed among controls and non encephalopathy group without a statistical significant difference at p-value=0.79 and 0.27 respectively. Vac A is a protein of 87 kDa which can vacuolize the gastric epithelial cells, and is known to be associated with peptic ulcer disease **[17]**.

As regards Ami E gene and Ami F genes, our results showed that they were expressed among almost all studied groups; with no statistical significant difference, indicating their essential role in the nitrogen metabolism of H pylori, similarly Skouloubris et al. demonstrated the important role of these genes in the nitrogen metabolism of H.pylori **[5]**.

It was stated that liver disease is associated with disrupted intestinal homeostasis and with dysbiotic changes in the intestinal microbiota with subsequent enhanced intestinal absorption of ammonia into the portal circulation **[18]**. However, our results raise another query concerning any relation between decompensated liver cirrhosis and expression of certain H pylori pathogenic genotypes.

Regarding the Relation between serum ammonia in the studied groups and genes expression, We found some H pylori genes that were expressed in all groups (100%) as Ure A, Ure B, whereas Ami E and Ami F genes were expressed in all controls and HE patients (100%), while Roc F and Vac A genes were only expressed in HE patients (100%).

Up to our knowledge till now, there were no studies done on different types of H pylori genes, their expression and their relation to serum ammonia among cirrhotic patients whether encephalopathy or not.

Conclusions:

Our data showed that despite serum ammonia was significantly higher among the cirrhotic patients with hepatic encephalopathy; it did not show a significant relation with different grades of hepatic encephalopathy. Also no statistical significant relation was found between serum ammonia and H. pylori genes expression.

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عدوى هيليكوباكتر بيلورى والجينات المصاحبة لها فى مرضى تليف الكبد المصريين

خلفية البحث: تنتج جينات الهليكوباكتر بيلورى H. Pylori كميات وفيرة من الأمونيا التى تعد من بين العوامل الرئيسية المسببة لاعتلال الدماغ الكبدى.

الهدف من البحث: لدراسة تأثير عدوى الهليكوباكتر بيلورى والجينات المرتبطة بها على حدوث وشدة الاعتلال الدماغى الكبدى.

المرضى وطرق البحث: اشتملت هذه الدراسة على ١٠٠ مريض يعانون من تليف الكبد وعدوى الهليكوباكتر بيلورى بالإضافة إلى ٢٠ شخصاً مصاباً بالبكتيريا الهليكوباكتر بيلورى بدون تليف الكبد كمجموعة ضابطة. تم إخضاعهم جميعاً لـ: اختبار اليورياز السريع، وقياس مستوى الأمونيا فى الدم، والتعبير الجينى (vacA، ureA/ureB، urel، amiE/amiF، rocF and nixA) عن طريق استخراج الحمض النووى الريبى.

النتائج: كان مستوى الأمونيا فى الدم أعلى عندما تم التعبير عن جينات Urel وAmi E وAmi F وRoc F بينما كان أقل عندما تم التعبير عن جينات Nix A وVac A فى بعض المجموعات المدروسة. فيما يتعلق بجين Nix A، كان مستوى الأمونيا فى الدم أعلى بشكل ملحوظ عند التعبير عن الجين بين مرضى التليف الكبدى غير المصابين بالاعتلال الدماغى الكبدى (قيمة p=0.017).

الخلاصة: أظهرت بياناتنا أنه على الرغم من أن الأمونيا فى الدم كانت أعلى بشكل ملحوظ بين مرضى التليف الكبدى المصابين بالاعتلال الدماغى الكبدى، إلا أنها لم تظهر علاقة معنوية مع درجات مختلفة من الاعتلال الدماغى الكبدى. كما لم يتم العثور على علاقة ذات دلالة إحصائية بين الأمونيا فى الدم وتعبير الجينات الهليكوبا كتر بيلورى H. pylori.