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Original Article

Insights into correlations between *H. pylori* infection and chronic hepatitis C disease progression: A Comparative study in the Egyptian population

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Abstract

Background: Accumulating evidence implicated *H. pylori* infection in the disease progression of patients with chronic liver. Highly virulent *H. pylori* mediate proinflammatory cytokines and ensuing inflammatory alterations. We aimed to assess the correlation between *H. pylori* infection and disease progression in chronic hepatitis C (HCV) patients with special concern on virulence traits and susceptibility patterns of isolated *H. pylori* strains.

Methods: After clinical, laboratory, and radiological evaluations, 189 chronic HCV patients were assigned into Group I (74 patients with chronic HCV), Group II (77 cirrhotic patients), and Group III (38 hepatocellular carcinoma (HCC) patients on top of cirrhosis). Fecal samples were analyzed using ELISA to detect *H. pylori* antigens. Upper gastrointestinal (GIT) gastric biopsies were processed to identify and isolate *H. pylori* strains. PCR assay verified *cagA*, *VacAs1* & *VacAs2* gens in isolated strains to be correlated with the degree of hepatic disease.

Results: *H. pylori* Ag in stool was identified in 73.02% of studied patients. *H. pylori* Ag was 55.4%, 84.4%, and 84.2% ($p < 0.001$); additionally, culture yielded viable *H. pylori* bacilli in 31.1%, 44.2%, and 55.3% ($p = 0.039$), and PCR assay revealed 47.8%, 85.3%, and 85.7% ($p = 0.004$) were positive for c*agA* and/or v*acAs2* virulence genes in chronic hepatitis, cirrhosis, and HCC patients, respectively. The presence of *H. pylori* positive culture and/or genetic profile is associated with advanced-stage liver disease, hepatic encephalopathy, and portal hypertensive gastropathy.

Conclusions: *H. pylori* infection should be assessed in all patients with liver impairment. Early *H. pylori* detection and subsequent eradication may lessen the severity of decompensation.

Key words: *H. pylori*; chronic liver disease; HCV; virulence genes.

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Background

The primary underlying cause of chronic hepatitis is usually a viral infection, which progresses slowly over time. Concerning the normal course of a chronic hepatitis C virus (HCV) infection, it is estimated that 10-20 percent of patients will develop cirrhosis and that the annual incidence of hepatocellular carcinoma (HCC) could range from 2 to 7% in those with cirrhosis. Worldwide, cirrhosis is a serious health issue with a considerable incidence and prevalence; in addition, it is linked to changes in the mucosa of the gastrointestinal tract [1, 2].

H. pylori are spiral microaerophilic Gram-negative fastidious rods that commonly colonize the gastric mucosa. Such a bacterium is the principal cause of peptic ulcer disease and may be linked to mucosa-associated lymphoid tissue lymphoma (MALT) and gastric cancer [3]. *H. pylori* infection is more prevalent in developing countries (80%) than in developed ones (25%). In Egypt, the prevalence of *H. pylori* infection is 50% or more [4].

The literature review highlights the ongoing interest in the relationship that exists between *H. pylori* infection, cirrhotic consequences, and liver disorders [5]. The incrimination of *H. pylori* in portal hypertensive gastropathy (PHG) has been postulated [2]. Nevertheless, however, the main mechanisms in the pathogenesis of PHG in cirrhotic patients are increased nitric oxide synthase (iNOS) release and consequently deterioration of the gastric defense [6]. Further evidence that coinfection with *H. pylori* may worsen the liver condition comes from detecting *H. pylori* DNA in

liver tissue in certain patients with chronic liver disease [7].

Concerning hepatocellular carcinoma, it has been speculated that there is a potential for *H. pylori* to play a role in HCC. Resected HCC specimens frequently incorporate *H. pylori* genes. In HepG2 hepatoma cells, *H. pylori* may increase the expression of specific proteins involved in signal transduction and gene transcription, potentially causing pathological changes [8]. Other studies addressed that histidine-rich protein (Hpn), from virulent type *H. pylori*, motivates apoptosis through activation of the P14-P53 signaling pathway in addition to suppression of ubiquitin-specific peptidase 5 (USP5) expressions [9]. An infection with *H. pylori* may intensify the oncogenic pathway that relies on transforming growth factor (TGF)-β1, resulting in a notable imbalance between the proliferation and apoptosis of hepatocytes [10].

The expression and intensity of virulence traits of *H. pylori* significantly affect the associated pathogenesis even outside the gastric mucosa. Extremely virulent *H. pylori* strains have been shown to carry a 40 kb region called the cytotoxin-associated genes pathogenicity island (*cagPAI*) that comprises 31 genes encoding the components of a type IV secretion system (T4SS) (pilus-like structure). Possibly one of the most well-known *H. pylori* virulence genes to date is c*agA* [11]. Being a well-known oncoprotein, *CagA* is introduced through T4SS into the host cell. Formerly, it was documented that *CagA* promotes the malignant transformation of gastric epithelial cells after being injected into host cells [12]. The cytotoxin produced by the vacuolating cytotoxin gene (v*acA*) is an additional extremely virulent component. It causes damage to epithelial cells via forming vacuoles, triggers an inflammatory response, and selectively prevents the activation and proliferation of the T-cells [13].

This study's primary goals were to reveal the relationship between *H. pylori* infection and the progression of chronic liver disease with particular attention to the virulence genetic profile and antimicrobial susceptibility pattern of *H. pylori.* The correlations between *H. pylori* infection and the clinical, endoscopic, and laboratory findings in chronic liver disease patients were also analyzed.

Methods

Study design and participants

Between March and October 2023, this cross-sectional comparative study was conducted at the Tropical Medicine Department, Medical Microbiology Immunology and Clinical Pathology Departments, Faculty of Medicine—Menoufia University in collaboration with the Internal Medicine Department, Faculty of Medicine—Benha University, and Hepatology, Gastroenterology Department, National Liver Institute—Menoufia University. The study enrolled 189 confirmed chronic HCV patients (141 males, 74.6% & 48 females, 25.4%); their ages ranged from 28 to 63 years old. The study participants were categorized into 3 groups: Group I was made up of 74 patients with chronic hepatitis C, Group II included 77 patients with HCV-related liver cirrhosis, and Group III consisted of 38 patients with HCC on top of liver cirrhosis.

Ethical considerations

Following an elaboration of the research questions and the research objectives, each participant was made aware of the research's nature and gave informed written consent prior to participating. The study was approved by the local ethics committee of the Faculty of Medicine, Menoufia University (IRB 3/2023TROP12), and was carried out in conformity with the Declaration of Helsinki.

Chronic hepatitis C was identified by the detection of HCV Ab for more than six months, which was confirmed by HCV RNA by real-time PCR. Liver cirrhosis was verified by clinical evaluations, laboratory investigations, and pelvic-abdominal ultrasound findings. Furthermore, triphasic CT of the abdomen was carried out in order to confirm the HCC diagnosis (arterial enhancement with delayed washout). Patients with chronic liver disease other than HCV, such as chronic hepatitis B, autoimmune hepatitis (AIH), metabolic hepatitis, and a history of alcohol consumption or hepatotoxic drugs, and those with hepatic focal lesions other than HCC (metastatic focal lesions, cholangiocarcinoma, hepatoblastoma, haemangioma, etc.), additionally, patients with a known history of *H. pylori* infection and/or treatment were excluded from this study.

Clinical evaluation

The following was applied to each patient: An accurate and precise history was taken, with particular regard to any history of abdominal pain, chronic urticaria, *H. pylori* testing and/or treatment, and drug history, in addition to a history of hepatic encephalopathy, jaundice, melena, and/or hematemesis.

Full physical examinations, including general examinations for signs of chronic liver diseases such as palmar erythema, spider nevai, jaundice, purpura, ecchymosis, flapping tremors, and lower limb edema as

well as signs of chronic urticaria. A local abdominal examination was performed to evaluate the liver, spleen, and ascites. The Child-Pugh classification has been applied to determine the severity of liver disease in cirrhotic patients in GII and GIII [14].

Laboratory investigations

Laboratory tests were carried out, which included complete blood counts and evaluations of the kidney and liver functions. ELISA (enzyme-linked immunosorbent assay) was used for determining HCV Ab and HBVsAg. Real-time PCR was used to quantitatively determine the HCV RNA level, with a detection threshold of 15 IU/mL. Moreover, by employing ELISA assay, serum AFP was quantitatively measured.

Imaging study

An abdominal ultrasound was performed for evaluating the liver, spleen, portal vein, ascites, and the hepatic focal lesion(s) (site, size, number, and the presence of abdominal lymph nodes). To confirm the presence of a hepatic focal lesion(s), triphasic CT was carried out. Additionally, CT identified the site, size, and vascularity of the focal lesion(s) and portal vein patency, along with the presence of abdominal lymph nodes.

Upper Gastrointestinal tract (GIT) endoscopy (Olympus endoscope)

All patients were evaluated for the existence and grading of esophageal varices as per Westaby *et al.* [15], portal hypertensive gastropathy (PHG) along with assessing other findings such as gastroduodenal ulcer(s), gastroduodenal erosions, and gastritis. Gathering of gastroduodenal biopsies for demonstration and isolation of *H. pylori* stains followed by molecular characterization of the target virulence genes.

Isolation of H. pylori from upper GIT biopsies

Sample collection

Biopsy specimens were obtained from 189 (141 males and 48 females) participants who underwent an upper gastrointestinal endoscopy at the Endoscopy Units. Specimens were collected and kept in tryptic soy broth as a transport media (Oxoid, UK) for preservation till processing at the Microbiology Laboratory. Eventually, biopsy specimens for molecular analysis of the target genes were kept frozen at −70°C in 15% tryptic soy broth until analyzed.

Culture and identification of *H. pylori*

For isolating *H. pylori,* the biopsy specimens were homogenized, ground, and subsequently inoculated onto Columbia blood agar supplemented with 10% horse blood, and the selective supplement of *H. pylori* (Skirrow's recipe supplements in the form of vancomycin 10 µg/mL, trimethoprim 5 µg/mL & polymyxin B 2.5 IU/mL, plus amphotericin B: Oxoid, UK) was added to inhibit the growth of associated bacterial pathogens. Then, the inoculated plates were incubated under microaerophilic conditions for 3 to 5 days at 37°C [16].

The identification of *H. pylori* was based on the cultural characteristics, colony morphology for red curved spiral bacilli, and the standard biochemical profile that involved positivity of all oxidase, catalase, and urease reactions [17]. Confirmed isolates were stored at −70°C in BHI (brain heart infusion broth; Oxoid, UK) containing 10% glycerol until performing the susceptibility test.

Antimicrobial susceptibility pattern

Under microaerophilic conditions and at 37°C, 5 mL of frozen isolates were subcultured onto BHI agar containing 10% defibrinated sheep blood and were incubated for three days. The colonies were inoculated into Mueller-Hinton agar plates (Oxoid, UK), and incubated at 37°C under microaerophilic conditions for 72h. Four antimicrobial agents [clarithromycin (15µg), amoxicillin (10µg), metronidazole (5µg) and ciprofloxacin (5µg)] were investigated.

Zone diameters were interpreted using the Clinical and Laboratory Standard Institute (CLSI) guidelines, alongside minor modifications based on preceding comparable research: a zone with a diameter less than 25 mm was identified as amoxicillin resistant, less than 16 mm for resistance for metronidazole [18], less than 30 mm for tetracycline resistance, less than 17 mm for ciprofloxacin [19]. In addition, less than 21 mm was identified for clarithromycin resistance and clarithromycin was susceptible ≥ 21 mm [18].

Virulence genetic profile analysis

The molecular analysis involved the demonstration of three virulence genes: c*agA*, *vacAs1*, and *vacAs2.* The molecular study involved the following steps:

Genomic DNA extraction

From the freshly cultured bacterial isolates, the total genomic DNA was extracted utilizing a commercial kit (QIAamp DNA Mini Kit; Qiagen, Hilden, Germany), as per the instructions of the manufacturer.

Table 1. Target genes sequence and amplified product size.

Target genes	Primers sequence (5′→3)	Expected product size	References
CagA- F	AATACACCAACGCCTCCAAG	496 bp	[20]
CagA-R	ATCTCAAGCTAACAGCCAAAA		
Cycling conditions: a reaction mixture composed of 1 µL of primer, 1 µL of extracted DNA, 12.5 µL PCR MasterMix, and ddH2O to get 25 µL as an entire volume. The cycling condition involved beginning through 5-minute initial denaturation at 95 °C, 30 cycles of 30 seconds at 94 °C, 30 seconds at 52 °C, 30 seconds at 72 °C and a final extension of 72 °C for 5 minutes. By agarose gel electrophoresis the amplified product was detected (Conventional PCR).			
VacAs1-F	5'-ATGGAAATACAACAAACACACCG-3'	338bp	[4]
VacAs1-R	5'-CAACCTCCATCAATCTTACTGGA-3'		
VacAs2-F	5’-ATGGAAATACAACAAACACAC-3’	286bp	
VacAs2-R	5’-CTGCTTGAATGCGCCAAAC-3’		
Cycling conditions: initial denaturation at 94 °C for 1 minute followed by 35 cycles at 95 °C for 1 minute, 53 °C for 1 minute, and 72 °C for 1 minute, with a final extension step of 72 °C for 7 minutes (Multiplex PCR).			

Table 2. Comparison between the three studied patient groups according to demographic, clinical and imaging data.

	Chronic hepatitis (n = 74)	Cirrhosis (n = 77)	HCC (n = 38)	Test of Sig.	*p*
Age (years)					
Mean ± SD.	50.1 ± 3.8	52.6 ± 7.2	54 ± 5.7	F = 6.535*	0.002*
Median (Min. – Max.)	50 (44 – 58)	54 (28 – 61)	54.5 (39 – 63)		
Sig. between Groups.		$p_1 = 0.023^*$, $p_2 = 0.003^*$, $p_3 = 0.468$			
Sex					
Male	56 (75.7%)	58 (75.3%)	27 (71.1%)	$\chi^2 = 0.319$	0.853
Female	18 (24.3%)	19 (24.7%)	11 (28.9%)		
Generalized weakness	8 (10.8%)	53 (68.8%)	30 (78.9%)	$\chi^2 = 68.954^*$	$< 0.001^*$
Right hypochondrial pain	16 (21.6%)	0 (0%)	4 (10.5%)	$\chi^2 = 18.644^*$	$< 0.001^*$
Anorexia	2 (2.7%)	22 (28.6%)	23 (60.5%)	$\chi^2 = 45.885^*$	$< 0.001^*$
Weight loss	0 (0%)	16 (20.8%)	21 (55.3%)	$\chi^2 = 48.821^*$	$< 0.001^*$
History of HE	–	17 (22.1%)	16 (42.1%)	$\chi^2 = 4.988^*$	0.026*
Upper GI bleeding	–	20 (26%)	4 (10.5%)	$\chi^2 = 2.042$	0.153
Cachexia	–	9 (11.7%)	5 (13.2%)	$\chi^2 = 0.051$	$^{FE}p = 1.000$
Ecchymosis	–	31 (40.3%)	13 (34.2%)	$\chi^2 = 0.394$	0.530
Lower limb oedema	–	47 (61%)	20 (52.6%)	$\chi^2 = 0.740$	0.390
Jaundice	–	55 (71.4%)	26 (68.4%)	$\chi^2 = 0.111$	0.740
Child Pugh classification					
A	–	23 (29.9%)	12 (31.6%)		
B	–	24 (31.2%)	13 (34.2%)	$\chi^2 = 0.251$	0.882
C	–	30 (39%)	13 (34.2%)		
Ascites by US					
No	–	19 (24.7%)	13 (34.2%)		
Mild to moderate	–	36 (46.7%)	17 (44.7%)	$\chi^2 = 1.4052$	0.495
Massive	–	22 (28.6%)	8 (21.1%)		
Spleen size by US					
Normal	69 (93.2%)	26 (33.8%)	13 (34.2%)	$\chi^2 = 64.721^*$	$< 0.001^*$
Enlarged	5 (6.8%)	51 (66.2%)	25 (65.8%)		
Splenic collaterals	–	24 (31.2%)	12 (31.6%)	$\chi^2 = 0.002$	0.964
Radiology Portal Vein (US)					
Normal	–	35 (45.5%)	15 (39.5%)	$\chi^2 = 0.370$	0.543
Dilated	–	42 (54.5%)	23 (60.5%)		
Triphasic CT Portal vein thrombosis					
Patent PV	–	76 (98.7%)	35 (92.1%)	$\chi^2 = 3.297$	$^{FE}p = 0.105$
Thrombosed	–	1 (1.3%)	3 (7.9%)		
Triphasic CT number of focal lesion (s)					
Single	–	–	29 (76.3%)	–	–
Multiple	–	–	9 (23.7%)		
Triphasic CT size (cm) of focal lesion					
Mean ± SD.	–	–	5.5 ± 2.9	–	–
Median (Min. – Max.)	–	–	5.3(1 – 10.5)		

HE: Hepatic encephalopathy; US: ultrasound; SD: Standard deviation; χ^2: Chi square test; MC: Monte Carlo; F: F for One way ANOVA test; Pairwise comparison between each 2 groups was done using Post Hoc Test (Tukey). *p*: *p* value for comparing between the three studied groups; p_1: *p* value for comparing between chronic hepatitis and Cirrhosis; p_2: *p* value for comparing between chronic hepatitis and HCC; p_3: *p* value for comparing between cirrhosis and HCC; *: Statistically significant at $p \leq 0.05$.

Until the target virulence genes were demonstrated, the extracted DNA was kept at -80°C.

PCR protocol

The primers and cycling conditions applied are shown in Table 1.

ELISA assay for *H. pylori* Ag detection in stool samples

As directed by the manufacturer, the ELISA kit (enzyme-linked immunosorbent assay) (EDITM Fecal *Helicobacter Pylori* Antigen ELISA Kit; Catalogue no. KT-826) was designed to detect the *H. pylori* antigen in feces. The results were read by a microwell reader and compared in a parallel manner with a calibrator and control.

Statistical analysis

The computer was supplied with data, and the IBM SPSS software package version 20.0 (Armonk, NY: IBM Corp) was used for analysis. Numerical and percentage representations were employed for categorical data. To compare between the two groups, the chi-square test was applied. When more than 20% of the cells had an expected count of fewer than 5, an alternative correction test, such as the Fisher Exact or Monte Carlo correction test, was implemented. The Kolmogorov-Smirnov test was employed to check for normality in continuous data. For the variables that are quantitatively represented, the range (minimum and maximum), mean, standard deviation, and median for quantitative variables that are not normally distributed. The student t-test was applied for comparing two groups, whereas a one-way ANOVA test was employed to compare the four patient groups and followed by a Post Hoc test (Tukey) for the pairwise comparison. Conversely, for non-normally distributed parameters, the Mann Whitney test has been utilized in comparing two patient groups, while, in order to compare different groups, we used the Kruskal Wallis test followed by the Post Hoc test (Dunn's for multiple comparisons test) for

Table 3. Comparison between the three studied patient groups according to laboratory investigations.

	Chronic hepatitis (n = 74)	Cirrhosis (n = 77)	HCC (n = 38)	Test of Sig.	*p*
HB (gm/dL)					
Mean ± SD.	13.8 ± 1.3	10 ± 1.1	10.1 ± 0.9	F = 236.661*	< 0.001*
Median (Min. – Max.)	13.9 (12 – 16.5)	9.9 (8.2 – 12.3)	10.5 (7.9 – 11)		
Sig. between Groups.		$p_1 < 0.001^*$, $p_2 < 0.001^*$, $p_3 = 0.723$			
TLC 10^3(mm^3)					
Mean ± SD.	7.3 ± 1.8	5.6 ± 1.6	5.4 ± 1.2	H = 37.034*	< 0.001*
Median (Min. – Max.)	7.5 (4.2 – 10.8)	5 (3.5 – 10.5)	5(3.7 – 8)		
Sig. between Groups.		$p_1 < 0.001^*$, $p_2 < 0.001^*$, $p_3 = 0.434$			
Platelet count 10^3(mm^3)					
Mean ± SD.	284.3 ± 60.1	137 ± 42.2	168.2 ± 75.8	H = 109.507*	< 0.001*
Median (Min. – Max.)	310 (179 – 350)	136 (65 – 216)	151.5 (86 – 312)		
Sig. between Groups.		$p_1 < 0.001^*$, $p_2 < 0.001^*$, $p_3 = 0.097$			
ALT (IU/L)					
Mean ± SD.	130.3 ± 54.6	35.7 ± 12.7	39.4 ± 15.8	H = 124.301*	< 0.001*
Median (Min. – Max.)	123 (47 – 224)	31 (20 – 76)	35 (14 – 90)		
Sig. between Groups.		$p_1 < 0.001^*$, $p_2 < 0.001^*$, $p_3 = 0.379$			
AST (IU/L)					
Mean ± SD.	108.9 ± 50.2	34 ± 15.9	43.5 ± 11.1	H = 119.011*	< 0.001*
Median (Min. – Max.)	103.5 (40 – 201)	31 (16 – 112)	41 (22 – 75)		
Sig. between Groups.		$p_1 < 0.001^*$, $p_2 < 0.001^*$, $p_3 = 0.006^*$			
Serum albumin (gm/dL)					
Mean ± SD.	4.4 ± 0.4	2.8 ± 0.8	2.6 ± 0.8	H = 132.501*	< 0.001*
Median (Min. – Max.)	4.2(4 – 5.5)	2.9 (1.5 – 4.1)	2.8 (1.2 – 4.8)		
Sig. between Grps.		$p_1 < 0.001^*$, $p_2 < 0.001^*$, $p_3 = 0.155$			
INR					
Mean ± SD.	1.0 ± 0.03	1.6 ± 0.3	1.7 ± 0.4	H = 133.531*	< 0.001*
Median (Min. – Max.)	1.0 (1 – 1.1)	1.5 (1.1 – 2.2)	1.6 (1 – 2.7)		
Sig. between Grps.		$p_1 < 0.001^*$, $p_2 < 0.001^*$, $p_3 = 0.314$			
Direct bilirubin (mg/dL)					
Mean ± SD.	0.2 ± 0.05	1.1 ± 0.7	1.4 ± 1.2	H = 112.052*	< 0.001*
Median (Min. – Max.)	0.2 (0.1 – 0.3)	1 (0.1 – 3.5)	0.8 (0.1 – 5.2)		
Sig. between Grps.		$p_1 < 0.001^*$, $p_2 < 0.001^*$, $p_3 = 0.961$			
Total bilirubin (mg/dL)					
Mean ± SD.	0.7 ± 0.1	3.4 ± 2.2	4.2 ± 3.4	H = 126.034*	< 0.001*
Median (Min. – Max.)	0.7 (0.2 – 0.8)	2.8 (0.5 – 9.9)	2.7 (0.8 – 14.5)		
Sig. between Groups.		$p_1 < 0.001^*$, $p_2 < 0.001^*$, $p_3 = 0.668$			
Alpha fetoprotein (AFP)					
Mean ± SD.	2.1 ± 0.5	17.73 ± 7.47	3570.03 ± 20.97	H = 147.492*	< 0.001*
Median (Min. – Max.)	2.21 (0.42 – 2.6)	12 (1.9 – 45)	2681 (20 – 5670)		
Sig. between Groups.		$p_1 = 0.001^*$, $p_2 = 0.001$, $p_3 < 0.001^*$			

HB: hemoglobin concentration; TLC: total leucocyte count; ALT: alanine transaminase; PT: prothrombin time; INR: International normalized ratio; SD: Standard deviation; χ^2: Chi square test; MC: Monte Carlo; H: H for Kruskal Wallis test; Pairwise comparison between each 2 groups was done using Post Hoc Test (Dunn's for multiple comparisons test). *p*: *p* value for comparing between the three studied groups; p_1: *p* value for comparing between chronic hepatitis and Cirrhosis; p_2: *p* value for comparing between chronic hepatitis and HCC; p_3: *p* value for comparing between cirrhosis and HCC; *: Statistically significant at $p < 0.05$.

pairwise comparison. The results' significance was determined at the level of 5%.

Results

189 confirmed chronic HCV patients, ages ranging from 28 to 63, were enrolled in this cross-sectional comparative study (141 males and 48 females). Three categories were used to group the study participants: Group I consisted of 74 patients suffering from chronic hepatitis C, Group II included 77 patients suffering from liver cirrhosis linked to HCV, and Group III included 38 patients suffering from HCC on top of liver cirrhosis. The demographic, clinical, and imaging features of the involved participants are shown in Table 2. Participants in the liver cirrhosis and HCC groups are older than those in the chronic hepatitis group (p = 0.002); however, there is no discernible gender difference. Furthermore, cirrhosis and HCC patients had higher rates of generalized weakness, anorexia, and weight loss than did patients with chronic hepatitis.

The clinical evaluation of the studied participants revealed no differences between the cirrhotic and HCC groups regarding the history of upper GIT bleeding, cachexia, ecchymosis, L.L. edema, jaundice, or Child Pugh grading (p = 0.86, 0.76, 0.79, and 0.35, respectively), while a history of hepatic encephalopathy was significantly observed in the HCC group (p = 0.026). The imaging characteristics of the hepatic focal lesions in the HCC group are demonstrated in Table 2.

In terms of laboratory investigations, there was a discernible difference in all complete blood count parameters between the patient groups. The cirrhotic and HCC groups had lower hemoglobin concentrations, platelet counts, and total leucocyte counts than the chronic hepatitis patients. Additionally, there were notable variations in alpha-fetoprotein (AFP) and liver function tests between the three groups (Table 3).

Figure 1. The results of disk diffusion test for demonstration of antimicrobial susceptibility.

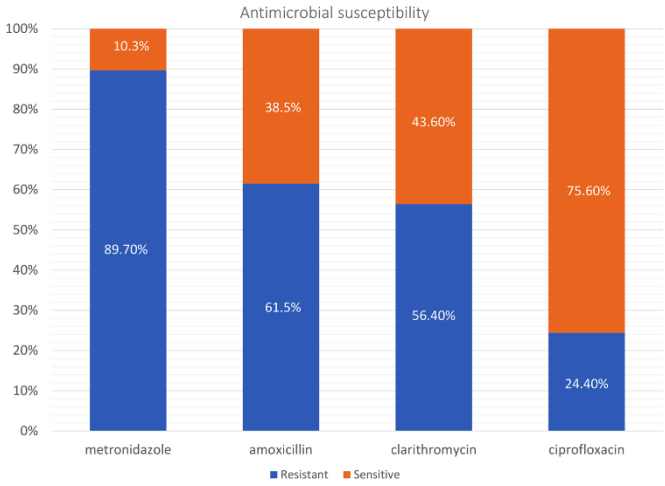

Table 4 presents the upper GIT endoscopy findings, including those related to portal hypertension. It indicates that there was no significant difference between the cirrhotic and HCC groups in terms of the presence or grade of esophageal varices. Furthermore, there was no discernible difference between the two groups in terms of portal hypertensive gastropathy (PHG) presence (p = 0.641). Gastroduodenal ulcers were detected in 5.4%, 11.7%, and 18.4% of chronic hepatitis, cirrhotic, and HCC patients, respectively.

The ELISA assay for *H. pylori* Ag detection in stool revealed the presence of *H. pylori* Ag in 73.02% (138/189) of the studied cases and was distributed as follows: 55.4%, 84.4%, and 84.2% in chronic hepatitis, cirrhosis, and HCC patients, respectively, with a statistically significant difference (p < 0.001). The microaerophilic culture techniques yielded viable *H. pylori* bacilli in only 41.3% (78/189) of the processed endoscopic biopsy specimens, where 31.1%, 44.2%,

Table 4. Comparison between the three studied groups according to upper GIT endoscopy findings.

	Chronic hepatitis (n = 74)	Cirrhosis (n = 77)	HCC (n = 38)	Test of Sig.	p
Esophageal varices					
No	–	30(39%)	14(36.8%)	χ^2 = 0.048	0.826
Yes	–	**47 (61%)**	**24 (63.2%)**		
Grade I	–	14 (29.8%)	7 (29.2%)		
Grade II	–	11 (23.4%)	4 (16.7%)	χ^2 = 6.133	0.105
Grade III	–	14 (29.8%)	3 (12.5%)		
Grade IV	–	8 (17%)	10 (41.7%)		
Upper GI Endoscopy PHG	–	52 (67.5%)	24 (63.2%)	χ^2 = 0.217	0.641
Other endoscopic finding					
No other findings	56 (75.7%)	56 (72.7%)	23 (60.5%)		
Gastroduodenal ulcer	4 (5.4%)	9 (11.7%)	7 (18.4%)		
Gastritis	9 (12.2%)	8 (10.4%)	6 (15.8%)	χ^2 = 5.8002	^{MC}p = 0.445
Gastroduodenal erosions	5 (6.7%)	4 (5.2%)	2 (5.3%)		

PHG: portal hypertensive gastropathy; SD: Standard deviation; χ^2: Chi square test; MC: Monte Carlo; F: F for One way ANOVA test; Pairwise comparison between each 2 groups was done using Post Hoc Test (Tukey); H: H for Kruskal Wallis test; Pairwise comparison between each 2 groups was done using Post Hoc Test (Dunn's for multiple comparisons test); p: p value for comparing between the three studied groups; p_1: p value for comparing between chronic hepatitis and Cirrhosis; p_2: p value for comparing between chronic hepatitis and HCC; p_3: p value for comparing between cirrhosis and HCC; *: Statistically significant at p < 0.05.

Table 5. Distribution of the target virulence genes among culture-positive cases.

	Chronic hepatitis (n = 74)	Cirrhosis (n = 77)	HCC (n = 38)	Test of Sig.	p
***H. Pylori* Ag in stool**					
Positive	41 (55.4%)	65 (84.4%)	32 (84.2%)	$\chi^2 = 19.144^*$	**< 0.001***
Negative	33 (44.6%)	12 (15.6%)	6 (15.8%)		
***H. pylori* culture**					
Positive culture	23 (31.1%)	34 (44.2%)	21 (55.3%)	$\chi^2 = 6.504^*$	**0.039***
Negative culture	51 (68.9%)	43 (55.8%)	17 (44.7%)		
Virulence genetic profile	**(n = 23)**	**(n = 34)**	**(n = 21)**		
Negative	12 (52.2%)	5 (14.7%)	3 (14.3%)		
Positive:	**11 (47.8%)**	**29 (85.3%)**	**18 (85.7%)**	$\chi^2 = 18.6735^*$	**$^{MC}p = 0.004^*$**
cagA alone	5 (21.7%)	10 (29.4%)	3 (14.3%)		
vacAs2 alone	3 (13.05%)	12 (35.3%)	5 (23.8%)		
Coexistence of *cagA* + v*acAs2*	3 (13.05%)	7 (20.6%)	10 (47.6%)		
$p_1 = 0.02^*$, $p_2 = 0.017^*$, $p_3 < 0.18$					

χ^2: Chi square test, MC: Monte Carlo; *p*: *p* value for comparing between the three studied groups; p_1: *p* value for comparing between chronic hepatitis and Cirrhosis; p_2: *p* value for comparing between chronic hepatitis and HCC; p_3: *p* value for comparing between cirrhosis and HCC; *: Statistically significant at $p < 0.05$.

and 55.3% of groups I, II, and III patients displayed positive *H. pylori* growth with a statistically significant difference ($p = 0.039$), as displayed in Table 5.

The performed disk diffusion test for demonstration of antimicrobial susceptibility showed that 91%, 61.4%, 57%, and 24.5% of the obtained *H. pylori* isolates were, respectively, resistant to metronidazole, amoxicillin, clarithromycin, and ciprofloxacin (Figure 1).

The molecular analysis of *H. pylori* virulence traits by PCR assay revealed that 47.8%, 85.3%, and 85.7% of groups I, II, and III patients were positive for c*agA* and/or *vacAs2* genes but with variable distributions and genotypes. For patients with chronic hepatitis, 21.7%, 13.05%, and 13.05% of their *H. pylori* isolates, respectively, had the cagA *gene, vacAs2* gene, and coexistence of c*agA* and *vacAs2*. Among the cirrhotic group, 29.4% of the patients were infected with c*agA* positive isolates, and about 35.3% proved *vacAs2* genotype. Moreover, 20.6% of the isolates exhibited coexistence of the two genes. Notably, 23.8% and 47.6% of HCC cases carried *vacAs2* and combined c*agA* + *vacAs2* genes, respectively. None of the investigated strains harbored *the vacA s1* gene, as shown in Table 5.

The relation between *H. pylori* stool antigen and *H. pylori* positive culture with clinical, endoscopic, and laboratory findings in chronic hepatitis patients is presented in Table 6. Statistical analysis revealed that chronic hepatitis C patients with gastroduodenal ulcers, gastritis, or gastroduodenal erosions were significantly positive for *H. pylori* culture. While there were no

Table 6. Relation between *H. Pylori* stool antigen and *H. Pylori* culture with clinical, endoscopic and laboratory findings in chronic hepatitis patients (n = 74).

	***H. Pylori* Ag in stool**				***H. pylori* culture**			
	Negative (n = 33)	Positive (n = 41)	Test of sig.	*p*	Negative (n = 51)	Positive (n = 23)	Test of sig.	*p*
Age (years)								
Mean ± SD.	50.4 ± 3.98	49.9 ± 3.70	t = 0.488	0.627	50.2 ± 3.54	50 ± 4.44	t = 0.183	0.855
Median (Min. – Max.)	49 (45 – 58)	50 (44 – 56)			50 (45 – 58)	50 (44 – 56)		
Sex								
Male	23 (69.7%)	33 (80.5%)	$\chi^2 = 1.157$	0.282	37 (72.5%)	19 (82.6%)	$\chi^2 = 0.871$	0.351
Female	10 (30.3%)	8 (19.5%)			14 (27.5%)	4 (17.4%)		
Other finding in upper GIT endoscopy								
Gastro No other finding	30 (75.6%)	26 (75.6%)	$\chi^2 = 7.7559$	$^{MC}p = 0.051$	43 (84.3%)	13 (56.5%)	$\chi^2 = 7.7925$	**$^{MC}p = 0.050$**
Gastric or duodenal ulcer	1 (6.1%)	3 (4.9 %)			2 (3.9 %)	2 (8.7 %)		
Gastritis	1 (6.1%)	8 (17.1%)			3 (5.9 %)	6 (26.1 %)		
Gastroduodenal erosions	1 (12.2%)	4 (2.40%)			3 (5.9 %)	2 (8.7 %)		
ALT (IU/L)								
Mean ± SD.	133.8 ± 52.8	127.5 ± 56.4	U = 616.0	0.510	133.2 ± 52.2	123.8 ± 60.1	U = 508.0	0.359
Median (Min. – Max.)	123 (53 – 220)	120 (47 – 224)			123 (47 – 224)	92 (52 – 216)		
Total bilirubin (mg/dL)								
Mean ± SD.	0.70 ± 0.11	0.65 ± 0.16	U = 585.0	0.301	0.69 ± 0.12	0.63 ± 0.18	U = 494.0	0.261
Median (Min. – Max.)	0.78 (0.50 – 0.80)	0.68 (0.20 – 0.80)			0.78 (0.50 – 0.80)	0.67 (0.20 – 0.80)		
Platelet count 10^3 (mm^3)								
Mean ± SD.	269.4 ± 63.4	296.3 ± 55.1	U = 516.0	0.079	276.8 ± 62.2	300.9 ± 52.6	U = 464.0	0.150
Median (Min. – Max.)	254 (179 – 350)	312 (190 – 350)			259 (179 – 350)	322 (199 – 350)		
Serum albumin (gm/dL)								
Mean ± SD.	4.36 ± 0.34	4.44 ± 0.46	U = 637.50	0.663	4.46 ± 0.42	4.28 ± 0.37	U = 452.0	0.107
Median (Min. – Max.)	4.20 (4 – 4.90)	4.20 (4 – 5.50)			4.50 (4 – 5.50)	4.20 (4 – 5)		
INR								
Mean ± SD.	1.03 ± 0.03	1.03 ± 0.02	U = 619.0	0.524	1.03 ± 0.03	1.02 ± 0.02	U = 447.0	0.097
Median (Min. – Max.)	1.03 (1 – 1.09)	1.03 (1 – 1.07)			1.03 (1 – 1.09)	1.02 (1.0 – 1.07)		

ALT: alanine transaminase; INR: International normalized ratio; χ^2: Chi square test; MC: Monte Carlo; t: Student t-test; U: Mann Whitney test. *p*: *p* value for comparison between the studied categories; *: Statistically significant at $p < 0.05$.

significant associations with other studied parameters, age, sex, ALT, serum albumin, total bilirubin, INR, or platelets.

According to Table 7, we noticed that cirrhotic patients with PHG displayed *H. pylori* antigen in stool in 72.3% versus 41.7 in those without PHG with a significant difference ($p = 0.049$). Although there is still a tendency for *H. pylori* antigen in stool to be more common in patients with Child Pugh grade B and C, history of hepatic encephalopathy (HE), the presence of esophageal varices, or other endoscopic findings, the differences were statistically insignificant. In relation to the *H. pylori* culture, it was found to be significantly positive in patients with cirrhosis who had Child Pugh grades B and C ($p = 0.006$), patients who had experienced a history of HE ($p = 0.012$), and patients who had gastritis, gastroduodenal erosions, or ulcers ($p = 0.003$).

In HCC patients, we found that those who had Child Pugh grades B and C and patients with a history of HE experienced *H. pylori* antigen more than those without. HCC patients who had PHG in upper endoscopy were significantly positive for both *H. pylori* stool antigen and *H. pylori* culture ($p = 0.018$ and 0.011, respectively) compared to those without (Table 8). Furthermore, Figures 2A and B showed the relationship between PHG and *H. pylori* virulence traits. We observed that PHG was substantially correlated with positive *cagA* and/or *vacAs2* gene expression in the cirrhotic and HCC groups. 94.1% of cirrhotic patients and 95.2% of HCC patients were positive for the *cagA* and/or *vacAs2* genes.

Discussion

Several clinical and experimental researches from various nations demonstrated a positive link between *H. pylori* infection and the development of liver disease and subsequent progression [21, 22]. Moreover, *H. pylori* could exacerbate extra-gastric organ disruptions, aggravating metabolic or cardiovascular disorders and impairing liver function, particularly among cirrhosis patients [23].

Table 7. Relation between *H. Pylori* stool antigen and *H. Pylori* culture with clinical, endoscopic and laboratory findings in cirrhotic patients (n = 74).

	***H. Pylori* Ag in stool**				***H. pylori* culture**			
	Negative (n = 12)	**Positive (n = 65)**	**Test of sig.**	***p***	**Negative (n = 43)**	**Positive (n = 34)**	**Test of sig.**	***p***
Age (years)								
Mean ± SD.	52.2 ± 6.5	52.7 ± 7.4	t = 0.237	0.813	52.8 ± 6.3	52.4 ± 8.4	t = 0.195	0.846
Median (Min. – Max.)	54 (42–61)	54 (28 –61)			55 (36 – 61)	54 (28–61)		
Sex								
Male	9 (75%)	49 (75.4%)	$\chi^2 = 0.001$	$^{FE}p = 1$	36 (83.7%)	22 (64.7%)	$\chi^2 = 3.694$	0.055
Female	3 (25%)	16 (24.6%)			7 (16.3%)	12 (35.3%)		
Child Pugh classification								
A	6 (50%)	17 (26.2%)	$\chi^2 = 2.592$	$^{MC}p = 0.271$	19 (44.2%)	4 (11.8 %)	$\chi^2 = 10.0682$	**0.006***
B	3 (25%)	21 (32.3%)			12 (27.9%)	12 (35.3 %)		
C	3 (25%)	27 (41.5%)			12 (27.9%)	18 (52.9 %)		
Hepatic encephalopathy (HE)								
No	11(91.7%)	49(75.4%)	$\chi^2 = 1796$	$^{FE}p = 0.281$	38(88.4%)	22(64.7%)	$\chi^2 = 6.1815$	**0.012***
Yes	1 (8.3%)	16 (24.6%)			5 (11.6 %)	12 (35.3 %)		
GI endoscopy esophageal varices								
No	8 (66.7%)	22 (33.8%)	$\chi^2 = 4.588$	$^{FE}p = 0.051$	19 (44.2%)	11 (32.4%)	$\chi^2 = 1.118$	0.29
Yes	4 (33.3%)	43 (66.2%)			24 (55.8%)	23 (67.6%)		
PHG								
No	7 (49.3%)	18 (27.7%)	$\chi^2 = 4.338^*$	$^{FE}p = 0.049^*$	12 (27.9%)	13(38.2%)	$\chi^2 = 0.924$	0.336
Yes	5 (41.7%)	47 (72.3%)			31 (72.1%)	21 (61.8%)		
Other finding								
No other finding	7 (75.0%)	47 (72.3%)	$\chi^2 = 0.4744$	$^{MC}p = 0.924$	38 (88.3%)	18 (52.9%)		
Gastroduodenal ulcer	1 (8.33%)	8 (12.3%)			2 (4.7%)	7 (20.6%)	$\chi^2 = 13.5539$	$^{MC}p = 0.003$*
Gastritis	1 (8.33%)	7 (10.8%)			1 (2.3%)	7 (20.6%)		
Gastroduodenal erosions	1 (8.34%)	3 (4.6%)			2 (4.7%)	2 (5.9%)		
ALT (IU/L)								
Mean ± SD.	38.5 ± 12.5	35.2 ± 12.8	U = 281.0	0.121	35.8 ± 11.9	35.6 ± 13.9	U = 674.00	0.554
Median (Min. – Max.)	37.5 (20– 68)	30 (20–76)			32 (21–76)	30.5 (20–72)		
Total bilirubin (mg/dL)								
Mean ± SD.	2.7 ± 2	3.5 ± 2.2	U = 284.0	0.136	3.5 ± 2.4	3.2 ± 1.9	U = 714.50	0.866
Median (Min. – Max.)	2 (0.7– 6.8)	2.8 (0.5–9.9)			2.8 (0.5–9.9)	2.8 (0.8–8.2)		
Platelet count 10^3(mm^3)								
Mean ± SD.	141.7 ± 50.2	136.1 ± 41	U = 362.50	0.699	137.3 ± 44.6	136.5 ± 39.7	U = 729.0	0.984
Median (Min. – Max.)	155 (69–216)	135 (65–216)			122 (67–216)	138 (65 – 216)		
Serum albumin (gm/dL)								
Mean ± SD.	2.9 ± 0.8	2.8 ± 0.8	U = 338.50	0.465	2.8 ± 0.8	2.8 ± 0.7	U = 702.0	0.764
Median (Min. – Max.)	3 (1.7–3.9)	2.9 (1.5–4.1)			2.9 (1.5–4.1)	2.9 (1.5– 3.8)		
INR								
Mean ± SD.	1.6 ± 0.4	1.6 ± 0.3	U = 381.0	0.899	1.6 ± 0.3	1.6 ± 0.3	U = 702.0	0.766
Median (Min. – Max.)	1.5 (0.9–2.2)	1.5 (1.1–1.9)			1.5 (0.9–2.2)	1.6 (1.1–2.2)		

PHG: portal hypertensive gastropathy, ALT: alanine transaminase, INR: International normalized ratio, χ^2: Chi square test, MC: Monte Carlo, t: Student t-test, U: Mann Whitney test; *p*: *p* value for comparison between the studied categories; *: Statistically significant at $p < 0.05$.

Figure 2. A: The relationship between PHG and *H. pylori* virulence traits in cirrhotic patients; **B:** The relationship between PHG and *H. pylori* virulence traits in HCC patients.

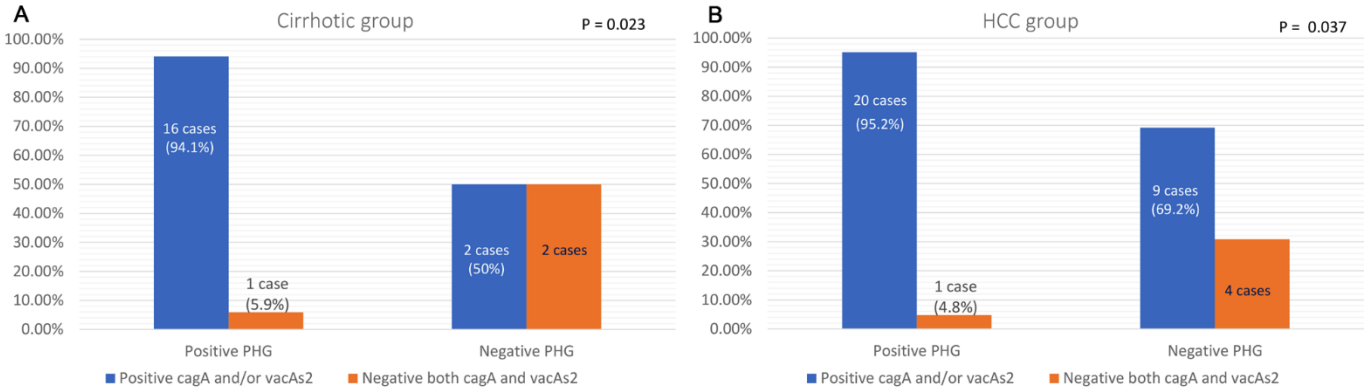

Table 8. Relation between *H. Pylori* stool antigen and *H. Pylori* culture with clinical, endoscopic and laboratory findings in HCC patients (n = 38).

	***H. Pylori* Ag in stool**				***H. pylori* culture**			
	Negative (n = 6)	**Positive (n = 32)**	**Test of sig.**	***p***	**Negative (n = 17)**	**Positive (n = 21)**	**Test of sig.**	***p***
Age (years)								
Mean ± SD.	50.7 ± 5.61	54.6 ± 5.58	t = 1.582	0.122	52.8 ± 6.3	52.4 ± 8.4	t = 0.371	0.713
Median (Min. – Max.)	53.5 (43 – 55)	55.5 (39 – 63)			55 (36 – 61)	54 (28–61)		
Sex								
Male	4 (66.7%)	23 (71.9%)	$\chi^2 = 0.067$	$^{FE}p = 1$	12 (70.6%)	15 (71.4%)	$\chi^2 = 0.003$	$^{FE}p = 1.000$
Female	2 (33.3%)	9 (28.1%)			5 (29.4%)	6 (28.6%)		
Child Pugh classification								
A	5 (83.3%)	7 (21.9%)	$\chi^2 = 7.565^*$	$^{MC}p = 0.008^*$	6 (35.3%)	6 (28.6%)	$\chi^2 = 0.352$	0.839
B	1 (16.7%)	12 (37.5%)			5 (29.4%)	8 (38.1%)		
C	0 (0.0%)	13 (40.6%)			6 (35.3%)	7 (33.3%)		
Hepatic encephalopathy (HE)	6(100%)	16 (50.0%)	$\chi^2 = 5.182^*$	$^{FE}p = 0.030^*$	11(64.7%)	11(52.4%)	$\chi^2 = 0.585$	0.444
	0 (0.0%)	16 (50.0%)			6 (35.3%)	10 (47.6%)		
GI endoscopy esophageal varices								
No	4 (66.7%)	10 (31.3%)	$\chi^2 = 2.724$	$^{FE}p = 0.167$	7 (41.2%)	7 (33.3%)	$\chi^2 = 0.248$	0.618
Yes	2 (33.3%)	22 (68.8%)			10 (58.8%)	14 (66.7%)		
PHG	5(83.3%)	9 (28.1%)	$\chi^2 = 6.618^*$	$^{FE}p = 0.018^*$	10 (58.8%)	4 (19.1%)	$\chi^2 = 6.3878$	**0.011***
	1 (16.7%)	23 (71.9%)			7 (41.2%)	17 (80.1%)		
Other Finding								
No other finding	5 (83.4%)	18 (56.2%)	NA	NA	12 (70.6%)	11 (52.4%)		
Gastroduodenal ulcer	1 (16.7%)	6 (18.8%)			3 (17.6%)	4 (19.0%)	$\chi^2 = 2.4592$	$^{MC}p = 0.827$
Gastritis	0 (0.0%)	6 (18.8%)			1 (5.9%)	5 (23.8%)		
Gastroduodenal erosions	0 (0.0%)	2 (6.2%)			1 (5.9%)	1 (4.8%)		
ALT (IU/L)								
Mean ± SD.	42.2 ± 12.8	38.9 ± 16.4	U = 75.0	0.422	40.1 ± 18.6	38.86 ± 13.5	U = 173.0	0.885
Median (Min. – Max.)	41 (24 – 60)	33.5 (14 – 90)			34 (19 – 90)	37 (14 – 68)		
Total bilirubin (mg/dL)								
Mean ± SD.	2.18 ± 0.80	4.55 ± 3.53	U = 62.500	0.185	4.89 ± 4.09	3.60 ± 2.59	U = 146.50	0.352
Median (Min. – Max.)	2.50 (0.80–3.0)	2.80 (0.80–14.5)			2.70 (1.3-14.5)	2.70 (0.8-8.7)		
Platelet count 10^3 (mm^3)								
Mean ± SD.	212.5 ± 89.1	159.9 ± 71.6	U = 60.500	0.159	180.8 ± 99	158.1 ± 50.6	U = 176.0	0.954
Median (Min. – Max.)	198.0 (105- 312)	149.5 (86 – 312)			120 (86 – 312)	157 (86 – 245)		
Serum albumin (gm/dL)								
Mean ± SD.	3.07 ± 0.41	2.55 ± 0.85	U = 60.0	0.159	2.68 ± 0.76	2.59 ± 0.87	U = 160.50	0.601
Median (Min. – Max.)	2.80 (2.80 – 3.60)	2.80 (1.20 – 4.80)			2.80 (1.40 – 3.60)	2.80 (1.20 – 4.80)		
INR								
Mean ± SD.	1.72 ± 0.66	1.71 ± 0.37	U = 84.0	0.653	1.71 ± 0.44	1.71 ± 0.40	U = 171.50	0.839
Median (Min. – Max.)	1.45 (1.11 – 2.70)	1.60 (1.04 – 2.57)			1.60 (1.04 – 2.57)	1.60 (1.11 – 2.70)		

PHG: portal hypertensive gastropathy; ALT: alanine transaminase; INR: International normalized ratio; χ^2: Chi square test; MC: Monte Carlo; t: Student t-test; U: Mann Whitney test; *p*: *p* value for comparison between the studied categories; *: Statistically significant at $p < 0.05$

H. pylori can enter the liver through retrograde transfer from the duodenum or through circulating phagocytes. Nonetheless, studies involving *H. pylori* cultures from liver biopsies of HCC patients provide evidence for the organism's actual hepatic colonization [24]. Finding out how *H. pylori* infection affects the progression of chronic liver disease was the main objective of this study. Special attention was paid to the virulence genetic profile and antimicrobial susceptibility pattern of *H. pylori*.

It is noteworthy that, in chronic HCV-infected patients, the *H. pylori* antigen was found in the stool of 55.4%, 84.4%, and 84.2% of patients with chronic hepatitis, liver cirrhosis, and HCC, respectively, in this study. A statistically significant difference ($p < 0.001$) was noticed among the three patient groups, denoting higher *H. pylori* prevalence among cirrhotic patients and those with HCC. These results agreed with a meta-analysis published in which investigators reported that *H. pylori* infection prevalence in individuals with cirrhosis has increased significantly worldwide due to viral etiology [25].

Waluga *et al.* found that cirrhotic patients had the highest rate of *H. pylori* infection (79.3%) when compared to control groups and patients with chronic viral hepatitis. They suggested that, in individuals with chronic viral hepatitis, the rate of *H. pylori* infection increased as the disease progressed. They proposed that *H. pylori*, in conjunction with chronic hepatitis, contributes to the pathophysiology of chronic liver disease [23].

In the same context, 31.1%, 44.2%, and 55.3% of the studied patients yielded positive culture for *H. pylori* in gastric specimens taken by upper GIT endoscopy. Although more time-consuming, the culture is a very specific diagnostic method that can screen for antibiotic susceptibility. Reductions in the number of culture-positive cases in comparison to stool *H. pylori* antigen detection can be attributed to a variety of factors such as the use of proton pump inhibitors (PPIs) and antimicrobials, inappropriate transportation circumstances, low or absent bacterial density in the biopsy specimens, and oxygen exposure-induced loss of microbe viability [23]. The majority of the variation in results between the two procedures could be explained by these considerations.

Wang *et al.* conducted a meta-analysis to determine *H. pylori* prevalence in 2377 control participants and 1449 chronic HCV patients. Compared to patients without chronic HCV, patients with chronic viral hepatitis had a considerably higher prevalence of *H. pylori* (odds ratio was 2.93). Furthermore, the odds ratios in a subgroup analysis were 4.48 for cirrhosis related to HCV and 5.45 for those with HCV-related HCC. Although these findings only demonstrated the presence of *H. pylori* and not its pathogenicity, they do suggest a connection between *Helicobacter species* and HCV-related disease progressions [27].

In patients with chronic HCV complicated by liver cirrhosis, the incidence of H. pylori infection rises in direct proportion to the severity of liver disease. Likewise, it has been demonstrated that among patients with chronic HCV, those who developed HCC had the highest percentage of *H. pylori* infection. Previous investigation indicates that *H. pylori* and HCV concomitant infection raises the likelihood of HCC [28].

Genes encoding the cytotoxin production are found in *H. pylori* DNA. About 40–60% of *H. pylori* strains have a variable gene system that encodes the *vacA* toxin, also known as the vacuolating toxin. *VacA* alters the structure of the cytoskeleton in epithelial cells. In addition, transcription, cell division, and the inflammatory response are all regulated by the toxin [24]. The *cagA* toxin has a significant role in the carcinogenesis of the stomach and other organs of which HCC is predicted when *cagA*-synthesizing *H. pylori* is discovered [26].

One of the major objectives of the current study was to explore the correlation between *H. pylori* virulence genetic profile and deleterious consequences of hepatic affection. Notably, genetic analysis of the isolated *H. pylori* strains revealed that 47.8%, 85.3%, and 85.7% of chronic hepatitis, cirrhosis, and HCC patients harbored *cagA* and *vacAs2* with varying percentages and genetic models. A key finding was that, in the isolated *H. pylori* strains, 47.6% of HCC patients displayed co-existence of both the *cagA* and v*acAs2* genes, which differed significantly from only 13.05% and 20.6% in the chronic hepatitis and cirrhotic groups, respectively.

Esmat *et al.* looked at the *cagA* gene expression in liver samples from people with chronic viral hepatitis or cirrhosis as part of their study into the link between *H. pylori* and these conditions. They found that the c*agA* gene was detected in 28.2% of cases with late fibrosis, compared to 5.9% in cirrhotic patients with early fibrosis with a significant difference [29]. However, Rocha *et al.* demonstrated the *cagA* gene in 3.5% and 4.2% of *H. pylori* isolates from non-cirrhotic chronic HCV patients and control participants, respectively. In addition, the *H. pylori vacAs1* gene was detected in 61.3% and 68.0% of patients with HCV with and without HCC, respectively [30].

Antibiotic resistance, which frequently correlates

with antibiotic use habits and varies within patient groups, is one of the main causes of *H. pylori* eradication failure. To provide the best clinical care, it is therefore critical to identify those who are more likely to have such problems. In that sense, we assessed the isolated strains for their antimicrobial susceptibility pattern and noticed that 91%, 61.4%, 57%, and 24.5% of the obtained *H. pylori* isolates were respectively resistant to metronidazole, amoxicillin, clarithromycin, and ciprofloxacin. Another study conducted by Mahmoud *et al*. in Egypt revealed that 100%, 68.8%, 68.8%, and 12.5% of *H. pylori* isolates were resistant to metronidazole, amoxicillin, clarithromycin, and ciprofloxacin, respectively [4].

Comparable resistance rates were noted by Fathi *et al.* [31]. However, Mergraud reported reduced resistance rates [32]. Given the high frequency of *H. pylori* infection and the high consumption pressure of these antibiotics, the high resistance of *H. pylori* to the majority of antimicrobial drugs could be explained. Worldwide, there is virtually minimal resistance to fluoroquinolones. When it comes to eliminating *H. pylori* in vitro, ciprofloxacin has low minimum inhibitory concentrations [33].

We noticed that patients with a history of HE and those with portal hypertensive gastropathy (PHG) in cirrhotic patients with or without HCC had higher rates of *H. pylori* prevalence in addition to higher expression of c*agA* and/or *VacAs2* genes in PHG patients.

In patients with cirrhotic or non-cirrhotic portal hypertension, PHG is a significant contributor to morbidity [34]. Those with PHG have mucosal friability and dilated blood vessels on the mucosal surface. Portal hypertension, which increases gastric blood flow and constricts mucosal and submucosal blood vessels, is a contributory factor of PHG [35]. PHG triggers a reduction in mucous secretion and local mucosal defense together with severe hemorrhagic congestion and mucosal edema, making the mucosa weaker and more vulnerable to harmful agents including *H. pylori* colonization as well as other factors such as nonsteroidal anti-inflammatory drugs [36].

H. pylori promotes the deterioration of hepatic cell function in the course of toxic injury and hyperinflammation. Additionally, *H. pylori* infections are particularly dangerous among patients with liver cirrhosis. It may significantly worsen the liver functions, causing hyperammonemia, and may have a role in elevated portal pressure, as well as esophageal varices development. Therefore, testing for *H. pylori* and the introduction of adequate treatment regimens among patients with liver cirrhosis is particularly important [21].

Conclusions

The presence of *H. pylori* infection should be assessed in all patients with liver impairment. Early *H. pylori* detection and subsequent eradication may lessen the severity of decompensation, including hepatic encephalopathy, portal hypertensive gastropathy, and esophageal varices. Larger-scale studies to address the direct role of *H. pylori* with variable virulence genotypes in the development of HCC are desperately needed. The ELISA technique for *H. pylori* Ag detection is a rapid, feasible, non-invasive, and sensitive technique that could be implemented for timely screening of chronic hepatic patients. Even though it was time-consuming, microbiological culture offered a higher opportunity to assess both the genetic background and antimicrobial susceptibility of *H. pylori* isolates.

Ethical considerations

Following an elaboration of the research questions and the research objectives, each participant was made aware of the research's nature and gave informed written consent prior to participating. The study was approved by the local ethics committee of the Faculty of Medicine, Menoufia University (IRB 3/2023TROP12), and was carried out in conformity with the Declaration of Helsinki.

Availability of data and material

All data generated or analyzed during this study are included in this published article.

Authors' contributions

All authors made significant contributions to the work presented. N.E., A.E., and M.O. selected the study design. A.R., A.E., M.R., and A.N. were responsible for the clinical evaluation of the involved patients, sample collection, and collecting the clinical and laboratory data of studied patients. A.E. and A.E. were responsible for laboratory investigations and data interpretation. S.E., S.H., and N.E. are responsible for data analysis and interpretation. N.E. and A.E. wrote the main manuscript text and S.H. revised the manuscript. All authors reviewed the manuscript.

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Conflict of interests

No conflict of interests is declared.

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