Serological and Immunohistochemical Detection of *Helicopacter pylori* Infection among Egyptian Hepatitis C Virus Patients

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Background and study aim: *Helicobacter pylori* infection is distributed worldwide. *H. pylori* colonize the liver and increase the severity of the liver pathogenesis. The aim of the present study was to detect the *H. pylori* in the HCV patients using serology and immunohistochemistry diagnostic methods. The aim was extended to evaluate the hematological and biochemical changes during the co-infection.

Materials and Methods: ELISA and immunohistochemistry diagnostic methods were utilized to examine patients chronically infected with HCV for *H. pylori*, and some hematological and biochemical parameters were detected.

Results: Overall prevalence of *H. pylori* infection was 10.81%, 8.00%, using ELISA, and immunohistochemistry, respectively. In the current study, no significant difference based on gender, residence, age, biochemical assessment and HCV RNA load was observed. Liver cirrhosis at patient co-infected with *H. pylori* and HCV recorded high percentage compared to those with chronic HCV mono-infection. A significant increase in the relative lymphocyte count was detected in patients with concomitant *H. pylori* and chronic HCV infections compared to patients with chronic HCV mono-infection.

Conclusion: Because *H. pylori* infection is frequent among Egyptian HCV infected patients, regular screening and treatment for *H. pylori* among this category is extremely important.

INTRODUCTION

In 1983, a Gram-negative bacterium, *Helicobacter pylori* (*H. pylori*), was firstly discovered **[1]**. Despite the infection *of H. pylori* is very common in developing countries, it was distributed worldwide **[2]**. Colonization of *H. pylori* in the stomach is associated with many pathogenic effects in the upper gastrointestinal tract, e.g. gastric cancer, peptic ulcer disease, mucosa-associated lymphoid tissue (MALT) lymphoma and chronic gastritis **[3]**. Moreover, *H. pylori* positivity has been linked to several diseases, such as idiopathic iron deficiency anemia **[4]**, idiopathic thrombocytopenic purpura **[5]**, ischemic heart disease **[6]**, auto-immune pancreatitis **[7-9]**, acute coronary syndromes **[10]**, and hepato-biliary diseases **[11-13]**.

Recent reviews have collected data emphasized the capability of *H. pylori* to induce and increase the severity of the liver pathogenesis **[1,14]**. Previous reports tried to explain how *H. pylori* colonize the liver. Some manuscripts clarified that upon the occurring of portal hypertension during the later stages of chronic liver disease, *H. pylori* could trans-located from the stomach into the blood through the portal system and then the bacteria DNA could be detected in the liver tissues **[15-17]**. Other reports mentioned that the *H. pylori* might use macrophages or circulating retrograde transfer from the duodenum to reach the liver **[18]**. *In vitro* studies using the human hepatic cell line (HepG2) emphasized the cytopathic effect of *H. pylori* in the damage of the hepatocytes **[19,20]**. The bacteria might exert its pathological

effect on HepG2 cells through the up-regulation of proteins incorporated in metabolism, signal transduction and transcription regulation **[21]**.

Around the world hepatitis C virus (HCV) affects one hundred seventy million persons and results in about five hundred thousand deaths/year **[22]**. The highest prevalence of HCV infection about 15% was recorded in Egypt **[23,24]**. Decompensated liver cirrhosis, hepatocellular carcinoma (HCC) and liver transplantation are associated with the hepatitis that caused by HCV infection **[25-27]**. Esmat and his colleagues demonstrated a significant association between *H. pylori* infection and severity of liver pathology in patients with HCV-related chronic hepatitis and cirrhosis with or without hepatocellular carcinoma **[28]**. Furthermore, in co-infected patients with HCV and *H. pylori*, more pronounced fibrosis stages and more cirrhotic nodules and impairment of hepatic parenchyma were detected than in HCV mono-infected patients **[29]**. Hence, the objective of the present study was to detect *H. pylori* in the HCV patients using serology and Immunohistochemistry diagnostic methods and evaluate the hematological and biochemical changes during the co-infection.

MATERIALS AND METHODS

Ethical statement

The present study was conducted in accordance with the Declaration of Helsinki and the Guidelines for Good Clinical Practice and approved by the ethical committee of Faculty of Medicine, Al-Azhar University and Kafer El Shiekh Liver and Heart Institute, Egypt. The purpose and procedures involved in the present study were explained and written informed consent was obtained from all participants.

Study population

One hundred eighty five patients chronically infected with HCV from Kafer El Shiekh Liver and Heart Institute, Egypt during the period between February 2015 and March 2016 were enrolled in the present study. The patients included 82 females and 103 males, with age range 20-57 with a mean of (40.90 ±7.90) years.

The study population was divided into three groups. Group-I: 121 patients with chronic HCV infection without *H. pylori* infection. Group-II: 18 patients with concomitant *H. pylori* and chronic HCV infections serologically detected. Group-III: 8 patients with concomitant *H. pylori* and chronic HCV infections immunohisto-chemically detected.

Exclusion criteria

Patients with malignancy, including HCC or renal, cardiopulmonary or autoimmune disorders and pregnant women were excluded from the study.

Detection of HCV antibodies and RNA

HCV antibodies were detected by EIA (COBAS-Amplicore, Germany). Qualitative evaluation of HCV-RNA by PCR was performed using a commercial kit (Roche Diagnostic, Branchburg, NJ) according to the manufacturer's instructions.

Serological analysis of *H. pylori* infection

H. pylori IgG antibodies were determined by the qualitative ELISA test using commercially available kit (Calbiotech Inc, CA, USA). Assays were done according to the manufacturer's instructions and results of *H. pylori* IgG were expressed as index values.

Immunohistochemical analysis of *H. pylori* infection

One hundred out of 185 HCV chronically infected patients performed a liver biopsy to investigate grading and staging of hepatic disease. In this category of patients, *H. pylori* was detected by an indirect labeling streptavidin-biotin immuno-histochemistry (IHC) [30] using a rabbit anti-*H. pylori*-specific antibody (Dako, Hamburg, Germany).

Hematological and biochemical analysis

Complete blood count (CBC) was determined using an automated hematology analyzer XP series (Sysmex, Japan). Direct, total bilirubin, albumin, fasting sugar, alanine transaminase (ALT), aspartate transaminase (AST) and creatinine were run on using ABX Pentra C400 clinical chemistry analyzer (Horiba ABX SAS, Montpellier, France). Alpha-fetoprotein (AFP) and antinuclear antibody (ANA) were determined using chemiluminescent immunoassay (Liaison, DiaSorin, Germany). Thyroid-stimulating hormone (TSH) was detected using (Teco Diagnostics, CA, USA). International normalized ratio (INR) was done automatically using a commercial kit (Siemens Healthcare Diagnostic Inc., Germany). In all 185 patients, compensated cirrhosis was determined by Fibro-scan™ >12.5 kPa. In the HCV chronically infected patients subjected to a liver biopsy compensated cirrhosis was also determined by biopsy of METAVIR 4 or Ishak more than or equal 5.

Statistical analysis

SPSS (IBM SPSS statistics for Windows, Armonk, NY) computer program was used for statistical analysis. Binary logistic regression was used to assess significant differences of *H. pylori* infection rate in HCV-infected patients of different age, localities, and sex. Hematological and biochemical changes were evaluated by using ANOVA test followed by post hoc analysis of group differences that was accomplished by the least significant differences (LSD) test; $p<0.05$ were considered to be statistically significant. Agreement between ELISA and immunohistochemistry was calculated according to Ibrahim et al. **[31,32]**.

RESULTS

Helicopacter pylori infection among Egyptian HCV patients was summarized in Table 1. Overall prevalence was 10.81%, and 8.00%, using ELISA, and IHC, respectively. The seroprevalence was significantly ($P<0.05$) increased among the HCV patients from Gharbiya province 15.66% when compared to Kafr El Sheikh province 6.86%. Similarly, IHC demonstrated significant higher levels of *H. pylori* infection among HCV patients Gharbiya province 10.53% compared to Kafr El Sheikh province 4.65%. *H. pylori* infection was detected in the liver of HCV patients as represented in Fig. 1.

During the *H. pylori* detection among Egyptian HCV patients, the results of the ELISA were cross-tabulated with those of IHC and summarized in Table 2. Among one hundred HCV patients, the agreement percentage between the results of ELISA, and those of IHC was 96%.

Table 3 demonstrated the relation between *H. pylori* positivity and age, gender, and residence among Egyptian HCV patients. According to residence, the bacteria prevalence was non-significantly lower ($P>0.05$) in the rural areas, 6.74%, 2.70% than the urban areas, 12.50%, and 11.11% using ELISA and IHC, respectively. Although, higher prevalence was recorded in male and younger patients compared to female and older patients, no significant difference was detected in the prevalence of *H. pylori* among HCV patients based on gender and age, using ELISA, or IHC.

Characteristics of cirrhosis, HCV RNA load and biochemical data of the study population were shown in Table 4. Liver cirrhosis and at patient with concomitant *H. pylori* and chronic HCV infections showed a higher percentage compared to those patients with chronic HCV mono-infection. Similar patterns were detected at the levels of HCV RNA load among the study populations. Furthermore, similar patterns were detected at the levels of AFP, TSH, glucose, INR, AST, ALT, direct, total bilirubin, albumin, creatinine and ANA among the study populations.

Hematological findings of the study population were illustrated in Tables 5. Minimal significant increase ($P<0.05$) was detected in the current study, at the level of relative lymphocyte counts in patients with concomitant *H. pylori* and chronic HCV infections immunohistochemically detected compared to those patients with chronic HCV mono-infection. No significant alterations were determined on the levels of the other examined hematological parameters (Table 5).

Table (1) : Prevalence of *Helicobacter pylori* infection among HCV infected patients from Egypt using ELISA and IHC

Regions	Total	ELISA	Total	IHC
Kafr-elshikh	102	7 (6.86%)	43	2 (4.65%)
Gharbiya	83	13 (15.66%)*	57	6 (10.53%)*
Total	185	20 (10.81%)	100	8 (8.00%)

* Prevalence of *H. pylori* is significantly different ($p < 0.05$, logistic regression test).

Table (2) : Summary on the detection of *Helicobacter pylori* infections

H. pylori	ELISA [a]		IHC [b]	
			(+)	(-)
	(+)	12	8	4
	(-)	88	0	88
	Total	100	8	92

[a] The frequencies of positive and negative samples as results of ELISA.
[b] The frequencies of positive and negative samples as results of IHC cross-tabulated with ELISA results.

Table (3) : Socio-demographic characteristics and prevalence of *Helicobacter pylori* infection among HCV infected patients using ELISA and IHC

Characteristics	Total	ELISA	Total	IHC
Age				
40 year or less	84	9 (10.71%)	53	5 (9.43%)
More than 40	101	9 (8.91%)	47	3 (3.38%)
Sex				
Male	103	13 (12.62%)	64	6 (9.38%)
Female	82	5 (6.10%)	36	2 (5.56%)
Residence				
Urban	96	12 (12.50%)	63	7 (11.11%)
Rural	89	6 (6.74%)	37	1 (2.70%)

Table (4) : Characteristics of cirrhosis, HCV RNA load and biochemical data of the study population

Parameter	Group I (HCV)	Group II (HCV+ *H. pylori*) ELISA	Group III (HCV+ *H. pylori*) IHC
Cirrhotic Liver	16 (13.22%)	3 (16.67%)	2 (25.00%)
HCV RNA (10^5 IU/ml)	19.69±2.44	20.85±6.16	22.54±13.09
AFP (ng/dl)	12.45±3.17	9.56±3.87	2.95±0.28
TSH (µlU/ml)	1.54±0.09	1.27±0.19	0.97±0.19
Glucose (mg/dl)	113.11±5.67	93.56±4.01	89.5±5.19
INR	1.08±0.01	1.13±0.04	1.14±0.08
ANA (Negative/Positive)	185(100%)/0(0%)	185(100%)/0(0%)	185(100%)/0(0%)
Creatinine (mg/dl)	0.74±0.01	0.73±0.03	0.68±0.04
Direct-Bil (g/dl)	0.39±0.04	0.27±0.03	0.23±0.03
T-Bil (mg/dl)	1.01±0.06	0.82±0.06	0.74±0.11
Albumin (g/dl)	3.94±0.04	4.26±0.07	4.15±0.11
ALT (U/L)	61.88±4.20	56.72±16.05	41.12±5.93
AST (U/L)	55.58±3.66	42.83±7.60	39.62±4.50

Data are expressed as: mean ± standard error (STE) or number (% among study population).

Table (5) : Hematological findings of different groups

	Study population		
	Group I (HCV)	**Group II (HCV+ *H. pylori*) ELISA**	**Group III (HCV+ *H. pylori*) IHC**
RBCs $\times 10^6$	4.83±0.09	5.36±0.14	5.30±0.15
PCV (%)	41.35±0.39	44.84±0.11	42.50±0.01
Hb (g/dl)	13.63±0.15	14.52±0.36	13.60±0.59
MCV (%)	83.69±0.55	84.10±0.99	80.95±1.66
MCH (%)	27.64±0.26	27.30±0.56	25.82±0.89
MCHC (%)	32.99±0.18	32.46±0.50	31.86±0.46
Platelets $\times 10^3$	185.31±7.38	203.7±14.90	192.62±20.63
WBCs $\times 10^3$	6.62±0.19	6.61±0.45	6.54±0.44
Lym (%)	37.67±0.01	39.28±0.02	43.25±0.02*
Neu (%)	51.40±0.01	50.11±0.02	47.38±0.03
Mon (%)	10.96±0.003	10.33±0.01	8.50±0.01

Data are expressed as: mean ± standard error (STE). * *P<0.05* indicate significant difference compared to the patients with chronic HCV mono-infection.

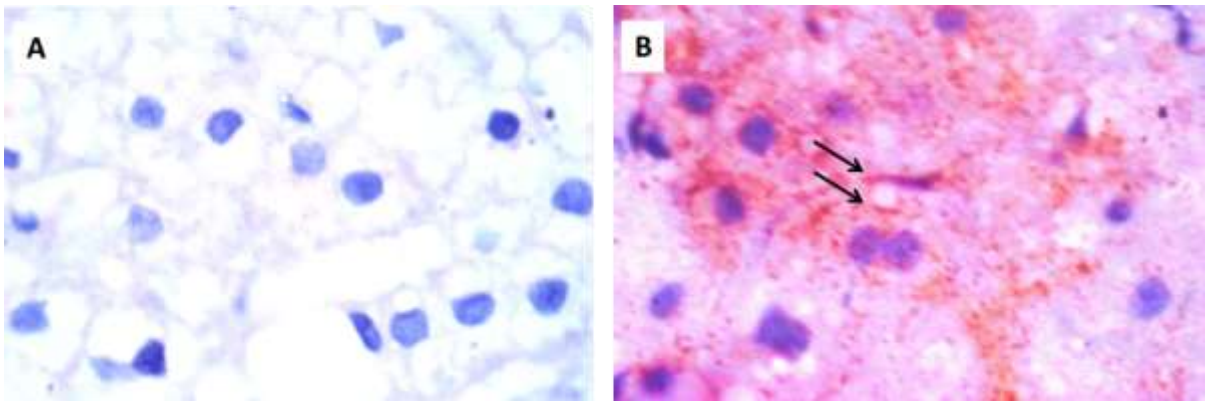

Fig. (1) : Identification of *Helicobacter pylori* in the liver from HCV infected patient. (A) Immunohistochemical section of the liver from HCV infected patient without any detection of *H. pylori* (B) Specific detection of *H. pylori* with anti- *H. pylori* antibody immuno-stained as a dark brown color, original magnification, ×1000.

DISCUSSION

In the current study, *H. pylori* prevalence in 185 HCV infected individuals from Gharbiya and Kafr El Sheikh provinces was examined by ELISA, and from those cases one hundred HCV infected patients were examined using IHC. The overall prevalence of *H. pylori* was 10.81%, and 8.00% using ELISA, and IHC, respectively. According to the area, significant high *H. pylori* prevalence was detected among patients from Gharbiya province compared to those from Kafr El Sheikh province using ELISA, and IHC, respectively. In Tanta City the capital of Gharbiya province, high prevalence of *H. pylori* 69.4% was demonstrated among patients with different gastrointestinal symptoms **[33]**. Wang et al. demonstrated that *H. pylori* prevalence was significantly higher in HCV infected patients than in those without chronic HCV infection **[34]**. Previous study detected *H. pylori* antibodies and DNA prevalence 61.7% and 10%, respectively, among chronic hepatitis C patients from Suez Canal areas (east of Egypt) **[35]**. Another Egyptian study from Alexandria, detected 76.90% *H. pylori* positivity in the stools of HCV patients using rapid test **[36]**. Molecular prevalence of *H. pylori* was demonstrated in 11.5% of chronic HCV patients from the Gastroenterology and Hepatology Unit of Suez Canal University Hospital **[37]**. Seroprevalence of *H. pylori* among HCV patients was 55.6% from Minufiya province, Egypt **[38]**.

Generally, low prevalence was detected for microbes using IHC or PCR compared to serological assays. The weak reliability of the IHC assays might be rendered to low numbers of the bacteria in the tested human tissues, small sample size of the collected tissue, and may be, randomized distribution of bacterial units. Although a high agreement between the results of ELISA, and IHC was demonstrated in this study, IHC detected lower positivity than ELISA. The lower positivity detected by the microscopic examination could be attributed to the previously mentioned sampling issues. Furthermore, the antibody response is always independent of bacterial burden. Pervious report recorded a good agreement between ELISA and histopathological methods with higher ELISA detection for *H. pylori* **[39]**. Moreover, low *H. pylori* prevalence was demonstrated by nested PCR when compared to *H. pylori* antibodies using ELISA **[35,37]**.

The *H. pylori* prevalence was higher in males, younger patients and urban area residents, however, no significant relation was demonstrated between the *H. pylori* positivity and age, gender and residence among HCV infected patients using ELISA and IHC. Moreover, in the current study, biochemical assessment and HCV RNA load at patient with concomitant *H. pylori* infection and chronic HCV infection was similar to patients with chronic HCV mono-infection. Several reports demonstrated that there was no statistical difference between ages, gender, liver function tests, AFP levels or viral load in the prevalence of *H. pylori* in HCV infected patients **[37,40,41]**.

In the current study, liver cirrhosis at patient with concomitant *H. pylori* and chronic HCV infections showed a high percentage compared to those patients with chronic HCV mono-infection. Several previous reports revealed a strong association between *H. pylori* infection and the progression of liver injuries such as cirrhosis and fibrosis among HCV infected patients **[28,34,35,37,38, 40,41]**.

Moreover, in the current study, a significant increase in the level of the relative lymphocyte count was detected in patients with concomitant *H. pylori* and chronic HCV infections compared to patients with chronic HCV mono-infection. Early report demonstrated that lymphocyte counts were increased in *H. pylori*-infected patients **[42]** Nagata et al. reported that lymphocyte counts were significantly elevated in *H. pylori*-infected patients compared to those in *H. pylori*-negative patients during the diagnosis of immune thrombocytopenic purpura **[43]**.

In conclusion, the present study indicated that *H. pylori* infection is frequent in Egypt, with noticeable prevalence among HCV patients. Regular screening and treatment for *H. pylori* among HCV patients is important.

Ethical approval: Approved .

Conflict of interest

The authors declare that they have no competing interests.

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