

## ROLE OF HEPATITIS C INFECTION IN CHRONIC LIVER DISEASE IN EGYPT

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**Abstract.** Hepatitis C virus (HCV) is considered the most common etiology of chronic liver disease (CLD) in Egypt, where prevalence of antibodies to HCV (anti-HCV) is ~ 10-fold greater than in the United States and Europe. Reported are results that show the role of HCV in both overt and occult CLD, the risk factors for CLD and for HCV infection, and the relative importance of chronic HCV, hepatitis B, or both in causing hepatic morbidity. Case patients included 237 new outpatients at the National Liver Institute. Controls comprised 212 sex- and age-matched neighbors without liver disease. Case patients were more likely than controls to report a history of blood transfusions, schistosomiasis, or parenteral therapy for schistosomiasis; to have anti-HCV, HCV RNA, hepatitis B surface antigen, and serum alanine aminotransferase (ALT) elevations; and to have abdominal ultrasound findings of cirrhosis, portal hypertension, and splenomegaly. Anti-HCV-positive case patients were more likely than anti-HCV-negative patients to be male, older, and farmers; to have received a blood transfusion or parenteral therapy for schistosomiasis; to have ALT elevations; and to have ultrasound findings of cirrhosis, portal hypertension, and spleen enlargement. Anti-HCV-positive controls were more likely than anti-HCV-negative controls to have received parenteral therapy for schistosomiasis. These data support the belief that HCV is the predominant cause of CLD in Egypt and suggest there is a large underlying reservoir of HCV-caused liver disease.

### INTRODUCTION

Egypt has the highest countrywide prevalence of hepatitis C virus (HCV) in the world, with an estimated 8–10 million among a population of 68 million having been exposed to the virus and 5–7 million active infections. An important cause for the high exposure to HCV was the establishment of a large reservoir of infection as a result of extensive schistosomiasis control programs that used intravenously administered tartar emetic 20–50 years ago.<sup>1</sup>

Only 15–20% of people infected with HCV have an acute viral hepatitis syndrome, but the majority develop chronic hepatitis that is usually asymptomatic and undetected for many years. Over a course of 20–40 years ~ 20% of those with HCV-caused chronic hepatitis progress to cirrhosis, and a proportion of these (possibly 2–3% per year) die as a result of complications of cirrhosis or hepatocellular carcinoma.<sup>2–13</sup>

The purpose of this study was to evaluate the frequency of HCV infection in patients seeking initial evaluation in a specialized hospital for chronic liver disease (CLD) and to compare risk factors for—and the magnitude of—HCV-caused CLD with those in sex- and age-matched neighborhood controls without a history of liver disease.

### PATIENTS AND METHODS

**Study subjects.** The National Liver Institute (NLI) is a hospital limited to providing medical care and researching patients with liver disease. Case patients were first-time attendees of the NLI outpatient clinic, regardless of eventual determination of CLD, who lived in the Nile Delta within a 50-km radius of Shibin El Kom. Schistosomiasis due to *Schistosoma mansoni* is endemic, but the majority of patients at the NLI seek care for symptoms of chronic hepatitis, cirrhosis, or both.<sup>14</sup> However, ~ 10% of referrals to the NLI are subjects found to be anti-HCV positive during a medical examination required for a work permit abroad, while being screened for blood donation, or for some other reason. A condition of enrollment was agreement on the part of case patients to recruit a neighbor of the same sex and within 5 years of age who did not have liver disease to become the controls. All

case patients and controls gave their informed consent to participate in the study, which was approved by the institutional review boards of the NLI and the University of Maryland School of Medicine.

**Evaluation.** All consenting case patients and controls responded to a questionnaire and underwent a medical examination. Venous blood was drawn for serum alanine aminotransferase (ALT) and viral hepatitis serology. The sera were separated from the cells, formed into aliquots, and labeled within 4 hours of collection, then stored at –70°C until testing for other than ALT, which was measured within 6 hours of drawing the blood. Levels above normal but < 1.5 times the upper limit of normal were considered minimally elevated, and levels > 1.5 times the upper range were considered moderately elevated. Stools were examined quantitatively for *Schistosoma mansoni* ova by a modified Kato procedure.<sup>15</sup>

**Hepatitis serology.** Serum samples were tested for anti-HCV according to the manufacturer's instructions with a second-generation enzyme immunoassay (EIA; Abbott GmbH, Delkenheim, Germany). Sera were also tested for hepatitis B surface antigen (HBsAg) via EIA (Abbott GmbH, Delkenheim, Germany) and for HCV RNA, amplified directly without isolation of RNA, by use of 1-step reverse transcriptase-polymerase chain reaction (RT-PCR).<sup>16</sup>

**Ultrasonographic examination.** Abdominal ultrasound was performed by an experienced hepatologist who was unaware of subject case control and serological status. Ultrasound interpretations were made according to published standardized criteria.<sup>17–24</sup> Hepatic cirrhosis was considered present when the liver had a coarse echo pattern with at least one of the following: nodularity of the surface, attenuation of hepatic veins, or enlargement of the caudate lobe. The spleen was considered enlarged if it was > 13 cm in length. Portal hypertension was diagnosed if the diameter of the portal vein was > 13 mm. Periportal fibrosis (PPF) was considered present if the mean total thickness of 3 peripherally located portal tracts was ≥ 4 mm. A “bright liver” had increased parenchymal echogenicity with a corresponding decrease in visualization of portal and hepatic veins.

**Statistical analysis.** All 449 case patients and controls answered the questionnaire and had their blood tested for anti-HCV, HCV RNA, HBsAg, and ALT. Adequate abdominal ultrasonography data were available from 383 to 387 subjects; stool parasitological exams were recorded for 426. Data were entered into a Microsoft Access database (Arabic version 97). Duplicate data entry was performed to ensure quality control. Data analysis was performed with a statistical package for personal computer (SPSS, version 9; SPSS, Inc., Chicago, IL) utilizing comparisons of means (Student's *t*-test) and proportions (chi-square) when appropriate. Odds ratios (OR) with 95% confidence intervals (CI) were calculated to assess associations.

To better define the magnitude of—and risk factors for—CLD in Egyptian communities, we compared findings between case patients and controls (Figure 1A), as well as analyzed case patients and controls separately to assess the role of HCV infection in overt (Figure 1B<sub>1</sub>) and covert (Figure 1B<sub>2</sub>) liver disease. In addition, hepatic morbidity was compared in subgroups having serological evidence of infection with hepatitis B virus (HBV) or HCV, or with neither or both of these hepatitis viruses, to assess the broader role of chronic viral hepatitis in CLD (Figure 1C).

## RESULTS

**Assessment according to case control status.** A total of 237 patients attending the NLI consented to participate and 212 provided age- and sex-matched controls. The mean age of participants was 37.4 years, and 56% were men. Case patients were more likely to have a history of blood transfusion, schistosomiasis, and parenteral therapy for schistosomiasis and to be anti-HCV positive than their controls (Table 1). However, 46.7% of controls were also anti-HCV positive. Fourteen case patients were HBsAg positive, in comparison with only 5 controls; 35.5% of case patients had elevations in their ALT levels in comparison with 27.4% of controls, with a larger difference in the proportion having ALT elevations > 1.5 times the upper limit of normal. The *S. mansoni* infection rate was similar in case patients and controls, and 9.4% of those having

stool examinations had ova detected. Abdominal ultrasound demonstrated differences between case patients and controls; 22.8% of case patients had changes characteristic of cirrhosis, compared with only 2.8% of controls. Dilated portal veins and splenomegaly were detected in 20.2 and 37.0% of case patients but only 3.7 and 11.5% of controls, respectively. However, PPF, almost always minimal grade 1 changes, was observed in about half of both case patients and controls (Table 1).

The prevalence of anti-HCV increased among the case patients until age 40, and the percentage of those who had anti-HCV within each 5-year age cohort was very similar in case patients and controls from age 25 until age 50 (Figure 2). However, the proportion of anti-HCV positive was much greater among the case patients in the 20–25-year cohort and in the 3 cohorts from age 50 to 65. The case patients in the latter 3 subgroups were ~ 60% anti-HCV positive; the controls dropped from ~ 40% in the 50–55 cohort to 20% in the 55–60 and 60–65 cohorts.

**Assessment of case patients according to anti-HCV status.** Anti-HCV-positive case patients were more likely than anti-HCV-negative case patients to be male, older, and farmers; to have received a blood transfusion; and to have had schistosomiasis and to have received parenteral therapy for it (Table 2). Only 16.1% of the anti-HCV-positive individuals had received a blood transfusion, and 65.9% received injection treatment for schistosomiasis.

We found HCV RNA was present in 69.6% of case patients who were anti-HCV positive and in 5.1% of those who were anti-HCV negative (Table 2). There was no difference in the HBsAg status between case patients that were anti-HCV positive or negative. The ALT levels were more likely to be elevated in anti-HCV positive than in anti-HCV-negative case patients.

Ultrasound findings compatible with cirrhosis, portal hypertension, and splenomegaly were more frequently noted in anti-HCV positive than in anti-HCV-negative case patients (Table 2). However, PPF was equally as common in case patients not having antibodies to HCV as in those that did, being present in about half of both groups.

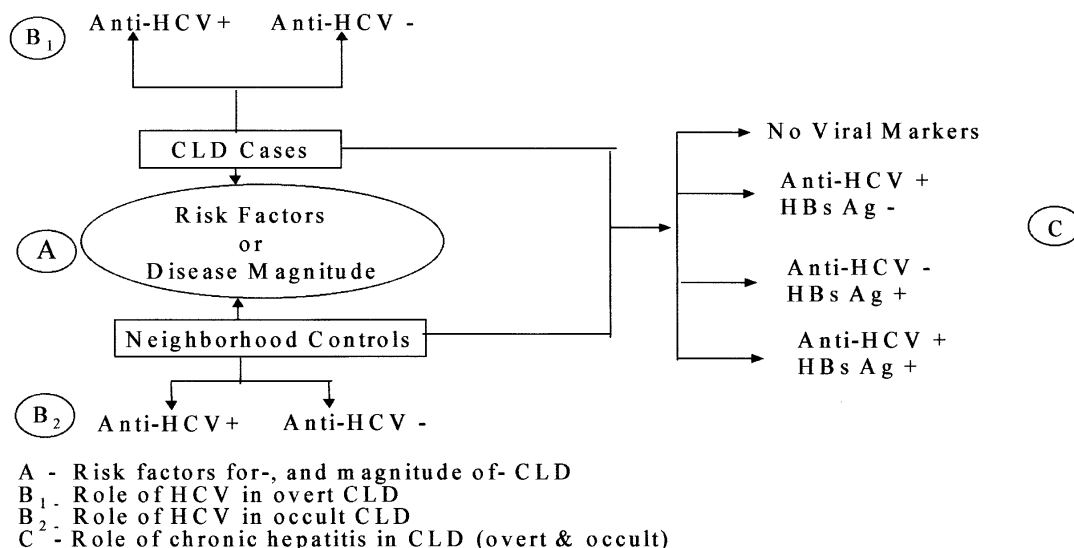


FIGURE 1. Algorithm describing how case patients and their matched neighborhood controls were evaluated.

TABLE 1  
Characteristics of the subjects according to recruitment status

Factor	Case patients (n = 237) n (%)	Controls (n = 212) n (%)	OR (95% CI)*	P value
Male sex	131 (55.3)	120 (56.6)	0.95 (0.65–1.38)	0.777
Age (mean years; $\pm$ SD)	38.1 $\pm$ 11.7	36.6 $\pm$ 10.7		0.172
Practicing agriculture	189 (80.8)	162 (78.6)	1.14 (0.72–1.82)	0.579
History of blood transfusion	26 (11.0)	10 (4.7)	2.50 (1.18–5.32)	0.014
History of schistosomiasis	154 (68.1)	111 (55.0)	1.75 (1.18–2.60)	0.005
Schistosomal injection therapy	108 (45.6)	76 (35.8)	1.50 (1.03–2.19)	0.037
Current <i>S. mansoni</i> infection	18 (7.9)	22 (10.6)	0.72 (0.37–1.38)	0.317
Anti-HCV positive	138 (58.2)	99 (46.7)	1.59 (1.10–2.31)	0.015
HCV-RNA positive	101 (42.6)	77 (36.3)	1.30 (0.89–1.91)	0.173
HBsAg positive	14 (5.9)	5 (2.4)	2.59 (0.92–7.31)	0.064
Serum ALT level			1.89 (1.07–3.36)†	0.028‡
Normal	153 (64.6)	154 (72.6)		
Minimally elevated	45 (19.0)	38 (17.9)		
Elevated	39 (16.5)	20 (9.4)	(1.07–3.36)	
Ultrasound				
Hepatic echogenic pattern‡			10.13 (4.26–24.1)§	<0.001§
Normal	78 (39.0)	114 (59.4)		
Bright	51 (25.5)	66 (34.4)		
Coarse	71 (48.0)	12 (15.6)		
Portal hypertension‡	40 (20.2)	7 (3.7)	6.58 (2.87–15.1)	<0.001
Splenomegaly‡	71 (37.0)	22 (11.5)	4.53 (2.66–7.72)	<0.001
Periportal fibrosis‡	98 (50.0)	92 (48.9)	1.04 (0.70–1.56)	0.835

\* 95% CI = 95% confidence interval; HBsAg = hepatitis B surface antigen; ALT = alanine aminotransferase; OR = odds ratio.

† Comparing the elevated group with normal and minimally elevated groups.

‡ Not all subjects had ultrasound data available (see text).

§ Comparing the group with coarse echogenic pattern to those with normal or bright patterns.

When age, sex, history of blood transfusion, schistosomiasis, and parenteral treatment of schistosomiasis were considered together in a logistic regression model, all of these remained associated with HCV infection except a history of schistosomiasis. The most significant associations were receiving a blood transfusion or parenteral treatment for schistosomiasis.

**Assessment of controls according to anti-HCV status.** Controls who were anti-HCV positive were more likely to be older, farmers, and to have had schistosomiasis and parenteral therapy for schistosomiasis than anti-HCV-negative controls (Table 3). Although they were more likely to have received a blood transfusion, this difference was not statistically significant. Whereas 49.5% anti-HCV-positive controls said they had received parenteral treatment for schistosomiasis, only 7.1% had blood transfusions.

When risk factors for HCV infection among controls was analyzed via logistic regression, only a history of receiving parenteral treatment for schistosomiasis remained associated with anti-HCV status.

HCV RNA was detected by RT-PCR in 74.7% controls who were anti-HCV positive and in 2.7% who were negative (Table 3). Four who had anti-HCV also had HBsAg in their sera. This compared with only one having HBsAg among controls who were anti-HCV negative. Elevations in ALT were present in 48.5% of the anti-HCV-positive controls and in only 8.9% of those who did not have anti-HCV.

As expected, ultrasound evidence of liver disease was not as frequent in the matched controls as in the case patients (Table 3). Five anti-HCV-positive controls had findings compatible with cirrhosis compared with only one who was anti-HCV negative. Changes of portal hypertension were also rare. Spleen enlargement was more frequent in those who were anti-HCV positive. As noted in the case patients, PPF

was present in half of the controls and was not associated with anti-HCV status.

**Assessment according to HCV RNA status.** When the above analyses were performed on the basis of RT-PCR results for HCV RNA, the conclusions were similar to those obtained when analysis was performed on the basis of anti-HCV status (data not shown).

**The ALT elevations and ultrasound morbidity according to HBV and HCV serological status.** Analysis of morbidity in all case patients and controls according to their viral hepatitis markers showed that ALT elevations and ultrasound evidence for cirrhosis, portal hypertension, and splenic enlargement were associated with the presence of anti-HCV, HBsAg, or both (Table 4). However, PPF was not associated with viral hepatitis markers. There were no differences between those infected with HCV, with HBV, or with both viruses. Cirrhosis was not detected in the 8 having only HBsAg, in comparison with it being present in 20.8% with anti-HCV and 27.3% having dual infections.

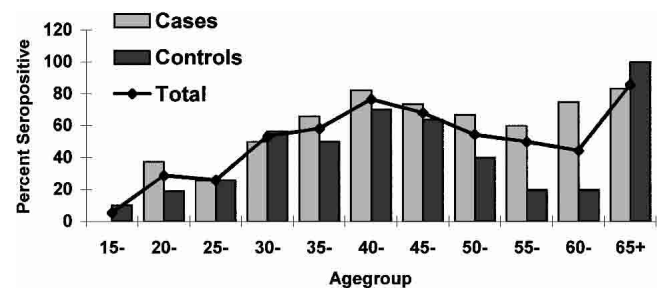


FIGURE 2. Anti-hepatitis C seroprevalence by age among case patients and controls.

TABLE 2  
Characteristics of the 237 case patients according to hepatitis C virus status

Factor	Positive (n = 138) n (%)	Negative (n = 99) n (%)	OR (95% CI)*	P value
Male sex	91 (65.9)	40 (40.4)	2.86 (1.67–4.87)	<0.001
Age (mean years; ± SD)	41.2 ± 10.4	33.8 ± 2.1		<0.001
Practicing agriculture	121 (88.3)	68 (70.1)	3.23 (1.64–6.36)	<0.001
History of blood transfusion	22 (16.1)	4 (4.0)	4.54 (1.51–13.6)	0.004
History of schistosomiasis	108 (81.2)	46 (49.5)	4.41 (2.43–8.01)	<0.001
Schistosomal injection therapy	91 (65.9)	17 (17.2)	9.34 (4.98–17.5)	<0.001
Current <i>S. mansoni</i> infection	9 (6.9)	9 (9.2)	0.73 (0.28–1.91)	0.520
HCV-RNA positive	96 (69.6)	5 (5.1)	43.0 (16.3–113)	<0.001
HbsAg positive	7 (5.1)	7 (7.1)	0.70 (0.24–2.07)	0.520
Serum ALT level			3.97 (1.67–9.42)†	0.001†
Normal	71 (51.4)	82 (82.8)		
Minimally elevated	35 (25.4)	10 (10.1)		
Elevated	32 (23.2)	7 (7.1)		
Ultrasound			4.84 (2.24–10.5)§	<0.001§
Hepatic echogenic pattern‡				
Normal	39 (31.7)	39 (50.6)		
Bright	25 (20.3)	26 (33.8)		
Coarse	59 (48.0)	12 (15.6)		
Portal hypertension‡	33 (27.3)	7 (9.1)	3.75 (1.57–8.99)	0.002
Splenomegaly‡	57 (49.1)	14 (18.4)	4.28 (2.18–8.49)	<0.001
Periportal fibrosis‡	61 (51.3)	37 (48.1)	1.14 (0.64–2.02)	0.661

\* 95% CI = 95% confidence interval; HbsAg = hepatitis B surface antigen; ALT = alanine aminotransferase; HCV = hepatitis C virus; OR = odds ratio.

† Comparing the elevated group with normal and minimally elevated groups.

‡ Not all subjects had ultrasound results recorded (see text).

§ Comparing coarse echogenic pattern group with normal and bright patterns groups.

## DISCUSSION

**Evidence for CLD.** Waked and others<sup>14</sup> reported the majority of 1023 outpatients referred to the NLI had clinical, biochemical, or ultrasonographic evidence of CLD. A subset of 100 of these patients had diagnoses established by liver biopsy: chronic hepatitis (n = 27), active cirrhosis (n = 39), hepatocellular carcinoma (n = 23), schistosomal hepatic fibrosis (n = 3), and other or unknown etiology (n = 8).

The average case patient was a middle-aged male farmer. One-sixth had elevated ALT levels > 1.5 times the upper

limits of normal. Abdominal ultrasonography showed that a quarter had findings characteristic of cirrhosis; one-fifth had dilation of the portal vein, which occurs with portal hypertension; and one-third had splenomegaly. These data, taken together, suggest that our case patients, defined as outpatients in a hospital specializing in liver disease, are a heterogeneous group, with many having findings compatible with chronic hepatitis, cirrhosis, or both.

**Role of HCV in CLD among the case patients.** Because 60% of the case patients were anti-HCV positive and 40% had HCV viremia, as compared with only 6% having HbsAg

TABLE 3  
Characteristics of the 212 controls according to hepatitis C virus status

Factor	Positive (n = 99), n(%)	Negative (n = 113), n(%)	OR (95% CI)*	P value
Male sex	59 (59.6)	61 (54.0)	1.26 (0.73–2.17)	0.411
Age (mean years; ± SD)	39.0 ± 8.7	34.6 ± 11.9		0.004
Practicing agriculture	87 (88.8)	75 (69.4)	3.48 (1.65–7.36)	0.001
History of blood transfusion	7 (7.1)	3 (2.7)	2.79 (0.70–11.1)	0.130
History of schistosomiasis	61 (62.9)	50 (47.6)	1.86 (1.06–3.27)	0.029
Schistosomal injection therapy	49 (49.5)	27 (23.9)	3.12 (1.74–5.60)	<0.001
Current <i>S. mansoni</i> infection	12 (12.5)	10 (9.0)	1.44 (0.59–3.51)	0.416
HCV-RNA positive	74 (74.7)	3 (2.7)	108.5 (31.6–372)	<0.001
HbsAg positive	4 (4.1)	1 (0.9)	4.77 (0.52–43.4)	0.128
Serum ALT level			7.60 (2.16–26.8)†	<0.001†
Normal	51 (51.5)	103 (91.2)		
Minimally elevated	31 (31.3)	7 (6.2)		
Elevated	17 (17.2)	3 (2.7)		
Ultrasound			5.96 (0.68–51.9)§	0.707§
Hepatic echogenic pattern‡				
Normal	53 (58.9)	61 (59.8)		
Bright	30 (33.3)	36 (35.3)		
Coarse	7 (7.8)	5 (4.9)		
Portal hypertension‡	4 (4.5)	3 (3.0)	1.52 (0.33–6.99)	0.587
Splenomegaly‡	14 (15.6)	8 (7.8)	1.92 (0.96–3.86)	0.063
Periportal fibrosis‡	42 (47.2)	50 (50.5)	0.88 (0.49–1.55)	0.650

\* 95% CI = 95% confidence interval; HbsAg = hepatitis B surface antigen; ALT = alanine aminotransferase; OR = odds ratio.

† Comparing the elevated group with normal and minimally elevated groups.

‡ Not all subjects had ultrasound results recorded (see text).

§ Comparing group with a coarse echogenic pattern with groups with normal and bright patterns.

TABLE 4  
Hepatic enzymes and ultrasonographic findings according to hepatitis infection status\*

Finding	No hepatitis (n = 204)	HCV alone (n = 226)	HBV alone (n = 8)	HCV + HBV (n = 11)	P value
Serum ALT level					<0.001
Normal	182 (89.2)	116 (51.3)	3 (37.5)	6 (54.5)	
Minimally elevated	15 (7.4)	63 (27.9)	2 (25.0)	3 (27.3)	
Elevated	7 (3.4)	47 (20.8)	3 (37.5)	2 (18.2)	
Cirrhosis	10 (4.9)	47 (20.8)	0 (0.0)	3 (27.3)	<0.001
Portal hypertension	9 (5.3)	34 (16.9)	1 (12.5)	3 (33.3)	0.001
Splenomegaly	20 (11.8)	67 (33.8)	2 (25.0)	4 (50.0)	<0.001
Periportal fibrosis	84 (50.0)	98 (49.2)	3 (37.5)	5 (55.6)	0.893

\* HBV = hepatitis B virus; HCV = hepatitis C virus; ALT = alanine aminotransferase

and 8% having *S. mansoni* ova, the predominant potential cause of CLD in these NLI outpatients was infection with HCV. Further support for HCV as a cause of CLD in NLI outpatients was noted when the case patients were stratified according to whether they were anti-HCV positive or negative. ALT elevations and ultrasound evidence of cirrhosis, portal hypertension, and splenomegaly were all ~ 3.5 to 4.5 times more frequent in anti-HCV positive than in anti-HCV-negative case patients. When the morbidity data among all 449 case patients and controls were analyzed according to HCV and HBV infection status, it was also obvious that elevated ALT levels, cirrhosis, portal hypertension, and splenomegaly were associated with HCV infection.

Stratifying anti-HCV prevalence by age suggested HCV infections were more likely to cause symptomatic CLD among the older population. A much greater proportion of case patients than controls older than 50 were anti-HCV positive. Lower prevalence of anti-HCV among older controls supports studies of the natural history of HCV that show that symptomatic CLD primarily occurs later in life, after many years of chronic infection.<sup>2-11</sup> We assume fewer older persons with HCV infections remained asymptomatic and therefore did not meet our criteria for controls (i.e., absence of diagnosed liver disease). Conversely, the higher prevalence of anti-HCV among the 20-25-year-old case patients resulted from evaluations of positive anti-HCV tests discovered during examinations of young adults seeking work visas for Saudi Arabia or another Gulf country.

**Magnitude of occult CLD.** Analyzing matched controls allows an estimate of the magnitude of CLD among the population of the Nile Delta. Approximately 10% of the controls had modest ALT elevations, 3.4% had ultrasound criteria for cirrhosis and portal hypertension, and almost 12% had splenic enlargement. Darwish and others<sup>25</sup> reported they detected ultrasound evidence of cirrhosis in 18 (or 5% of those older than 30) among a household-stratified sample of inhabitants of a village in the Nile Delta. These results are similar to ours even though they did not exclude subjects seeking medical care for liver disease.

**Role of HCV infection in occult CLD.** Almost half of the matched controls were anti-HCV positive, and three-quarters of these also had HCV RNA evidence for active infections. This compares with only 1 in 20 who were HBsAg positive and 1 in 10 who had active schistosomiasis mansoni. Therefore, HCV was by far the most common potential cause of liver disease among the controls. When the controls were stratified by whether they were anti-HCV positive or negative, the majority of the detected morbidity was present in the 99 controls who had HCV infections. One in 6 had

modest ALT elevations, splenic enlargement, or both, and ~ 5% had evidence for cirrhosis and portal hypertension, abnormalities that were rare in controls who did not have anti-HCV.

Fourteen (78%) of patients with ultrasound-detected cirrhosis in an Egyptian cross-sectional survey were anti-HCV positive.<sup>25</sup> This compared with a 22% HBsAg-positive rate. The adjusted OR for cirrhosis was 3.6 (95% CI, 1.1-12.2) for anti-HCV and 2.3 (95% CI, 0.7-7.8) for HBsAg. HCV was associated with cirrhosis 3.5 times more frequently than HBV in their community.

**Risk factors for HCV infection.** Because the case patients and controls were defined by whether they sought evaluation of liver disease, rather than whether or not they were anti-HCV positive, the case patients and controls were separately analyzed for anti-HCV risk factors. Among the case patients, when analyzed via logistic regression, male sex, age, farming, history of a blood transfusion, or injection therapy for schistosomiasis were all associated with anti-HCV. Although these risks were less significant because of the lower prevalence of anti-HCV, analysis of the 212 controls showed similar relationships. Male sex and history of a blood transfusion were no longer significant risks, and with logistic regression, only parenteral therapy of schistosomiasis remained a risk. These data corroborate our community-based investigations of risk factors for anti-HCV.<sup>26-28</sup> Other than blood transfusions, percutaneous risk factors in our subjects differ from those in developed countries,<sup>29-35</sup> with intravenous drug abuse being very rare in the Nile Delta.

Darwish and others<sup>25</sup> also studied correlates of HCV infection; 40% of their 796 subjects were anti-HCV positive. The inhabitants having anti-HCV were older than those that were antibody negative. They queried their subjects about almost all of the same exposures as we did. Only a history of schistosomiasis (OR = 1.8) was associated with anti-HCV-positive case patients. Their data differed from ours in that ultrasound findings compatible with PPF had marginal significance. Their sample size was too small to demonstrate a relationship between prior injection therapy for schistosomiasis and anti-HCV (age-adjusted OR = 1.5, 0.8, 2.9). They also confirmed the absence of exposures to intravenous drug abuse and alcohol. We did not ask these questions because we knew these exposures in rural communities in the Nile Delta would be absent or very low; we knew we would not receive a truthful answer if the question were asked; and we thought it would likely disturb our subjects and reduce their cooperation.

The data from this report further supports the supposition by Frank and others<sup>1</sup> that the large reservoir of HCV infec-

tion in Egypt was primarily built during schistosomiasis control campaigns conducted 20–50 years ago by use of intravenously administered tartar emetic because more than half of those who were anti-HCV positive reported a history of this exposure. The Nile Delta, the site of the NLI, was (and remains) highly endemic for *S. mansoni*, which would account for the persistence of ultrasound changes of PPF in half our subjects.<sup>36</sup>

**Limitations and advantages of the study design.** Because of the high prevalence of anti-HCV among the controls, we considered the possibility that the controls included many subjects who had liver disease. Although it was carefully explained to each case patient that controls should be neighbors not having a history of liver disease, there was some concern that this criteria might be ignored to obtain clinical evaluation for neighbors with liver disease. However, this is unlikely. With the exception of the serum anti-HCV and HCV RNA markers of HCV infection and ultrasound evidence for PPF, bright liver echo pattern, and splenomegaly, evidence of hepatic morbidity was much less common in the controls than in the case patients. Also, the prevalence of anti-HCV in adults living in rural areas of the Nile Delta, where most of the controls resided, is similar to the 46.7% of our controls.<sup>25–28,37–39</sup>

Liver biopsy is the most reliable means to diagnose hepatic morbidity. However, it is unethical to perform a biopsy unless it will assist in patient care. Sometimes biopsies are needed to confirm a diagnosis. This would increase the number of patients with unusual or more severe clinical presentations. Also, liver biopsies are indicated for patients who are candidates for antiviral therapy. However, antiviral therapy for either HBV or HCV was not given at the NLI in 1992<sup>14</sup> and was rarely used in 1997 at the time of this study. Therefore, we based our evidence for CLD on biochemical and ultrasound results. If some case patients did not have CLD, it would reduce the significance of our findings, but it should not lead to false-positive results.

The reliability of ultrasound to detect hepatic morbidity is often questioned. The ultrasound exams were performed under the supervision of the same person (T.S.), a gastroenterologist with extensive experience with abdominal ultrasonography. She performed the examination, as opposed to a technician who takes pictures to be read later by a radiologist, and was not aware if the subject was a case patient or control or the patient's anti-HCV status. There are many potential causes, including fatty liver, obesity and diabetes, of a bright echogenic pattern in addition to chronic hepatitis.<sup>20–22</sup> Minimal PPF is present in many circumstances,<sup>40</sup> and PPF could not be used to detect HCV infections in our subjects. However, its use to detect prior morbidity from *S. mansoni* has been described.<sup>18</sup> Our data further confirm this: 57.4% of subjects having a history of schistosomiasis had periportal thickening  $\geq 4$  mm, compared with only 42.6% of subjects without a history of schistosomiasis ( $P = 0.028$ ). This contrasts with finding no difference ( $P = 0.41$ ) in the frequency of PPF among those with, and those without, *S. mansoni* ova in their stools. The high prevalence of splenomegaly in the case patients could partially be due to relatively large spleens among this population with frequent exposure to *Schistosoma mansoni*, hepatitis viruses, and other infectious agents, as well as due to the increased sensitivity of directly measuring spleen length by ultrasound.

Another potential bias is related to the selection of case patients. In our desire to have the broadest spectrum of CLD patients among the case patients, we included all subjects seeking evaluation at the NLI. This includes some patients who, as a result of inappropriate referral or misdiagnosis, may not have liver disease at all; 10% of case patients were referred for evaluation of positive anti-HCV EIAs detected during screening examinations. Because these subjects were usually asymptomatic and were, as a group, younger than our total case patient population, they would less likely to have detectable morbidity than case patients evaluated because they exhibited symptoms. In addition, they would increase the proportion of case patients who were anti-HCV positive. This bias reducing case patient morbidity would lessen support for our conclusion that morbidity in our subjects is associated with HCV infection. The increase in anti-HCV prevalence bias would increase the prevalence of anti-HCV among case patients.

Despite our difficulty establishing a diagnosis of CLD and evaluating morbidity, this study shows hepatic morbidity in those presenting for evaluation of liver disease in this region of Egypt is primarily associated with HCV infection. Neighborhood controls who denied having a history of liver disease also had a high prevalence of exposure to HCV, and some of these HCV-positive controls also had elevated serum ALT and ultrasound abnormalities compatible with CLD.

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## REFERENCES

1. Frank C, Mohamed MK, Strickland GT, Lavanchy D, Arthur RR, Magder LS, El Khoby T, Abdel-Wahab Y, Ohn ESA, Anwar W, Sallam I, 2000. The role of parenteral antischistosomal therapy in the spread of hepatitis C virus in Egypt. *Lancet* 355: 887–891.
2. Alter MJ, Margolis HS, Krawczynski K, Judson FN, Mares A, Alexander WJ, Hu PY, Miller JK, Gerber MA, Sampliner RE, 1992. The natural history of community-acquired hepatitis C in the United States: the Sentinel Counties Chronic Non-A, Non-B Hepatitis Study Team. *N Engl J Med* 327: 1899–1905.
3. Di Bisceglie AM, 1997. Hepatitis C and hepatocellular carcinoma. *Hepatology* 26: 34S–38S.

4. Di Bisceglie AM, Goodman ZD, Ishak KG, Hoofnagle JH, Melpolder JJ, Alter HJ, 1991. Long-term clinical and histopathological follow-up of chronic posttransfusion hepatitis. *Hepatology* 14: 969-974.
5. Poinard T, Bedossa P, Opolon P, 1997. Natural history of liver fibrosis progression in patients with chronic hepatitis C: the OBSVIRC, METAVIR, CLINIVIR, and DOSVIRC groups. *Lancet* 349: 825-832.
6. Rodger AJ, Roberts S, Lanigan A, Bowden S, Brown T, Crofts N, 2000. Assessment of long-term outcomes of community-acquired hepatitis C infection in a cohort with sera stored from 1971 to 1975. *Hepatology* 32: 582-587.
7. Roudot-Thoraval F, Bastie A, Pawlotsky JM, Dhumeaux D, 1997. Epidemiological factors affecting the severity of hepatitis C virus-related liver disease: a French survey of 6,664 patients: the Study Group for the Prevalence and the Epidemiology of Hepatitis C Virus. *Hepatology* 26: 485-490.
8. Gordon SC, Bayati N, Silverman AL, 1998. Clinical outcome of hepatitis C as a function of mode of transmission. *Hepatology* 28: 562-567.
9. Niederau C, Lange S, Heintges T, Erhardt A, Buschkamp M, Hurter D, Nawrocki M, Kruska L, Hensel P, Petry W, Hausinger D, 1998. Prognosis of chronic hepatitis C: results of a large, prospective cohort study. *Hepatology* 28: 1687-1695.
10. Datz C, Cramp M, Haas T, Dietze O, Nitschko H, Froesner G, Muss N, Sandhofer F, Vogel W, 1999. The natural course of hepatitis C virus infection 18 years after an epidemic 7. outbreak of non-A, non-B hepatitis in a plasmapheresis centre. *Gut* 44: 563-567.
11. Alberti A, Chemello L, Benvegna L, 1999. Natural history of hepatitis C. *J Hepatol* 31: 17-24.
12. Hu KQ, Tong MJ, 1999. The long-term outcomes of patients with compensated hepatitis C virus-related cirrhosis and history of parenteral exposure in the United States. *Hepatology* 29: 1311-1316.
13. Thomas DL, Astemborski J, Rai RM, Anania FA, Schaeffer M, Galai N, Nolt K, Nelson KE, Strathdee SA, Johnson L, Laeyendecker O, Boitnott J, Wilson LE, Vlahov D, 2000. The natural history of hepatitis C virus infection: host, viral, and environmental factors. *JAMA* 284: 450-456.
14. Waked IA, Saleh SM, Moustafa MS, Raouf AA, Thomas DL, Strickland GT, 1995. High prevalence of hepatitis C in Egyptian patients with chronic liver disease. *Gut* 37: 105-107.
15. Peters PA, El Alamy M, Warren KS, Mahmoud AA, 1980. Quick Kato smear for field quantification of *Schistosoma mansoni* eggs. *Am J Trop Med Hyg* 29: 217-219.
16. Abdel-Hamid M, Edelman DC, Highsmith WE, Constantine NT, 1997. Optimization, assessment, and proposed use of a direct nested reverse transcription-polymerase chain reaction protocol for the detection of hepatitis C virus. *J Hum Virol* 1: 58-65.
17. Needleman L, Kurtz AB, Rifkin MD, Cooper HS, Pasto ME, Goldberg BB, 1986. Sonography of diffuse benign liver disease: accuracy of pattern recognition and grading. *AJR Am J Roentgenol* 146: 1011-1015.
18. Abdel-Wahab MF, Esmat G, Milad M, Abdel-Razek S, Strickland GT, 1989. Characteristic sonographic pattern of schistosomal hepatic fibrosis. *Am J Trop Med Hyg* 40: 72-76.
19. Zwiebel WJ, 1995. Sonographic diagnosis of hepatic vascular disorders. *Semin Ultrasound CT MR* 16: 34-48.
20. Zwiebel WJ, 1995. Sonographic diagnosis of diffuse liver disease. *Semin Ultrasound CT MR* 16: 8-15.
21. Kurtz AB, Rubin CS, Cooper HS, Nisenbaum HL, Cole-Beuglet C, Medoff J, Goldberg BB, 1980. Ultrasound findings in hepatitis. *Radiology* 136: 717-723.
22. Needleman L, 1988. Diffuse benign liver disease. *Clin Diagn Ultrasound* 23: 57-73.
23. Giorgio A, Amoroso P, Lettieri G, Fico P, de Stefano G, Finelli L, Scala V, Tarantino L, Pierri P, Pesce G, 1986. Cirrhosis: value of caudate to right lobe ratio in diagnosis with US. *Radiology* 161: 443-445.
24. Joseph AE, Saverymattu SH, 1991. Ultrasound in the assessment of diffuse parenchymal liver disease. *Clin Radiol* 44: 219-221.
25. Darwish MA, Faris R, Darwish N, Shouman A, Gadallah M, El-Sharkawy MS, Edelman R, Grumbach K, Rao MR, Clemens JD, 2001. Hepatitis C and cirrhotic liver disease in the Nile Delta of Egypt: a community-based study. *Am J Trop Med Hyg* 64: 147-153.
26. Abdel-Aziz F, Habib M, Mohamed MK, Abdel-Hamid M, Gamil F, Madkour S, Mikhail NN, Thomas D, Fix AD, Strickland GT, Anwar W, Sallam I, 2000. Hepatitis C virus (HCV) infection in a community in the Nile Delta: population description and HCV prevalence. *Hepatology* 32: 111-115.
27. Habib M, Mohamed MK, Abdel-Aziz F, Magder LS, Abdel-Hamid M, Gamil F, Madkour S, Mikhail NN, Anwar W, Strickland GT, Fix AD, Sallam I, 2001. Hepatitis C virus infection in a community in the Nile Delta: risk factors for seropositivity. *Hepatology* 33: 248-253.
28. Nafeh MA, Medhat A, Shehata M, Mikhail NN, Swifee Y, Abdel-Hamid M, Watts S, Fix AD, Strickland GT, Anwar W, Sallam I, 2000. Hepatitis C in a community in Upper Egypt: 1. Cross-sectional survey. *Am J Trop Med Hyg* 63: 236-241.
29. Alter MJ, Hadler SC, Judson FN, Mares A, Alexander WJ, Hu PY, Miller JK, Moyer LA, Fields HA, Bradley DW, 1990. Risk factors for acute non-A, non-B hepatitis in the United States and association with hepatitis C virus infection. *JAMA* 264: 2231-2235.
30. Alter MJ, 1999. Hepatitis C virus infection in the United States. *J Hepatol* 31: 88-91.
31. Guadagnino V, Stroffolini T, Rapicetta M, Costantino A, Kondili LA, Menniti-Ippolito F, Caroleo B, Costa C, Griffo G, Loiacono L, Pisani V, Foca A, Piazza M, 1997. Prevalence, risk factors, and genotype distribution of hepatitis C virus infection in the general population: a community-based survey in southern Italy. *Hepatology* 26: 1006-1011.
32. Noguchi S, Sata M, Suzuki H, Mizokami M, Tanikawa K, 1997. Routes of transmission of hepatitis C virus in an endemic rural area of Japan: molecular epidemiologic study of hepatitis C virus infection. *Scand J Infect Dis* 29: 23-28.
33. Murphy EL, Bryzman SM, Glynn SA, Ameti DJ, Thomson RA, Williams AE, Nass CC, Ownby HE, Schreiber GB, Kong F, Neal KR, Nemo GJ, 2000. Risk factors for hepatitis C virus infection in United States blood donors. NHLBI Retrovirus Epidemiology Donor Study (REDS). *Hepatology* 31: 756-762.
34. Sun CA, Chen HC, Lu CF, You SL, Mau YC, Ho MS, Lin SH, Chen CJ, 1999. Transmission of hepatitis C virus in Taiwan: prevalence and risk factors based on a nationwide survey. *J Med Virol* 59: 290-296.
35. Sanchez JL, Sjogren MH, Callahan JD, Watts DM, Lucas C, Abdel-Hamid M, Constantine NT, Hyams KC, Hinostroza S, Figueroa-Barrios R, Cuthie JC, 2000. Hepatitis C in Peru: risk factors for infection, potential iatrogenic transmission, and genotype distribution. *Am J Trop Med Hyg* 63: 242-248.
36. Abdel-Wahab MF, Esmat G, Medhat E, Narooz S, Ramzy I, El-Boraey Y, Strickland GT, 2000. The epidemiology of schistosomiasis in Egypt: Menofia Governorate. *Am J Trop Med Hyg* 62: 28-34.
37. Abdel-Wahab MF, Zakaria S, Kamel M, Abdel-Khalik MK, Mabrouk MA, Salama H, Esmat G, Thomas DL, Strickland GT, 1994. High seroprevalence of hepatitis C infection among risk groups in Egypt. *Am J Trop Med Hyg* 51: 563-567.
38. Darwish MA, Faris R, Clemens JD, Rao MR, Edelman R, 1996. High seroprevalence of hepatitis A, B, C, and E viruses in residents in an Egyptian village in the Nile Delta: a pilot study. *Am J Trop Med Hyg* 54: 554-558.
39. Kamel MA, Miller FD, el Masry AG, Zakaria S, Khattab M, Esmat G, Ghaffer YA, 1994. The epidemiology of *Schistosoma mansoni*, hepatitis B and hepatitis C infection in Egypt. *Ann Trop Med Parasitol* 88: 501-509.
40. Medhat A, Nafeh M, Swifee Y, Helmy A, Zaki S, Shehata M, Ibrahim S, Abdel-Kader DA, Strickland GT, 1998. Ultrasound-detected hepatic periportal thickening in patients with prolonged pyrexia. *Am J Trop Med Hyg* 59: 45-48.