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Schistosomiasis Does Not Affect the Outcome of HCV Infection in Genotype 4-Infected Patients

Walaa R. Allam, Ahmed Barakat, Zainab Zakaria, Gehan Galal, Tamer S. Abdel-Ghafar, Mohamed El-Tabbakh, Nabiel Mikhail, Imam Waked, and Sayed F. Abdelwahab*

Egyptian Company for Blood Transfusion Services (Egyblood), Agouza, Giza, Egypt; Department of Microbiology, Faculty of Science, Ain Shams University, Abbasia, Cairo, Egypt; Department of Hepatology, National Liver Institute, Menoufiya University, Menoufiya, Egypt; South Egypt Cancer Institute, Assuit, Egypt; Department of Microbiology and Immunology, Faculty of Medicine, Minia University, Minia, Egypt

Abstract. Although reports suggest that *Schistosoma mansoni* increases hepatitis C virus (HCV) morbidity and chronicity, its impact on HCV spontaneous resolution is not clear. HCV genotype, viral load, abdominal ultrasonographic findings, and HCV-specific cell-mediated immunity (CMI) were examined among 141 healthcare workers infected with HCV (68 workers with and 73 workers without *S. mansoni*). HCV genotype 4 was dominate, and viral loads were 2.62 ± 0.69 × 10^6 and 4.24 ± 1.4 × 10^6 IU/mL among patients with and without coinfection, respectively ($P = 0.309$); 23.5% with and 32.9% without coinfection had spontaneously resolved HCV infection ($P = 0.297$). Interferon-γ spot-forming cells/10^6 peripheral blood mononuclear cells among responding viremic patients with and without coinfection were 716 ± 194 and 587 ± 162, whereas among aviremic patients, it was 794 ± 272 and 365 ± 36 ($P > 0.05$), respectively. In conclusion, there was no statistical difference in HCV spontaneous resolution, viral load, liver pathology, or CMI in patients with or without *S. mansoni* coinfection, suggesting that it did not impact the outcome of HCV infection.

INTRODUCTION

Hepatitis C virus (HCV) is a major causative agent of chronic hepatitis. Approximately 70% of infected persons develop chronic hepatitis C, and 20–30% may progress to cirrhosis and hepatocellular carcinoma.[1–3] The prevalence of HCV infection in Egypt is the highest in the world, where ~15% of the general population have HCV antibodies and two-thirds of those individuals have active HCV infection or viremia.[4] The progression of liver disease in HCV-infected patients may be affected by viral factors (e.g., viral load, viral genotype, and quasispecies diversity).[5] Also, many host factors have been observed to increase the risk of progression of liver disease, including race, male sex, older age at infection, and immunosuppressed states.[6] Other complicating factors include schistosomiasis, human immunodeficiency virus (HIV) coinfections, and alcohol intake.[2,7]

Concomitant schistosomiasis and HCV infections are common in Egypt and many developing countries, where schistosomiasis is endemic,[8] especially among persons in contact with river water and individuals working in agriculture. Several reports suggest that *Schistosoma mansoni* increases HCV morbidity and chronicity, leading to viral persistence and accelerated progression of hepatic complications. In this regard, compared with patients infected with HCV alone, the majority of HCV and *Schistosoma* coinfected patients failed to clear HCV infection and progressed to chronic hepatitis, with a more severe clinical course,[9] higher HCV-RNA titers,[10,11] higher incidence of cirrhosis and hepatocellular carcinoma,[12–15] poor response to interferon (IFN) therapy,[16,17] a higher treatment relapse rate,[17] and higher mortality rates.[11,18]

Little is known about the mechanisms of HCV and *S. mansoni* interactions, and the immunological changes that occur during such a coinfection are not clearly defined,[19] mainly because of the lack of a small animal models that can support both infections. Spontaneous resolution of HCV monoinfection is associated with strong $CD4^+$ and $CD8^+$ T-cell responses.[20–25] Chronic infection with schistosomiasis induces a T-helper 2 (Th-2) cell-mediated immune (CMI) response while suppressing Th-1 response, which may be the cause for a poor prognosis of HCV infection.[26] This immunosuppression caused by *S. mansoni* coinfection may even persist beyond having active schistosomiasis (i.e., having viable adult worms).[27] How coinfection impacts the clearance or persistence of HCV infection is not clear and understudied. In this study, the effect of *S. mansoni* infection on HCV clearance, viral load, liver pathology, and HCV-specific CMI response was examined in patients with and without coinfection in a cross-sectional survey of 141 Egyptian healthcare workers (HCWs) at the National Liver Institute (NLI) in the Nile Delta.

SUBJECTS AND METHODS

Study subjects. We previously reported the prevalence[28] and incidence[29] of HCV markers among 859 HCWs at the NLI at Menoufiya University; 141 (16.6%) HCWs were positive for HCV antibodies, 13 (1.5%) HCWs were positive for hepatitis B virus (HBV) surface antigen (HBsAg), and 2 (0.02%) HCWs were positive for both viruses. Antibodies to *S. mansoni* were detected in 297 (34.6%) of these HCWs. In this study, we examine the impact of schistosomiasis on 141 HCV antibody-positive HCWs. They were 92 males and 49 females, with a mean age of 41.2 ± 10.2 years. At the time of this study, none of the HCV-infected subjects were aware of HCV status and did not receive any standard of care treatment for the infection. According to *S. mansoni* antibody status, these 141 HCWs were classified into two categories. The first category included 68 coinfected patients who were positive for both HCV and *S. mansoni* antibodies, and the second category included 73 patients infected with HCV alone who were positive for HCV antibodies but not *S. mansoni* antibodies. Data regarding age, sex, residence, education, job category, and clinical history were obtained using standard demographic questionnaires. Blood samples were collected for laboratory testing and immunological studies. The study protocol was approved by the ethical committee of the NLI, and each participant signed a consent form before enrollment.

*Address correspondence to Sayed F. Abdelwahab, Department of Microbiology and Immunology, Faculty of Medicine, Minia University, Minia 61511, Egypt. E-mail: sayed.awahab@mu.edu.eg

Detection of HCV, *S. mansoni*, and other biomarkers. Serum samples were collected in plain vacutainer tubes, and serum alanine aminotransferase (ALT) levels were measured using routine clinical test kits (HUMAN Gesellschaft für Biochemica und Diagnostica mbH, Wiesbaden, Germany). Anti-HCV was tested using a third generation enzyme immunoassay (EIA; Murex Anti-HCV, version 4.0, Abbott Park, IL) according to the manufacturer's instructions. HBsAg (Murex) and HBV core antibody (HBc; total anti-HBc immunoglobulin M [IgM] and IgG; Adaltis, Milan, Italy) EIAs were performed according to the manufacturers' instructions and as previously described.[28] Detection and quantification of HCV-RNA was performed on subjects' sera after extraction of RNA using the Qiagen viral RNA extraction kit (QIAgen, Limburg, Netherlands) and a quantitative real-time reverse transcriptase polymerase chain reaction (RT-PCR) by strand-specific AgPath-ID one-step assay according to the manufacturer's instructions (Applied Biosystems-Life Technologies Corporation, Foster City, CA). The kit uses HCV-specific primers and probes as well as internal controls. Detection of *S. mansoni*-specific IgG was performed by EIA according to the manufacturer's instructions (Novatec Immunodiagnostica GmbH, Germany, Diezenbach, Germany).

HCV genotyping was conducted by restriction fragment-length polymorphism (RFLP) analysis of the 5′ non-coding region using two sets of restriction endonucleases, *MvaI/HinfI* and *RsaI/HaeIII*, as previously described.[30] The genotyping data were confirmed by conventional PCR using genotype-specific primers as previously described.[31]

Abdominal ultrasonography. Routine abdominal ultrasound was performed for all subjects included in the study.

Synthetic HCV antigens and other control antigens. HCV genotype 4a isolate ED43 peptide antigens composed of 15 amino acids (15mer) and overlapped by 11 amino acids were used in this study. These synthetic peptides were obtained from the National Institute of Allergy and Infectious Diseases' Biodefense and Emerging Infections Research Resources Repository and were at least 80% pure. There were 585 peptides that were combined in seven pools and labeled with alphabetical letters as follows: E2", representing the viral envelope protein E2 (92 peptides); F", comprising the N-terminal one-half of the NS3 protein (78 peptides); G", comprising the remaining one-half of NS3 (78 peptides); H", comprising the NS4a and NS4b proteins (79 peptides); I", comprising the NS5a protein (111 peptides); L", representing the first one-half of NS5b (75 peptides); M", covering the remainder of NS5b protein (72 peptides). Negative control cultures included cells stimulated with culture medium alone but containing the solvent used for the preparation of the peptides (dimethyl sulfoxide). Cytomegalovirus lysate (Virusys Corporation) and Cytomegalovirus, Epstein-Barr Virus and Influenza Virus (CEF) peptide pool (Pantecs GmbH, Germany) were used as positive controls for antigen-specific responses, whereas staphylocoocal enterotoxin B (SEB; Sigma, MO) was used as a polyclonal positive control.

IFN-γ ELISpot assay. To examine if there is a difference in HCV-specific CMI responses among those subjects with HCV alone and those subjects with concomitant *S. mansoni* infection (active or inactive; because antibodies cannot differentiate current from past infection), we performed an IFN-γ enzyme linked immunospot (Elispot) assay to assess HCV-specific CMI response. A convenience sample of 56 patients was examined for HCV-specific CMI responses. These subjects were 27 coinfected patients (20 patients with and 7 patients without HCV viremia) and 29 subjects who were positive for HCV infection alone (14 patients with and 15 patients without HCV viremia). Approximately 15 mL whole blood were collected into ethylene diamine tetraacetic acid (EDTA) vacutainer tubes (Becton Dickinson Biosciences, NJ). Peripheral blood mononuclear cells (PBMCs) were isolated by Ficoll-Hypaque density gradient centrifugation, and viability was determined by the trypan blue exclusion method. The ELISpot assay is described elsewhere.[32] Briefly, PBMCs (2×10^5/well) were incubated in triplicate cultures in the ELISpot plates (Whatman Unifilter, Pittsburgh, PA) coated with anti-human IFN-γ antibody (Mabtech, Stockholm, Sweden) and incubated for ~16 hours with or without recombinant HCV antigens at 3 μg/mL for each single peptide in complete RMPI-1640 medium. Negative and positive controls included medium containing dimethyl sulfoxide alone and 0.1 μg/mL SEB, respectively. At the end of the incubation period, the assay was developed until the appearance of spots, and then, the wells were rinsed with tap water to stop the reaction. The number of spots per well was counted using an automated ELISpot reader (Cellular Technology Ltd., Cleveland, OH). Mean numbers of IFN-γ spot-forming cells (SFCs) in control wells were subtracted from antigen-stimulated wells to correct for background cytokine production and are expressed as SFCs per 1 million PBMCs. A positive HCV antigen-specific response was considered if the SFCs in the presence of antigen were at least threefold the number of SFCs in the medium control and if there was > 55 SFCs/million PBMCs as we previously reported.[33]

Statistical analysis. All data were entered into a Microsoft Access database (Redmond, WA). Duplicate data entry was performed to ensure quality control. Analysis was done on SPSS package version 17.0. (SPSS Inc., IL). Fisher exact test was performed for categorical data, whereas Student's *t* test (or Kruskal–Wallis *U* test, when appropriate) was performed for comparison of continuous data.

RESULTS

Characterization of the study population. This study was conducted on 141 HCWs from the NLI at Menoufiya University and included 68 patients who were coinfected with HCV and *S. mansoni*, of whom 16 (23.5%) patients had spontaneously resolved HCV infection (i.e., negative HCV-RNA), and 73 patients who were positive for HCV infection alone, of whom 24 (32.9%) patients had spontaneously resolved HCV infection. There was no significant difference in the percentage of subjects who spontaneously resolved HCV infection in the presence or absence of *S. mansoni* ($P = 0.297$). The subjects have an overall mean age of 41.2 ± 10.2 years. Among them, 92 (65.2%) patients were males ($P = 0.081$), and 69.8% of patients were rural residents ($P = 0.793$). The job category and detailed demographic characteristics of the study subjects with different HCV and *S. mansoni* infection states are shown in Table 1.

HCV-RNA loads are not significantly different between those subjects infected with HCV alone or combined with *S. mansoni* infection. All subjects positive for HCV antibodies were tested for HCV current infection and viral load by RT-PCR, and 101 of 141 HCV-antibody positive HCWs (71.6%)

TABLE 1
Demographic characteristics of patients infected with HCV and/or *S. mansoni*

Characteristic	HCV and *S. mansoni* coinfection		HCV alone		*P* value
	Chronic (*N* = 52; %)	Resolved (*N* = 16; %)	Chronic (*N* = 49; %)	Resolved (*N* = 24; %)	
Male sex	38 (73)	12 (75)	25 (52)	17 (71)	0.081
Mean age (years)	42 ± 9.6	39 ± 10	40 ± 10	43 ± 9	0.335
Rural residence	41(79)	13 (81)	35 (71)	18 (75)	0.793
Occupational category					
Physician/medical staff/student	1 (1.9)	1 (6.3)	1 (2.0)	3 (12.5)	0.147
Senior nurse/nurse/nursing student	4 (7.7)	4 (25)	13 (26.5)	6 (25)	
Housekeeping	30 (57.7)	8 (50.0)	18 (36.7)	8 (33.3)	
Administrative and pharmacy staff	15 (28.8)	3 (18.8)	15 (30.6)	5 (20.8)	

Age is shown as mean ± SD.

were also HCV-RNA–positive. Unexpectedly, the average HCV-RNA loads among 52 viremic patients infected with both HCV and schistosomiasis ($2.62 \pm 0.69 \times 10^6$; range = $2.29–2{,}360 \times 10^3$ IU/mL) were slightly lower than the loads measured in 49 viremic patients with HCV infection alone ($4.24 \pm 1.4 \times 10^6$; range = $0.357–4{,}770 \times 10^3$ IU/mL) (Figure 1). The difference in HCV viral loads was not statistically significant ($P = 0.309$) (Table 2). No correlation could be established between HCV RNA titers and serum ALT levels (below).

HCV genotyping showed that five HCV-RNA samples (5%) were non-typable by our method. The great majority of 96 typed samples (95%) belonged to HCV genotype 4. HCV genotype 4 was found in almost all typed samples either as a single genotype in 89 (93%) of 96 subjects (96% in HCV and *S. mansoni* coinfected subjects and 83% in HCV monoinfected subjects) or with other HCV genotypes. Genotype 1 was found combined with genotype 4 in three (6.4%) of the HCV monoinfected subjects but not in the HCV coinfected subjects. Also, genotype 2 was combined with genotype 4 in two (4%) of the coinfected subjects and five (10.2%) of the monoinfected subjects (Table 2).

Liver inflammation as measured by ALT levels and ultrasound in the study subjects. As shown in Table 2, we did not find a significant difference in ALT levels between the viremic subjects infected with HCV alone and those subjects coinfected with *S. mansoni* ($P = 0.456$), and this finding was also true for those subjects without HCV-RNA ($P > 0.05$). These results are consistent with the HCV viral load data. There were significantly higher ALT levels in chronic subjects with and without *S. mansoni* coinfection compared with those subjects who resolved HCV infection ($P < 0.05$) (Table 2).

Additional examination of the liver of viremic subjects by ultrasound imaging showed that 27% and 39% of those patients with and without *S. mansoni* coinfection, respectively, had normal abdominal ultrasound findings. The abnormal findings among those viremic subjects with and without *S. mansoni* coinfection ranged from coarse liver and splenomegaly (29% versus 25%, respectively) to liver cirrhosis and splenomegaly (19% versus 11%, respectively). Also, periportal fibrosis was found in 25% of the viremic subjects with *S. mansoni* coinfection, whereas echogenic liver was found in 25% of those subjects infected with HCV alone.

However, 58% and 62% of those subjects who had resolved HCV infection with and without *S. mansoni* coinfection had normal abdominal ultrasound findings, respectively. The abnormal findings among those aviremic subjects with and without *S. mansoni* coinfection were echogenic liver (25% also associated with periportal fibrosis versus 38%, respectively). Also, periportal fibrosis was found in 17% of those aviremic coinfected subjects. No significant differences were found in the ultrasound findings between those subjects with and without *S. mansoni* coinfection among the total, viremic, or aviremic subjects ($P > 0.05$).

HCV-specific CMI responses among the study subjects. A convenience sample of the study subjects was tested for HCV-specific CMI and included 27 coinfected patients (20 patients with and 7 patients without HCV-RNA) and 29 subjects who were positive for HCV monoinfection (14 subjects with and 15 subjects without HCV-RNA). Only 8 of 20 (40%) and 11 of 14 (79%) viremic patients with and without *Schistosoma*, respectively, had a positive HCV-specific CMI response to at least one of seven HCV antigen pools tested ($P = 0.026$). However, 4 of 7 (57%) and 9 of 15 (60%) aviremic patients with and without *Schistosoma*, respectively, had a positive HCV-specific CMI response to at least one of seven HCV antigen pools tested ($P = 0.327$) (Figure 2). The magnitude of the HCV-specific CMI response measured by comparing the total mean (± SEM) of SFCs among viremic (chronic) patients with and without *Schistosoma* coinfection was 716 ± 194 and 587 ± 162 ($P = 0.617$), respectively, whereas among aviremic (resolved) patients, it was 794 ± 272 and 365 ± 36 ($P = 0.036$), respectively (Figure 2), showing higher CMI response among coinfected patients. No significant differences ($P > 0.05$) were

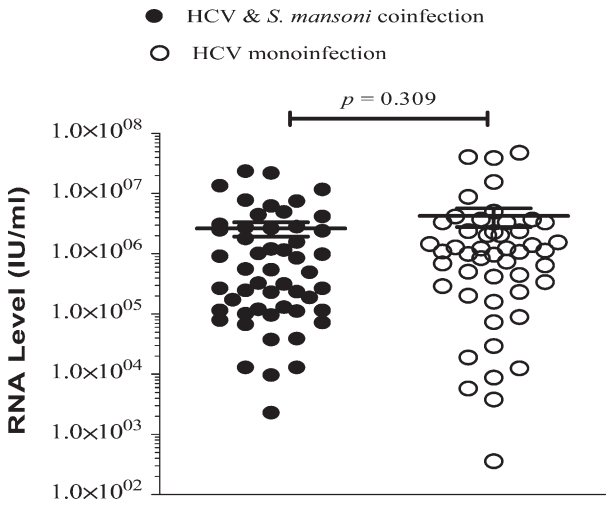

FIGURE 1. Serum levels of HCV RNA in HCV-infected patients without and with *S. mansoni* coinfection. Data are expressed as scatter plot and the bars represent the means ± SEMs.

TABLE 2
Laboratory data of patients infected with HCV or *S. mansoni* alone or combined

Characteristic	HCV and *S. mansoni* coinfection		HCV alone		*P* value
	Chronic (*N* = 52; %)	Resolved (*N* = 16; %)	Chronic (*N* = 49; %)	Resolved (*N* = 24; %)	
RNA titer (× 10^6 IU/mL)*	2.6 ± 0.69	N/A	4.2 ± 1.45	N/A	0.309
HCV genotype					
Genotype 4	47 (90%)	N/A	39 (80)	N/A	0.905
Genotype 1	1 (2%)	N/A	0	N/A	
Mixed genotypes 1 and 4	0	N/A	3 (6.0)	N/A	
Mixed genotypes 2 and 4	1 (2%)	N/A	5 (10)	N/A	
Non-typable	3 (6%)	N/A	2 (4)	N/A	
Serum ALT level (U/L)	45 ± 24	27 ± 12	50 ± 40	27 ± 17	0.0001*

Data are shown as mean ± SEM unless otherwise indicated. N/A = not applicable.
*Statistically significant.

found in the breadth or quality of the response (number of responding antigen pools in each subject) to seven antigen pools tested among the coinfected and HCV moninfected subjects with or without HCV RNA (data not shown).

DISCUSSION

This cross-sectional survey examined the effect of *S. mansoni* infection on HCV clearance, viral load, liver inflammation, and HCV-specific CMI responses in coinfected patients and compared it with the effect in those patients infected with HCV alone among 141 Egyptian HCWs at the NLI in the Nile Delta, where HCV antibody prevalence reaches 24% in the rural areas.[34,35] HCV genotype 4 existed in almost all HCV infections, with about 11% coinfections with other HCV genotypes (mainly genotypes 1 and 2). We show that there was no statistical difference in HCV spontaneous resolution, viral load, liver pathology, or CMI in patients with or without *S. mansoni* coinfection, suggesting that it did not impact the outcome of HCV infection. Several aspects of these data warrant additional discussion.

First, the subjects included in this study were 68 HCWs infected with both HCV and *S. mansoni* and 73 HCV-infected HCWs without evidence of *S. mansoni* infection. Notably, there was no statistical difference in HCV spontaneous clearance in patients infected with HCV alone or coinfected with *S. mansoni*. In this regard, 16 (23.5%) of the coinfected subjects and 24 (32.9%) of the HCV-infected patients spontaneously resolved HCV infection ($P = 0.297$), suggesting no impact of schistosomiasis on HCV spontaneous resolution. Also, the average RNA load in coinfected and HCV monoinfected patients was statistically non-significant. In addition, there were no significant differences in the mean IFN-γ SFCs/10^6 PBMCs among viremic patients coinfected with *S. mansoni* and those patients infected with HCV alone. Unexpectedly, among aviremic subjects, there was a statistically higher frequency of HCV-specific IFN-γ SFCs in coinfected patients among the total responding subjects ($P = 0.036$). These data suggest that *S. mansoni* does not impact the outcome of HCV infection.

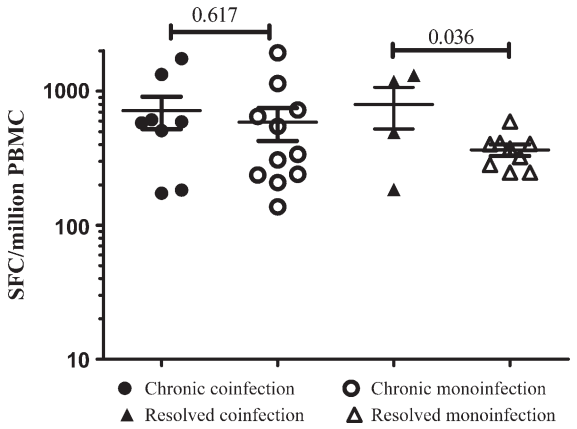

FIGURE 2. IFN-γ ELISPOT responses in the study subjects. PBMCs were stimulated in triplicates with seven sets of pooled overlapping 15mer HCV genotype 4a peptide antigens for 16–18 hours as described. The subjects were classified into four groups: HCV and schistosoma coninfected patients with and without HCV-RNA and HCV monoinfected patients with and without RNA. The total cumulative number of HCV-specific IFN-γ SFCs for the seven pools per 10^6 PBMCs is shown for the responding subjects only. The lines represent the means ± SEMs.

Second, it was reported that patients coinfected with HCV and schistosomiasis have higher viral loads, higher incidence of cirrhosis, hepatocellular carcinoma, poor response to IFN therapy, and higher mortality rates because of liver-related causes compared with patients infected with HCV alone.[9,11,36] Also, immunosuppression was sometimes observed in *Schistosoma* and HCV coinfection.[37,38] This immunosuppression was explained by a shift in T-cell response by down-regulating the Th-1 cytokines while favoring a Th-2 environment and response caused by chronic *Schistosoma* infection in experimental mouse model.[39,40] In this regard, our data did not show any significant increase in the spontaneous resolution of HCV infection among HCV monoinfected patients compared with those patients coninfected with *S. mansoni*. However, because the liver is the main target for both pathogens, it was suggested that coinfection with *S. mansoni* might cause localized suppression of CMI in the liver, inducing viral persistence and more severe hepatic complications.[11] Our data did not indicate an increase in viral load in HCV and *S. mansoni* coinfected patients compared with HCV monoinfected patients. Actually, HCV-RNA titers were higher in subjects infected with HCV alone compared with coinfected patients. However, this increase was not significant.

Third, ALT data did not reflect any more severe liver inflammation caused by *Schistosoma* coinfection. ALT levels were not significantly higher in coinfected subjects, whether viremic or aviremic, compared with subjects with HCV infection alone. However, ALT levels were significantly higher in viremic subjects with and without concomitant *S. mansoni* infection compared with patients who resolved HCV infection.

In this regard, a study by Derbala and others[41] suggested a lack of association of ALT levels with schistosomiasis. Consistent with ALT results and similar to our ultrasound findings of comparable liver pathology and cirrhosis proportions among subjects with and without *Schistosoma* infection (whether viremic or aviremic), Derbala and others[41] also found that chronic HCV patients with or without schistosomiasis had similar fibrosis and response to treatment rates and that schistosomiasis coinfection is not a surrogate of poor response. Data by Kamal and others[11] contradict our findings, because higher ALT levels were found among coinfected subjects. This contradiction could be attributed to proven active schistosomal infection in their study,[11] which was based on long-term follow-up, whereas our study was a cross-sectional survey. Our findings were confirmed with comparable abdominal ultrasound findings among coinfected patients and those patients infected with HCV alone.

Fourth, the CMI data presented in this study show that there is no decrease in the frequency of IFN-γ SFCs by cells isolated from patients coinfected with *S. mansoni* compared with those cells of patients infected with HCV alone. This finding was true for the magnitude and breadth of HCV-specific CMI responses (Figure 2) (data not shown). Others have reported dysfunctional HCV-specific T cells that were unable to secret IFN-γ in HCV and *S. mansoni* coinfected patients.[21,26,42] However, another study had findings[43] similar to ours where no significant differences in CMI responses were found between those infected with HCV alone and those infected with HCV combined with schistosomiasis. Of note, in this study, there was a significant increase in the number of subjects responding to HCV antigens by IFN-γ secretion in HCV monoinfection patients compared with coinfected subjects with viremia (79% versus 40%; $P = 0.026$), which may explain reports of decreased response in coinfected subjects compared with HCV monoinfected subjects. This difference could be attributed to the limited number of subjects tested in this report. Importantly, there was no significant difference in the quality (breadth) of the CMI responses among the responding subjects examined in this study.

Fifth, we measured *S. mansoni* infection using IgG EIA, which does not differentiate between current and previous infection. Although it is considered a limitation in this study, the objective of our study was to see any difference in the spontaneous resolution in coinfected subjects compared with subjects infected with HCV alone, regardless of active *S. mansoni* infection, which is not clear and understudied. In this regard, the immunosuppression caused by *S. mansoni* coinfection may not be limited to active infections only (i.e., having viable adult worms).[27] Another limitation of this study is that it is a cross-sectional study, which could explain the differences in the outcomes compared with other prospective or longitudinal studies.[11,44,45] Also, we evaluated liver damage in both groups using ultrasonography and not the more accurate fibrotest/fibroscan (they were not available) or the more invasive liver biopsy, which would have been refused by most subjects. Finally, the measurement of HCV-specific CMI responses to pooled HCV peptides and not fine mapping of the responses to individual peptides is another limitation. It could be examined in future studies. Notably, among those subjects without viremia, there was a statistically higher frequency of HCV-specific IFN-γ SFCs in coinfected patients among the total subjects. We speculate that coinfection with *S. mansoni* may actually increase the lifespan of HCV-specific memory cells.

In conclusion, HCV genotype 4-infected Egyptian HCWs previously or currently infected with *S. mansoni* did not have suppressed viral clearance, increased RNA levels, or liver inflammation, suggesting that the clearance of HCV is not impaired by the presence of *S. mansoni*. Also, *Schistosoma* infection did not suppress HCV-specific CMI in either magnitude or breadth of the response compared with HCV infection alone.

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Authors' addresses: Walaa R. Allam, Zainab Zakaria, and Gehan Galal, Egyptian Company for Blood Transfusion Services (Egyblood), Agouza, Giza, Egypt, E-mails: walaaramadan@gmail.com, zainab_ali52@hotmail.com, and gehan_galal@hotmail.com. Ahmed Barakat, Department of Microbiology, Faculty of Science, Ain Shams University, Cairo, Egypt, E-mail: barakat.ainshams@gmail.com. Tamer S. Abdel-Ghafar, Mohamed El-Tabbakh, and Imam Waked, Department of Hepatology, National Liver Institute, Menoufiya University, Menoufiya, Egypt, E-mails: tamerghfar@yahoo.com, meltabbakh@liver-eg.org, and iwaked@liver-eg.org. Nabeil Mikhail, South Egypt Cancer Institute, Assuit, Egypt, E-mail: nabiel.mikhail@gmail.com. Sayed F. Abdelwahab, Department of Microbiology and Immunology, Faculty of Medicine, Minia University, Minia, Egypt, E-mail: sayed.awahab@mu.edu.eg.

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