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Original Article 1

Evaluation of APRI Score-based hepatic fibrosis and cirrhosis among hepatitis C patients co-infected with schistosomiasis

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Background
Hepatitis C virus (HCV) causes severe liver pathology, and schistosomiasis (SCH), a neglected tropical disease, leads to hepatic damage.
Objective
This study aimed to report the incidence of SCH in HCV patients through serological detection of corresponding antibodies and to investigate the clinical outcomes of HCV/SCH co-infection, revealing its possible associations with aspartate aminotransferase-to-platelet ratio index (APRI) score-based hepatic fibrosis and the precence of cirrhosis.
Materials and methods
This study involved 132 participants (70 HCV patients and 62 controls). All participants underwent measurements of clinical parameters, including HCV RNA level. The APRI score was calculated to assess liver fibrosis and cirrhosis. Serologically, serum samples were examined for schistosomal antibodies**.**
Results and conclusion
HCV patients had a significantly higher incidence of SCH (38/70, 60%) compared to controls (6/62, 9.68%) ($P < 0.001$). HCV/SCH co-infected patients showed significantly higher levels of total bilirubin, direct bilirubin, prothrombin time (PT), international normalized ratio (INR), and APRI score compared to those with HCV mono-infection (P 0.002, 0.004, 0.006, 0.001, and 0.013, respectively). Likewise, albumin and prothrombin concentration (PC) levels decreased significantly in HCV/SCH co-infection (P 0.020 and 0.001, respectively). PC level, followed by APRI score, was the parameter most highly associated with HCV/SCH co-infection. Nevertheless, the diagnostic utility of APRI score to identify liver fibrosis and cirrhosis at cutoff values of 1.5 and 2, respectively, revealed no significant difference in hepatic fibrosis status between HCV/SCH co-infected patients compared to HCV mono-infected patients, nor in cirrhosis presence across the two groups. But, at the APRI score cutoff value of 1, HCV/SCH co-infected patients had a significantly lower likelihood of ruling out cirrhosis. In HCV/SCH co-infection, schistosomal antibody titres were significantly different regarding liver fibrosis status and cirrhosis (P 0.001 and < 0.001, respectively). It is concluded that the increased incidence of schistosomal antibodies in HCV patients is a well-defined pathologic issue that is not associated with liver fibrosis progression or cirrhosis, as assessed by the APRI score. However, HCV/SCH co-infection is associated with a reduced likelihood of ruling out liver cirrhosis.

Keywords: Hepatitis C virus, schistosomiasis, co-infection, APRI biomarker, liver fibrosis, cirrhosis.

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Introduction

Hepatitis C virus (HCV) is still a major hazard to public health in many places, even with massive global efforts to contain its spread. An estimated 242,000 deaths worldwide in 2022 were attributed to HCV consequences, such as cirrhosis and liver cancer. With the appearance of direct-acting antivirals (DAAs), which are efficacious for treating the majority of hepatitis C infections, the incidence of chronic HCV infection has decreased to 50 million cases worldwide [1]. In fact, Egypt is the first country to attain gold-tier status according to World Health Organization (WHO) guidelines. Egypt has met the programming standards

necessary to reduce the number of new cases of HCV infections and deaths to achieve the end of the HCV epidemic and reach all elimination targets before 2030 [2]. Indeed, patients with chronic HCV infection cannot clear this virus throughout the illness's acute stage. Consequently, liver inflammation, fibrosis, and cirrhosis are obvious complications [3–5], leading to hepatic dysfunction [4,5]. Although the recent advances in HCV treatment were established with proven success, many patients are still at risk of chronic liver disease progression to cirrhosis and hepatocellular carcinoma (HCC) at different rates [6–9].

Schistosomiasis (SCH) is one of the most widespread neglected tropical illnesses in the rural areas of developing nations [10–12]. Worldwide, nearly 240 million people are currently infected. On an annual basis, 800 million people are exposed to infection in 78 countries, and 200 thousand deaths are caused by the disease [13–15]. Indeed, SCH is a parasitic disease [16], which may progress from of acute to chronic disease [14,15]. The condition mostly affects the liver, gut, urogenital tract, and blood system. It is also known to cause liver fibrosis [16]. It is caused by different species of *Schistosoma (S)*, each of which is responsible for different manifestations [15]. In Egypt, the diseases caused by both *S. mansoni* and *S. haematobium* have been endemic since ancient times [15,17]. Afterwards, *S. mansoni* has completely replaced *S. haematobium* in the Nile Delta and several other regions [15,18]. The incidence of *S. haematobium* infection has decreased by less than 0.2% since 2016. Moreover, the remarkable decrease in prevalence of SCH in Egypt has been observed according to the emerging SCH agenda for 2017-2020, which directs efforts to turn from control toward final elimination. Despite the observed significant fall in SCH, there are about 300 villages in Egypt with an incidence of more than 3%, requiring further efforts for continuous examination as well as prolonged treatment [19,20]. In general, as of 2022, it is estimated that 251 million people require preventive chemotherapy for SCH, 90% of whom live in Africa [21].

Serology provides a more sensitive tool for diagnosing SCH than parasitology, which depends on a direct examination of the parasite's eggs [20, 22,23]. However, serological approaches still have the limitation of not distinguishing between past and current exposure, but they are very useful in less endemic areas [24,25]. Indirect hemagglutination assay (IHA) is the main immunodiagnostic technique for SCH that is designed for quantitative detection of antibodies in the serum of infected people [20,25,26]. The IHA test is quite beneficial in epidemiological and clinical contexts [25], has high accuracy, and is superior to the enzyme-linked immunosorbent assay (ELISA) method [25,27].

During the course of its co-infection, SCH potentiates, initiates, and prolongs the liver injury induced by infection with HCV [15,28]. The chronicity of HCV is increased by *S. mansoni* infection [29–32]. The pathogenesis of HCV with *S. mansoni* co-infection on liver fibrosis is well established [33–36], referring to hepatic damage due to different immunological responses. This co-infection may be responsible for the disease progression to advanced liver disease and cirrhosis [36–38].

Non-invasive biomarkers have been developed and validated to diagnose liver fibrosis accurately [39–41]. In this regard, the aspartate aminotransferase-to-platelet ratio index (APRI) is an important biomarker. This is considered a serum biomarker supposed to facilitate stratification during different disease stages and is used as a crucial biomarker to diagnose liver fibrosis and cirrhosis [42–44]. It is confirmed that the APRI score is able to identify severe fibrosis and cirrhosis during chronic HCV infection [41]. The goal of the current study is to record the incidence of schistosomal antibodies in HCV patients, to highlight HCV/SCH co-infection, and to investigate the clinical outcomes referring to the possible association between this pathologic pattern and APRI score-based hepatic fibrosis and cirrhosis.

Materials and methods

Study population

One hundred thirty-two Egyptian participants were included in the current study. The study's participants were divided into two groups: 62 controls and 70 patients who had an HCV infection. Prior to the collection of blood samples, written informed consent was obtained from each participant. The study was conducted in accordance with the World Medical Association's Declaration of Helsinki guidelines for research involving human beings published in 1964 and its later amendments, as well as the guidelines of the ethics committee of National Research Centre, approval number10231. **Inclusion criteria**: The group of patients included adult subjects of both sexes and different ages. They were confirmed to have HCV infection by the presence of HCV antibodies in their sera, whereas the group of control individuals included adult healthy subjects of both sexes and various adult ages. They were free from HCV infection and had negative HCV antibodies in their sera as well as negative HCV RNA. **Exclusion criteria:** Pediatric subjects were omitted from both controls and HCV patients. Subjects who tested positive for the hepatitis B surface antigen (HBsAg) or who tested positive for the human immunodeficiency virus (HIV) based on the detection of HIV antibodies, regardless of whether they were patients or controls, were excluded from the study.

Control participants and patients were evaluated by routine clinical biochemical parameters of liver function, including alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), gamma-glutamyl transferase (GGT), total protein (T protein), albumin, total bilirubin (T Bil), and direct bilirubin (D Bil). Additionally, hematological parameters, including platelet count (PLC), prothrombin time (PT), prothrombin concentration (PC), and international normalized ratio (INR), were also estimated. All subjects were assessed for schistosomal antibodies serologically using the IHA technique. Considerably, the APRI score was calculated to identify liver fibrosis and cirrhosis.

Quantitative determination of hepatitis C virus ribonucleic acid (**HCV RNA**)

Real-time polymerase chain reaction (PCR) was used to detect and quantify HCV RNA. Briefly, the QIAamp Viral RNA kit (Qiagen, Santa Clarita, CA) was used to perform the extraction of HCV RNA from serum samples in compliance with the manufacturer's standard instructions. The Artus HCV QS RGQ Kit (Qiagen, Santa Clarita, CA) was used to perform a one-step, real-time RT-PCR to confirm the HCV detection according to the protocol of the manufacturer. The following was the amplification thermal profile: an initial incubation period of 30 minutes at 51°C was followed by 10 minutes at 95°C, 50 cycles of 30 seconds at 95°C and 1 minute at 60°C, 40 cycles at 95°C for 15 seconds, 60°C for 1 minute, and 72°C for 30 seconds. At the cycle's annealing/extension stage, detection of fluorescence signals was monitored. Rotor Gene real-time PCR (Qiagen, Santa Clarita, CA) was applied to amplify and quantify HCV RNA [45].

Schistosomal serological diagnosis

The IHA technique was used for the diagnosis of SCH through the measurement of schistosomal antibodies. This assay was performed using a diagnostic kit made by Fumouze Diagnostics (Levallois-Perret, France). The kit included sensitized red blood cells composed of sheep red blood cells coated with *Schistosoma mansoni* antigen. As per the manufacturer's instructions, a stock dilution of 1/40 of test serum was prepared by mixing 0.05 ml of test serum with 1.95 ml of buffer solution. In the microplate reaction, 50 µl of buffer solution (sample diluent) was delivered into eight wells. The serial dilution stage began with the addition of 50 ul of serum stock dilution to the first well, mixed with buffer, then 50 µl from the first well moved to the second well and mixed, and so on until the micropipetted 50 µl was discarded from the sixth well. A negative control was prepared by adding 50 µl of serum stock dilution to the seventh well, mixing with buffer, and discarding 50 µl. This was the serum control with a 1:80 dilution. The dilutions were set for the corresponding wells from the first to the sixth, with an ascending dilution of 1:80, 1:160, to 1:2560. Then, one drop (16.66 µl) of sensitized red blood cells was distributed in the first six wells, followed by the addition of one drop of non-sensitized red blood cells in the seventh well (serum control). Additionally, one drop of sensitized red blood cells was added in the eighth well, which constituted the reagent control, ensuring the validity of the buffer and sensitized red blood cells. Next, the reaction was observed 2 hours later. The hemagglutination reaction with a titer of less than 1:160 was interpreted as a negative result, whereas titers equal to or more than 1:160 indicated a positive result [20].

Calculation of aspartate aminotransferase-to-platelet ratio index (APRI) score

The non-invasive liver cirrhosis estimation was carried out by calculating APRI score according to the formula originally reported by Wai *et al.* (2003) [46]. APRI = [(AST/upper limit of normal) × 100]/platelet count 10^9/L [44,46,47]. The upper limit of normal for AST was taken as 40 U/L, while the normal platelet count range was taken as 150,000 to 450,000/mm^3. According to METAVIR (Meta-analysis of Histological Data in Viral Hepatitis) scoring system, liver fibrosis included four successive stages: F1, mild fibrosis (portal fibrosis without septa); F2, moderate fibrosis (portal fibrosis with rare septa); F3, severe fibrosis (numerous septa without cirrhosis); and F4, cirrhosis [44,48]. The increase in liver fibrosis stage referred to a worsening prognosis [49]. Two APRI cutoff values, known as higher WHO cutoff values, were considered, thereby indicating significant fibrosis and cirrhosis. An APRI score ≥ cutoff value of 1.5 was used to diagnose patients with significant fibrosis (≥ F2), whereas an APRI score ≥ cutoff value of 2 was used to predict patients with cirrhosis (F4). Furthermore, another two APRI cutoff values, known as lower WHO cutoff values, could rule out significant fibrosis and cirrhosis. An APRI score < cutoff value of 0.5 was used to rule out significant fibrosis, whereas an APRI score < cutoff value of 1 was used to rule out liver cirrhosis [41,50–52].

Statistical analysis

A Microsoft Excel worksheet was used to collect the data, which were then imported into the statistical program for social science (SPSS) software version 25 (IBM SPSS Statistics, version 25; IBM Corp., Armonk, NY, USA) to be analyzed. The *t*-test was used to examine the quantitative data, which were represented as mean (M) and standard deviation (SD) values. The chi-square test was used to analyze the qualitative data, which were represented as numbers and percentages. Binary logistic regression was used to determine

the outcome and identify the association between independent variables and a single dichotomous dependent variable. The beta (ß) coefficient, which refers to the variables having the greatest association, was used in multivariate linear regression analysis. In all statistical analyses, a *P* value of ≤ 0.05 denoted significant results.

Results and discussions

Demographic data and clinical features of the study population

The baseline demographic and clinical characteristics of the study cohort were summarized in Table 1. The study subjects included controls (n = 62) and HCV patients (n = 70). Except for sex, the analyzed data referred to significant differences in all the evaluated baseline parameters between HCV patients and controls. In HCV patients, a significant elevation was recorded for ALT, AST, AST/ALT ratio, GGT, ALP, T Bil, D Bil, I Bil, PT, INR, and APRI (P < 0.001) compared to controls. Likewise, a significant decline was reported for T protein, albumin, PLC, and PC (P 0.004 and < 0.001 for T protein and the rest of the other parameters, respectively).

Table 1 Demographic and clinical characteristics of hepatitis C virus (HCV) infected patients and controls.

Parameters	Study population			*P*-value
	Controls, n = 62	HCV Patients, n = 70		
Sex (M/F)	48(77.419%)/14(22.851%)	56(80%)/14(20%)		0.717
Age (years)	29.387 ± 8.572	48.514 ± 6.131		< 0.001*
HCV RNA (IU/ml)	-	447226.86 ± 852861.13		-
ALT (U/L)	23.115 ± 11.490	37.724 ± 20.752		< 0.001*
AST (U/L)	22.654 ± 8.378	54.207 ± 32.687		< 0.001*
AST/ALT ratio	1.099 ± 0.341	1.556 ± 0.602		< 0.001*
GGT (U/L)	23.556 ± 10.339	64.852 ± 72.154		< 0.001*
ALK (U/L)	74.963 ± 18.560	133.074 ± 66.246		< 0.001*
T protein (g/dL)	7.424 ± 0.520	7.074 ± 0.670		0.004*
Albumin (g/dL)	4.644 ± 0.304	2.838 ± 0.761		< 0.001*
T Bil (mg/dL)	0.654 ± 0.304	3.297 ± 1.578		< 0.001*
D Bil (mg/dL)	0.221 ± 0.123	1.960 ± 1.250		< 0.001*
I Bil (mg/dL)	0.433 ± 0.199	1.337 ± 0.776		< 0.001*
PLC (10^3/cmm)	257.480 ± 61.967	115.241 ± 64.252		< 0.001*
PT (sec)	12.425 ± 1.107	17.243 ± 4.116		< 0.001*
PC (%)	94.264 ± 8.403	54.509 ± 17.489		< 0.001*
INR	1.035 ± 0.055	1.582 ± 0.405		< 0.001*
APRI score	0.230 ± 0.100	1.617 ± 1.164		< 0.001*

Where: M: male, F: female, ALT: alanine aminotransferase, AST: aspartate aminotransferase, GGT: gamma-glutamate transferase, ALK: alkaline phosphatase, T protein: total protein, T Bil: total bilirubin, D Bil: directbilirubin, I Bil: indirect bilirubin, PLC: platelet count, PT: prothrombin time, PC: prothrombin concentration, INR: international normalized ratio, APRI: AST- to-platelet ratio index, *n*: number of subjects, and *: significant value. Normal ranges were as follows: HCV RNA positive results > 34 IU/ml (HCV RNA was detected above the detection limit in 58/70 of HCV patients), AST: from 0 to 40 U/L, ALT: from 0 to 40 U/L, ALP: from 30 to 120 U/L, GGT: from 10 to 55 U/L, T protein: from 6 to 8 g/dL, albumin: from 3.5 to 5 g/dL, T.Bil: from 0 to 1.2 mg/dL, D.Bil; from 0.1 to 0.03 mg/dL, I.Bil: from 0.1 to 0.9 mg/dL, PLC: from 150 to 450 10^3/cmm, PT: from 11 to 13 sec, PC: from 70 to 120%, and INR: from 0.8 to 1.1, age ranged from 21 to 53 years in controls and from 35 to 61 years in HCV patients. The chi-square test was applied to analyze sex, whereas the *t*-test was used to analyze the data for all of the other parameters, which were expressed as M and SD values.

Incidence of schistosomiasis (SCH) among the entire cohort of study

The data regarding the incidence of schistosomal antibodies among controls and HCV patients were analyzed and reported in Table 2. The positive cases of schistosomal antibodies were recorded in 6/62 (9.68%) of controls, whereas they were reported in 42/75 (60%) of HCV patients. The chi-square analysis referred to a significant increase in the incidence of schistosomal antibodies in HCV patients compared to controls ($P < 0.001$).

Table 2 Incidence of schistosomiasis (SCH) among hepatitis C virus (HCV) infected patients and controls

Study population	Schsitosomal antibodies						Chi-Square	
	Negative		Positive		Total			
	n	%	*n*	%	*n*	%	X^2	*P*-value
Controls, *n* = 62	56	90.32	6	9.68	62	100	35.981	< 0.001*
HCV Patients, *n* = 70	28	40.00	42	60	70	100		

In recent years, the incidence of SCH has increased, even in areas that are not considered endemic [53,54]. Herein, our findings were somewhat in agreement with different reports. Omar *et al.* observed high seroprevalence of schistosomal antibodies among a cohort of HCV patients. They recorded the incidence of SCH in 94/200 (47%) of HCV patients in a study designed to investigate the impact of SCH on the increased incidence of occult HBV [55]. Furthermore, Abbas *et al.* recorded a high incidence of schistosomal antibodies in 11/23 (47.82%) of HCV patients in their study on the impact of HCV/SCH co-infection on different clinical pathological parameters [56]. However, our findings differed from those reported by Abdel-Rahman *et al.*, who found SCH in 983/3596 (27.3%) of HCV patients. In this large cohort study, serological screening for schistosomal antibodies among HCV patients was conducted to investigate the prevalence of HCV/SCH co-infection and its impact on liver fibrosis and treatment response [48]. Additionally, Paye *et al.*'s findings, which recorded schistosomal antibodies in 97/110 (88%) Zambian patients with hepatosplenic disease—the majority of whom had hepatomegaly—were incosistent with ours [57].

Indeed, the increased frequency of SCH among HCV patients in our study may be attributed to the natural history of HCV infection. Actually, the HCV epidemic in Egypt emerged through intravenous SCH treatment in rural areas between the 1960s and 1980s. Throughout this previous period, SCH was regarded as a major dangerous threat to public health. Between the 1950s and the 1980s, *Schistosoma mansoni* was considered the main etiology of liver disease. Intravenous tartar emetic was used by the Egyptian Ministry of Health as the standard treatment for SCH [58]. Unfortunately, this effort has established a very intensive reservoir of HCV. By the mid-1980s, praziquantel (PZQ) was used instead of tartar emetic for the treatment of SCH as an oral drug with high efficacy. This not only reduced SCH but also interrupted the epidemic of "occult" HCV. As diagnostic serology became available in the 1990s, it was evident that SCH had been displaced by HCV as a cause of CLD [59]. As a result, the majority of HCV patients over this period may have acted as reservoirs for schistosomal antibodies.

Baseline characteristic features among hepatitis C virus (HCV) mono-infected patients and hepatitis C virus/schistosomiasis (HCV/SCH) co-infected patients

According to the incidence of the schistosomal antibodies, HCV patients were divided into those with negative schistosomal antibodies (HCV mono-infection) and those with positive schistosomal antibodies (HCV/SCH co-infection). In Table 3, chi-square analysis revealed a significant increase in the positivity of schistosomal antibodies in males compared to females (*P* 0.007). Furthermore, *t*-test analysis referred to a significant increase in T Bil, D Bil, PT, INR, and APRI score levels (*P* 0.002, 0.004, 0.006, 0.001, and 0.013, respectively) among HCV/SCH co-infectied patients compared to those with HCV mono-infection. Likewise, a significant decrease in age, albumin, and PC levels (*P* 0.006, 0.020, and 0.001, respectively) was also reported in HCV/SCH co-infected patients.

Table 3 Comparison between hepatitis C virus (HCV) mono-infected patients and hepatitis C virus/schistosomiasis (HCV/SCH) co-infected patients.

Parameters	Schistosomal antibodies in HCV infected patients						*P*-value
	Negative, *n* = 28			Positive, *n* = 42			
Sex (M/F)	18(64.29%)/10(35.71%)			38(90.48%)/4(9.52%)			0.007*
Age (years)	50.929	±	5.805	46.905	±	5.868	0.006*
HCV RNA (IU/ml)	776050.45	±	1120653.66	246297.11	±	567063.22	0.016*
ALT (U/L)	39.615	±	27.260	36.188	±	13.679	0.536
AST (U/L)	49.692	±	36.074	57.875	±	29.734	0.348
AST/ALT ratio	1.515	±	0.784	1.589	±	0.411	0.646
GGT (U/L)	71.400	±	88.653	61.000	±	61.604	0.614
ALP (U/L)	116.000	±	67.245	146.733	±	63.245	0.090
T protein (g/dL)	7.227	±	0.594	6.969	±	0.708	0.166
Albumin (g/dL)	3.100	±	0.824	2.614	±	0.635	0.020*
T Bil (mg/dL)	2.558	±	1.591	3.878	±	1.325	0.002*
D Bil (mg/dL)	1.403	±	1.307	2.399	±	1.027	0.004*
I Bil (mg/dL)	1.155	±	0.855	1.479	±	0.690	0.145
PLC (10^3/cmm)	130.429	±	65.554	101.067	±	60.686	0.082
PT (sec)	15.400	±	3.717	18.662	±	3.896	0.006*
PC (%)	63.950	±	20.544	47.246	±	10.179	0.001*
INR	1.363	±	0.303	1.750	±	0.398	0.001*
APRI score	1.242	±	0.824	1.867	±	1.293	0.013*

Where: M: male, F: female, ALT: alanine aminotransferase, AST: aspartate aminotransferase, GGT: gamma-glutamate transferase, ALK: alkaline phosphatase, T protein: total protein, T Bil: total bilirubin, D Bil: direct bilirubin, I Bil: indirect bilirubin, PLC: platelet count, PT: prothrombin time, PC: prothrombin concentration, INR: international normalized ratio, APRI: AST-to-platelet ratio index, *n*: number of patients, and *: significant value. Normal ranges were as follows: HCV RNA positive results > 34 IU/ml (HCV RNA was detected above the detection limit in 22/28 of HCV mono-infected patients and in 36/42 of HCV/SCH co-infected others), AST: from 0 to 40 U/L, ALT: from 0 to 40 U/L, ALP: from 30 to 120 U/L, GGT: from 10 to 55 U/L, T protein: from 6 to 8 g/dL, albumin: from 3.5 to 5 g/dL, T.Bil: from 0 to 1.2 mg/dL, D.Bil; from 0.1 to 0.03 mg/dL, I.Bil: from 0.1 to 0.9 mg/dL, PLC: from 150 to 450 10^3 /cmm, PT: from 11 to 13 sec, PC: from 70 to 120%, and INR: from 0.8 to 1.1. The chi-square test was applied to analyze sex, whereas the *t*-test was used to analyze the data for all of the other parameters, which were expressed as M and SD values.

Multivariate linear regression analysis using the beta (ß) coefficient in hepatitis C virus (HCV) infected patients

Depending on the preceding results, a significant difference was recorded in sex, age, albumin, T Bil, D Bil, PT, INR, and APRI between HCV/SCH co-infected patients and HCV mono-infection patients. Those parameters were subjected to further investigation using beta (ß) regression analysis, revealing the variables with the strongest association with HCV/SCH co-infection. In Table 4, where the dependent variable was the

schistosomal antibodies, a significant association between schistosomal antibodies and other independent variables including sex, age, PC, and APRI was reported (*P* 0.029, 0.047, 0.001, and 0.003, respectively). The corresponding ß values for those variables were -0.347, -0.340, -0.603, and 0.483, respectively. Depending on the direction of the relationship, the highest the ß value was, the closer value to 1 or -1, which referred to the strongest relationship with schistosomal antibodies. Thus, PC (ß -0.603) followed by APRI score (ß 0.483) referred to the strongest associated variable with HCV/SCH co-infection.

Table 4 Multivariate beta (ß) regression analysis in hepatitis C virus (HCV) infected patients referring to schistosomal antibodies as a dependent variable

Independent variables	Unstandardized coefficient		Standardized coefficients	*t*	*P*- value
	B	SE B	ß		
Sex (M/F)	-0.383	0.168	-0.347	-2.286	0.029*
Age (years)	-0.027	0.013	-0.340	-2.066	0.047*
Albumin (g/dL)	0.152	0.120	0.207	1.270	0.213
T Bil (mg/dL)	-0.113	0.096	-0.265	-1.185	0.245
D Bil (mg/dL)	0.184	0.118	0.276	1.549	0.131
PT (sec)	0.023	0.049	0.186	0.475	0.638
PC (%)	-0.017	0.008	-0.603	-2.025	0.001*
INR	-0.692	0.709	-0.241	-0.976	0.336
APRI score	0.193	0.061	0.483	3.184	0.003*
The dependent variable is schistosomal antibodies					

Where: T Bil: total bilirubin, D Bil: direct bilirubin, PT: prothrombin time, PC: prothrombin concentration, INR: international normalized ratio, APRI: aspartate aminotransferase–to-platelet ratio.

Our study covered different factors to assess the role of HCV/SCH co-infection in liver disease deterioration and progression. First of all, demographic factor investigations revealed a significant increase (*P* 0.007) in the incidence of HCV/SCH co-infection in males (90.48%) compared to females (9.52%). This finding agreed with that reported by Abdel-Rahman *et al*., who observed a higher elevation (*P* 0.002) of HCV/SCH co-infection in males (84.1%) compared to females (15.9%) [48]. The increased incidence of HCV/SCH co-infection in males than in females may be explained by those work activities related to males more than females, which render males at an increased rate of being subjected to the sources of schistosomal infection. In contrast, our results disagreed with those of Abbas *et al*., who found no significant difference in the incidence of HCV/SCH co-infection between males and females in their study [56]. Moreover, our findings referred to a significant decrease in age among HCV/SCH co-infected patients compared to HCV mono-infected patients. This finding differed from that reported by Abdel-Rahman *et al.* and Abbas *et al.*, who did not record a significant change in patients' ages in this regard [48, 56]. In our study, males were associated with HCV/SCH co-infection more than females. Also, there was a reverse association between age and the incidence of schistosomal antibodies.

The next factor that was investigated in studying the outcome of HCV/SCH co-infection was the clinical biochemical signs. Biochemical changes in different liver function parameters due to HCV/SCH co-infection were reported. Our findings revealed that HCV/SCH co-infected patients experienced a significant decline in albumin, demonstrating hypoalbuminemia, as well as a significant elevation in total and direct bilirubin,

indicating hyperbilirubinemia when compared to HCV mono-infected patients. These findings agreed with those of Fahim *et al*., who investigated the biochemical alterations in a cohort of 60 Egyptian male patients divided into 30 patients with SCH and another 30 patients with HCV/SCH co-infection, as well as 10 individuals as a control group [60]. However, Abbas *et al*.'s findings, which showed no change in albumin and bilirubin levels in patients with HCV/SCH and those with HCV mono-infection, did not match ours.[56]. Similarly, our findings differed from those reported by Abdel-Rahman *et al*., who found no significant variation for albumin in this regard [48]. Additionally, our findings revealed no significant differences in liver enzymes, particularly ALT and AST levels, between patients with HCV/SCH co-infection and those with HCV mono-infection.These findings were consistent with those of Abdel-Rahman *et al.*, and Abbas *et al.*, who observed no significant change for ALT and AST in this regard [48,56]. Likewise, Allam *et al.* found no significant difference in ALT level between HCV/SCH co-infected patients and HCV mono-infected patients in their study of a cohort consisting of 141 HCV-infected healthcare workers, 68 of whom had *Schsitosoma mansoni* co-infection [61]. In contrast, our results were inconsistent with those reported by Fahim *et al*., who studied the biochemical changes in patients with HCV/SCH co-infection and recorded significant differences in both ALT and AST levels between co-infected patients and HCV mono-infected patients [60].

Regarding the hematological signs, our results referred to a tendency to decrease in platelet count (PLC) among HCV/SCH co-infected patients compared to HCV mono-infected patients. Moreover, the significant changes in the coagulation profile were highlighted. Those changes included a significant decrease in PC level and a significant elevation in both PT and international INR levels among HCV/SCH co-infected patients compared to HCV mono-infected patients. Consequently, our findings referred to deteriorations of PLC, PT, PC, and INR levels in HCV/SCH co-infected patients. Indeed, Khedr *et al.* referred to an elevation in coagulation abnormalities in HCV patients relative to healthy controls. Their findings included an increase in PT and INR, along with a decrease in PC levels among HCV patients with low PLC level [5]. Furthermore, Eyayu *et al.* referred to an elevation in coagulation abnormalities in cases of SCH compared to controls. Their findings included an increase in PT and INR levels, as well as a decrease in PLC level in adult cases infected with *Schistosoma mansoni* [62]. The findings of the former study referred to coagulation deterioration in cases of HCV infection [5], whereas the findings of the latter one referred to coagulation deterioration in cases of SCH [62]. Therefore, our findings may be explained by the probable synergistic interaction of both HCV and SCH, leading to a greater deterioration in HCV/SCH co-infected patients than in those with HCV mono-infection. In this study, PC level revealed the strongest association with HCV/SCH co-infection with a negative relationship.

Incidence of schistosomiasis (SCH) according to hepatitis C virus ribonucleic acid (HCV RNA) quantitative levels in hepatitis C virus (HCV) infected patients

Based on the real-time PCR quantitative results of HCV RNA, HCV patients were divided into 3 groups: group 1 (G1), HCV RNA < 34 IU/ml (below the detection limit, n = 12); group 2 (G2), from 34 to 100,000 IU/ml (low viral load, n = 28); and group 3 (G3), > 100,000 IU/ml (high viral load, n = 30). In Table 5, the results of the chi-square test referred to a significant increase in the positivity of schistosomal antibodies in G2 of HCV patients with a lower HCV RNA viral load (P 0.001).

Additionally, the M and SD for the overall detectable level of HCV RNA in both groups 2 and 3 (n = 58) were reported as 447226.86 ± 852861.13 IU/ml. Moreover, M and SD of HCV RNA level in HCV mono-infected patients (n = 22) were reported as 776050.45 ± 1120653.66 IU/ml. On the other hand, M and SD of HCV RNA level in HCV/SCH co-infected patients (n = 36) were reported as 246297.11 ± 567063.22 IU/ml, with a significant decrease compared to those with HCV mono-infection (P 0.016) (Tables 1 and 3).

As a viral factor, HCV RNA quantitative level was studied in our study. HCV patients were grouped into those with a lower viral load, considered at HCV RNA range 34–100,000 IU/ml, and those with a higher viral load were considered at HCV RNA > 100,000 IU/ml [63]. Regarding HCV RNA groups, our investigations found a significant change in the incidence of HCV/SCH co-infection. A significant elevation in the incidence of HCV/SCH co-infection was observed in the group of lower viral load (< 100,000 IU/ml) compared to the other group of higher viral load (> 100,000 IU/ml), as well as the group of viral load below detection (< 34 IU/ml). The HCV RNA in the latter group may have been present below our detection limit of investigation or in peripheral blood mononuclear cells (PBMCs), referring to an intracellular HCV infection, supported by the previous findings reported by AbdAlla and EL Awady [64]. Unexpectedly, HCV RNA titre showed a significant elevation in HCV mono-infected patients compared to HCV/SCH co-

infected patients. This finding was somewhat in agreement with those of Allam *et al.*, who reported a tendency for elevation of HCV RNA titre in HCV mono-infected patients compared to those with HCV/SCH co-infection [61]. Also, our findings contrasted with that recorded by Abdel-Rahman *et al.*, who reported a tendency for elevation in HCV RNA titre among HCV/SCH co-infected patients compared to those with HCV mono-infection [48].

Table 5 Chi-square analysis of the incidence of schistosomiasis (SCH) in relation to the hepatitis C virus ribonucleic acid (HCV RNA) groups in hepatitis C virus (HCV) infected patients.

HCV RNA (IU/ml)		Schsitosomal antibodies						Chi-Square	
		Negative		Positive		Total			
		n	%	*n*	%	*n*	%	X^2	*P*-value
HCV Patients, *n* = 70	G1 (< 34)	6	50.00	6	50.00	12	100	21.984	0.001*
	G2 (34-100,000)	2	7.14	26	92.86	28	100		
	G3 (>100,000)	20	66.67	10	33.33	30	100		

Where: *n*: number of patients and *: significant value.

Indeed, co-infection of hepatitis C and SCH may cause hepatic illness deterioration and worsen the clinical outcome, particularly with elevated HCV RNA titres, which may increase the rate of mortality by an increase in the frequency of advanced liver fibrosis, cirrhosis and liver cancer [65–67]. Furthermore, this pattern of co-infection may be accompanied by a decline in the immune system's capability of spontaneously resolving this viral infection. In addition, it may accelerate the course of hepatic disease due to the synergetic interaction of HCV pathogenicity and SCH in hepatic disease deterioration [67,68].

Relationships between the incidence of schistosomiasis (SCH) with liver fibrosis and cirrhosis in hepatitis C virus (HCV) infected patients

Different cutoff values of the APRI score were associated with liver fibrosis and cirrhosis in HCV patients. Based on an APRI score cutoff value of 1.5, HCV patients were distinguished for liver fibrosis into two groups: one group with an APRI score ≥ 1.5 was identified as having significant fibrosis (≥ F2, *n* = 34), and another group with an APRI score < 1.5 was identified as having non-significant fibrosis (< F2, *n* = 36). On the other hand, depending on an APRI score cutoff value of 2, patients were distinguished for liver cirrhosis into two groups: one group with an APRI score ≥ 2 was identified as having cirrhosis, F4 (*n* = 24), and another group with an APRI score < 2 was identified as having non-cirrhosis (*n* = 46). In Table 6, the chi-square and logistic regression analyses were reported with insignificant *P* values (*P* > 0.05), demonstrating no associations between HCV/SCH co-infection and either liver fibrosis or cirrhosis. However, the tendency for an elevation in the incidence of HCV/SCH co-infection was recorded for each of the significant fibrosis and cirrhosis groups of HCV patients.

Additionally, at an APRI score cutoff value of 0.5, HCV patients could be distinguished into two groups: one group with APRI score < 0.5 was identified as having a high probability to rule out significant fibrosis (*n* = 10) and another group with APRI score > 0.5 was identified as having a low probability to rule out significant (*n* = 60). A tendency in the decrease in the high probability to rule out significant fibrosis was reported in HCV/SCH co-infection. Also, at an APRI score cutoff value of 1, HCV patients could be distinguished into two groups: one group with APRI score < 1 was identified as having a high probability to rule out liver cirrhosis (*n* = 24) and another group with APRI score > 1 was identified as having a low probability to rule out liver cirrhosis (*n* = 46). A significant decrease in the high probability to rule out liver cirrhosis among HCV patients was associated with HCV/SCH co-infection (Odd's ratio 0.312, 95% C.I. 0.1120 to 0.8720, and *P* 0.026) (Table 6).

Table 6 Chi-square and logistic regression analyses of the incidence of schistosomiasis (SCH) in relation to liver fibrosis and cirrhosis based on different aspartate aminotransferase-to-platelet ratio index (APRI) score cutoffs in hepatitis C virus (HCV) infected patients.

APRI score cutoff			Schistosomal antibodies			Chi-Square		Logistic Regression		
			Negative	Positive	Total					
			n (%)	*n* (%)	*n* (%)	X^2	*P*-value	Odd's ratio	95% C.I.	*P*-value
Liver fibrosis in HCV patients, *n* = 70	APRI < 1.5	Non-significant fibrosis	16 (44.45%)	20 (55.55%)	36 (100%)	0.610	0.435	1.467	0.5600 to 3.8411	0.436
	APRI ≥ 1.5	Significant fibrosis	12 (35.29%)	22 (64.71%)	34 (100%)					
Liver cirrhosis in HCV patients, *n* = 70	APRI < 2	No cirrhosis	20 (43.48%)	26 (56.52%)	46 (100%)	0.676	0.411	1.538	0.5494 to 4.3077	0.412
	APRI ≥ 2	Cirrhosis	8 (33.33%)	16 (66.67%)	24 (100%)					
Rule out significant fibrosis in HCV patients, *n* = 70	APRI < 0.5	High probability	6 (60.00%)	4 (40.00%)	10 (100%)	1.944	0.163	0.386	0.0981 to 1.5586	0.173
	APRI > 0.5	Low probability	22 (36.67%	38 (63.33)	60 (100%)					
Rule out cirrhosis in HCV patients, *n* = 70	APRI < 1	High probability	14 (58.33%)	10 (41.67%)	24 (100%)	5.115	0.024*	0.312	0.1120 to 0.8720	0.026*
	APRI > 1	Low probability	14 (31.82%)	32 (72.73%)	46 (100%)					

Where: *n*: number of recruited patients, C.I.: refers to confidence intervals (determined as 95% for Odd's ratio).

The most important factors covered by our study in order to investigate the role of HCV/SCH co-infection in liver pathology were hepatic fibrosis and cirrhosis. In this regard, a non-invasive biomarker for liver fibrosis and cirrhosis, the APRI score, was used to discriminate among HCV patients for liver fibrosis and cirrhosis. The APRI score cutoff values included higher WHO cutoff values (1.5 and 2 for liver fibrosis and cirrhosis, respectively) with high specificity and lower WHO cutoff values (0.5 and 1 for liver fibrosis and cirrhosis, respectively) with high sensitivity. The employment of higher and lower APRI cutoff values provided the highest sensitivity, more than 90%, and the highest specificity, more than 80%, for diagnosing liver fibrosis and cirrhosis [50–52,69]. In addition, our decision to rely on those non-invasive tools in this work was further supported by the previous finding obtained by Derbala *et al.*, who suggested that the non-invasive APRI biomarker could be noted for its high sensitivity and specificity for diagnosing liver fibrosis and cirrhosis among HCV/SCH co-infected patients compared to liver biopsy. In this regard, they underlined that the APRI biomarker diagnostic accuracy was unaffected by the incidence of schistosomal antibodies among HCV patients [70]. In our statistical analysis, *t*-test results for quantitative data referred to a significant increase in the APRI score in HCV/SCH co-infected patients compared to HCV mono-infected patients. Moreover, the APRI score showed a significant association with HCV/HSC co-infection. These findings differed from those of Abbas *et al.*, who report no significant increase in the APRI score in HCV/SCH co-infected patients compared to those with HCV mono-infection. Instead, they observed a tendency for an elevation in the APRI score in HCV/SCH co-infected patients [56].

Additionally, the data obtained from the chi-square analysis at the APRI score cutoff value of 1.5 revealed no significant difference in the incidence of HCV/SCH co-infection between HCV patients without significant fibrosis and those with significant fibrosis. Likewise, our findings at the APRI score cutoff value of 2 referred to a non-significant difference in the incidence of HCV/SCH co-infection among HCV patients without cirrhosis and those with cirrhosis. Moreover, further investigations using logistic regression analysis revealed no significant effect of the incidence of HCV/SCH co-infection on either hepatic fibrosis or

cirrhosis in HCV patients. Indeed, our findings were consistent with those of Abdel-Rahman *et al.*, who found no significant effect of HCV/SCH co-infection on liver fibrosis among HCV patients [48]. In contrast, our findings differed from those of Abbas *et al.*, who observed a significant increase in the incidence of HCV/SCH co-infection towards the lower stages of liver fibrosis among HCV patients [56]. Consequently, our findings referred to the minimal role of HCV/SCH co-infection in liver fibrosis progression and the presence of cirrhosis. However, HCV/SCH co-infection was associated with a decrease in the high probability of ruling out liver cirrhosis based on the discriminating capacity of the APRI score cutoff value of 1.

Variation in the incidence of schistosomiasis (SCH) based on different schistosomal antibody titres in relation to liver fibrosis and cirrhosis in hepatitis C virus/schistosomiasis (HCV/SCH) co-infected patients

Based on the different titres of schistosomal antibodies, the chi-square test was used to demonstrate the variation in positivity for SCH among HCV/SCH co-infected patients. The data of patients per each of the schistosomal antibody titres were summarized in Table 7. The significant variation in the positivity of the incidence of SCH based on different schistosomal antibody titres was recorded in relation to liver fibrosis and cirrhosis among HCV/SCH co-infected patients (*P* 0.001 and < 0.001, respectively).

Table 7 Chi-square analysis of the incidence of schistosomiasis (SCH) based on different schistosomal antibody titres versus liver fibrosis and cirrhosis among hepatitis C virus/schistosomiasis (HCV/SCH) co-infected patients

Liver Fibrosis and cirrhosis		Different schistosomal antibody titres					Chi-Square	
		1⁄160	1⁄320	1⁄640	1⁄1280	Total		
		n(%)	*n*(%)	*n*(%)	*n*(%)	*n*(%)	X^2	*P*-value
Liver Fibrosis in HCV/SCH co-infected patients, *n* = 42	Non-significant	0 (0%)	2 (10%)	8 (40%)	10 (50%)	20 (100%)	17.258	0.001*
	Significant	4 (18.18%)	12 (54.54%)	2 (9.10%)	4 (18.18 %)	22 (100%)		
Liver cirrhosis in HCV/SCH co-infected patients, *n* = 42	No cirrhosis	0 (0%)	4 (15.38%)	8 (30.77%)	14 (53.85%)	26 (100%)	23.100	< 0.001*
	Cirrhosis	4 (25%)	10 (62.5%)	2 (12.5%)	0 (0%)	16 (100%)		

Where: *n*: number of patients and *: significant value.

Our further investigations included schistosomal antibody titre analysis in relation to liver fibrosis and cirrhosis among HCV/SCH co-infected patients. In this regard, our findings referred to a significant variation in the incidence of different titres of schistosomal antibodies in HCV/SCH co-infected patients with and without significant fibrosis, as well as those with and without cirrhosis. In HCV patients with significant fibrosis and those with cirrhosis, the highest incidence of HCV/SCH co-infection was recorded at the schistosomal antibody titre of 1/320 with percentages of 54.54% and 62.5% in patients with significant fibrosis and others with cirrhosis, respectively. On the other hand, the highest incidence of HCV/SCH co-infection was reported at the schistosomal antibody titre of 1/1280 with percentages of 50.00% and 53.85% in patients without significant fibrosis and others without cirrhosis, respectively. This finding may be explained due to pathological and immune reactions exerted in those HCV patients. Actually, there is not much available information about schistosomal antibody titre analysis. Our findings differed from those of Liang-Jun *et al.*, who reported the incidence of schistosomal antibody at titres greater than that of 1/320 in 135 patients with acute SCH. In their study, the schistosomal antibody was detected at a starting titre of 1/640, followed by 1/1280 with percentages of 1.48% and 28.15%, respectively, of the total incidence of those antibody titres [71].

To explain the preceding findings, the mechanism underlying liver fibrosis formation in SCH and its relation to HCV co-infection are taken into consideration. This mechanism is attributed to chronic inflammatory disease due to the continuous deposition of parasite eggs. In hepatic SCH, the immunological response can be triggered from an allergy to the egg antigen of *Schistosoma mansoni* due to the continuous laying of eggs in the liver for about 3 weeks. This may induce the development of a non-ending hypersensitivity state [67,72]. The immune reactions involved the stimulation of a type 1 helper (Th1) response [67,73]. Furthermore, a

dominant type 2 helper (Th2) immunological response can be triggered as a result of the egg-derived antigens stimulation, leading to eosinophil recruitment and subsequent granuloma formation, which may initiate the fibrogenic process of the liver [67,74–76]. As a result of this immune response, pathological changes were formed, including granuloma formation. Although these changes can cause blocking in the hepatotoxic influences of the parasite eggs as an antigen, this immune reaction may result in severalharmful alterations leading to liver fibrosis development with an overabundance of collagen and extracellular matrix in the periportal space [67,77]. In addition, numerous pathological alterations can lead to irreversible fibrosis; consequently, this may cause the development of severe portal hypertension [67,78]. In the co-infection of SCH and HCV, the influences of the hepatotropic virus co-infection may alter the Th2-dominated granulomatous phase during schistosomal infection. They trigger a highly specific T cell response, resulting in the infiltration of many IFN-γ-producing CD8+ cells into the liver cells. Furthermore, these immune alterations may result in decreasing cytokine levels by downregulating Th2 cytokine production, which is dominant during the period of SCH, and prompting hepatotoxicity-related morbidity [65–67]. Additionally, it is supposed that in advanced liver fibrosis or cirrhosis, the replication rate of HCV RNA may be decreased as a result of the decrease of vital hepatocytes in the liver tissue. Similarly, the deposition of parasite eggs may decrease due to the decline in hepatocytes. Consequently, the immune reactions, which result in an increase in fibrosis accumulations as well as schistosomal antibodies, are diminished, leading to a decline in the level of schistosomal antibodies. Therefore, in HCV cases with significant fibrosis or cirrhosis, the increased incidence of SCH may be observed with lower schistosomal antibody titres than higher titres, and vice versa.

Conclusion

The increased incidence of schistosomal antibodies is well established among HCV patients, indicating a high rate of HCV/SCH co-infection. The clinical signs of this co-infection include hyper-bilirubinemia, hypoalbuminemia, and impaired coagulation. Despite the strong association between the APRI score and HCV/SCH co-infection, the use of higher WHO APRI cutoff values for differentiating liver fibrosis and cirrhosis indicates no association between HCV/SCH co-infection and hepatic fibrosis progression or the presence of cirrhosis. However, the association between HCV/SCH co-infection and a decreased likelihood of ruling out liver cirrhosis has been identified. The incidence of SCH in HCV patients varies dramatically depending on the titre of schistosomal antibodies.

Abbreviations

HCV: Hepatitis C virus
SCH: Schistosomiasis
APRI: Aspartate Aminotransferase-to-Platelet Ratio Index
WHO: World Health Organization
CLD: Chronic Liver Disease

Author contributions

A.K.: Conceptualization, methodology, data collection, data analysis and interpretation, writing the manuscript, and revision for important intellectual content.
M.M.: Patient recruitment, resources and validation.
All authors approved the manuscript.

Conflict of Interest

The authors have no conflict of interest.

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