The Impact of Schistosomal and Hepatitis C Virus Co-infection on Some Clinic-pathological Parameters

Iman M. Abbas, Ahmed M. Ghoneim, Khaled Farid, Abdelaziz khidr

Abstract—Both hepatitis C virus (HCV) and schistosomiasis are highly endemic in Egypt and co-infection is frequently encountered. The impact of such co-infection on hepatic fibrosis and necro-inflammatory grades is worthy of investigation. In this study, we compare the degree of hepatic fibrosis and necro-inflammatory activity in liver tissue samples in two groups of HCV patients: positive and negative to shcistosoma antibodies. In addition, we investigate the effect of co-infection on clinic-pathological parameters in serum samples of both groups such as Aspartate aminotransferase (AST); Alanine aminotransferase (ALT); Bilirubin; Albumin and AST to platelets ratio index (APRI). Our data reveal that positive schistosomal serology has a significant impact on fibrosis stage and necro-inflammatory activity but no statistically significant differences were found in serum clinic-pathological parameters.

1 Introduction

Egypt has the highest prevalence of infection with HCV genotype 4 in the world, which is considered a major cause of chronic hepatitis, liver cirrhosis, HCC, and liver transplantation in the country [1], [2]. In Egypt, Schistosomiasis is a major public health problem.

Hepatocellular damage occurs when the cell infected with HCV is recognized by the immune system and destroyed. This process is extremely variable and dynamic, resulting in different intensities of hepatic necrosis and inflammation. Thus, this continuous inflammatory process is responsible for fibrogenesis [3], [4], [5]. During HCV infection, co-infection with schistosomiasis could be linked to histological severity [6], [7]. The presence of both HCV and *Schistosoma* spp. is of significant concern as patients with coinfections have been shown to have higher HCV RNA titers, increased histological activity, greater incidence of cirrhosis/HCC, and higher mortality rates than patients suffering from single infections [7].

Liver biopsy is the gold-standard method to stage fibrosis; however, it is an invasive procedure and is potentially dangerous. Therefore, this study is considered as an attempt to provide a non-invasive tool to predict fibrosis. This study investigates whether co-infection has effect on some serum biological markers (like AST, ALT, bilirubin and albumin) that could be used to predict the severity of hepatic fibrosis in schistosomiasis and HCV as isolated diseases or co-infections. APRI score is an example of one of these non-invasive modalities. It constitutes the AST to platelets ratio index. This score has been studied to assess the degree of fibrosis in patients with HCV with proven efficacy [8]. The relationship between platelet count and the stages of fibrosis in chronic HCV hepatitis have been examined previously; however few reports have studied this association in patients infected with *Schistosoma mansoni* [9]. In a meta-analysis of 40 studies, investigators concluded that an APRI score greater than 1.0 had a sensitivity of 76% and specificity of 72% for predicting cirrhosis. In addition, they concluded that APRI score greater than 0.7 had a sensitivity of 77% and specificity of 72% for predicting significant hepatic fibrosis [10]. For detection of cirrhosis, the use of an APRI cutoff score of 2.0 was more specific (91%) but less sensitive (46%). The lower the APRI score (less than 0.5), the greater the negative predictive value (and ability to rule out cirrhosis) and the higher the value (greater than 1.5) the greater the positive predictive value (and ability to rule in cirrhosis); midrange values are less helpful (Reference required). The APRI alone is likely not sufficiently sensitive to rule out significant disease. Some evidence suggests that the use of multiple indices in combination (such as APRI plus FibroTest) or an algorithmic approach may result in higher diagnostic accuracy than using APRI alone [11]. In addition, this study may offer deeper understanding to the possible impact of *schistosoma* and HCV co-infection on degree of necro-inflammatory and fibrosis stages in human liver.

2 PATIENTS AND METHODS

2.1 Patients

Liver biopsies and serum samples were collected from 23 HCV patients. All patients underwent liver biopsy and were tested for schistosomal antibodies before HCV treatment. The serology results were used to categorize the patients into group I (11 patients positive to schistosomal antibodies) and group II (12 patients negative to schistosomal antibodies).

- *Iman abbas is currently pursuing masters degree program at zoology department, faculty of science, in Dmietta University, Egypt, PH-01063782009. E-mail: imanabbas1@yahoo.com*
- *Ahmed M. Ghoneim is currently associate professor at zoology department in faculty of science, in Damietta University, Egypt, PH-01093052742. E-mail: am_goneim@du.edu.eg*
- *Khaled Farid is currently proffesor at Tropical Medicine Department, Faculty of Medicine in Mansoura University, Egypt, PH- 01222844484. E-mail: khaled_som@mans.edu.eg*
- *Abdelaziz khid is currently professor at zoology department in faculty of science, in Damietta University, Egypt, PH- 0128708701. E-mail: akhidr@du.edu.eg*

2.2 Detection of Schistosoma antibodies by immunoassay

By using Fumouze kits (Schistosomiasis Fumouze AWA/IHAT Paris/France), the test procedure was as follows. Fifty microliters of a 1:40 initial dilution of each serum was subjected to further twofold serial dilutions, and 10 µl of sheep red blood cells sensitized with *S. mansoni* adult worm antigen was added to each diluted sample. Positive and negative control sera and nonsensitized red blood cells were included in each test as controls for naturally occurring antibodies. After incubation for 2 h at room temperature, the titer in the test serum was recorded as one dilution before that which yielded a clear sharp dark spot similar to those in the negative control.

2.3 *Histopathological evaluation*

The histopathological assessment of necroinflammatory grade and fibrosis stage was scored using the METAVAIR system [12]. Fibrosis was, therefore, scored on a scale from 0 to 4 (F0 = no fibrosis, F1 = portal fibrosis without septa, F2 = portal fibrosis and few septa, F3 = numerous septa without cirrhosis, F4 = cirrhosis), and activity on a scale from 0 to 3 (A0 = none, A1 = mild, A2 = moderate, A3 = severe). For statistical convenience, patients were divided into two groups according to the severity of their histological lesions: non significant or mild fibrosis (F0-F1) and significant or severe fibrosis (F2-F4). This ichotomization was performed because F2 fibrosis is generally considered the threshold for the initiation of antiviral treatment of chronic HCV. These patients were also analyzed according to the presence of necroinflammatory activity and divided into two groups: non significant activity (A0-A1) and significant activity (A2-A3).

2.4 Laboratory Tests of Liver Disease

Serum AST, ALT, albumin and bilirubin concentrations, and APRI were determined. Serum HCV RNA was estimated by Polymerase chain reaction PCR.

2.5 Statistical analysis

Results were tested for significance by chi-squared test using SPSS (ver. 18) programme

3 Results

Out of 23 patients (13 males and 10 females), Schistosomal antibody was positive in 47.82% of patients (72.7% males and 27.3% females) and was negative in 52.17% of patients (41.66% males and 58.33% female). The patients in group I were older (P = 0.368) and had a higher proportion of males (p=0.14) than the patients in group II table 1. Table 2 shows that there was increase in proportion of significant fibrosis stage in group I (P = 0.042) (Fig.1) as well as increase in the proportion of necroinflammatory activity in group I (P= 0.056) than group II (Fig.2). Serum biochemical parameters of patients in both groups are shown in table 1 that illustrates non significant higher proportion in group II in all parameters except in serum albumin which is

TABLE 1
CHARACTERISTICS AND BIOCHEMICAL PARAMETERS OF PATIENTS

	Sch + ve (n = 11)	Sch - ve (n = 12)	P value
Age	42.3636±10.45	40.25±8.43	0.368
Male (%)/Female (%)	72.7/27.2	41.66/58.33	0.14
AST (ref.: 40 IU/L)	48.52±27	41±17.76	0.244
ALT (ref.: 40 IU/L)	42.29±24.5	39.16±19.66	0.19
Bilirubin [0.3-1.2]mg/dl	0.985±0.3	0.575±0.18	0.182
Albumin(ref.:3.5-5.5 mg/dL)	4.38±0.36	4.17±0.5	0.707
HCV RNA, IU/mL	$0.764\times10^{-6}\pm 1.723\times10^{-6}$	$0.39\times10^{-6}\pm0.36\times10^{-6}$	0.394
APRI	0.7044±0.46	0.5197±0.38	0.256

Data are expressed as mean ± SD. (+SCH) = positive to schistosoma antibodies, (-SCH) = negative to schistosoma antibodies, (AST) = Aspartate aminotransferase; (ALT) = Alanine aminotransferase; (HCV) = Hepatitis, APRI: Aspartate aminotransferase to platelets ratio index

TABLE 2
METAVIR FIBROSIS STAGES AND NECROINFALMMATORY ACTIVITY DEGREE IN SCHISTOSOMA POSITIVE (+SCH) AND SCHISTOSOMA NEGATIVE (-SCH) HCV PATIENTS.

Fibrosis stage	Schisto + ve	Schisto - ve	P value
(F0-F1) %	54.54%	91.16%	0.042
(F2-F3-F4)%	45.45%	8.33%	
Activity grade%			
(A0-A1)%	45.45%	83.33%	0.056
(A2-A3)%	54.54%	16.66%	

Data are expressed as percent. (+SCH) = positive to schistosoma antibodies, (-SCH) = negative to schistosoma antibodies, (F) = fibrosis according to metavir scoring system; (A) = necroinflammatory degree according to metavir scoring system.

non-sifnificantly lower in group I than group II. The comparison of AST and ALT in both groups is shown in Fig. 3 and the comparison of albumin and bilirubin in the two groups is illustrated in Fig.4.

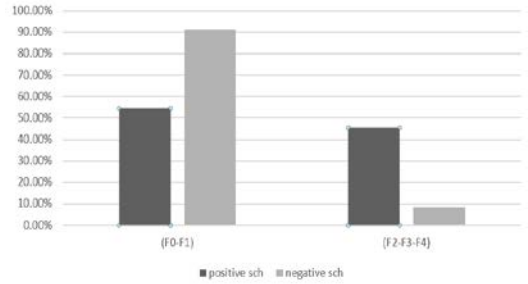

Fig.1. Comparison between significant (F2-F3-F4) and non significant (F0-F1) Fibrosis stages in Schistosoma positive (+Sch) and Schistosoma negative (-Sch) HCV patients.

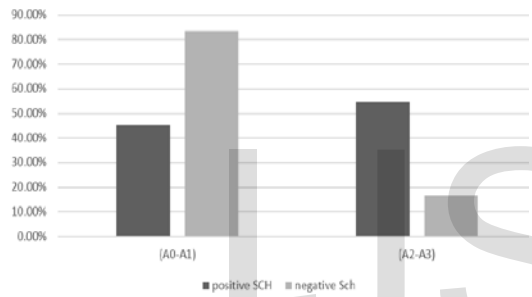

Fig. 2. Comparison between significant (A2-A3) and non significant (A0-A1)Necroinfalmmatory activity in Schistosoma positive (+Sch) and Schistosoma negative (-Sch) HCV patients.

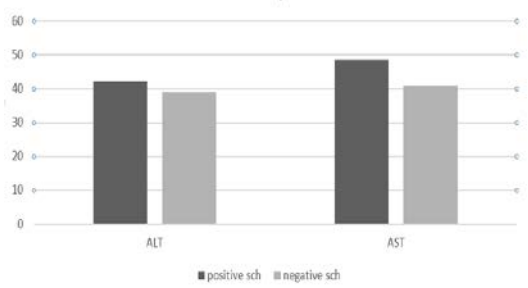

Fig. 3. Comparison of AST and ALT between Schistosoma positive (+Sch) and Schistosoma negative (-Sch) HCV patients.

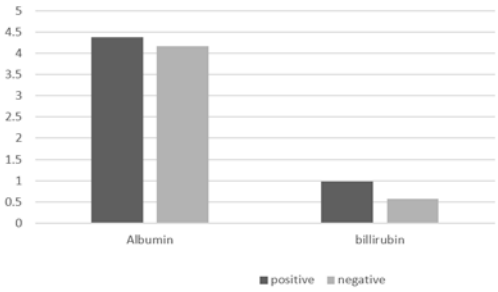

Fig. 4. Comparison of Albumin and Bilirubin between Schistosoma positive (+Sch) and Schistosoma negative (-Sch) HCV patients.

4 Discussion

Clinical studies in Egypt showed that 70-90% of patients with chronic hepatitis, cirrhosis, or HCC are co-infected with schistosomiasis and HCV [13], [14], [15], [16], [17]. It was suggested that the combination of chronic schistosomiasis caused by *S. mansoni* and HCV may cause a higher risk of HCC due to the increased viral load in co-infected patients, leading to higher inflammatory activity and more advanced disease state [18]. Fibrosis is the major cause of mortality and morbidity related to both schistosomiasis and HC, although the pattern of fibrosis and underlying immunological mechanisms are different [19]. According to Kamal et al. (2004, 2006) [7], [20], co-infection shows a specific clinical, virological and histological pattern characterized by virus persistence with high HCV RNA titres and higher necroinflammatory and fibrosis scores in liver biopsies. Our results are consistent with Kamal et al. (2004, 2006) study as group I in the current study shows higher proportion of necroinflammatory and fibrosis scores as well as higher RNA titres. However, Abdel-Rahman et al. [21] showed no significant difference between samples positive to schistosoma antibodies compared to negative samples in terms of fibrosis staging. In addition, de Morais et al [22] showed no statistically significant differences in fibrosis degree on histology evaluation when fibrosis of HCV + hepatosplenic schistosomiasis patients were compared to HCV patients alone. Ahmad et al [23] showed that schistosomiasis coinfection with HCV and/or non-alcoholic steatohepatitis had no significant impact on fibrosis stage

The possible synergistic relationship between HCV and schistosomiasis is controversial. Also, the degree of necroinflammatory injury and the stage of fibrosis in patients with mixed schistosomiasis and chronic HCV remains unclear. Results involving differences in fibrosis stages may be explained by several factors. Genetic predisposition may play a role, whereby only a minority of the individuals infected with *Schistosoma mansoni* may develop hepatic fibrosis or be more

sensitive to infection(s). Due to the discripant results in literature, further detailed studies, including larger number of patients is a demand.

Andrade [24] and Dusek et al. [25] emphasized that the association between chronic hepatitis and schistosomiasis aggravated liver disease. Our results confirm this finding as all biochemical parameters show higher proportions in the co-infection group than mono-infection group. Derbala et al., [26] suggested that noninvasive biochemical markers like APRI are sensitive and specific in diagnosing the degree of fibrosis and cirrhosis in patients with co-infection of HCV and schistosomiasis as compared to biopsy. The current results further support this suggestion as our results show higher APRI in the co-infection group than mono-infection group.

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