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Biochemical and Histopathological changes in Egyptian patients with Hepatitis C and Bilharziasis

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Abstract

Viral hepatitis and infection with *Schistosoma mansoni* are the main causes of chronic liver disease and liver cirrhosis. Infection with hepatitis C virus (HCV) is the most important public health problem in Egypt. This study was done to determine the association between *Schistosoma* and hepatitis C, by testing liver function using the following biochemical tests: Total and direct bilirubin, TB-DB, alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), serum albumin, and liver biopsy, and make comparison between them. The results indicated that 60 % of cases HCV and Bilharzias reveal abnormal results accompanied by chronic liver disease, when compared to the 40% of normal result. All cases were subjected to routine biochemical liver function tests and serum antibodies to bilharziasis. The results showed that, ALT and AST were highly significant difference in group hepatitis C only compared with control group which showed normal results. The other liver functions such as ALP, TB and DB were high in patients with hepatitis C and Biharziasis compared with control group with statically significant difference $P < 0.05$. Also, the results showed histopathological changes (fibrosis and cirrhosis) in patients

with Hepatitis C and Hepatitis C and Biharziasis after taking liver biopsy from both patients.

Keywords: Hepatitis C, *Schistosoma mansoni*, Liver function tests, Histopathology, Co infection

1 Introduction

HCV infection is one of the most causes of chronic hepatitis, cirrhosis, and hepatocellular carcinoma which globally spreading (Rao et al., 2002 and National Institute of health, 2007). Since 1989 the scientists was discover the HCV viral infection at the Centers for Disease Control and Prevention in the USA when it was considered cause of non-A, non-B hepatitis (National Institute of health, 2007). Hepatitis C viral infection has a variable course that may be adversely affected by alcohol consumption and other factors, such as concomitant diseases.

HCV is an RNA virus that has 6 major genotypes and more than 50 subtypes (Ballester et al., 2005). This extensive genetic variation may explain the difficulties in developing a vaccine and the lack of response to therapy. The HCV RNA genotype that is most common in Egypt is

genotype 4a, representing 85% of cases (National Institute of health, 2007). One of the most methods of transmitting the infection of HCV is infected blood which caused by using injection drug and blood transfusion (Madwar et al., 1998). The incubation period of HCV RNA in the blood after infection is one to three weeks. Rising in the level of ALT in the serum of individual occurs within four to twelve weeks on average, plurality of infected individual ranged between (60 to 85%) accompanied chronic infection (National Institute of health, 2007 and National center for complementary, 2007). A problem is that about 30% of patients with chronic HCV have normal ALT. A liver biopsy cannot serve to diagnose HCV infection, farther more it can provide useful histological information on liver injury. According to the NIH Consensus Statement approve treatment for chronic hepatitis C may begin when patients have three investigation of HCV:

Abnormal ALT for over Six months, Positive HCV RNA and Liver biopsy has fibrosis and signs of hepatocyte necrosis and inflammation (National Institute of health, 2007).

Schistosomiasis, also known as bilharzia, snail fever, and Katayama fever, is a disease caused by parasitic flat worms of the Schistosoma type. The disease is spread by contact with water contaminated with the parasites. These parasites are released from infected freshwater snails (WHO, 2014) and found in tropical countries in Africa, Asia and in the Middle East. Schistosoma is common in about 75 developing countries and mainly affects people living in rural agricultural and periurban areas (Oliveira et al., 2004 and Neglected tropical disease, 2014). Schistosomiasis affects almost 210 million people worldwide, (Fenwick, 2012) and an estimated 12,000 (Lozano et al., 2012) to 200,000 people die from it a year (Thetiot-laurant et al., 2013). A particularly useful diagnostic tool is abdominal ultrasonography, which allows accurate measurements of liver and spleen size, grading of hepatic fibrosis, and detection of complications of portal hypertension. (Hatz et al., 1992 and Hatz and Murakami, 1992).

In many parts of the world, HCV is not the only infection which affects chronically the liver. Mediterranean region, especially Egypt; patients with HCV are commonly found to be co-infected with schistosomiasis. Both conditions can distinguish portal hypertension with its consequences which includes splenomegaly hypersplenism with pancytopenia (Dai et al., 2010).

Schistosomiasis and HCV co-infection is most endemic in Egypt. The relation between the schistosomiasis and HCV was studied (Rao et al., 2002). Individual which infected with HCV and Schistosoma mansoni more intensely exposed to chronic liver disease such as liver fibrosis and cirrhosis than which infected with HCV alone (Kamal et al., 2004).

The infection with S. mansoni increases HCV morbidity and chronicity, which might be referred to previous liver failure (Mohamed et al., 1998, Gad et al., 2001, Emam et al., 2004). Higher mortality rates were noticed in patients with co-infection (Kamal, 2001). The aim of this work

was to study the effect of schistosomiasis and HCV on Egyptian patients.

2 Materials and Methods

An Approval from the research ethics committee in ministry health was obtained to conduct this study. Official permission from the hospital administrator was also obtained to facilitate the work. An informed consent was obtained from all participants. It includes all details about the study (title, objectives, method, expected benefits).

Technical design :

This cross-sectional study that was conducted on one hundred Persons 40 Patients as control (21 Male and 19 Female), 20 Patient with HCV only (10 male and 10 female), 20 patient with bilharzias only (15 male and 5 female) and 20 patient with bilharzias and HCV (11 male and 9 female). The aged of the patients ranged from 20 to 60 years old. They attending the center of hepatic (Benha fever hospital) and University Hospital, Benha, Egypt. During the period of 2012–2014, start of October 2012 till end of February 2014. Negative for HBs antigens and HBs antibodies were screened for anti-HCV antibodies from each patient.

Specimen Collection:

Under complete aseptic technique, 10 ml of blood was taken from all subjects. Blood was allowed to clot naturally in a test tube after taking 2 ml for complete blood count. Serum was separated, divided into small aliquots and stored at –60 °C till being tested.

Histological evaluation of biops samples:

The histological evaluation of paraffin-embedded liver specimens was carried out at the Pathology Department; Benha Faculty of Medicine. Liver fibrosis was estimated according to METAVIR scoring system. Histological grades depend on the degree of fibrosis have five degrees of fibrosis: as F0 (no fibrosis), F1 (mild portal fibrosis without septa), F2 (moderate periportal fibrosis with few septa), F3 (severe fibrosis, fibrous septa with architectural distortion but with no obvious cirrhosis (bridging fibrosis) and F4 (cirrhosis).

Biochemical , Hematological study and Biomarkers fibrosis :

According to the National Egyptian Program for the treatment of chronic HCV infection, we reviewed all studied subjects with history taking and were examined for clinical features of liver disease. EDTA blood samples were subjected to complete blood counts as (HB, Platelets, WBCs), by automated RT 7600. Serum were collected and aspartate aminotransferase (AST), ALT, total bilirubin, alkaline phosphatases (ALP) and Creatinine (Cr) and serum albumin, (Alb) was determined. Fasting blood sugar were measured using semi automated method RT 9200 analyzer Prothrombin time (Pt) INR by techo coagulation analyzer and, HBs Ag, Anti Nuclear Antibodies (ANA), TSH Alpha feto- protein (AFP), by using mini vidas technique anti bilharzial anti body, quantitative HCV PCR, pregnancy test in married females in child bearing period and liver biopsy was determined.

Statistically analysis

The collected data were tabulated. All data was presented as mean values. Spearman's rank correlation was used to assess the significant association between continuous variables and liver fibrosis stages. The student t-test was used to compare arithmetic means and parameters while Chi-square (X²) test was used to compare categorical data, correlation with Fisher's exact test was used when appropriate. Patients were divided into three main groups as, patients with no or minimal fibrosis (F1), patients with advanced fibrosis (F2-F3) and patients with cirrhosis (F4). The independently distinguished values of biochemical marker.

3 Results

1. Characteristics of the study group:

The groups of this study (Table 1) were as follow: 47.5% females ,52.5% males in group one (control), 50% females , 50% males in group two (Hepatitis C only), 45% females ,55% males in group three (patient with hepatitis Hcv& Bilharziasis) and 25 % females 75% males in group four (Bilharziasis) (P> 0.05). There were no statically significant difference between the percentage of study group.

2. Alanine aminotransferase (ALT):

The result of ALT was expressed as mean \pm SD (Table 2) showed that: there was high level of ALT in group 2 Hepatitis C only (62 ± 19.2), and group 3 Hepatitis C & B (44.38 ± 27.33) and group 4 (bilharzias) (36.17 ± 17.11), while low level was detected in control group (22.19 ± 6.22) there were high w statically significant difference (P< 0.001).

3. Aspartate aminotransferase(AST):

Also (Table 2) showed high level in group two (Hepatitis C only) (61.9 ± 20.57), group three (Hepatitis C & B) (45.63 ± 22.33) and group four (bilharzias) (36.28 ± 22.79). on the other hand, low level was observed in control group (21.78 ± 4.29) as normal result which statically were highly significant (P< 0.001).

4. Albumin (ALB):

The result of ALB (Table 2) illustrated that there was low level of activity in group three (Hepatitis C & bilharziasis) (3.47 ± 0.51) and high level in control group as normal result which statically were highly significant (p<0.001).

5. Alkaline phosphatases (ALP):

The result of ALP showed in Table (2) reflected a high level of activity in group three (Hepatitis C & B) (128.8 ± 19.92), group two (Hepatitis C only) (105.45 ± 14.78) and group four (bilharzias) (97.65 ± 11.04) while it showed low level in control group (92.55 ± 15.16) as normal result which statically were highly significant (P < 0.001)

6. Total Biliruben (TB) and Direct Blilruben (DB).

The result of (TB) (Table 2) showed that there was high level in group three (Hepatitis C & bilharzias) (1.07 ± 0.81) and low level in control group as normal result (0.58 ± 0.11) which statically were highly significant (P< 0.001).

The result of (DB) illustrated high level in group three (Hepatitis C & bilharzias) (0.26 ± 0.27) (Table 2) and low level of activity in control group as normal result (0.12 ± 0.03) which statically were highly significant (P< 0.001).

7. Histopathological changes:

From another point ,in this study Table (3) showed some histopathological changes in group two (Hepatitis C only) which showed 100 % grade Two of fibrosis , group Two (patient with HCV & bilharziasis) showed 45% grade two of fibrosis , 25 % grade three of fibrosis and 30 % grade four of fibrosis that lead to cirrhosis of liver and chronic liver disease (Hepato cellular carcinoma) and finally group four (patient of bilharzias) showed grade (one) 100% of fibrosis.

4 Discussion

Although schistosomiasis was the major public health problem in Egypt in the past, HCV currently is the major problem (Andrade et al.,1992 and Hibbs et al.,1993). Co-occurrence of these two infectious agents was previously reported (Darwish et al.,1993,Neely et al.,1990 and Alter et al.,1989). In the present study, a high predominance of bilharziasis and HCV was encountered among patients with chronic liver disease, followed by those with other chronic diseases, in comparison to normal controls.

One hundred percent of patients with liver disease were positive for at least one of the studied infectious agents. Concurrent infection with two or three of the studied agents was statistically higher in patients with liver disease than other individuals. An obvious association was found between the studied viruses and bilharziasis, which is in agreement with the recently published report (Aceti et al.,1993). This high association could be attributed to the transmission of the viruses via sharing contaminated syringes used to inject tarter emetic, which was prescribed to treat patients with bilharziasis about 25 years ago. a marked association between bilharziasis and HCV was noted in patients with chronic liver disease compared to the other groups .infection with HCV was found to cause a more severe and irreversible form of liver damage than schistosomal infection.

Therefore, HCV over-infection may explain the rapid progress toward end-stage liver disease and the early death of some patients with bilharziasis (Aceti et al.,1993).In the present study, because some patients with established chronic liver disease had abnormal liver function tests, we could not distinguish the impact of the studied infectious agents on liver pathogenesis. However, in patients with chronic diseases other than liver disease and apparently healthy individuals, HCV seems to be much more aggressive than bilharziasis. One hundred percent of patients with HCV or mixed infection with HCV and bilharziasis could be discriminated by laboratory findings, compared with 20.8% of patients with bilharziasis only.

Table (1): Characteristics of the study group.

study group		(1) Control group		(2) Patient with hepatitis C		(3) Patient with HCV & Bilharzias		(4) Patient with Bilharzias		Test of significant
Variable		NO	%	NO	%	NO	%	NO	%	
1- Sex	Female	19	47.5	10	50	9	45	5	25	$\chi^2 = 3.4$ $P > 0.05$
	Male	21	52.5	10	50	11	55	15	75	
2- Mean \pm SD Age		28.98 \pm 9.94		39.05 \pm 9.02		45.85 \pm 10.5		37 \pm 12.6		F=12.5 P < 0.001

Table (2): Descriptive laboratory data of four groups.

study group	(1) Control group (N= 40) $\bar{x} \pm SD$	(2) Patient with hepatitis C (N= 20) $\bar{x} \pm SD$	(3) Patient with HCV & Bilharzias (N=20) $\bar{x} \pm SD$	(4) Patient with Bilharzias (N= 20) $\bar{x} \pm SD$	Test of significant
Liver function					
1-Gpt (ALT)	22.19 \pm 6.22	62 \pm 19.2	44.38 \pm 27.33	36.17 \pm 17.11	F=25.3 (P< 0.001)
2-Got (AST)	21.78 \pm 4.29	61.9 \pm 20.57	45.63 \pm 22.33	36.28 \pm 22.79	F=26.4 (P< 0.001)
3-ALB	4.28 \pm 0.29	4.1 \pm 0.48	3.47 \pm 0.51	4.14 \pm 0.68	F=13.5 (P< 0.001)
4-ALP	92.55 \pm 15.16	105.45 \pm 14.78	128.8 \pm 19.92	97.65 \pm 11.04	F=25.6
5- Total Bili (TB)	0.58 \pm 0.11	1.07 \pm 0.35	1.07 \pm 0.81	0.67 \pm 0.19	F= 10.5 (P < 0.001)
6- Direct Bili (DB)	0.12 \pm 0.03	0.17 \pm 0.07	0.26 \pm 0.27	0.13 \pm 0.05	F=6.3 (P< 0.01)

Data are expressed as mean \pm SD. P<0.01 : P < 0.001= Highly Significant. AST : aspartate transaminase; ALT : alanine transaminase; ALP : alkaline phosphatase; TB : total bilirubin; DB : direct bilirubin ; ALB : Albumin .

Table(3): Risk factors and selected histopathological changes in patients according to probable etiology of liver disease.

Study group	Patient with Hepatitis C		Patient with HCV & Bilharzias		Patient with Bilharzias	
	NO	(%)	NO	(%)	NO	(%)
F 1	0	0	0	0	20	100
F 2	20	100	9	45	0	0
F 3	0	0	5	25	0	0
F 4	0	0	6	30	0	0
Total	20	100	20	100	20	100

F1=Grade 1 of fibrosis, F2= Grade 2 of fibrosis, F3= Grade 3 of fibrosis, F4 =Grade 4 of fibrosi

Table (4): frequency distribution of the study group regarding ultrasound

	Frequency	Percent
Enlarged Coarse Liver With Splenomegaly	1	1.0
Mild Liver Cirrhosis - Splenomegaly	1	1.0
Mild Liver Cirrhosis Hepatomegaly	2	2.0
Enlarged Coarse Liver	16	16.0
Bilharzias Liver	20	20.0
Small enlarged Liver	20	20.0
Normal Ultra Sound	40	40.0
Total	100	100.0

*This table shows that 60% of causes reveal abnormal signs while 40% are normal ultrasound

The type of relationship between HCV infection and schistosomiasis has been the subject of much controversy. Some studies suggested a causative relationship between both diseases, because anti schistosomal therapy, which was given as multiple injections in the past, (Patel et al.,1991). could have transmitted the HCV infection through contaminated needles and syringes (Patel et al.,1991, and Bassily et al.,1992). Nevertheless, the demonstration of actual HCV histopathological changes in the liver biopsy specimens of all the two group included in our study makes this latter assumption seem unlikely. In this study Our data were further strengthened by the high levels of ALT in the serum of the group three (Hepatitis C with *Shistosoma*) with highly significant difference between (the Hepatitis C only)and (Hepatitis C with *Shistosoma*). Although, (Helal et al., 1998). indicated high level of Alt in the group of S with no significant between S Group an NS Group. This enzyme has been considered as asurrogate marker of HCV infection (Helal et al., 1998). agreement with our study.

Another point of controversy is the pathophysiological relationship between HCV and schistosomiasis. Although (Bassily et al., (1992, and Uemura et al.,(1992). reported asynergistic relationship between both conditions, while (Kamel et al.,1994). failed to obtain such an association.

The current study indicated that antischistosomal seropositivity enhance the severity of HCV hepaticpathology as evidenced by the lack of a statistically significant difference between the S and NS groups. Although the the (Helal et al., 1998). Indicate didn't antischistosoma enhance the severity of HCV hepaticpathology.

The current study and (Helal et al.,1998). which analyzed the effect of Chornic schistosomiasis on the severity of HCV hepatitis.

From the histological point of view,in contrast to the reports of (Bassily et al.,1992 and Uemura et al.,1992). investigated the effect of HCV infection on the course of chronic schistosomiasis. The development of Abnormal sign such as cirrhosis and fibrosis was noticed in 60 %of all patients.

CONCLUSION

In conclusion, bilharziasis, HCV were associated with chronic liver disease in patients in Egypt, with HCV having the greatest impact. Coinfection with two or more of these infectious agents may potentiate pathogenesis of the liver disease. The HCV genotype 4a is the most common genotype in Egypt and type-specific PCR is a valid, simple technique for HCV genotyping. Another study including a large number of samples from different locations in Egypt is recommended to draw a map for the incidence of the different HCV genotypes in Egypt.

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