Med. J. Cairo Univ., Vol. 62, No. 3, September: 803 - 811, 1994

Clinical, Laboratory and Histopathological Features of Chronic Hepatitis C Virus Infection in Egypt

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Abstract

Thirty patients referred consecutively from the laboratory, diagnosed as hepatitis-C virus infection both by second generation ELISA and RIBA-II methods, were analyzed. All had elevated serum aminotransferases more than 6 months. Patients were frequently diagnosed incidentally, about one-third had no symptoms of liver disease and were asymptomatic or had only fatigue. On routine screening raised serum level of aminotransferase enzymes was detected. On careful abdominal palpation, hepatomegaly was detected in 6% of cases. Patients presented with liver cirrhosis, splenomegaly and endoscopic evidence of varices were all above 40 years and represent 20% of cases. Four patients had positive antinuclear and antismooth muscle antibodies. Hepatic histopathological changes frequently observed in such patients included: portal tract lymphoid aggregates, bile duct affection, free sinusoidal lymphocytes and reactive kupffer cells. Hepatic cell changes included single cell acidophilia, fatty change, dysplasia and multinucleation. Such features were observed in addition to the classic morphological changes of chronic persistent, lobular and active hepatitis. Estimation of serum aminotransferases should be a part of routine laboratory screening tests in Egypt. Detection of immunological markers is important in hepatitis-C positive cases, as autoimmune chronic hepatitis may coexist with chronic hepatitis-C. Liver biopsy is indicated not only for etiological diagnosis, but also for determining disease activity and stage.

Introduction

THE cloning of hepatitis-C virus and the subsequent detection of serological markers allow an accurate diagnosis of hepatitis C [1].

Hepatitis-C virus(HCV) infection progresses to chronic liver disease and cirrhosis in 20-40% of cases [2]. Diagnosis of chronic hepatitis-C depends on detection of serum antibodies (anti-HCV) by first and second generation ELISA test. About 80% of patients with classic post transfusion non-A, non B hepatitis have a positive test [3].

However, positive results also occur in many liver diseases including alcoholic liver disease, primary biliary cirrhosis and au-

toimmune chronic active hepatitis [4]. Even by the confirmation test for HCV, the second generation RIBA-II, anti-HCV antibodies could be detected in 23% of patients with type I autoimmune chronic active hepatitis [5]. In a recent study, anti-HCV antibodies could also be detected in a proportion of patients with type II-autoimmune chronic active hepatitis [6]. Such patients had positive liver-kidney microsomal antibodies, (LKM-1).

Even by the use of the polymerase chain reach in (PCR) which detects HCV-RNA, 13% of patients with type I-autoimmune chronic active hepatitis have a positive result. Ludwig termed such cases as cryptogenic chronic hepatitis (unclassified type) [7].

The prevalence of HCV positivity in blood donors in Egypt was 10.9% in one study [8]. At least 50% of them are at high risk of cirrhosis in 3-5 years [2].

Histopathological studies to date, do not specifically diagnose chronic hepatitis-C. In case of hepatitis B virus infection, ground glass inclusions within hepatocytes serve as a histological marker of hepatitis B surface antigen in chronic hepatitis-B virus carriers [9]. Although no analogous cellular marker of HBV infection has been shown to date, a number of histological features were described as potentially diagnostic [10].

The aim of the present work is to describe the clinical, laboratory and histopathological features of chronic-hepatitis C-virus infection.

Patients and Methods

Thirty patients with chronic hepatitis-C (20 males, 10 females with a mean age 46) were studied retrospectively. They were consecutively referred from the laboratory,testing positive for HCV antibodies, by the first and subsequently by second generation enzyme-linked immunoassay ELISA. ALl patients had persistent elevation of serum alanine aminotransferase (ALT) levels at least 1.5 times the upper normal for at least 6 months.

Full history including the presenting symptoms, duration of disease, possible methods of exposure and complications were analyzed, as well as full physical examination at presentation.

No patient had received antiviral therapy, the following investigations were done for all patients:

1- Ultrasonographic imaging of the abdomen.

2- Upper endoscopy.

3- Full blood picture and platelet count.

4- Complete liver function tests including serum albumin (using bromocresol green method) [11], prothrombin time (measured by thrombotest technique) [12], serum total bilirubin and serum alanine and aspartate aminotransferases (done by a calorimetric method) [13].

5- Serum samples were tested for anti-UCV antibodies using commercially available Ortho-anti HCV and Abott-anti HCV ELISA test systems [14]. These assays test for antibody C_{100-3}, C_{33}, C_{200} and C_{22-3} antigens. The first three are non-structural proteins derived from the hepatitis-C viral genome and the last is a structural protein derived from the core region of the viral genome. Patients with a positive anti-HCV test were only included if it could be verified by a repeated positive test by both Abott and Ortho assays. Confirmation was done by lhe second generation test for

HCV antibodies, the recombinant immunoblot assay (RIBA-II) [15].

Serum was tested for hepatitis-B surface antigen (HBsAg) by ELISA technique using kits (Inzyanost HBsAg micro) supplied from Behering institute [16].

6- Estimation of antinuclear antibodies and anti smooth muscle antibodies by indirect immunofluorescence technique [17].

7- Liver biopsy was done after doing prothrombin time and in absence of a ascites. Liver biopsy specimens were fixed in 10% neutral buffered formalin. Sections were cut and stained with H and E., masson trichrome for fibrous tissue identification and Gordon and Sweets silver stain for identification of reticulin fibers.

Results

Clinical data (shown in table 1):

20% of patients were asymptomatic and discovered by routine analysis of serum aminotransferases. The commonest presenting symptoms were fatigue 33.3% and abdominal pain 13.3%. The most common physical sign was hepatomegaly (60%). Manifestations of liver cell failure and splenomegaly with esophageal varices occurred in 20% of patients, all of them were above 40 years.

History of blood transfusion or past surgery was present in one third of cases.

Laboratory findings (Table 2):

3 patients were HBsAg positive. 6 patients tested for A.N.A. s positive, 4 of them had positive anti smooth muscle antibodies.

Histopathological findings:

All liver biopsy specimens were classified according to the type of hepatitis (chronic persistent, chronic lobular, and chronic active hepatitis) and graded according to the knodell histological activity index [18]. The grading was according to the degree of periportal inflammation, lobular and limiting plate necrosis and the degree of fibrosis (Table 3).

The following histological features were identified (Table 4), (Figs. 1,2,3,4,5).

1- Lymphoid follicles and or aggregates in portal tracts.
2- Bile duct damage.
3- Fatty changes with vacuoles of lipid in hepatocytes.
4- Single acidophilic material within liver cell cytoplasm.
5- Liver cell multinucleation.
6- Liver cell dysplasia: enlarged hepatocytes with atypical nuclei.
7- Sinusoidal inflammatory cells mainly lymphocytes and hyperplasia of kupffer cells.

Table (1): Clinical and Endoscopy Data at First Presentation of 30 Patients with Chronic Hepatitis-C.

Mean age	46 ± 12.4 years
Sex	20 males, 10 females
Past blood transfusion	3 cases
Past history of jaundice	7 cases
Diagnosed incidentally	15 cases
Fatigue	6 cases (20%)
Abdominal pain	10 cases (33.3%)
Jaundice	4 cases (13.3%)
Ascites and edema	5 cases (16.7%)
Haematemesis and melaena	1 cases (3.3%)
Hepatogemaly	2 cases (6.7%)
Splenomegaly	18 cases 960%)
Spider naevi	5 cases (20%)
Endoscopy: Esophageal varices	5 cases (20%)
Gastritis or peptic ulcer	7 cases (23.3%)

Table (2): Laboratory Data of 30 Patients with Chronic Hepatitis-C.

	Mean ± S.D.
Serum bilirubin	2.3 ± 0.8 mg/100 ml.
Serum ALT	165 ± 118 IU/L (Reference value up to 45 U/L)
Serum AST.	141 ± 56 IU/L (Reference value up to 40 U/L)
Serum albumin	3.6 ± 1.1 gm% (Reference value 3.7-5.3 g/dl)
Prothrombin time	11 ± 3.6 sec. (normal value 10.5-12.5 seconds)
ANAs	Positive in 6 cases (20%)
Antismooth muscle antibodies	Positive in 4 cases (13.3%)
HBsAg	Positive in 3 cases (10%)
Hemoglobin	13.1 ± 4.8 gm/dL.
White cell count	4.7 ± 1.3 /Cmm.
platelet count	220 ± 101 10^9 /Cmm.

Table (3): Histopathological Classification of 30 Patients with Chronic Hepatitis.

	Number of cases
Type of hepatitis:	
Chronic persistent hepatitis	3 (10%)
Chronic lobular hepatitis	4 (13.3%)
Chronic active hepatitis (CAH).	14 (46.7%)
Chronic hepatitis and cirrhosis.	5 (16.7%)
Chronic mixed hepatitis (unclassified as to viral or autoimmune aetiology)	4 (13.3%)
Grade of inflammatory activity:	
Grade I: portal and lobular inflammation (No necrosis)	7 (23.3%)
Grade II: Mild limiting plate and lobular focal necrosis	9 (30%)
Grade III: Moderate limiting plate necrosis and severe focal cell damage (moderate CAH).	10 (33.3%)
Grade IV: Severe limiting plate necrosis and lobular damage including bridging necrosis (severe CAH)	4 (13.3%)
Stage / degree of fibrosis:	
Stage I: No fibrosis	8 (26.6%)
Stage II: Periportal fibrosis but intact architecture	14 (46.7%)
Stage III: Septal fibrosis with architectural distortion but no obvious cirrhosis.	3 (10%)
Stage IV: Cirrhosis	5 (16.7%)

Table (4): Liver Biopsy Features of Chronic Hepatitis-C.

Biopsy feature	Number of cases
Bile duct damage	10 (33.3%)
Lymphoid follicles	15 (50%)
Focal fatty change	24 (80%)
Acidophilic heptocytes	6 (20%)
Liver cell dysplasia	3 (23.3%)
Sinusoidal lymphocytes and kupffer cell hyperplasia	20 (66.7)

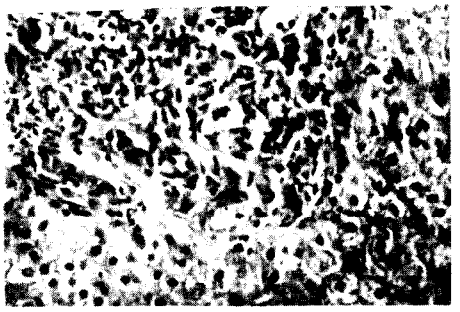

Fig. (1): Portal tract with a heavy lymphoid infiltrate and evidence of bile duct damage (x 400 Masson chrome).

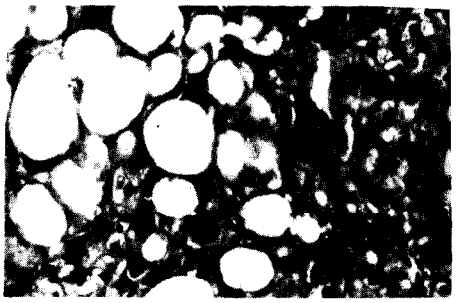

Fig. (2): Focal fatty change showing both micro and macro vascular fatty change (H and E).

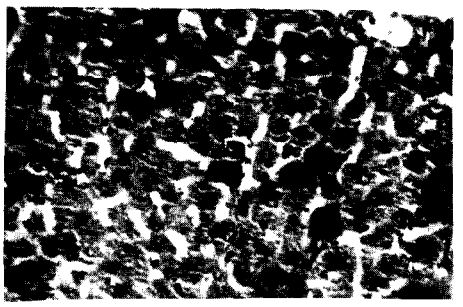

Fig. (3): Scattered single acidophilic cells within liver cell plates with pyknotic nucleus (arrow) (H and E x 400).

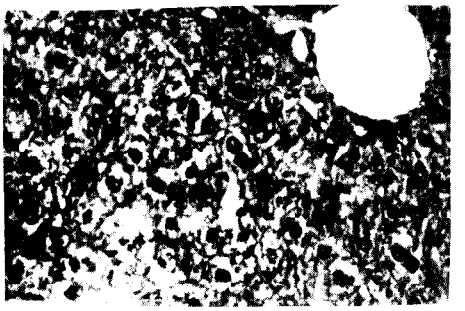

Fig. (4): Binucleated hepatocyte showing features of nuclear dysplasia (arrow) and other scattered dysplastic cells (H and E x 400).

Fig. (5): Mononuclear cells in sinusoids (arrow) (H and E x 400.)

Discussion

After acute infection by HCV, chronic hepatitis develops in approximately 50% of patients and at least 20% of these cases progress to cirrhosis [2].

There have been relatively few studies focusing on clinical presentations of chronic hepatitis C in patients identified by serological markers [19]. We have catalogued the clinical, laboratory and histological features in patients with clear evidence of chronic hepatitis. The results suggest that most of these patients have mild symptoms and a relatively few patients had sought medical attention for specific hepatic disease as jaundice or ascites.

Kamel et al. [20] examined HCV antibodies in 2164 apparently healthy male University Egyptian students, aged 20-27 years, who each donated one unit of blood in 1992. They found positive anti-HCV antibodies by Abbott ELISA in 10.9% of cases, 89% of which were confirmed by supplemental tests. In other countries, the prevalence of HCV antibodies in healthy blood donors ranged from zero to 2% [21].

About 400.000 units of blood are transfused annually in Egypt. Since ELISA confirmed anti HCV positivity is frequently viraemic [22], potentially 10% of the recipients could contract HCV infection.

About 60% of our patients had palpable hepatomegaly and careful physical examination remains important in drawing attention to possible chronic hepatitis C . About 20% of patients in this study had advanced liver disease, 16.7% had histological evidence of cirrhosis. They had splenomegaly, endoscopy proven varices and impaired hepatocellular functions. All of them were above 40 years of age. Disease duration is not important in developing cirrhosis, as it may develop 3 to 5 years after HCV infection [2]. All per se may be one determining factor for prognosis of hepatitis C infection. This may be due to the result of metabolic or immunological changes associated with aging or it may be due to difference in the strains of HCV that infect older patients [23]. Concurrent hepatitis B-infection may be an important factor for the development of cirrhosis. In our study, three patients had both infection with hepatitis B and C.

Hepatitis B is responsible for 40-60% of patients who presented with sporadic acute viral hepatitis in Egypt [24]. The carrier rate for HBsAg ranged between 1.5-10% in Egypt depending upon the age,sex, locality whether rural or urban [25].

Anti-HCV was more likely to be positive in patients positive for any hepatitis B (HBV) markers than in negative subjects [26]. HCV can be transmitted in the same manner as HBV in endemic areas for HBV infection [27].

In our study, few patients had associated positive A.N.A.'s and antismooth muscle antibodies. Ludwig et al. [7] have postulated a recent etiological classification of

chronic hepatitis and patients with both HCV antibodies and positive immunological markers of autoimmune chronic hepatitis were termed cryptogenic or mixed chronic hepatitis. In the study of Nishiguchi et al. [28] of a group of patients with chronic active hepatitis type I, 39% were positive for HCV antibodies by RIBA-II, however, HCV-RNA was detected in only 13% of these patients. Even autoimmune hepatitis type II characterized by positive (LKM-1) was associated with the presence of HCV infection [6]. Papo et al. [29] showed that treatment of autoimmune chronic hepatitis with alpha-interferon on the basis of positive HCV antibodies was followed by aggravation of hepatitis with a rise of serum ALT enzyme and resolved on discontinuation of treatment. Nishiguchi et al. [30] proposed certain recommendations for the use of alpha-interferon in patients with mixed hepatitis. A 4 to 6 month trial of corticosteroids should be considered. In most cases of autoimmune hepatitis, a prompte response will occur, if there is no response, steroids should be "weaned-off" and interferon instituted later. There appears to be no harm in treating chronic hepatitis C with corticosteroids.

To date, HCV has not been detected by electron microscopy in liver cells and there is no analogous hepatocellular changes to the ground-glass HBsAg inclusion by which chronic HCV can be recognized by light microscopy. Histological markers of chronic HCV infection are therefore needed. This study showed, although not exclusive to chronic HCV, the presence of lymphoid aggregated in portal tract, bile duct damage, sinusoidal free lymphocytes, reactive kupffer cells, prominent steatosis and acidophilic changes in hepatocytes increases the likelihood of diagnosis of HCV chronic hepatitis [10]. Pathological differentiation between chronic hepatitis C and autoimmune chronic active hepatitis may be difficult, but the presence of severe piecemeal necrosis, broad areas of parenchymal collapse and plasma cell in the periportal infiltrate all favour autoimmune disease [10].

The pathogenesis of histological lesions in chronic hepatitis C is unknown. Lymphoid aggregates are a feature of a variety of chronic autoimmune diseases and their presence in chronic hepatitis C reflects an immunological reaction. Bile duct damage may be immunologically mediated reaction to bile duct epithelial antigen. Fatty change and acidophilic material in hepatocytes reflect a direct cytopathic effect of HCV on hepatocytes [31].

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