Prevalence of hepatitis C virus among patients with liver disease in the Republic of Yemen

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الخلاصة: قمنا باستقصاء 143 من المرضى بمختلف فئات مرض الكبد و120 من الأشخاص الأصحاء، بحثاً عن واصمات مصلية لفيروسَيْ التهاب الكبد "سي" C و"بي" B. وتبيَّن لنا أن معدل انتشار واصمات أضداد فيروس التهاب الكبد "سي" بلغ 37.1% مقابل 4.2% في المجموعة الشاهدة. وتم اكتشاف وجود المستضد السطحي لفيروس التهاب الكبد "بي" في 33.6% من الحالات، 13.3% في المجموعة الشاهدة. كما تم اكتشاف وجود أضداد فيروس التهاب الكبد "سي" C والمستضد السطحي لالتهاب الكبد "بي" في 7.7% من الحالات، في حين لم يُكتشف وجودهما في المجموعة الشاهدة. كذلك تم اكتشاف وجود أضداد فيروس التهاب الكبد "سي" C، والمستضد السطحي لالتهاب الكبد "بي" HBsAg، وأضداد المستضد اللبي لالتهاب الكبد "بي" B، وأضداد التهاب الكبد "دي" D في ثلاثة مرضى. واتَّضح من نتائج الرحلان الكهربائي لبروتين المصل ارتفاع مستوى الغاماغلوبلين وانخفاض مستوى الألبومين في صفوف المرضى الإيجابيين لأضداد فيروس التهاب الكبد "سي" C من مرضى الكبد.

ABSTRACT We investigated 143 patients with various classes of liver disease and 120 healthy subjects for serological markers of hepatitis C and B viruses. We found a prevalence rate of 37.1% of anti-HCV markers in patients with liver disease (cases), and 4.2% and in the control group. HBsAg was detected in 33.6% of cases and 13.3% of the controls. Anti-HCV and HBsAg were detected in 7.7% of cases but were not detected in the controls. Anti-HCV, HBsAg, anti-HBe and anti-HDV were detected in three patients. Serum protein electrophoresis results showed elevated gammaglobulin and low albumin in patients positive for anti-HCV who had liver disease.

Prévalence du virus de l'hépatite C chez les patients atteints de maladie hépatique en République du Yémen

RESUME Nous avons examiné 143 patients atteints de différentes sortes de maladies hépatiques et 120 sujets en bonne santé à la recherche de marqueurs sérologiques des virus de l'hépatite C et B. Nous avons trouvé un taux de prévalence de 37,1 % des marqueurs anti-VHC chez les patients atteints de maladies hépatiques (cas), et de 4,2 % dans le groupe des témoins. L'antigène de surface de l'hépatite B (HBsAg) a été détecté dans 33,6 % des cas et chez 13,3 % des témoins. Des anticorps anti-VHC et des HBsAg ont été détectés dans 7,7 % des cas mais n'ont pas été détectés chez des témoins. Des anticorps anti-VHC, des HBsAg, des anticorps anti-HBe et anti-HDV ont été détectés chez trois patients. Les résultats de l'électrophorèse des protéines sériques ont montré des gammaglobulines élevées et une albumine faible chez les patients positifs pour l'anti-VHC qui avaient une maladie hépatique.

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Introduction

Hepatitis C virus (HCV), a blood-transmitted non-A, non-B hepatitis (NANBH) virus, is believed to be an important causative agent of liver disease [*1*]. HCV and hepatitis B virus (HBV) infections have been reported all over the world. In Saudi Arabia, anti-HCV has been detected in serum samples of 1.2%–1.7% of adult blood donors and children [*2–4*], compared to 1.9%–3.9% in other countries bordering the Republic of Yemen [*5,6*].

Compared to HCV rates in the normal population, much higher rates have been reported in patients with chronic hepatitis and hepatocellular carcinoma (HCC). A study in Japan showed that 25% of HCC patients were positive for hepatitis B surface antigen (HbsAg). By contrast, 50%–76% of HBsAg negative patients were positive for HCV antibodies (anti-HCV) [7]. Further, a study in patients with HCC in Saudi Arabia reported that the prevalence of anti-HCV was 39.5% [*8*]. In a study conducted in Iraq, anti-HCV was reported in 16.2% of acute viral hepatitis patients compared to 42.8% in patients with chronic viral hepatitis [*9*]. As there are yet no data on the significance of HCV as a causative agent of acute and chronic hepatitis in the Republic of Yemen, we sought to investigate the prevalence of anti-HCV in liver disease patients in the country, and to determine the role of HCV — alone or in conjunction with other hepatitis viruses — in liver diseases.

Methods

Serum samples from 143 patients (92 males and 51 females) with acute and chronic liver diseases were collected at the University Hospital and private clinics in Sana'a, Republic of Yemen. Patients ranged in age from 12 years to 62 years (mean 42.3 ± 13.5 years) [*10*]. Diagnoses of acute viral hepatitis and chronic viral hepatitis were made on the basis of clinical assessment, duration of illness, laboratory investigations, abdominal ultrasounds and, in some cases, gastroscopy and liver biopsy. Samples from 120 healthy controls (72 males and 48 females) with no history of liver disease were also collected. The age range of the control group was 9–61 years (mean age: 31.5 ± 12.6 years) [*10*].

Serum samples collected were tested as follows

- Micro ELISA assay using two different kits: one kit (Randox Laboratories Limited, United Kingdom) was used for the detection of anti-HCV, HBsAg, hepatitis D virus antibodies (anti-HDV) and anti-HBe markers, and another kit was obtained from the Crescent Company (Saudi Arabia) for the detection of anti-HCV.
- Liver function and total serum bilirubin tests were performed using kits obtained from Randox Laboratory Limite. Abnormal serum protein patterns were studied electrophoretically as previously described [*11*].

Results

Serum anti-HCV in patients with liver disease

Serum samples from 143 patients with liver disease were tested for the presence of anti-HCV. Table 1 shows the prevalence of HCV among patients with liver disease and the control group. A significantly higher percentage of cases (37.1%) showed the presence of anti-HCV compared to controls (4.2%) ($\chi^2_1 = 41.08$, $P = 0.0000$). Moreover, the presence of liver disease was 13.54 times more likely to be associat-

Table 1 Prevalence of anti-HCV in 143 patients with liver disease

Disease status	Anti-HCV			
	Positive		Negative	
	No.	%	No.	%
Liver disease	53	37.1	90	62.9
Acute viral hepatitis	12	21.4	44	78.6
Chronic viral hepatitis	38	61.3	24	38.7
Others[a]	3	12.0	22	88.0
Controls	5	4.2	115	95.9

[a]*Patients with cryptic hepatitis or autoimmune disease.*
χ^2_1 – *41.00*, P – *0.0000*.

ed with anti-HCV positivity (odds ratio = 13.54, 95% confidence interval; 5.1–44.84). Positivity for anti-HCV was more frequently observed among patients with chronic viral hepatitis (61.3%) followed by those with acute viral hepatitis (21.4%). However, only 12.0% of patients with cryptic hepatitis or autoimmune disease were positive for anti-HCV. Relative to the latter, there was a twofold risk of anti-HCV among those with acute viral hepatitis and a greater than elevenfold risk among those with chronic viral hepatitis (Table 1).

Table 2 shows that anti-HCV in the acute viral hepatitis group were detected in 7 males and 5 females (mean age: 25.6 years), compared to 26 males and 12 females (mean age: 38.9 years) in the chronic viral hepatitis group. Anti-HCV in other groups of hepatitis was detected in three patients (two males and one female; mean age: 41.2 years), albeit in a small sample.

Table 2 **Distribution of the 53 patients with liver disease positive for anti-HCV and controls according to age and sex**

Disease	No.	Sex		Mean age (years)
		Male	Female	
Liver disease	53	35	18	35.2
Acute viral hepatitis	12	7	5	25.6
Chronic viral hepatitis	38	26	12	38.9
Others	3	2	1	41.2
Controls	5	3	2	32.4

Serological patterns in patients with liver disease

Table 3 shows that the four different patterns of serological markers depicted by anti-HCV, HBsAg, anti-HBe and anti-HDV were found. Pattern 1: anti-HCV was the only positive marker in 53 (37.1%) patients with liver disease; pattern 2: Anti-HCV and HBsAg were the two markers in 11 (7.7%) patients with acute viral hepatitis and chronic viral hepatitis; pattern 3: HBsAg is the only positive marker detected in 48 (33.6%) patients with acute viral hepatitis and chronic viral hepatitis; pattern 4: anti-HCV, HBsAg, anti-HBe and anti-HDV markers were concomitantly shown in three (2.1%) patients with chronic viral hepatitis.

Liver function tests

Tables 4 and 5 show the mean values of alanine aminotransferase (ALT) (IU/L) and total serum bilirubin (TSB) (mg/dL) respectively in patients with liver disease who were positive for one or more of the serological markers anti-HCV, HBsAg, and anti-HDV. Mean ALT and TSB values for patients with acute viral hepatitis who were positive for anti-HCV, HBsAg, or both anti-HCV and HBsAg were 98.2, 81.5 and 79.0 IU/L respectively, and 78.4, 70.6 and 61.5 IU/L respectively in chronic viral hepatitis patients. The three markers were concom-

Table 3 **Serological pattern in patients with liver disease**

Pattern	No. of patients	Anti-HCV	HBsAg	HBeAg	Anti-HBe	Anti-HBc	Anti-HBs	Anti-HDV
1	53	+	–	–	–	–	–	–
2	11	+	+	–	–	–	–	–
3	48	–	+	–	–	–	–	–
4	3	+	+	–	+	–	–	+

HBsAg was detected in 16/120 (13.3%) of the controls.

Table 4 **Mean values of alanine aminotransferase (ALT) in patients with liver disease according to serological pattern**

Disease type	No. of patients	Anti-HCV		HBsAg		Anti HCV, HBsAg		Anti-HCV, HBsAg and anti-HDV	
		No.	ALT	No.	ALT	No.	ALT	No.	ALT
AVH	56	12	98.2	23	81.5	2	79.0	–	–
CVH	62	38	78.4	17	70.6	9	61.5	3	6.8
Others	25	3	61.4	–	–	–	–	–	–

ALT measured in U/L; normal value of ALT = 10–20 U/L.
AVH = acute viral hepatitis.
CVH = chronic viral hepatitis.

itantly positive in only three chronic viral hepatitis patients with a mean ALT value of 6.8 IU/L (Table 4). Mean TSB levels in acute viral hepatitis patients who were positive for anti-HCV, HBsAg, and anti-HCV and HBsAg were 10.8, 6.4, and 6.8 mg/dL respectively, and 6.8, 4.7 and 3.8 mg/dL respectively in chronic viral hepatitis patients (Table 5). Similar to Table 4, the three markers were co-positive in only three CVH patients (mean 4.6 mg/dL). Finally, mean ALT and TSB values of 61.4 IU/L and 3.6 mg/dL respectively were shown in the "others" patient group.

Serum protein electrophoresis patterns in HCV patients

Electrophoretic protein abnormalities are frequently encountered in liver disease patients. Compared to a normal individual (Figure 1), elevated levels of gammaglobulins and albumin, depicting a cirrhotic pattern, were seen in HCV-positive patients (Figure 2).

Discussion

Hepatitis virus and liver disease

Previous studies have shown that HCV is an important agent in causing serious liver disease [*12*]. The prevalence of anti-HCV in the general population of the Republic of Yemen has not been established and is the subject of the current study. Results obtained in our study indicate that the prevalence of HCV, as depicted by anti-HCV, in patients with various classes of liver dis-

Table 5 **Mean values of total serum bilirubin (TSB) in patients with liver disease according to serological pattern**

Disease type	No. of patients	Anti-HCV		HBsAg		Anti-HCV, HBsAg		Anti-HCV, HBsAg and anti-HDV	
		No.	TSB	No.	TSB	No.	TSB	No.	TSB
AVH	56	12	10.8	23	6.4	2	6.8	–	–
CVH	62	38	6.8	25	4.7	9	3.8	3	4.6
Others	25	3	3.6	–	–	–	–	–	–

TSB measured in mg/dL; normal value of TSB = 0.1–1.0 mg/dL.
AVH = acute viral hepatitis.
CVH = chronic viral hepatitis.

ease is significantly higher than that seen in the control group; (53/143) 37.1% and 5/120 (4.2%) respectively (Table 1). This finding suggests that HCV infection may play an important role in patients with liver disease in the Republic of Yemen, which is in keeping with earlier observations in European and Middle Eastern patients [*8,13–15*]. This frequency is also similar to that found in a number of tropical areas such as Cameroon and in Iraq, where anti-HCV was detected in 15% and 16.2% of patients with acute viral hepatitis respectively [*9,16*]. This may be explained by the similarity in contracting the virus under similar exposure conditions. However, the above observations contrast with data from the United States, where anti-HCV has been detected in less than 3% of acute viral hepatitis cases [*16*]. This discrepancy could be due to the introduction of the screening programme for blood donors and donors' blood units in the United States, and subsequent elimination of anti-HCV-positive blood units, thereby decreasing the prevalence of HCV in acute viral hepatitis patients in the United States [*16*]. As HCV is a persistent infection, in previous studies the disease has been reported in at least half of all chronic hepatitis patients [*17,18*]. This is in agreement with our findings (Table 1), in which we found a prevalence of HCV in chronic viral hepatitis patients of 61.3% (38/62). It also accords with the 65% prevalence reported in south-western Saudi Arabia [*19*], which can be explained by the high endemicity of the virus in that area. The prevalence of HCV in 12% of the "others" group (autoimmune or cryptic hepatitis) warrants further investigation to clearly identify the causative agents.

Serological markers anti-HCV and anti-HDV

The well-known feature of concomitant infection by multiple hepatotropic viruses such as hepatitis A, B and D agents is supported by the results obtained in our study (Table 3). HBsAg alone was detected in 48 patients with acute viral hepatitis and chronic viral hepatitis. Moreover, three patients were shown to be positive for anti-HCV, HBsAg, anti-HBe and anti-HDV. From these results, we noted four different patterns as shown in Table 3. Coexistence of anti-HCV and HBsAg with no other markers of HBV (pattern 2) suggests that there might be super-infection by HCV. This requires further investigation of HBV DNA to demonstrate whether the virus is replicat-

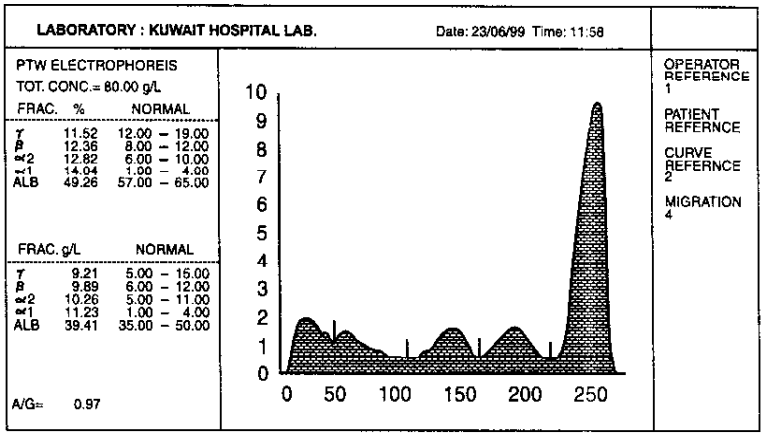

Figure 1 **Serum protein electrophoresis for healthy individuals**

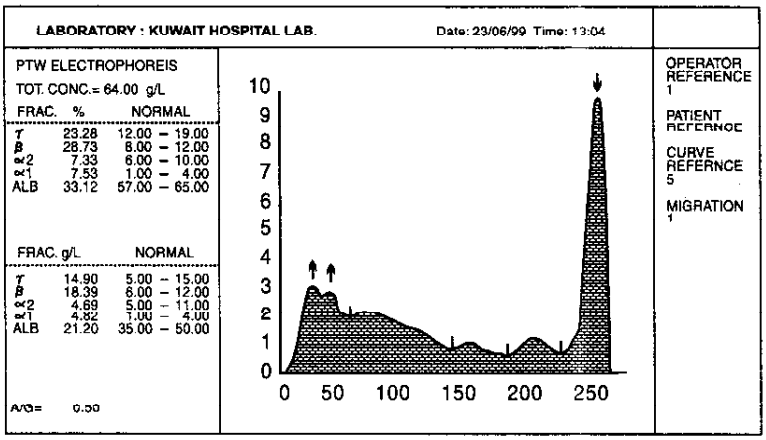

Figure 2 **Serum protein electrophoresis for patients with liver disease and positive for HCV antibody**

ing or not [*20*]. Pattern 4 (anti HCV, HBsAg, anti-HBe and anti-HDV) shows the coexistence of three virus markers in the same patient. The significance of such findings has not, to the best of our knowledge, been reported in previous investigations, although infection by multiple hepatotropic viruses such as HAV, HBV and HDV is well known [*9*]. Further, the detection of anti-HDV (Table 3) indicates the

possibility of chronic HDV-agent infection followed by super-infection by HCV, possibly occurring through the use of contaminated blood. Co-infection with HCV cannot be excluded, but this needs the development of specific testing which can differentiate between markers of recent or past HCV infections [*12*]. In such cases, we propose that HCV and HDV may synchronize their harmful effects on the liver. Also, the presence of HBsAg may be due to the fact that HDV is a defective virus that depends on HBV for its survival [*21*]. Anti-HBe were detected in the sera of some patients, which indicates that HBV was not in a replicative state due to suppression by HDV, but this suppression is usually transient [*22,23*]. From the above findings, it could be suggested that infection with both viruses may suppress HBV replication.

The liver function test results (ALT and TSB) obtained in our study (Tables 4 and 5) for anti-HCV-positive patients can be explained in that HCV may exist in two forms. It either plays an oncogenic role, with infected hepatocytes rendered neoplastic and the patient may progress to HCC, or the infected hepatocytes that were injured will progress to an inflammatory reaction resulting in hepatitis and its sequelae. The results could also suggest different individual responses to HCV infection possibly due to genetic predisposing factors [*22*]. Most patients who were anti-HCV-positive often had elevated levels of ALT and TSB. This finding could be indicative of liver cell damage, although anti-HCV was not necessarily coincident with elevated ALT and TSB, as there is a considerable interval "window" between HCV exposure and subsequent detection of anti-HCV in patients with acute HVC infection [*23*].

Serum protein electrophoresis

Results in Figure 2 show that patients with different types of liver disease, especially those with hepatic cirrhosis and who had anti-HCV and/or anti-HCV, HBV and anti-HDV, can have polyclonal gammopathy with a broad elevation of gammaglobulins with reduction of albumin. These results are in agreement with previous results [*11*], in which elevations of gammaglobulins with concomitant reduction of albumin was observed in hepatic cirrhosis due to HCV infection.

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