ACTA MÉDICA PORTUGUESA 1994; 7: 31-34

HEPATITIS C VIRUS ANTIBODIES IN ASYMPTOMATIC CHRONIC CARRIERS OF HEPATITIS B SURFACE ANTIGEN

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SUMMARY

The objectives of the study were to evaluate the prevalence, incidence and clinical significance of antibodies to hepatites C virus in HBsAg chronic carriers. The evaluation of stored sera was combined with the follow up of a cohort of cases observed in a referral-based university hospital. A total of 183 HBsAg asymptomatic chronic carriers were indentified during routine sceening and followed for a mean period of 3.8 years. Stored sera and sera obtained during follow-up were tested for anti-HCV using ELISA. Second generation RIBA (Ortho) was used as a possible confirmatory test. Demographic data and risk factors were assessed using a standard questionnaire. The prevalence of HCV infection in HBsAg chronic carriers was 2,7% (95% CI: 1,2%-6 3%), higher in males than females (3,1% vs 1,8%, p=0,52) and also higher than that found in voluntary blood donors from the same region. Only 3 out of 5 ELISA-positive cases were RIBA-positive. Patients positive for both types of virus more frequentely admitted drugabusers. The presence of anti-HCV was not significantly related to the histological severity. During follow-up no new cases of infection were found. Conclusions: The prevalence of HCV infection in asymptomatic chronic carriers is higher than in blood donors but lower than previously reported for other populations of chronic hepatitis B cases. HCV infection was not found responsible for the frequency or the type of lesions observed in these HBsAg chronic carriers.

INTRODUCTION

When it was possible to show, in an unequivocal way, the existence of acute and chronic hepatitis occurring in the absence of serum markers of infection by the hepatitis B virus (HBV), hepatitis A [1,2] or other known viral agents, and after ruling out toxic, metabolic or auto-immune causes, different epidemiological studies enhance similarities between hepatitis B and some of these forms of hepatitis designated as non-A, non-B (NANB). In western countries the importance of NANB hepatitis was particularly recognized in relation with blood transfusions and its products. However, in a high proportion of the cases diagnosed there was no history of transfusions nor apparent risk of parenteral transmission [3]. Recently, it was possible to characterize the genome of the agent responsible for the majority, if not the totality, of NANB infections transmitted by parenteral routes, designated as the hepatitis C virus (HCV), and develop serologic tests for the identification of anti-HCV antibodies [4,5].

With the successive generations of laboratory techniques available [6], it was possible to confirm that, as what occurs with HBV, HCV is transmitted by parenteral routes but is also frequently present in the absence of a history of transfusions, hemodialysis, intravenous drug abuse or occupational exposure [7,9]. It is possible that family or sexual transmission may play an important role in maintaining the infection, however, as yet there are no studies which allow a conclusive quantification of the risk of infection by HCV in these circumstances [10].

The epidemiological similarities would lead to the supposition that HCV infection would be frequently detected in subjects with HBV infection. In fact the initial studies [11-16] showed a high prevalence of anti-HCV in patients who were positive for HBV surface antigen (HBsAg) even suggesting that HCV suppressed HBV replication, its combined presence being associated with more severe hepatic lesions [14,15]. Also in Portugal, in a group of 121 patients with chronic hepatitis B, a prevalence of anti-HCV of 16,4% [16] was shown by first generation ELISA.

Received for publication: 20 August, 1992

In this paper we assessed the prevalence and eventual clinical significance of anti-HCV antibodies in chronic asymptomatic carriers of HBsAg, as well as the incidence of HCV infection after the identification of the state of chronic carrier of HBsAg.

METHODS

We studied 183 chronic asymptomatic carriers of ABsAg, identified by family screening, during voluntary blood donations, the prenatal period or in routine tests. Only cases detected before the systematic research of anti-HCV were included to avoid a selection bias. All the subjects were given a detailed questionnaire to assess the risk factors for infections by HBV and by HCV. None of the patients admitted homosexual or bisexual behavior. The average follow up time was 3,8 years (from 16 months to 64 months), corresponding to 695 persons - year.

Anti-HCV was retrospectively tested on serum kept at -20ºC, not previously defrosted, obtained on the date the cases were identified. Serums gathered during the follow up of the patients were also tested.

The biochemical parameters were obtained by routine automated techniques. The dosage of hialuronate (HA) was measured by a sequential radiometric method (HA Pharmacy Test, Uppsala, Sweden) based on a matching test. The serum markers of HBV infection and hepatitis delta virus (HDV) and of human immunodeficiency (HIV1 and HIV2) were studied by micro-ELISA (ORGANON TEKNICA or BEHRING). Anti-HCV was studied by ELISA (first and second generation) and the positive cases, as well as a sample of negatives, assessed by second generation *Recombinant immunoblot assay* (RIBA) (ORTHODIAGNOSTIC).

A liver biopsy was performed on 86 patients (47%). The histological findings were classified according to the recommendations of an international group and graded according to the index of activity proposed by Knodell [17,18]. The HBV core antigen (HBcAg) was studied, in fixed sections, by immuno-histochemistry (avidine-biotine complex).

The study of the results was made with the Epi Info program [19]. Continuous variables were compared by the Wilcoxon test and the frequencies by the exact Fisher test. The associations were assessed by the odds ratio (OR) and respective 95% confidence intervals.

RESULTS

183 chronic asymptomatic carriers of HBsAg were assessed, 56 women and 127 men, with a mean age $\pm$ standard deviation of 30,1 $\pm$ 10,9 and 32,6 $\pm$ 11,5 years respectively (p=0,19). Anti-HDV and antibodies for HIV1 and HIV2 were absent in all cases. In all of the chronic HBsAg carriers, HBeAg was present in 34 (18,6%) and transaminase activity was abnormal in 59 (32,2%).

Anti-HCV, studied by ELISA, was present in 5 cases (2,7%, 95% confidence interval: 1,2% - 6,3%). The comparison of the anti-HCV prevalences in men (4/127, 3,1%, 95% confidence interval: 1,2 - 8,0%) and women (1/56, 1,8%, 95% confidence interval: 0,3% - 9,7%) did not reveal any significant differences between sexes (p=0,52). The five positive cases (4 men and 1 woman) presented reactivity in the first and second generation ELISA tests; 3 were also positive by RIBA, one was undetermined (the only female case) and the other negative. RIBA was also negative in 34 cases in which the ELISA tests had not been positive. Only acknowledging as true positives those in which the anti-HCV study is confirmed by RIBA, the prevalence of HCV infection in chronic HBsAg carriers was only 1,5% (3/183, 95% confidence interval: 0,5% - 4,8%)

No new case positive for anti-HCV was observed during the follow up. The 95% confidence interval for the incidence observed (0/695) varies between 0 and 0,1 $*10^{-3}$ year $^{-1}$.

The demographic, biochemical, serologic and histologic characteristics of the cases studied are presented in Table 1 according to the result of the anti-HCV study by ELISA. Due to the small number of positive subjects, the cases were not compared separately according to RIBA reactivity. In this cohort of chronic HBsAg carriers only the intravenous use of illegal drugs was significantly more frequent in anti-HCV positives, no significant differences being detected in relation to the other parameters assessed.

Table 1 – Demographic, biochemical and serologic characteristics of 183 chronic HBsAg carriers, in relation to the result of the antibody study for HCV (ELISA)

	HCV positive n=5	**HCV negative n=178**	**p**	**OR (SM at 95%)**
Age	30,2+3,7	31,8+11,5	0,98	1,8(0,2-90)
M/F	4/1	123/55	0,52	2,0(0,2-99)
Married	4/5	119/178	0,47	0,0(0,0-10)
Tattoos	0/5	18/178	0,59	0,0(0,0-21)
Transfusions	0/5	2/178	0,94	
IV drugs	1/5	0/178	0,027	
ALT1	49,2+20,1	45,1+48,3	0,14	
AST1	33,0+7,0	32,7+21,7	0,20	
GT1	26,4+12,0	31,4+39,9	0,60	
Bilirrubin2	0,6+0,0	0,7+0,3	0,82	
Albumin3	52,6+6,0	47,0+3,0	0,22	
Gamma Corp.3	10,0+2,4	10,5+2,7	0,78	
Hialuronate4	13,7+3,9	29,8+43,2	0,23	
HBeAg pos.	1/5	33/178	0,65	1,1(0,0-12)

1UI/L 2mg/L 3g/L 4ug/L

In the same way for the variables described in Table 1, no significant differences were observed in the proportion of cases with alanine aminotransferases (3/5 vs 50/178, p=0,15) and aspartate (1/5 vs 36/178, p=0,73) or gamma glutamiltranferase (0/5 vs 23/178, p=0,51)

above the normal limits. No significant differences were detected in the distribution of cases by histological results (Table 2) nor in the proportion of those with abnormal histology, that is, cirrhosis, chronic active or persistent hepatitis, and normal histology or presenting non-specific alterations, in relation to anti-HCV reactivity (4:1 in the positives vs 37:44 in the negatives). Withal, the OR calculated is 4,8 (95% confidence interval: 0,4-239), clearly deviating to the right, suggesting a limitation of the sample size in the detection of a true difference.

DISCUSSION

In this cohort of chronic asymptomatic HBsAg carriers, accidentally detected, the prevalence of HCV antibodies, assessed by ELISA, was 2,7%. This figure is inferior to those described in other populations of patients with chronic hepatitis B in which prevalences of 11%[15], 16%[11], 17%[12], 25%[13], 26%[16] and 36%[14] were stated. Withal, in those studies, the samples assessed were above all composed of clinically recognized patients, some cases with acute hepatitis, HIV infection or hepatoma, without an assessment of the ensuing risk of eventual hospitalizations and transfusions which the gravity of the disease may have determined. In the cases in which they were considered separately in the so called healthy carriers, they presented distinctly inferior prevalences[1]. In the previous Portugese study [16], in which the prevalence of anti-HCV was approximately 10 times higher than what we now found, patients with normal histology were not included and the proportion of drug addicts was higher than in our sample.

Table 2 – Relationship between the histologic characteristics and anti-HCV reactivity (second generation ELISA) in chronic asymptomatic HBsAg carriers

	HCV positive n=5	HCV negative n=81	p	OR (SM at 95%)
Cirrhosis	0	8		
Active HC	0	2		
Persist. HC	4	27		
Minimal lesions	0	33		
Normal	1	11	0,23	
HBcAg positive	0/3	16/68	0,46	0,0(0,0-11)
Knodell index	1,3+1,5	2,1+1,9	0,46	

The frequency of HCV infection detected by us could be due to the fact that the population assessed consists of asymptomatic HBsAg carriers and therefore naturally implies a biased selection favouring the less serious cases, those expected to correspond to the ones in which a single agent of infection is present. However, it is worth pointing out that in this sample 10% of the patients who were submitted to liver biopsy presented cirrhosis, although no significantly more severe histologic forms were detected in the cases in which the anti-HCV was present (Table 1). In addition, the average value of hialuronate, a useful marker in the assessment of hepatic fibrosis [20], was similar in both groups. This study can not exclude the possibility of a true disadvantage existing for the cases with infection by both viruses, which presented an OR of 4,8 for severe histologic lesions. In patients with chronic renal insufficiency, for example, we observed that the association of HBsAg and anti-HCV was followed by more severe hepatic disease, measured according to biochemical and histologic indexes [21].

It was not possible to do a serum determination of the HBV DNA, so there is no direct measure of the eventual effect of the presence of HCV infection in the replication of HBV. However, we studied the presence of HBcAg in the liver, detecting higher percentages of positivity in the cases negative for anti-HCV (24% vs 0%). However, this difference was not significant, both groups having a similar serum prevalence of HBeAg (18% vs 20%). Previous studies conducted on experimentally infected chimpanzees [22] and observations of humans infected with both viruses suggested that hepatitis C could suppress HBV replication [14,16]. Our results were not able to confirm this influence of HCV infection in the expression of HBV markers, although they should be assessed with caution due to the low proportion of positive anti-HCV cases detected by us as well as the well known disparity which exists between replication and HBV antigen expression [23].

The prevalence of anti-HCV in this population of chronic HBsAg carriers was higher than that observed in a group of 4924 volunteer blood donors, assessed at the Immuno-hemotherapy Department, S. João Hospital (H. Alves, H. Barros, C. Koch, et al. Anti-HCV ELISA and RIBA reactivity in Portuguese blood donors and dialysis patients, submitted). Despite eventual differences in the general characteristics of the population considered, it seems possible to affirm that the presence of HBsAg is associated with a greater risk of HCV infection (5/183 vs 50/4924, OR=2,8 (95% confidence interval: 1,0-7,3, p=0,045). However, no new case of HCV infection was detected, suggesting that the risk of exposure is relatively low, although higher than that of groups such as volunteer blood donors.

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