short report
Haematologica 1996; 81:162-164

EVALUATION OF HEPATITIS B AND C VIRUS INFECTIONS IN PATIENTS WITH NON-HODGKIN'S LYMPHOMA AND WITHOUT LIVER DISEASE

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ABSTRACT

Hepatitis B virus (HBV) and hepatitis C virus (HCV) are hepatotropic and lymphotropic viruses endemic to Sicily. To evaluate whether these viruses may chronically infect patients with non-Hodgkin's lymphoma (NHL) and without liver disease, we examined serum samples from 24 such patients. Five cases (20.8%) revealed HCV infection, as shown by the detection of viral RNA through the polymerase chain reaction technique, while HBV-DNA was not found in any of them by the same method. These results provide one more epidemiological element supporting the hypothesis that the association between HCV infection and lymphoproliferative diseases is not a casual event, and show that HCV may chronically infect patients with NHL without producing liver damage.

Key words: hepatitis B and C virus, non Hodgkin's lymphoma

Hepatitis B virus (HBV) and hepatitis C virus (HCV) are recognized to be responsible for most of the cases of acute and chronic hepatitis worldwide. Both these viruses are also lymphotropic[1,2] and may be associated with mixed cryoglobulinemia (MC),[3-5] which is a lymphoproliferative disease that evolves, in a certain number of cases, into non-Hodgkin's lymphoma (NHL). In particular, HCV is believed to be the major causative agent of MC;[4,5] furthermore, Ferri *et al*. have recently found a high prevalence of markers of HCV infection in patients with non-Hodgkin's lymphoma and biochemical features of liver disease.[6] In the above mentioned studies, HCV infection was evaluated through detection of both specific antibodies (anti-HCV) and viral RNA (HCV-RNA) in the blood of the patients. In contrast, HBV infection has been investigated only by testing serum HB surface antigen (HBsAg), whereas it is well known that this infection may go unnoticed and HBV may persist in HBsAg-negative subjects at low levels of replication.[7]

In the present study we evaluated whether HCV and/or HBV infections, which are endemic to our geographic area, may exist in patients with NHL even in the absence of liver damage and, in this case, whether the prevalence of evident or inapparent HBV infection is comparable to that of HCV infection.

Patients and Methods

We retrospectively examined serum samples from 24 patients admitted to our Department because of NHL (see Table) and selected on the basis of: a) the absence of a history of major surgery, blood transfusions, drug or alcohol abuse; b) negativity for human immunodeficiency virus antibodies; c) persistently normal liver biochemistry and normal liver sonography. Three different tubes, containing 1 mL of serum each, had been stored (–20°C) for each

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Received November 27, 1995; accepted February 15, 1996.

Table 1. Clinical features and serum markers of HBV and HCV infection in the study group.

		NHL	*HBV*			*HCV*	
N°	Age/Sex	Grade	HBsAg	Anti–HBc	HBV–DNA	RIBA	HCV–RNA
1	54/F	I	–	–	–	+	+
2	81/F	L	–	+	–	–	–
3	88/M	L	–	+	–	–	–
4	55/M	I	–	–	–	–	–
5	46/F	I	–	–	–	–	–
6	71/M	L	–	–	–	–	+
7	66/M	L	–	–	–	–	–
8	27/F	H	–	–	–	–	–
9	63/F	L	–	–	–	–	–
10	19/F	L	–	–	–	–	–
11	84/M	L	–	–	–	–	–
12	82/F	L	–	–	–	–	–
13	69/F	L	–	+	–	–	–
14	48/M	I	–	+	–	–	+
15	78/F	L	–	–	–	–	–
16	45/M	L	–	–	–	–	–
17	62/F	L	–	–	–	–	–
18	72/M	L	–	–	–	–	–
19	58/F	L	–	–	–	–	–
20	47/F	L	–	+	–	–	–
21	36/F	L	–	–	–	–	–
22	57/M	L	–	–	–	+	+
23	56/F	L	–	+	–	–	+
24	50/M	I	–	–	–	–	–

L=low; I=intermediate; H=high.

patient at the time of NHL diagnosis, which was made by examination of lymph node biopsy specimens evaluated according to the Working Formulation classification.

All the serum samples were tested for anti-HCV antibodies by the RIBA test (Chiron Corp., Emeryville, CA, USA) and for HCV-RNA through reverse transcription of RNA extracts followed by *nested* polymerase chain reaction (PCR) amplification using 2 sets of primers from the 5' non-translated region of the viral genome. Only those samples found to be HCV-RNA positive in three distinct experiments performed on sera collected in different tubes were considered as true positive cases. Moreover, HCV was genotyped according to Okamoto *et al.*[8] The serum samples were also tested for HBsAg and antibody to the HB core antigen (anti-HBc) by commercial kits (Abbott, Chicago, IL, USA), and for HBV-DNA through a PCR technique using two sets of primers encompassing, respectively, the preC/C and the preS regions of the viral genome. The HBV primers had the following 5'- and 3'- nucleotide positions: precore/core amplification = 1777-1805, 2477-2558; preS amplification = 1928-1945, 174-154 (numbering from the Eco R1 unique site of the viral genome).

Results and Discussion

Anti-HCV was detected in 2 cases (8.3%), while 5 patients (20.8%), including the two anti-HCV-positive subjects, proved to be positive for HCV-RNA (see Table). One of the two individuals found to be anti-HCV/HCV-RNA positive agreed to undergo liver needle biopsy, and histological examination revealed normal liver tissue. The finding that 3 out of the 5 HCV-RNA-positive cases were anti-HCV negative might be due to defects in the host immune response, commonly observed in patients with lymphoma, and confirms the relevance of molecular biology techniques for correct evaluation of viral infections in this kind of patient. HCV genotyping showed that genotype II, which is the one most commonly observed in Sicily,[9] was detected in all 5 positive cases (a mixture of genotype II + IV was found in 1 case). In contrast, HBV analyses led us to exclude both *apparent* and *inapparent* HBV infection in all 24 patients, since neither HBsAg nor HBV-DNA was found in the serum of any of them and the anti-HBc positivity detected in 6 cases (see Table) was, presumably, just a marker of previous exposure to HBV.

Summarizing, these results show a high prevalence of HCV infection in patients with NHL, while none of these patients presented evidence of persistent HBV infection. Surprisingly, none of the HCV carriers developed liver disease, although all of them were infected by viral genotype II, which has been reported to be associated with the most severe forms of type C chronic hepatitis.[10] Finally, considering that the prevalence of HCV and HBV infections are very

similar in the general Sicilian population but completely different in patients with NHL, our study provides one more epidemiological element supporting the hypothesis that HCV may be involved in the development of NHL, although more extensive studies are necessary to clarify the possible role played by the virus in the pathogenesis of this disease.

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