Histological and immunohistochemical study of hepatitis B virus in human immunodeficiency virus infection

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Abstract

Because the risk factors for human immunodeficiency virus (HIV) infection and hepatitis B (HBV) are similar and therefore coinfection is not uncommon, a detailed histological and immunohistochemical study of chronic hepatitis B infection in a group of 20 HIV positive Caucasian males (who did not have AIDS) and 30 HIV negative controls was undertaken. Using both the conventional histological classification and the Knodell histological activity index it was shown that HIV negative patients were more likely to have active disease and also more scarring than HIV positive patients. Hepatitis B surface antigen (HBsAg) expression was not significantly different between the two groups but expression of hepatitis Be antigen (HBeAg) and HBV-DNA polymerase was greater in those who were HIV positive. HIV positive patients are therefore more likely to have immunohistochemical markers of active viral replication, although histologically, liver disease is less severe.

These findings have important implications for assessing the biopsy specimens in this group of patients and for treatment strategies aimed at improving their immune function.

Liver injury sustained as a result of hepatitis B virus (HBV) infection depends on viral replication and immune lysis of infected hepatocytes by T lymphocytes.¹ Infection with HBV and human immunodeficiency virus (HIV) are found in similar risk populations such as homosexuals and intravenous drug addicts and coinfection with these viruses is therefore common. It is known that infection with HIV produces immunosuppression and, in particular, results in diminished T lymphocyte function. Coinfection with HIV may therefore modify the natural history of the HBV infection. Studies have shown that in HIV positive patients there is increased HBV antigen display with a trend towards a milder histological grade of liver disease and improvement in biochemical markers of liver cell damage.²⁻⁵

Our aim was to undertake a detailed histological and immunohistochemical study of the effect of coinfection with HBV and HIV to see in which ways the histological picture of liver disease varied and how markers of HBV infection and replication differed.

Methods

Patients chosen for the study were male Caucasians who were known to have had positive serology HBV surface antigen (HBsAg) for longer than six months. The patients had been tested for HIV antibodies using a commercial enzyme linked immunosorbent assay (ELISA) technique (Wellcome Laboratories, Beckenham, Kent). These comprised 20 HIV positive men (age range 27-45 years, mean 43 years) and 30 HIV negative men (age range 34-63 years, mean 48 years). None of the HIV positive patients had AIDS as defined by the American Centers for Disease Control criteria.⁶

Liver biopsy was performed under local anaesthetic with a Menghini needle. The core of liver was fixed for at least 24 hours in 10% unbuffered formal-saline, processed, embedded in paraffin wax and 20 sections 6 µm thick were cut. Sections were stained with haematoxylin and eosin, haematoxylin van Gieson, diastase and periodic acid Schiff, orcein, Masson's trichrome, reticulin and Perls's stain. The biopsy specimens were assessed histologically and placed into standard disease categories (chronic persistent hepatitis (CPH), chronic lobular hepatitis (CLH), and chronic active hepatitis (CAH) with or without cirrhosis).⁷ Each biopsy specimen was scored separately (0-4) for necrosis, inflammation, and fibrosis to give a total Knodell histological activity index ranging from 0-12.⁸

IMMUNOHISTOCHEMICAL PROCEDURES

Unstained paraffin wax embedded sections were dewaxed in xylene and decreasing concentrations of alcohol to water. Endogenous peroxidase was blocked by incubation of the sections with 1% hydrogen peroxide in methanol for 15 minutes. No protease or trypsin digestion was required. Sections were washed in TRIS-buffered saline (TBS), 0.05M (pH 7.6) and then incubated in normal swine serum (diluted 1/5 in TBS) for 15 minutes, and then washed again in TBS three times. The sections were incubated with undiluted primary antibody for two hours. The antibodies used were murine monoclonal antibodies prepared against HBsAg and HBeAg⁹ and a rabbit polyclonal antibody prepared against HBV DNA polymerase. After another wash in TBS the sections were incubated for 30 minutes with 1/300 biotinylated rabbit anti-mouse immunoglobulin or swine anti-rabbit (both from Dako), washed again in TBS, and incubated for a further 30 minutes with avidin

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Table 1 Comparison of Knodell histological activity index in HIV positive and negative male Caucasians with chronic HBV infection

Histological activity index	HIV positive (n = 20)	HIV negative (n = 30)
Inflammation necrosis	Median = 3 (range 1-5)	Median = 6 (range 2- 8)
Fibrosis	Median = 0 (range 0-4)	Median = 1.5 (range 0- 4)
Total histological activity index*	Median = 3 (range 1-7)	Median = 9 (range 3-11)

*p < 0.01.

biotin complex (1 µl of avidin and 1 µl of peroxidase conjugated biotin in 125 µl of TBS). The slides were washed in TBS and the peroxidase was developed with a solution of 10 mg of diaminobenzidine in 10 ml of TBS and 0.08 ml of 3% hydrogen peroxide for 10 minutes. A known positive and negative control were incorporated in each batch. The sections were counterstained with haematoxylin, dehydrated, and mounted.

The results of the immunohistochemical techniques were graded semiquantitatively depending on the proportion of hepatocytes that stained (0 = no staining, 1 = 0-10% of cells, 2 = 10-25% of cells, 3 = 25-50% of cells and 4 = more than 50% of cells). Sections were also stained with a polyclonal antibody raised against the δ antigen of the hepatitis δ virus (HDV). No cases of positive staining for δ antigen were found. Staining with a murine anti-HBc antibody was unsuccessful as would be expected on formalin fixed tissue rather than frozen sections.^{2 10}

The results were analysed using the Mann-Whitney U test for non-parametric data, a χ^2 test, and a rank correlation coefficient.^{7 11}

Results

The histological findings are summarised in tables 1 and 2 and the immunohistochemical findings in table 3.

Male Caucasians coinfecting with HIV had a significantly lower Knodell histological activity index (p < 0.01) (table 1) than HIV negative controls. A significant association between HIV antibody state and histological grade of chronic liver disease was shown, with CPH being more common and CAH being less common in the HIV positive than in the HIV negative men ($\chi^2 = 12.58$, p < 0.001). Six of the HIV negative men had cirrhosis compared with only one of the 20 HIV positive men, but the numbers involved are too small to be analysed. The amount of lobular inflammation did not differ between the two groups.

Liver biopsy specimens from HIV positive patients showed significantly greater expression of HBeAg and HBV DNA polymerase compared with HIV negative controls, as

Table 2 Histological categories in HIV positive and negative Caucasian men with chronic HBV infection

Histology	HIV positive (n = 20)	HIV negative (n = 30)
CPH	16	10
CAH (without cirrhosis)	2	14
CLH	1	0
Cirrhosis with CAH	0	4
Cirrhosis	1	2

Table 3 Hepatitis B viral antigen and DNA polymerase expression in HIV positive and HIV negative Caucasian men

Viral antigen expression	HIV positive	HIV negative
HBsAg*		
0	5	14
1+	14	11
2+	1	5
3+	0	0
4+	0	0
HBeAg†		
0	3	22
1+	5	7
2+	6	1
3+	6	0
4+	0	0
HBV DNA polymerase†		
0	4	24
1+	2	3
2+	8	3
3+	5	0
4+	1	0

*Not significant. †p < 0.01.

assessed by immunohistochemistry (p < 0.01) (table 3). The expression of HBsAg does not, however, seem to be influenced by HIV state. There was a strong positive correlation between immunohistochemical staining for HBeAg and HBV DNA polymerase expression as would be expected as both are markers of HBV replication (rank correlation coefficient r = 0.73, p < 0.001) (table 3).

Discussion

The aim of this study was to assess the effect of coinfection with HIV on the histological and immunohistochemical changes seen in chronic HBV infection. Only Caucasians were studied to exclude those cases, especially common in the Chinese, who may have acquired their HBV by vertical transmission and who tend to have less severe disease as assessed histologically. Patients with AIDS were excluded because it is more difficult to assess the effects of the many other disease processes and their treatments, which may coexist.

Although other groups of workers have looked at the association of HBV and HIV, these have not looked at the histological findings in the liver in detail. Chronic active hepatitis, with and without cirrhosis, is more common in HIV negative patients, and the score for the inflammatory component of the Knodell index is higher. Thus two different methods of assessing the activity of the HBV infection show that it is greater in HIV negative patients. Furthermore, the degree of scarring was also greater in HIV negative patients as measured by the fibrosis component of the Knodell index or the incidence of cirrhosis.

An immunohistochemical study of HBV antigen expression shows that HBeAg and HBV DNA polymerase expression is significantly increased in HIV positive patients (p < 0.01) (table 3). This indicates that viral replication is greater in these patients. The HBV DNA polymerase is a new immunohistochemical marker for HBV replication and it correlates well with HBeAg expression.

The low histological activity indices in the presence of high levels of HBV replication can be explained by the fact that hepatocyte

damage in HBV depends on cytotoxic T lymphocyte activity directed against hepatocytes expressing HBcAg,¹ and it is likely that HIV positive patients have subclinical immunosuppression even if they don't have AIDS.^{2,3} Long term follow up studies will be necessary to assess the effect of these changes on the natural history of the disease process.

These findings have two important implications. Firstly, conventional histological grading will underestimate the amount of HBV and level of HBV replication in the absence of immunohistochemical techniques to show the presence of viral antigens. Secondly, any treatment for HIV which improves T lymphocyte function may increase the histological activity of chronic HBV infection of the liver.

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