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Prevalence and Significance of Anti-HDV Antibodies in Hbsag-Negative Subjects

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Abstract

Objective: The presence of anti-HDV antibodies in HBsAg-positive subjects suggests HBV/HDV co-infection. Anti-HDV antibodies may remain after recovery and the disappearance of HBsAg, but active HDV and/or HCV infections can also sometimes decrease HBsAg production. However, there are few epidemiological data concerning anti-HDV antibodies in HBsAg-negative subjects. The aims of this study were to determine the prevalence of anti-HDV antibodies in HBsAg-negative subjects with other markers of HBV infection in an urban area of northern Italy in order to evaluate their significance in the presence/absence of HCV, and to contribute to HDV epidemiology by comparing the results with those obtained in a previous study of HBsAg-positive subjects in the same area.

Methods: Anti-HDV antibodies were retrospectively investigated in 1287 HBsAg-negative/anti-HBc-positive subjects who hade been examined for HBV markers. Signs of possible liver alterations and HCV and HIV infections were sought in the clinical records of the anti-HDV-positive patients, and HBV-DNA, anti-HBc IgM, anti-HDV IgM and HDV-RNA were detected.

Results: Thirteen samples (1.0%) were anti-HDV-positive. The clinical evaluation of 12 of these patients showed that nine had had previous HBV/HDV co-infections; two had concomitant HCV infection, and one occult HBV infection. The prevalence of past HDV infection was 0.7%. The prevalence of anti-HDV in HBsAg-positive patients in the same area was 4.9%.

Conclusion: The prevalence of past HDV infection in the study area is low, but interference from HCV is possible in a few cases. Comparison with data obtained from HBsAg-positive subjects suggests that about one-quarter of HBV/HDV co-infections resolve naturally.

Keywords

Anti-HDV antibodies; Epidemiology; Co-infections; Interference; Past infections; HBsAg positive; HBV/HDV

Introduction

Hepatitis delta virus (HDV), a defective RNA virus that requires hepatitis B virus (HBV) to be able to replicate [1], is responsible for both acute and chronic infections [2-4]. HDV disease is usually severe and rapidly progresses to cirrhosis, although it may sometimes be asymptomatic and not progressive [5,6]. It has been estimated that 18-20 million people throughout the world are chronically infected with HDV (i.e. approximately 5% of all HBV carriers) [7]. The infection is endemic in sub-Saharan areas, the Middle East, central Asia, the South Pacific, the Amazon, the Mediterranean basin, and Eastern Europe [8]. In Europe as a whole, it is present in 8-12% of the carriers of HBV surface antigen (HBsAg) [9-13], but the recent substantial immigration of people from highly endemic areas [11,14] may lead to a rapid change in its epidemiology.

HDV infection can be transmitted simultaneously with HBV infection (co-infection), or transmitted subsequently to patients who are already infected with HBV (super-infection). Co-infections range from mild to severe and fulminant [15], but are frequently self-limiting and only 20% of affected patients progress to cirrhosis; however, in the case of super-infection, hepatitis may be exacerbated in asymptomatic HBsAg-positive patients, becomes chronic in 90% of cases, and leads to cirrhosis in 70% [16].

The first diagnostic markers of co-infection to appear are those of acute HBV (HBsAg and anti-antigen of HBV core IgM antibodies [anti-HBc IgM]) and HDV infection (anti-HDV IgM antibodies that subsequently convert to IgG) [17]. In the case of super-infections, anti-HDV IgM antibodies are also present before the appearance of IgG, but anti-HBc antibodies are only IgG [17]. The persistence of anti-HDV IgM indicates chronic infection, and is related to the inflammatory activity of the underlying liver disease [18]. Anti-HDV IgG (and some IgM) antibodies therefore persist for a long time in the case of super-infections, but the antibody response (also IgG) is shorter in the case of self-limiting co-infections [19]. Consequently, in order to identify acute or chronic HDV infections, anti-HDV antibodies are normally only sought in HBsAg-positive subjects [19] but, as HDV interference with HBV can suppress the production of HBsAg [20,21], HDV may also be present in apparently HBsAg-negative subjects [22,23]. Furthermore, as other viruses such as HCV may also interfere with HBV replication [24,25] and some HBV/HDV co-infections may be clinically asymptomatic [2,26], a diagnosis of HDV infection requires more than the mere detection of anti-HDV antibodies in HBsAg-positive subjects.

Many clinical and epidemiological studies have investigated the presence of anti-HDV antibodies in HBsAg-positive subjects, but the few studies of HBsAg-negative subjects in Western countries are not recent and have mainly investigated high-risk patient groups with the aim of assessing the prevalence of previous infections and/or interactions with other hepatitis viruses [27-30]. However, some more recent studies carried out in highly endemic Asian countries have found that more than 10% of asymptomatic HBsAg-negative subjects are positive for anti-HDV antibodies [31,32].

The aims of this study were to determine the prevalence of anti-HDV antibodies in Italian and non-Italian HBsAg-negative subjects

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with other markers of HBV infection (anti-HBc with or without anti-HBs or anti-HBe) in an urban area of northern Italy in order to evaluate past infections and the possible interference of other virus such as HIV or HCV, and to contribute to HDV epidemiology by comparing the results with those obtained in a previous study of HBsAg-positive subjects in the same area [33].

Methods

Legnano Hospital, which is located in an urban area of northern Italy near Milan, has a catchment area of about 250,000 people. It has both general and specialist surgical and medical departments, and is equipped with points for the collection of patients samples sent by external general practitioners for testing by the hospital laboratory.

Anti-HDV antibodies were retrospectively investigated in all of the 1,287 consecutive frozen and stored HBsAg-negative and total anti-HBc-positive serum samples taken from subjects that were sent by their general or specialist practitioners to our laboratory with a request for a search for HBV markers between 1 January 2013 and 31 December 2014. The samples were taken from 751 males and 536 females (718 in-patients and 569 outpatients) who had a mean age of 65.6 years (range 18-96).

An enzyme immunoassay (ETI-AB-DELTAK-2, DiaSorin, Saluggia, Italy) was used to determine total anti-HDV antibody levels. The medical and laboratory records of the patients whose samples were anti-HDV positive were consulted for signs of possible chronic liver disease which, if a liver biopsy had not been performed, consisted of the presence of fluctuating or persistently high aminotransferase levels (upper limits of normal 40 U//L for aspartate aminotransferase and 45 U/L for alanine aminotransferase) and/or ultrasound signs of abnormal hepatic echo structure. The presence of HIV and HCV co-infection was also sought. Whenever possible, if there were no anti-HBc IgM and anti-HDV IgM data in the patients' medical records, a chemiluminescence assay (VITROS Anti-HBc IgM, Ortho Clinical Diagnostics, Raritan, NJ, USA) was used to search for anti-HBc IgM, and an enzyme immunoassay (ETI DELTAK-IGMK-2, DiaSorin) was used to search for anti-HDV IgM. Anti-HBc IgM or anti-HDV IgM negativity indicates that that any anti-HBc or anti-HDV antibodies are only IgG. Molecular biology methods were used to search for HBV-DNA (AmpliPrep/COBAS TaqMan HBV Test, Roche Diagnostics, Mannheim, Germany) and HDV-RNA (HDV RNA Quantitation, DIAPRO, Sesto San Giovanni, Milan, Italy). The HDV-RNA test has a dynamic range of between 10^2 and 10^7 copies/mL; the HBV-DNA test has a dynamic range of between 20 and $17x10^7$ IU/mL, but can also detect the presence of <20 IU/mL of HBV DNA albeit without determining the titre.

The patients were divided on the basis of gender, type (in-patients and outpatients), country of origin (Italian and non-Italian), age, and serological HBV patterns. The data were statistically analysed using Fisher's exact test, analysis of variance (ANOVA) and linear regression using SPSS software (Version 16.0, SPSS Inc., Chicago, IL).

Results

Thirteen (1%) of the 1,287 samples were anti-HDV positive. They were taken from five of the 718 in-patients (0.7%), and eight of the 569 outpatients (1.4%) (p=0.26), who had a mean age of 55.6 years (range 39-83). Ten were Italian males and three non-Italian females. Table 1 shows the prevalence of HDV antibody positivity by gender and origin: there was a statistically significant difference between the Italian males and the Italian females (p=0.007) and between the Italian and non-Italian females (p=0.002), but not between the Italian and non-Italian males (p=0.61) or between the non-Italian males and non-Italian females (p=0.11).

The samples were divided into three groups on the basis of the markers of HBV infection: 954 (74.1%) were HBsAg negative, and anti-HBs and total anti-HBc positive (group A); 239 (18.6%) were HBsAg, anti-HBs and anti-HBe negative, and total anti-HBc positive (group B); and 94 (7.3%) were HBsAg and anti-HBs negative, and anti-HBe and total anti-HBc positive (group C). Eight of the 13 anti-HDV positive samples (8/954, 0.8%) were in group A, three (3/239, 1.3%) in group B, and two (2/94, 2.1%) in group C (Table 2), with no statistically significant difference between the groups (p=0.45).

Table 3 shows the prevalence of anti-HDV antibodies by patient age group, and that the prevalence was significantly higher in the patients aged 41-60 years (p=0.004).

The results relating to one of the 13 anti-HDV positive patients were inconclusive as the stored serum sample was insufficient for further investigations, and no additional data were collected.

The other 12 anti-HDV positive samples were all negative for anti-HBc IgM and HDV-RNA; only one was positive for HBV-DNA (<20 IU/mL) and another was positive for anti-HDV IgM (all of the other samples were negative for both). Consultation of the laboratory and medical records of the 12 anti-HDV positive subjects showed that none of them was anti-HIV positive (two were ex-intravenous drug addicts), but three were anti-HCV reactive to a screening test (the others were anti-HCV negative). One of the three anti-HCV reactive subjects was weakly reactive, but indeterminate upon immunoblotting and negative for HCV RNA; on the basis of these and the clinical data, he was considered as having had a previous HCV infection or a non-specific antibody result. The other two were highly reactive to screening and immunoblot tests; one was negative for HCV RNA; the other had a high viral load and was genotype 1a.

The laboratory and archived clinical data indicated that nine of the 12 anti-HDV positive subjects (three females and six males, including the case with past HCV infection or a non-specific antibody result) had had a past HDV infection (9/1,287=0.7%, CI 95% 0.24-

Table 1: Prevalence of anti-HDV antibodies in HBsAg-negative subjects by gender and origin.

	Males		**Females**		**P-value**	**Total**	
	No.	95% CI	No.	95% CI			95% CI
Italians	10/682 (1.5%)	0.59-2.41	0/473 (0%)	0.00-0.00	0.007	10/1155 (0.9%)	0.36-1.44
Non-Italians	0/69 (0%)	0.00-0.00	3/63 (4.8%)	0.00-10.08	0.11	3/132 (2.3%)	0.00-4.86
P-value	0.61		0.002			0.14	
Total	10/751 (1.3%)	0.49-2.11	3/536 (0.6%)	0.00-1.25	0.26	13/1287 (1.0%)	0.46-1.54

Table 2: Results of the anti-HDV antibody search in HBsAg-negative subjects with different HBV markers.

Group	HBV markers	Anti-HDV positive	
		No.	95% CI
A	Anti-HBs positive, anti-HBc positive	8/954 (0.8%)	0.23-1.37
B	Anti-HBs negative, anti-HBe negative, anti-HBc positive	3/239 (1.3%)	0.00-2.74
C	Anti-HBs negative, anti-HBe positive, anti-HBc positive	2/94 (2.1%)	0.00-5.00
Total		13/1287(1.0%)	0.46-1.54

Table 3: Prevalence of anti-HDV antibodies by age group.

	Anti-HDV positive	
Age groups (years)	No.	95% CI
<20	0/11 (0%)	0.00-0.00
21-40	1/94 (1.1%)	0.00-3.21
41-60	8/313 (2.6%)	0.84-4.36
61-80	3/629 (0.5%)	0.00-1.05
>80	1/240 (0.4%)	0.00-1.20

1.16), but the situation was more complex in the remaining three cases. One was a male ex-drug addict with HCV-induced liver disease (a high HCV RNA titre and genotype 1a); he was anti-HBs and anti-HBe negative, and anti-HBc positive. The second was a man with decompensated liver cirrhosis who was receiving anti-HBV prophylaxis with lamivudine while awaiting transplantation, but was virologically cleared (HCV-RNA, HBV-DNA and anti-HBs negative, and anti-HBe and anti-HBc positive). His records showed chronic active HBV/HDV hepatitis in 1985, and related cirrhosis and HBV/HDV/HCV infection in 2005, when he was anti-HDV IgM positive; he was still anti-HDV IgM positive at the time of the study. The third was a hospitalised man with a blastoid variant of mantle cell lymphoma B who was receiving R-COMP immunochemotherapy. He was anti-HBs and anti-HBc positive, anti-HCV antibody negative, and the only subject who was positive for HBV-DNA (albeit with a titre of <20 IU/mL) and taking lamivudine prophylaxis.

Discussion

In the early 2000s, the endemicity of HBV infection in Italy was considered to be low because the prevalence of HBsAg carriers had fallen to <2% [34] and the prevalence of HDV infection in HBsAg-positive subjects was about 8%, although there are geographical differences between the northern, central and southern regions [35,36]. In the area of Legnano, the current prevalence of HBsAg carriers is 2.1% (1.8% among Italians and 6.5% among non-Italians) [37], and the prevalence of HDV infection in HBsAg-positive subjects is 4.9% [33].

The findings of this study show that the prevalence of anti-HDV antibodies in HBsAg-negative subjects is 1%, with no significant difference between Italians and non-Italians. This figure is in line those found in a previously published study of Western countries [27].

There was no significant difference in the profiles of HBV markers, thus indicating that the HBV antibody pattern is not associated with HDV infection. Furthermore, the fact that the tests for HDV-RNA and anti-HBc IgM and anti-HDV IgM antibodies were negative (except for the case with HBV/HCV/HDV cirrhosis) indicates that there were no cases of acute HBsAg-negative co-infection or super-infection.

It was found that only about 75% of the cases (9/12) could be definitely identified as having had past HDV infections (i.e. 0.7% of all of the HBsAg-negative subjects with other markers of HBV infection) [38]. However, it should be borne in mind that these figures may be underestimated because the absence of anti-HDV antibodies can be the result of their disappearance over time, especially in the case of self-limiting co-infections [19], and the limitation of this study is that it is not known when these patients first developed HBV infection, or whether they were affected by co-infection from the onset.

The possibility of HCV interference [31,39] can be considered in two cases. One of these was a man who was positive for HCV-RNA and negative for HDV-RNA and HBV-DNA, but no search was made of his liver, and it has been reported that patients with HCV/anti-HBc positive (but HBsAg negative) infections are more positive for HBV-DNA in the liver than in serum [40]. The other case was characterised by virologically cleared HCV/HBV/HDV infection and previously documented active HBV/HDV replication years ago, but the current presence of anti-HDV IgM confirmed the chronicity of the disease [18].

Finally, the patient whose chemotherapy-induced immunosuppression was prophylactically treated with lamivudine presented occult HBV infection without any clinical signs of liver disease: it can be presumed that HDV infection had resolved naturally because he was HDV-RNA negative despite immunosuppressive chemotherapy but, given his critical condition and as lamivudine has no effect on HDV [17], clinical and serological follow-up for both HDV and HBV would be appropriate.

The findings of this study were compared with those of a previously published study of anti-HDV antibodies in HBsAg-positive subjects in the same geographical area [33]. Both studies found a statistically significant difference between men and women, and that the age group in which anti-HDV antibody-positive subjects are most represented is 41-60 years. It is therefore possible that, regardless of their HBsAg status, the anti-HDV antibody-positive subjects belong to the same population of survivors of the epidemic that occurred in the 1970s and 1980s [11,14]. There is no difference between the two studies in terms of HCV infection, but there is a difference in terms of the prevalence of anti-HIV antibodies (about 43% in the anti-HDV/HBsAg-positive subjects and 0% in the HBsAg-negative subjects in this study). This may be due to the fact that about half of the HBsAg-positive subjects in the previous study were ex-intravenous drug addicts, against 15% of the HBsAg-negative subjects in the present study, thus suggesting a difference in the route of transmission.

On a speculative level, the two studies could be combined insofar as both were carried out over the same period of time (two years) in the same area and included sequential samples sent for analysis at the same centre. The fact that the two studies were conducted about five years apart should not substantially affect the epidemiological findings despite the recent influx of immigrants because the percentage of non-Italians was similar in both. Considering only the definitely identified cases of past HDV infection in this study, it can therefore be suggested that at least a quarter of the HDV infections resolved naturally.

In conclusion, the prevalence of past HDV infection in the study area is at least 0.7%, and it can be hypothesised that this represents no less than a quarter of all of the HDV infections occurring in the same area; the situation is more complex in a small percentage of cases because of possible interference from HCV. Despite the influx of immigrants from countries in which HBV and HDV infections are highly endemic, no differences were found between Italian and non-Italian patients (or at least those who are formally resident in Italy, who are the only immigrants with access to the study centre). Nevertheless, the continuing increase in the number of immigrants who will become resident in the future could lead to changes in this epidemiology.

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