

## CLINICAL RESEARCH

## Increased severity and morbidity of acute hepatitis in drug abusers with simultaneously acquired hepatitis B and hepatitis D virus infections

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### Abstract

Hepatitis D virus (delta agent) markers were present in 111 (36%) of 308 intravenous drug abusers who were positive for hepatitis B surface antigen (HBsAg), 52 of these having hepatitis D virus antigenaemia. IgM antibody to hepatitis B core antigen (anti-HBc IgM) was present in 92 out of 95 subjects tested, indicating that hepatitis D virus and hepatitis B virus infections had been acquired simultaneously. Hepatitis D virus markers were present in three out of four patients with fulminant hepatitis, in seven of 11 (64%) with severe hepatitis, and in 80 of 223 (36%) with mild or moderate hepatitis compared with four of 29 (14%) of those who were asymptomatic. These proportional differences were significant ( $p < 0.001$ ). Hepatitis D virus markers were present in twice as many patients positive for anti-HBc IgM requiring admission to hospital with

acute hepatitis compared with outpatients attending a drug treatment centre. Tests on one patient showed complete disappearance of HBsAg, but hepatitis D antigen (HDAg or delta antigen) and hepatitis B e antigen (HBeAg) were still present in serum samples.

All five patients with chronic active hepatitis had hepatitis D antibody (anti-HD) compared with seven of 24 (29%) with chronic persistent hepatitis ( $p < 0.008$ ). Blocking anti-HD persisted for long periods after simultaneous infections with hepatitis B virus and hepatitis D virus but at lower titres than in patients with chronic liver disease.

### Introduction

Superinfection of carriers of hepatitis B virus by hepatitis D virus (delta agent) may produce more severe hepatitis and lead to chronic active hepatitis and cirrhosis more often than with hepatitis B infection alone.<sup>1-4</sup> This may occur because in a patient with previously established hepatitis B virus infection replication of hepatitis D virus occurs more quickly, causing a more severe infection than in patients with hepatitis B alone.<sup>1</sup> Reports from Italy,<sup>2</sup> California,<sup>3</sup> and a study of Venezuelan Indians<sup>4</sup> have shown an increased incidence of hepatitis D virus markers in carriers of hepatitis B surface antigen (HBsAg) with fulminant hepatitis and in patients progressing to chronic active hepatitis and cirrhosis. Studies in the United Kingdom,<sup>5</sup> Ireland,<sup>6</sup> Greece,<sup>7</sup> Sweden,<sup>10</sup> and the United States of America,<sup>11</sup> however, have suggested that simultaneous infection with hepatitis D and hepatitis B virus does not necessarily produce increased clinical severity compared with hepatitis B virus infection alone.

The opportunity to investigate the role of hepatitis D virus in exacerbating acute hepatitis when hepatitis B and hepatitis D viruses are acquired at roughly the same time was provided by a large continuing outbreak of hepatitis B and hepatitis D virus infection in drug abusers in Dublin, which started in October 1980.<sup>12</sup>

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### Patients and methods

A total of 308 intravenous drug abusers who were positive for HBsAg were examined for hepatitis D virus and hepatitis B virus markers. These comprised 148 patients admitted to hospitals in Dublin with acute hepatitis and 160 patients who were detected by routine screening of outpatients attending the Drug Advisory and Treatment Centre in Dublin.

Two hundred and sixty seven patients had acute or asymptomatic hepatitis; follow up serum samples were available from 195 of these over a period ranging from two months to four years. Serum samples that were positive for hepatitis D virus markers (74) and a random sample of those negative for hepatitis D virus markers (21) were also tested for anti-HBc IgM. Eleven patients were severely ill with transaminase activities greater than 10 times normal for more than seven days and with raised prothrombin ratios ( $>1.25$ ) and four patients had fulminant hepatitis. Patients in whom illness was classified as mild or moderate ( $n=223$ ) had transaminase activities two to 10 times normal, while patients classified as asymptomatic ( $n=29$ ) showed no or only slight increases in transaminase values ( $<$  twice normal) and cleared HBeAg and HBsAg within normal lengths of time. Forty one patients, 40 of whom were from the drug treatment centre, had chronic liver disease diagnosed histologically in accordance with the criteria suggested by an international group in 1977.<sup>13</sup>

HBsAg and anti-HBs were detected by radioimmunoassay (RIA, Abbott Laboratories, Chicago). Hepatitis B e antigen (HBeAg) and anti-HBe were detected by enzyme immunoassay.<sup>14</sup> Tests for anti-HBc IgM were carried out by Dr R Tedder at Middlesex Hospital, London, using radioimmunoassay.

HDAg and blocking (total) anti-HD were detected by enzyme immunoassay using HDAg extracted from serum, as previously described.<sup>15</sup> IgM anti-HD was detected by an IgM class capture enzyme immunoassay, also using HDAg extracted from serum.

A test for linear trend in proportions was applied to the data on patients classified by severity of illness.<sup>16</sup> The  $\chi^2$  test with Yates's correction was applied to data on patients admitted to hospital and Fisher's exact test was used on the data on patients with chronic liver disease.

### Results

Table I shows the results of tests for hepatitis D virus markers on 308 drug abusers with hepatitis B virus markers. Anti-HBc IgM was present in 92 of 95 patients tested (50 of 51 HDAg positive patients; the one other patient positive for HDAg had had acute hepatitis B four months earlier (IgM positive)). Only a single acute phase serum sample was available from 24 of the 52 patients positive for HDAg because of either early discharge from hospital by the patient or late return to the drug treatment centre. Eight of these patients were simultaneously positive for anti-HD IgM. Anti-HBc IgM was found in 21 of 23 anti-HD positive patients tested. The mean age of the study group was 21.7 years and the male to female ratio was 4.4:1. The male to female ratio and the mean ages (data not shown) were similar in those with and without hepatitis D virus markers.

TABLE I—Results of tests for hepatitis D markers on 308 drug abusers with hepatitis B markers

	Total (percentage)	Male	Female
HDAg positive and seroconversions	52 (16.9)	44	8
Anti-HD positive only	59 (19.1)	48	11
Hepatitis D marker negative	197 (64.0)	160	37
Total	308 (100)	252	56

Serial specimens from 25 patients were available over a mean period of 18 months after hepatitis D virus antigenaemia. The mean duration of hepatitis D virus antigenaemia was 11 days, range three to 21 days. All 25 patients developed anti-HD after a mean of 29 days, range 10-60 days; 24 remained anti-HD positive for the duration of testing (mean 18 months, range two months to four years) and one patient became negative after three months. Most of these patients had anti-HD titres of  $<1/200$ .

Table II gives the results of the clinical analysis of 267 drug abusers with acute hepatitis. Differences in the proportion with D markers between groups with hepatitis of varying severity was highly significant ( $p < 0.001$ ).

TABLE II—Clinical analysis of 267 drug abusers with hepatitis

Severity of hepatitis	HDV positive (percentage)	HDV negative	Total
Fulminant	3 (75.0)	1	4
Severe	7 (63.6)	4	11
Mild or moderate	80 (35.8)	143	223
Asymptomatic	4 (13.8)	25	29
Total	94 (35.2)	173	267

$Z = 3.51$ ,  $p < 0.001$  (test for linear trend in proportions).<sup>16</sup>

An analysis of 82 patients who were positive for anti-HBc IgM with hepatitis showed that twice as many of those admitted to hospital had anti-HD compared with those who attended the drug treatment centre—that is, with mild or asymptomatic hepatitis ( $p < 0.1$ ) (table III). HDAg positive patients were excluded from this comparison because of the relatively transient nature of serum HDAg.

TABLE III—Hepatitis D virus infection among 82 anti-HBc IgM positive drug abusers with hepatitis

	Admitted to hospital with clinical hepatitis	Attended drug advisory and treatment centre only*	Total
Anti-HD positive	41	20	61
Hepatitis D marker negative	9	12	21
Total	50	32	82

$\chi^2 = 2.94$  (for 1 df with Yates's correction);  $p < 0.1$ .

\*With mild or asymptomatic hepatitis.

Of four patients with fulminant hepatitis, two died and only one had superinfection with hepatitis D virus; two had HDAg and seroconverted to anti-HD. Neither of the two patients who died had HDAg or blocking anti-HD, but one had anti-HD IgM. No serum sample was available from the fourth patient for retrospective testing for anti-HD IgM and therefore hepatitis D virus could not be excluded.

All five carriers of HBsAg with histologically diagnosed chronic active hepatitis had high titres of anti-HD ( $>1/5000$ ). This incidence was significantly different from that in non-HBsAg carriers with chronic liver disease and in those with chronic persistent hepatitis ( $p = 0.008$ ) (table IV).

TABLE IV—Hepatitis D virus markers in 41 drug abusers with chronic liver disease

	Anti-HD positive (percentage)	HDV negative	Total
Chronic active hepatitis HBsAg carriers	5 (100) <sup>†</sup>		5
Chronic active hepatitis or cirrhosis*	5 (42)	7	12
Chronic persistent hepatitis, asymptomatic HBsAg carriers, or both	7 (29)	17	24
Total	17 (41)	24	41

\*Non-HBsAg carriers but had hepatitis B markers.

<sup>†</sup> $p = 0.008$  (Fisher's exact test) compared with other groups of patients with chronic liver disease combined.

Figure 1 shows the typical serological course found in patients in whom HDAg was detected in their serum samples taken in the acute phase of simultaneously acquired hepatitis B and hepatitis D virus infections.

Two patients with hepatitis D virus superinfection and moderate hepatitis became hepatitis D virus antigenaemic about five months

after acute hepatitis B virus infection. In these patients tests for HBeAg became negative at the time of hepatitis D virus antigenaemia (fig 2). A decrease in the amount of HBsAg was also seen in some patients positive for HDAg; in one patient with moderate hepatitis HBsAg became completely undetectable, but tests for HDAg and HBeAg remained positive and he seroconverted to anti-HD and anti-HBe 18 days later but remained negative for HBsAg (fig 3). The only patient with fulminant hepatitis D virus superinfection also showed a decrease in the amount of HBsAg when positive for HDAg and in coma five months after acute hepatitis B (fig 4). Recovery from coma was accompanied by the disappearance of HDAg and a rise in the amount of HBeAg in this and one other patient.

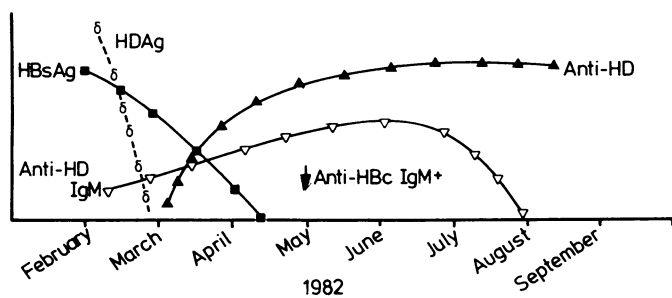


FIG 1—Typical serological course found in simultaneously acquired hepatitis D and hepatitis B infection in a patient with moderate hepatitis.

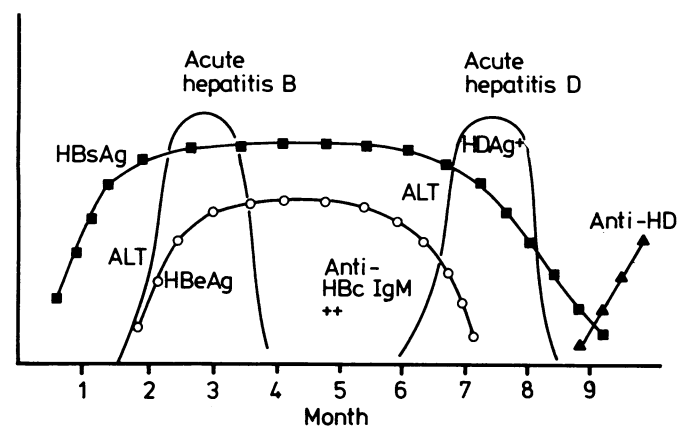


FIG 2—Hepatitis D virus superinfection about five months after hepatitis B in two patients with moderate hepatitis.

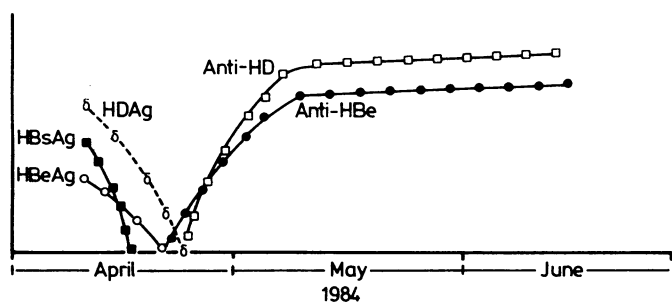


FIG 3—Serological course of simultaneously acquired hepatitis D and hepatitis B infection with HDAg and HBeAg remaining after total loss of HBsAg in a patient with moderate hepatitis.

## Discussion

As in Sweden,<sup>10</sup> it appears that hepatitis D virus infection has only recently been introduced into Ireland since hepatitis D virus markers have not been detected in serum samples stored before 1973 (AGS, unpublished data). Tests for anti-HBc IgM indicated that most cases of hepatitis B in this outbreak were acute and that where hepatitis D virus infection also occurred this was acquired simultaneously. The number of drug abusers

in Dublin who were carriers of HBsAg was low at this acute stage of the epidemic, which probably accounts for the low incidence of hepatitis D virus superinfections recorded in this study.

Although we have noted transient anti-HD antibody in some patients previously,<sup>6</sup> in this study seroconversion to blocking anti-HD usually occurred early (mean 29 days) and remained positive in 24 of 25 cases for the duration of testing (mean 18 months). This contrasts with two studies that found seroconversion to anti-HD to be transient or absent after simultaneously acquired hepatitis D and hepatitis B virus infection in three and 20 patients, respectively.<sup>17, 18</sup> HDAg derived from liver was used in these two studies, whereas HDAg derived from serum, which has been found to be more sensitive for the detection of anti-HD,<sup>15</sup> was used in our study. In most patients anti-HD was detectable only at a relatively low dilution (up to 1/200), whereas, as observed by Smedile *et al*,<sup>17</sup> those

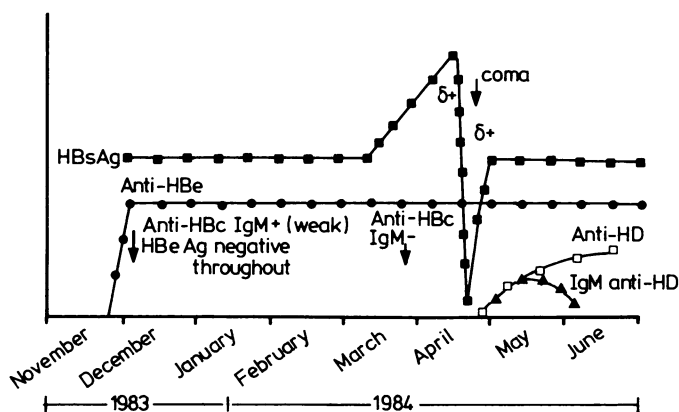


FIG 4—Decrease of HBsAg in a patient with fulminant hepatitis D virus superinfection.

who were positive for anti-HD with chronic liver disease had much higher titres (>1/5000). Sensitivity of the test system is therefore more important in detecting anti-HD after acute hepatitis D virus infection than in chronic cases.

Hepatitis D virus antigenaemia is common in drug abusers in Dublin<sup>6, 12, 15</sup> and occurred as the initial marker in 47% of those with hepatitis D virus infection in this study. It was found for an average of 11 days and for up to three weeks after the time of admission to hospital. Although this was transient, hepatitis D virus antigenaemia cannot be described as rare as reported previously.<sup>18-20</sup> A high incidence of hepatitis D virus antigenaemia has also been found in small groups of Scottish,<sup>6, 15</sup> Australian,<sup>21</sup> and Swiss<sup>22</sup> drug abusers. Enzyme immunoassay may be more sensitive than radioimmunoassay for the detection of HDAg,<sup>15, 23</sup> which may contribute to the higher incidence found in this study.

Rapid and pronounced fluctuations in concentrations of HBsAg were seen in patients with hepatitis D virus antigenaemia and, usually, moderate or severe hepatitis. Although a decrease in the concentration of HBsAg after hepatitis D virus antigenaemia has been reported,<sup>10</sup> total loss of HBsAg during hepatitis D virus and HBe antigenaemia has not been recorded previously. In one patient total loss of HBsAg occurred while HDAg and HBeAg remained positive (fig 3). Furthermore, 24 of our patients from whom only single specimens were obtained had hepatitis D virus antigenaemia, and eight were also positive for anti-HD IgM. If a blocking anti-HD test had been the only test used these hepatitis D virus infections would have been missed. This and the finding of anti-HD IgM alone in one of our fulminant cases suggest that all drug abusers with hepatitis should be screened for all three hepatitis D virus markers and secondary hepatitis B virus markers.

Hepatitis D virus markers occurred in three of four (75%) patients with fulminant hepatitis, in seven of 11 (64%) with severe hepatitis, in 80 of 223 (36%) with mild or moderate hepatitis, and in four of 29 (14%) of those with asymptomatic hepatitis; these proportional differences were highly significant. Furthermore, among 82 patients with diagnosed acute or recent hepatitis B infection (anti-HBc IgM positive) twice as many of those admitted to hospital had hepatitis D virus infection compared with asymptomatic anti-HBc IgM positive patients who attended the drug treatment centre. Although this finding was not significant at the 5% level ( $p < 0.1$ ), the cumulative findings suggest a strong association between simultaneously acquired hepatitis D and hepatitis B virus infection and the severity of hepatitis. Thus patients with hepatitis D virus infection required admission to hospital more often than those with hepatitis B virus infection alone. These findings are consistent with results of our previous reports,<sup>6,7</sup> which were confined to patients attending the centre, and provide the first evidence confirming that hepatitis D virus can cause a more severe acute infection when it is acquired simultaneously with hepatitis B virus.

Despite the differences in severity and morbidity noted above most patients appeared to make a complete recovery. Further follow up studies are required to establish whether infection with hepatitis D virus during the acute phase of hepatitis B virus infection predisposes to the development of chronic liver disease. In this study 41 patients were shown to have chronic liver disease and all five carriers of HBsAg with chronic active hepatitis had anti-HD in high titre. Furthermore, seven of 24 (29%) drug abusers with chronic persistent hepatitis had anti-HD, and these are being followed up for possible progression to chronic active hepatitis; progression from chronic persistent hepatitis to chronic active hepatitis is more common in drug abusers than in people who do not use drugs,<sup>24</sup> and this might be attributable to hepatitis D virus infection. Hepatitis non-A, non-B infections also cause chronic liver disease in drug abusers,<sup>25</sup> making it difficult to assess the contribution of each agent to chronic liver disease. Nevertheless, the association between hepatitis D virus and chronic active hepatitis appears to be established.<sup>1,4,12</sup>

In conclusion, our data suggest that even simultaneous infection with hepatitis D virus and hepatitis B virus causes increased severity and morbidity, in addition to the acknowledged role of hepatitis D virus in chronic liver disease and increased severity after hepatitis D virus superinfection. Thus hepatitis D virus infections may lead to increased severity in all clinical situations.

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## 100 YEARS AGO

Whilst all who read the newspapers in any country must have learnt, with regret, that the gallant general who saved his country from disunion, and guided its destinies for so many years, is suffering from a painful and deadly malady, it is very advisable that capital should not be made by a certain party out of the alleged cause of his illness. It has been distinctly reported in several journals that General Grant is suffering from cancer of the tongue caused by smoking. A little knowledge of pathology is sufficient to demonstrate that smoking cannot cause cancer, although the irritation of a pipe sometimes sets up ulceration of the lip, which, when of very long standing, may become cancerous, provided that the patient has a hereditary tendency to cancer. There is no evidence whatever that cigar-smoking causes cancer of the tongue. Mr. Butlin, the author of some of the most recent observations and statistics on cancer of the tongue, has shown that the proportion of men to women suffering from that disease is nearly six to one, but that it occurs in men who neither drink nor smoke, whilst it is as rare among women of the most masculine habits as amongst other females. Even the irritation of a broken or decaying tooth can only be an occasional exciting cause, since this condition is as common amongst women as amongst men, whilst cancer of the tongue is, fortunately, rare, out of all proportion to cases of decayed teeth. There can be no doubt that a man with a tooth irritating his tongue ought to have it removed. It is equally certain that no smoker who has a sore on his tongue ought to persist in the use of tobacco until that sore is cured, but the risk of cancer through smoking is so infinitesimal, as to be perfectly useless as an argument for the antitobaccoists. (*British Medical Journal* 1885;ii:551.)

Mr. Davey was successful on the 12th instant, in obtaining the insertion, on the report of the English Registration of Voters Bill, of a clause, which had been previously rejected in Committee, providing that medical or surgical relief, or the giving of medicine, shall not be deemed to constitute parochial relief, so as to disqualify the recipient from exercising the franchise. We have no intention, at the present moment, of going into the ethics of this question; but clearly what was considered by the Government right in Ireland can hardly be wrong in England. Much of the debate on the several occasions when this question has been recently under discussion has been, as might be anticipated, beside the point. There can be no doubt, however, that such "relief" as isolation in a rate-supported infectious hospital, for the benefit and protection of the community at large quite as much as of the patient himself, ought not to deprive a man of any electoral privileges. And if a dweller in a town can get, without legal branding as a pauper, the benefits of free hospital advice, it is hardly fair that the rural artisan, who has no hospital to fly to, should be deprived, with one hand, of the vote which has just been given to him with the other, because he gets from the parish a black draught or a pot of ointment. (*British Medical Journal* 1885;ii:1007.)