Incidence of infection with hepatitis B virus in 56 patients with haemophilia A 1971-1979

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SUMMARY During each of the four-year periods 1971-1975 and 1975-1979, the annual incidence of hepatitis B infection has been assessed in 56 patients with haemophilia A by measuring plasma HBsAg, anti-HBs and anti-HBc levels. Infection rates of 7% and 9.5% per annum respectively were observed for each four-year period despite the screening of individual blood donations for HBsAg by techniques up to the sensitivity of reversed passive haemagglutination. The highest incidence of seroconversion was amongst severe haemophiliacs many of whom had received treatment predominantly with cryoprecipitate. Of the 16 patients in whom serological evidence of hepatitis B infection was obtained only one had an accompanying clinical episode of hepatitis. We conclude that haemophiliacs are still at high risk of infection by hepatitis B virus despite the screening of individual blood donors for HBsAg.

Despite the development of techniques for screening individual blood donations for evidence of hepatitis B infectivity it is clear that haemophiliacs are still at risk of developing hepatitis. Whilst some episodes of hepatitis may be related to non A non B viruses, others are clearly related to hepatitis B virus (HBV) and these may be asymptomatic particularly in patients with severe haemophilia who receive large quantities of transfused blood products.¹⁻⁴ During the period of this study from 1971 to 1979 two changes have taken place which might influence the rate of hepatitis B infection in haemophiliacs. The development of more sensitive tests to screen individual blood donations for HBsAg has led to a reduction in post transfusion hepatitis B in non-haemophiliac patients during the past 10 years.⁵ It might be anticipated, therefore, that patients with haemophilia would also demonstrate a reduced frequency of infection. Mitigating against such a reduction, however, is the gradual change from the use of cryoprecipitate to factor VIII concentrate. Whereas each unit of cryoprecipitate is derived from a single blood donation each bottle of factor VIII concentrate is made from a pool of several hundred or thousand donations and therefore has a much greater likelihood of containing infective viral contamination.^{6,7} The study reported in this communication is

a sequel to a previous study of Edinburgh patients in which the HBV infection rate was 36% of patients at risk over the four-year period 1971-1975.⁸ To follow up this study we not only collected further blood samples in 1979 from haemophiliacs but we have retested deep frozen serum samples from the same patients from 1971 and 1975, for anti-HBs and also anti-HBc, by radioimmunoassay. In addition to the original patients studied in 1971-75 we have examined sera from additional patients in the period 1975-79. The testing of all serum samples from each patient collected on different occasions in a single assay makes it easier to determine whether an individual patient has been infected by hepatitis B during the period of study.

Patients and methods

PATIENTS

The total number of haemophilia A patients of varying degrees of severity was 56 (Table 1). All patients had received blood products prior to 1971. Serum samples were available for the whole period of study from 42 patients. Eleven additional haemophiliacs were studied 1971-1975 and four other individuals were included in the period 1975-1979. Therapy for these haemophiliacs was "on demand" and during the initial period of study was predominantly by cryoprecipitate although during the later period of

Table 1 Patients studied 1971-75

Category	No	Severity of haemophilia			Mean age (yr) (range)
		Severe	Moderate	Mild	
1 Infected before 1971	24	18	3	3	25.5 (12-44)
2 Seroconverted	8 (5)*	7 (4)	1	—	13 (5-19)
3 No evidence of infection	21	8	5	8	22 (3-53)

*Of the 26 (21 + 5) seronegative patients in 1971, 5 became seropositive by 1975 ie 5/26 (19%). An additional 3 patients were infected a short time prior to collection of the initial samples in 1971 (see results) and if these are included the seroconversion rate is 8/29 (28%).

investigation factor VIII concentrate (Scottish National Blood Transfusion Service) was given increasingly to patients.

SERUM SAMPLES

These were collected during 1971, 1975 and 1979 and stored at -20°C .

TESTS FOR HEPATITIS B INFECTION

When received patients' sera were screened for hepatitis B surface antigen (HBsAg) by reverse passive haemagglutination.⁹ All sera were tested later in parallel for antibody to HBsAg (anti-HBs) by radioimmunoprecipitation,¹⁰ and for antibody to hepatitis B core antigen (anti-HBc) by a commercial assay (Corab, Abbott) or a radioimmunoassay using HBcAg produced in *E. coli*.¹¹ At the Scottish Regional Blood Transfusion Centres individual blood donations were tested for HBsAg by techniques up to the sensitivity of reversed passive haemagglutination.

Results

The results of the anti-HBs assay can be scored as weak, moderate and strong, corresponding to 0.01, 0.1 and 1.0 IU of antibody per ml. The cut off in the anti-HBc assays was calculated as the mid-point of the means of negative and positive controls. Sera were considered to show evidence of past hepatitis B infection if anti-HBs and anti-HBc were both present, the anti-HBc in all cases being at a level close to the positive control serum (Abbott). Weak anti-HBs levels were detected in some patients in the absence of anti-HBc: these were considered to be negative.

One patient was HBsAg-positive throughout the study.

On this basis, the patients were classified for each period as (1) previously exposed to hepatitis B, (2) at risk if they were seronegative at the start of each quadrennium, (3) showing evidence of infection if anti-HBs and anti-HBc were found in the later sample. The results are summarised in Tables 1 and 2. The mean ages are given for the mid-points of each period, 1973 and 1977. Of the 53 patients followed between 1971 and 1975, 24 had evidence of previous infection, 26 patients were negative in 1971, but by 1975, five (19%) of these patients at risk had converted serologically. A further three patients must have been infected a short time before collection of the first sample as initially they had no anti-HBs, but did have anti-HBc. The follow-up samples were positive for both antibodies. Interestingly the anti-HBc results in two of these cases were close to the cut off in the first sample, but were clearly positive later. If these three patients are included as infections in the first period, the rate becomes 28% of those at risk. All of the eight patients classified as seroconverting from 1971 to 1975 had maintained both anti-HBs and anti-HBc when retested in 1977. Forty-six patients were followed from 1975-1979; 25 had been infected before 1975. Of the 21 seronegative cases, eight (38%) converted by 1979. The difference between the two rates of infection is not statistically significant.

CLINICAL EVIDENCE OF HEPATITIS

Of the 16 patients who seroconverted for hepatitis B during the study, only one had clinical jaundice the remaining 15 individuals being asymptomatic.

Table 2 Patients studied 1975-79

Category	No	Severity of haemophilia			Mean age (yr) (range)
		Severe	Moderate	Mild	
1 Infected before 1975	25	20	4	1	26.3 (9-44)
2 Seroconverted	8	8	—	—	20.6 (7-43)
3 No evidence of infection	13	5	4	4	25.1 (11-57)

Of the 21 seronegative patients in 1975, 8 became seropositive by 1979 ie 8/21 (38%).

Discussion

The study reported here is a follow-up to an investigation of haemophiliacs treated at the Edinburgh Haemophilia Centre between 1971 and 1975.¹⁰ Serum from this former period was available for many of the patients for retesting concomitantly with an extra sample collected in 1979. The initial survey only assessed patients' HBsAg and anti-HBs levels by reversed passive haemagglutination and radioimmunoprecipitation techniques respectively, but in this follow-up study, anti-HBc had been measured. In the initial study evidence of infection included not only those individuals who seroconverted but also those whose antibody level rose by at least 100-fold during the period of study. In the current report we have only considered patients as showing evidence of infection if they were initially negative for all markers and subsequently became positive by both antibody tests. The possibility that the patients acquired their anti-HBs and anti-HBc by passive immunisation from either cryoprecipitate (particularly as this may be enriched in immunoglobulins compared to plasma) or factor VIII concentrates must be considered. From a previous study, one in a thousand Scottish blood donors¹² had an anti-HBs level of 1–10 IU/ml and if a patient received 50 ml of plasma (the volume of one unit cryoprecipitate) from such a donor the immediate post transfusion level would be approximately 0.1–0.2 IU/ml. But even a severe haemophiliac is unlikely to receive cryoprecipitate from more than 500 donors per year and if the half-life of infused anti-HBs is one month, then the chance of any of our patients having acquired a detectable passively transfused antibody must be very small. In all cases scored as positive for both antibodies in the first sample, the later samples were also positive and the actual results in good agreement. At most there was a tenfold variation in anti-HBs levels in a four-year period. In total six patients had a weak anti-HBs level in the absence of anti-HBc in one or more samples. These results were discounted as the antibody might have been acquired from their therapy. In total, 11 patients had low levels of anti-HBs but were also clearly anti-HBc positive. Interpretation of these anti-HBs results alone would be difficult, however, the consistent presence of anti-HBc strongly suggests that these antibodies resulted from infection.

Our patients have received almost exclusively SNBTS factor VIII which contains no detectable anti-HBs, unlike some commercial concentrates which have appreciable levels of this antibody (R Hopkins, personal communication, 1982). As most severe haemophiliacs in the UK are likely to receive

commercial concentrates for a significant part of their treatment, a study such as ours would not be possible at other Haemophilia Centres.

It is clear from this study that hepatitis B infection continued to occur throughout the period 1971–79 at an annual rate of approximately 8% and presumably patients with evidence of immunity were also continuing to have this boosted. As might be expected the rate of conversion is highest amongst young severe haemophiliacs and it is interesting to note that this was despite the predominant use of cryoprecipitate which might be considered to carry a lower risk of transmitting viral infection compared to factor VIII concentrates which are prepared from large plasma pools derived from many hundreds or thousands of donors.⁶ It is interesting that despite the evidence presented here that over half the patients have evidence of previous hepatitis B infection and frequently abnormal liver function tests¹³ none has symptoms referable to chronic liver disease. For the time being HBV will continue to infect haemophiliacs but it is hoped that the risk may decline in the foreseeable future. Firstly since January 1980 all blood donations in Scotland are being screened for HBsAg by radioimmunoassay. Secondly it is possible that hepatitis-free clotting factor concentrates may become available¹⁴ and thirdly active immunisation against hepatitis B may be introduced.¹⁵ Mitigating against a future reduction in infection is the fact that the plasma pool size for manufacture of factor VIII is increasing. It is therefore important that studies such as this should continue and we hope to report a further follow-up in due course to include patients who currently do not demonstrate evidence of HBV infection and newly diagnosed haemophiliacs.

Addendum

Since this manuscript was prepared a paper, *Hepatitis and haemophilia therapy in Australia* by Dr KA Rickard and colleagues (*Lancet* 1982;ii:146) has appeared. The incidence of hepatitis B infection in Australian haemophiliacs treated predominantly with cryoprecipitate was assessed and found to be identical to the incidence we observed in our Scottish patients.

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