

Serological and histological follow up of chronic hepatitis B infection

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SUMMARY In order to study the clinical, serological, and morphological evolution of chronic hepatitis B virus infection in childhood, a prospective study has been carried out. A total of 90 children with a chronic infection were followed up for a mean (SD) of 3 (1.8) years. At the beginning of the study, 61 children were asymptomatic and 77 were household contacts of chronic carriers. Serologically 77 were hepatitis B e antigen (HBeAg) positive and 71 of them were positive to hepatitis B virus DNA. The mean alanine aminotransferase activities were higher among HBeAg positive patients than in antihepatitis B e (anti-HBe) positive ones. The most severe histological damage was also found among HBeAg positive patients. The annual seroconversion rate was 14%. A significant increase in the alanine aminotransferase activity was observed 13 (5.6) months before appearance of anti-HBe in the 85% of cases. Among anti-HBe positive patients, the alanine aminotransferase activities were normal in all except three (19%), two of whom had intrahepatic delta antigen. An increase in the histological activity was observed among patients who maintained HBeAg presence while an amelioration of liver damage was observed in anti-HBe carriers.

Chronic hepatitis B virus infection has two distinguishable phases. During the first phase there are circulating Dane particles, hepatitis B e antigen (HBeAg) and hepatitis B virus DNA (HBV-DNA). Usually there is biochemical or pathological evidence of disease activity, or both. Later, either spontaneously or with antiviral treatment, those particles disappear from the circulation, HBeAg and HBV-DNA become undetectable, and antihepatitis B e (anti-HBe) develops.¹

Several reports on the natural history of chronic hepatitis B infection in children have been published with differing results.²⁻⁸ Cirrhosis has been reported in 3%⁷ to 25%.^{4, 5} Because of these varying findings we have carried out a prospective study in children with chronic hepatitis B virus infection.

Patients and methods

Our study comprised 134 children who were positive carriers for hepatitis B and who attended our hospital from 1979 to 1986; 44 were not available for long term follow up. We studied the remaining 90 children: all were hepatitis B surface antigen (HBsAg) and antihepatitis B c (anti-HBc) positive

with raised alanine aminotransferase activities for at least six months and were available for long term follow up. Other causes of liver disease were ruled out (α_1 antitrypsin deficiency, Wilson's disease, autoimmune chronic hepatitis, etc). The clinical, serological, and biochemical features are summarised in table 1. The children ranged from 6 months to 14 years of age (mean (SD) 6 (3) years). There were 55 boys and 35 girls. A clinical and serological follow up (every four months) was performed during a mean of three years (range six months to seven years). At the beginning of the study, 29 (32%) children had one or more of the following clinical symptoms of liver disease: asthenia (n=14, 15%), anorexia (n=11, 12%), cholestasis (n=10, 11%), abdominal pain (n=9, 10%), vomiting (n=7, 8%), and fever (n=5, 6%); the other 61 children were symptomless. Details of results of investigations for serology of HBeAg and anti-HBe, activity of alanine aminotransferase, and liver histology are shown in table 2. Of the symptomatic patients, 24 (83%) had hepatomegaly and three (10%) splenomegaly compared with four (6%) and none, respectively in the asymptomatic ones. In 77 children the infection was acquired by

Table 1 Baseline clinical and serological features of children studied (n=90)

	Serological markers				
	HbeAg+ HBV-DNA+ HBV-DNAp+	HbeAg+ HBV-DNA+ HBV-DNAp-	HbeAg+ HBV-DNA- HBV-DNAp-	Anti-HBe+	Anti-HBe+ Anti-HD+
No (%) of children	67 (74)	4 (4)	6 (7)	11 (12)	2 (2)
Male/female	44/23	2/2	2/4	6/5	1/1
Mean (SD) age (years)	6.0 (3.0)	6.1 (6.0)	6.2 (1.7)	6.4 (3.3)	4.7 (0.4)
Mean (SD) alanine aminotransferase (IU/l)	232 (289)	293 (218)	147 (148)	37 (14)	87 (40)
No (%) with symptoms	17 (25)	2 (50)	1 (17)	7 (64)	2 (100)
No (%) with hepatomegaly	21 (31)	1 (25)	1 (17)	3 (27)	2 (100)

HBV-DNAp=hepatitis B virus DNA polymerase.

Table 2 Basal features of symptomatic and asymptomatic patients

	Asymptomatic patients (n=61)	Symptomatic patients (n=29)
No (%) HBeAg positive	57 (93)	20 (69)
No (%) anti-HBe positive	4 (7)	9 (31)
Mean (SD) alanine aminotransferase (IU/l)	210 (273)	183 (254)
No (%) with diagnosis from histology of:		
Chronic active hepatitis	39 (79)	17 (59)
Chronic persistent hepatitis	8 (16)	9 (31)
Cirrhosis	1 (2)	2 (7)
Minimal changes	1 (2)	1 (3)

household contact. Two children had a previous transfusion, and in 11 children the cause of the infection was unknown.

Serology for the presence of markers for hepatitis B virus, anti-HIV, antihepatitis delta (anti-HD), and liver function tests were performed every four months using standard methods (Abbott commercial assays and SMAC Technicon). HBV-DNA was tested by dot-blot hybridisation, using a HBV-DNA probe isolated from the pBH20-HBV plasmid. The specific activity of the ^{32}P -HBV-DNA after nick-translation was 7×10^7 – 3×10^8 cpm/ μg DNA. This technique detected less than 0.5 pg of HBV-DNA.

At the beginning of the study a liver biopsy was performed under general anaesthesia in 78 of the 90 children using a Tru-Cut needle. A second liver biopsy was performed during a mean follow up of three years (range from eight months to seven years) in 27 children. The presence of liver HDag among anti-HD positive patients was detected by immunoperoxidase staining using a conjugated anti-HD antibody (anti-HD titre: 1/1000). Liver HBsAg and

hepatitis B core antigen (HBcAg) were also tested by immunoperoxidase technique (Dakopatts a/s).

Results

In relation to the 'e' system, 77 children were HBeAg positive and 13 had anti-HBe. A total of 71 of the HBeAg positive children had circulating HBV-DNA. HBeAg positive patients had a significantly higher mean (SD) initial alanine aminotransferase activity than those with anti-HBe (232 (289) compared with 37 (14) IU/l). During follow up 24 became negative for HBeAg and HBV-DNA and 21 developed anti-HBe. The time from the beginning of the study until the seroconversion to anti-HBe varied from six months to five (2.3 (1.4)) years. The annual seroconversion rate was 14%. None of the anti-HBe children had HBV-DNA, although two were anti-HD positive.

At the end of the study all the children were clinically asymptomatic. With respect to the physical signs the frequency of hepatomegaly increased in the children who remained with HBeAg (basal 13 (24%) compared with final 31 (58%)), while a decrease was observed in children who seroconverted to anti-HBe (basal 10 (47%) compared with final three (14%)). No changes were observed in the anti-HBe children (basal five (38%) compared with final two (15%)).

Among the 21 children who seroconverted to anti-HBe, an increase of at least three times the basal alanine aminotransferase value (18 (12) times the normal value) before seroconversion was observed in 18 cases (85%). The time between the alanine aminotransferase peak and the appearance of anti-HBe was 13 (5.6) months (range: 6–28 months). These alanine aminotransferase peaks were similar to those found in acute hepatitis, although the children did not suffer any symptom or clinical complication. All the children who seroconverted to anti-HBe had normal alanine aminotransferase by

4.3 (5.8) months (range: 0–17 months) and remained so during the follow up (two years). Eight children who were anti-HBe positive initially had normal alanine aminotransferase activities. Two children returned to normal in 12 months but three remained abnormal in follow up periods of 38 to 72 months. Two of the latter had anti-HD in serum and HDAg in the liver biopsy specimen.

A basal liver biopsy was performed on 68 of the 71 children with viral replication markers (HBeAg, HBV-DNA). Although most of the children (n=54) had a chronic active hepatitis, 11 had a chronic persistent hepatitis, two had liver active cirrhosis, and one had minimal changes. After the beginning of the study, a second liver biopsy was obtained in 24 children. Of the 17 who remained positive to HBeAg, the histological picture was the same in nine cases. The other eight children showed a worsening of the histological lesion (table 3). All 17

children remained positive to liver HBsAg and HBeAg, most of them (15/17) showed a diffuse pattern of HBeAg.

A second liver biopsy was also performed on seven children who seroconverted to anti-HBe during the three and five year periods (mean 3.8 (0.8) years). Five cases showed an improvement in the histology and in two cases the same liver lesion was observed. The basal liver biopsy specimen in these seven children was positive to HBsAg and HBeAg. In the second biopsy specimen, however, HBeAg was negative in all these cases and HBsAg remained positive. All but one had a predominantly focal pattern of HBeAg in the liver cell. The study of the HBsAg and HBeAg expression in the rest of the patients who had a liver biopsy was not available.

The basal histology of the 10 anti-HBe positive patients was two with chronic active hepatitis, six with chronic persistent hepatitis, one with inactive cirrhosis, and one with minimal changes. The liver histology from the second biopsy specimen obtained in the three anti-HBe children who remained with an abnormal alanine aminotransferase activity, resulted in the same histological findings in two cases (HDAg positive). In the HDAg negative patient, an amelioration of the histological lesion was observed.

Table 3 Histological evolution of chronic hepatitis B in children

	Liver biopsy	
	Basal	Final
No (%) children who remained HBeAg positive during the study (n=17):*		
Chronic active hepatitis	10 (59) (9 mild, 1 moderate)	11 (65) (7 mild, 4 moderate)
Chronic persistent hepatitis	6 (35)	3 (18)
Active cirrhosis	1 (6)	3 (18)
No (%) children who seroconverted from HBeAg to anti-HBe (n=7):†		
Chronic active hepatitis	5 (72)	—
Chronic persistent hepatitis	2 (28)	4 (57)
Minimal changes	—	3 (43)
No (%) children who remained anti-Hbe positive (n=3):‡		
Chronic active hepatitis	2 (67) (HDAg+) (1 mild, 1 moderate)	2 (67) (HDAg+) (2 moderate)
Chronic persistent hepatitis	1 (33)	—
Minimal changes	—	1 (33)

Mean (SD) time between basal and final liver biopsies: *2.7 (1.5) years; †3.8 (0.8) years; and ‡4.1 (0.6) years.

Discussion

In our experience children with hepatitis B virus replication markers suffered a more active liver disease biochemically and histologically than those without replication. These results are in accordance with previous reports.^{7,8} Although at the end of the study all the children were asymptomatic, in those who seroconverted to anti-HBe a decrease in the frequency of hepatomegaly was observed, with an important histological improvement. In contrast, children who remained HBeAg positive had an increase in the frequency of hepatomegaly and a progression of the histological lesion (in some cases to liver active cirrhosis). This result is lower than those reported by other authors,^{4,5} and different from that of Bortolotti *et al* who did not find progression to cirrhosis.^{2,7} A possible explanation could be the technical differences in the performance of the liver biopsies. Dupuy *et al* obtained the biopsy specimens under laparoscope control,⁴ which could increase the probability of a diagnosis of cirrhosis.⁸ We used a blind biopsy technique with a Tru-Cut rather than a Menghini needle, as was used by Bortolotti *et al*.⁷ Therefore, laparoscope and the longer cylinder obtained with our needle will most likely account for the differences.

Additionally, our data differ from that of Lai *et*

al.⁹ Our children with hepatitis B virus replication markers had an abnormal alanine aminotransferase activity, while most of their Chinese children had normal activities. This variation could be explained by racial and epidemiological differences. Chinese children acquired the infection at birth, while ours did not. The interferon concentrations in lymphocytes of Chinese people are normal but are abnormal in white races.¹⁰

All the children from our study who seroconverted remained HBsAg positive; this is in accordance with previous studies.^{6,8} Thus the risk of the development of hepatoma probably persists in these children.¹¹ Further studies are required to indicate if HBV-DNA remains in an integrated form after seroconversion.

The children with persistent viral replication had a diffuse pattern of HBcAg in the liver. In contrast, those children who seroconverted to anti-HBe showed a focal pattern. These results agree with those of Bortolotti *et al*,¹² and so testing for HBcAg in the liver could be useful as a prognosis indicator.

During seroconversion, an increase in alanine aminotransferase activity similar to that in acute hepatitis was observed in most of the children. In our experience, however, only 50% of the adults who seroconverted had a previous alanine aminotransferase increase.¹³ Although we are unsure as to the reason for this difference between adults and children from the same population, a possible explanation is the difference in their immune systems. In support of this theory, we observed an important regression of liver disease in adults during seroconversion while none was detected in the children.¹⁴ This variation could be due to the fact that liver cirrhosis is less common in children, or also that they regenerate the liver cells more quickly than adults.

With the exception of two patients with delta superinfection, all the children with anti-HBe and abnormal alanine aminotransferase activity at the beginning of the study had normal alanine aminotransferase at the end of the follow up period. Therefore, the anti-HBe status in children correlated with a normal alanine aminotransferase activity and good prognosis in the absence of the delta virus. During the follow up reactivation of viral replication in the anti-HBe children did not take place as all the children remained HBV-DNA negative in contrast with that reported in adults.¹⁵ A similar lack of HBV-DNA among anti-HBe children has been reported.^{2,5} This suggests that clearance of all HBV-DNA replicative forms in hepatocytes occurred in these children. In adults, however, HBV-DNA supercoiled could remain in hepatocytes due to the lack of elimination and

consequently reactivation takes place in about 40% of anti-HBe cases (unpublished data).

This study confirms the high frequency of advanced liver disease in association with chronic hepatitis B virus infection and a persistently raised serum transaminase activity for a period of more than six months. Such patients should be considered for treatment with antiviral agents both to limit progression of liver disease and to reduce the risks of HBV-DNA integration and the subsequent development of hepatoma.^{16,17} In such a case, if the treatment is efficient, the prognosis of the disease will be more favourable.¹⁸

JB was supported by a grant from the Fondo de Investigaciones Sanitarias de la Seguridad Social, Insalud.

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Accepted 16 March 1989