Long-Term Outcome in Children with Chronic Hepatitis B: A 24-Year Observation Period

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Background. Chronic hepatitis B seems to manifest as mild disease in children and young adults. However, data regarding the long-term course of hepatitis B in untreated and interferon-treated children are still scarce. This study investigates the long-term outcome of disease in a large series of untreated and treated children with hepatitis B virus (HBV) infection.

Methods. Clinical, biochemical, virological, and histological features were evaluated in children (age range, 2–18 years) with chronic HBV infection who did not have concomitant chronic systemic diseases other than HBV infection and who were admitted to the liver unit in the Department of Pediatrics at University “Federico II” (Naples, Italy) during the period 1981–2005.

Results. One hundred eight consecutive patients observed for up to 24 years were studied. During the observation period, 67 children remained untreated, and 41 were treated with interferon-α. After a median period of observation of 12.1 years (range, 5–23 years), hepatitis B early antigen loss and serum HBV DNA clearance occurred in 43 untreated patients (69.3%) who were hepatitis B early antigen positive at study entry and in 33 treated children (80%; the P value is not statistically significant). In addition, 6 untreated patients (9.7%) and 4 treated patients (9.7%) became hepatitis B surface antigen positive at the end of the follow-up period. Histological assessment, evaluated for 57 children, showed mild-to-moderate disease in 91.2% of cases of HBV infection. No patient developed end-stage liver disease or hepatocellular carcinoma.

Conclusions. Children with chronic HBV infection are symptom free, with morphologically mild liver disease. Considering that the overall long-term outcomes did not differ between treated and untreated patients, the real impact of therapy on the long-term course of HBV infection remains to be established. Additional studies are needed to confirm our conclusions.
have been reported in 23% of treated children, compared with 13% of untreated control subjects [16].

In contrast with chronic hepatitis C virus infection, in which a sustained clearance of serum hepatitis C virus RNA is obtained in 50%–60% of patients treated with pegylated IFN plus ribavirin [17], in patients with CHB, a definitive clearance of serum HBV DNA is more rarely observed in instances of favorable response to therapy.

The ultimate goal of therapy is to prevent morbidity and mortality related to the development of cirrhosis and HCC. The parameters used to assess treatment response include HBeAg clearance and decrease in serum HBV DNA level [18]. Data regarding differences between spontaneous seroconversion to anti-HBe and treatment-induced seroconversion during the long-term course of infection are still scarce.

The aim of this study was to provide information on long-term outcome after spontaneous and treatment-induced HBeAg seroconversion in patients with chronic HBV infection acquired during childhood who were observed regularly from childhood to young adulthood. For this purpose, treated and untreated patients observed at the liver unit in the Department of Pediatrics at University “Frederico II” (Naples, Italy) over a 24-year period were retrospectively evaluated and compared for the clinical, biochemical, and virological outcomes.

PATIENTS AND METHODS

Patients. All children with CHB (defined as the presence of hepatitis B surface antigen [HBsAg] in serum for ≥6 months) who were observed for >5 years and presented at the liver unit in the Department of Pediatrics at University “Frederico II” during the period 1981–2005 were enrolled in our study. Exclusion criteria included presence of concomitant systemic diseases, concurrent hepatitis C virus, hepatitis delta virus, HIV infection, or other liver disease (e.g., α-1-antitrypsin deficiency, Wilson disease, autoimmune hepatitis, cystic fibrosis, and celiac disease–related liver damage).

At each evaluation, symptoms and health-related quality-of-life data were recorded by clinical examination and appropriate interview; growth was evaluated using standard height and weight charting [19]. At baseline, all patients underwent physical examination and liver function tests and were investigated for clinical history, risk factors for HBV infection, age at infection, clinical signs of liver disease, blood cell count, virological markers (i.e., HBsAg, antibody to HBsAg, HBeAg, anti-HBe, and serum HBV DNA levels), α-fetoprotein level, serum immunoglobulin level, and non–organ-specific autoantibody levels. Serum aspartate aminotransferase and alanine aminotransferase (ALT) levels were evaluated using standard methods (upper limit of normal, 50 IU/L).

Thereafter, all patients were monitored every 3–6 months by physical examination, liver function tests, virological tests, and α-fetoprotein level determination. At 6–12-month intervals, autoimmunity markers were determined for treated patients. Ultrasonographic examination of the liver, biliary tract, spleen, and portal vein was performed at 12-month intervals.

Liver biopsy specimens, obtained after receiving informed consent from parents or legal guardians, were analyzed by the same liver pathologist, who was blinded to clinical and biochemical data. Specimens were scored with regard to hepatitis activity (grades, 0–18) and fibrotic changes (stages, 0–6), according to Ishak et al. [20].

Data on IFN treatment were collected. Complete response to therapy was defined as a decrease in transaminase levels (within the normal range), an HBV DNA level <10^5 copies/mL, clearance of HBeAg, and development of anti-HBe within 12 months after stopping treatment [18].

For untreated children, biochemical and virological aspects were monitored throughout the follow-up period. Inactive carrier state was defined as a detectable HBsAg level, an undetectable HBeAg level, a detectable anti-HBe level, a serum HBV DNA level <10^4 copies/mL, and persistently normal ALT and aspartate aminotransferase levels. Resolved hepatitis B state was defined as previous HBV infection, with an undetectable HBsAg level, a normal ALT level, and an undetectable serum HBV DNA level [18]. This study was performed in accordance with the Helsinki Declaration.

Laboratory procedures. At study entry and at each visit, a serum sample was collected and stored at −80°C. Biochemical and virological tests were performed on fresh or frozen samples. Viral markers (HBsAg, antibody to HBsAg, HBeAg, and anti-HBe levels) were measured using commercial immunoassay kits (Abbott Diagnostics). Serum HBV DNA level was quantitatively investigated, depending on the time, with a commercial hybridization method (cutoff value, 5 pg/mL; Abbott Diagnostics) or with a commercial PCR assay (Amplircor HBV-Monitor kit; Roche Diagnostic System).

Statistical analysis. All data are expressed as median and range or mean ± SD. Comparison of categorical variables was performed using the χ² test or Fisher’s exact test. Comparison of continuous data was performed using the Mann-Whitney U test and the Kruskal-Wallis test. A P value <.05 was considered to be statistically significant.

RESULTS

One hundred eight consecutive patients (65 male patients; median age at last observation, 17.9 years; range, 6–34.2 years) observed over a period of up to 24 years were enrolled in the study. The course of CHB was evaluated for a median period of 12.1 years (range, 5–23 years).

During the observation period, 67 children remained untreated, and 41 were treated with IFN-α; these patients had been included in clinical trials performed at our department.
in previous years [21]. At baseline, treated and untreated patients were comparable with regard to demographic, clinical, and laboratory features (table 1). All but 1 patient were white.

A total of 57 liver biopsy specimens were obtained from 37 treated and 20 untreated children. For treated patients, biopsy was performed before starting IFN-α therapy at a mean duration of HBV infection of 6.4 ± 2.9 years. The mean duration of disease at histological evaluation for untreated children was 7.2 ± 2.9 years (the P value is not statistically significant). Histological assessment revealed the presence of mild disease in most patients. In particular, 33 children (57.9%; 19 treated and 14 untreated; the P value is not statistically significant) had minimal hepatitis, and 19 patients (33.3%; 14 treated and 5 untreated; the P value is not statistically significant) had moderate hepatitis. Severe hepatitis was present in 4 patients (7.1%; 3 treated and 1 untreated; the P value is not statistically significant), and features of micronodular cirrhosis were detected in 1 child (1.7%; an 8-year-old boy with unknown duration of disease who subsequently received IFN-α treatment).

All patients were asymptomatic at presentation and remained symptom-free throughout the observation period, with the exception of IFN-α–related adverse effects among the treated patients. None of the patients showed abnormal growth. None of the patients showed signs of hepatic decompensation, and all of the patients had normal levels of albumin and international normalized ratio. Monitoring of a-fetoprotein levels and ultrasonographic findings did not reveal signs of HCC in any of the patients.

Treated patients. The median duration of follow-up for 41 IFN-treated children was 13.7 years (range, 5.4–20.9 years). On the basis of trials in which children had been included, 13 patients received recombinant IFN-α-2b therapy (5–10 MU/m², 3 times per week for 6–12 months), and 28 were treated with α-lymphoblastoid IFN (5 MU/m², 3 times per week for 6–12 months). No statistically significant difference was found between the 2 groups (divided according to different IFN-α treatments) with regard to sex, route of infection, age at the start of therapy, and duration of disease (data not shown); a statistically significant difference was found for basal ALT level, which was higher in patients who received α-lymphoblastoid IFN (ALT × normal value, 2.1; range, 1.3–7.9) than in those treated with recombinant IFN-α-2b (ALT × normal value, 1.5; range, 1.3–2.3; P = .035). Treatment was stopped before the end of the established period because of adverse events or significant elevation of transaminase levels in 4 patients. The median duration of posttherapy follow-up for 41 patients was 10.2 years (range, 3–15.5 years). Only 3 children had a posttherapy observation period that was a duration of <5 years (median; range, 3–4 years).

A complete response to treatment occurred in 9 patients (21.9%; 6 treated with α-lymphoblastoid IFN and 3 treated with recombinant IFN-α 2b; the P value is not statistically significant). At the end of the observation period (figure 1), 23 patients (56%) achieved seroconversion to anti-HBe, an HBV DNA level <10⁴ copies/mL, and a normal ALT level (i.e., inactive carrier state). In 4 patients (9.8%) who achieved seroconversion to anti-HBe and normal transaminase levels, the HBV DNA level was 10⁴ copies/mL to 10⁷ copies/mL. HBV infection resolved in 4 patients (9.8%) who seroconverted to antibody to HBsAg. Six patients (14.8%) maintained detectable HBeAg and HBV DNA levels, as well as hypertransaminasemia. Two children (4.8%) seroconverted to anti-HBe, despite the persistence

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>IFN-treated children (n = 41)</th>
<th>Untreated children (n = 67)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex, M:F</td>
<td>26:15</td>
<td>39:28</td>
</tr>
<tr>
<td>Age, median years (range)</td>
<td>5.7 (1–14.7)</td>
<td>6.7 (1.1–13.2)</td>
</tr>
<tr>
<td>Route of infection⁴</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vertical</td>
<td>14 (34.1)</td>
<td>32 (47.8)</td>
</tr>
<tr>
<td>Horizontal</td>
<td>15 (36.6)</td>
<td>13 (19.4)</td>
</tr>
<tr>
<td>Unknown</td>
<td>12 (29.3)</td>
<td>22 (32.8)</td>
</tr>
<tr>
<td>Age at diagnosis, median years (range)</td>
<td>3.9 (0.3–14)</td>
<td>4 (0.2–12)</td>
</tr>
<tr>
<td>ALT level</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>5 (12.2)</td>
<td>18 (26.9)</td>
</tr>
<tr>
<td>Elevated</td>
<td>36 (87.8)</td>
<td>49 (73.1)</td>
</tr>
<tr>
<td>Median ALT level × ULN (range)</td>
<td>2 (1.4–7.9)</td>
<td>2.2 (1.1–26)</td>
</tr>
<tr>
<td>Detectable HBeAg and HBV DNA levels</td>
<td>41 (100)</td>
<td>62 (92.5)</td>
</tr>
</tbody>
</table>

NOTE. Data are no. (%) of patients, unless otherwise indicated. P > .05 for all variables. ALT, alanine aminotransferase; HBeAg, hepatitis B early antigen; ULN, upper limit of normal.

⁴ Vertical transmission was defined as transmission of HBV infection from a mother with a detectable HBsAg level in the absence of other risk factors for HBV infection.
of elevated ALT levels and viral loads >10\(^5\) copies/mL. This serological pattern was suggestive of HBeAg-negative CHB (i.e., CHB in the absence of a detectable HBeAg level). Two patients (4.8%) who achieved clearance of HBeAg and undetectable HBV DNA levels presented with mild ALT level abnormalities, in the absence of other known causes of liver damage.

**Untreated patients.** Sixty-seven untreated children were observed for a median duration of 10 years (range, 5–23 years). At baseline, 62 children had a detectable HBeAg level. Among this group, at the end of the observation period (figure 2), 30 patients (48.4%) became inactive carriers; 4 (6.4%) achieved seroconversion to anti-HBe, normal ALT levels, and HBV DNA levels of 10\(^4\) copies/mL to 10\(^5\) copies/mL; and 6 (9.7%) achieved clearance of HBsAg (i.e., resolved hepatitis B) and seroconverted to antibody to HBsAg. In contrast, 16 patients (25.9%) continued to have detectable HBeAg and serum HBV DNA levels, as well as elevated transaminase levels. Three patients (4.8%) had a detectable HBV DNA level in serum but an undetectable HBeAg level in serum (i.e., HBeAg-negative CHB). Three patients (4.8%) who achieved seroconversion to anti-HBe had mild ALT level abnormalities during follow-up, in the absence of viremia. In this subgroup, coinfections, metabolic diseases, and autoimmunity were ruled out. Five untreated children were already HBeAg negative before first observation; 1 child experienced spontaneous HBsAg seroclearance during the follow-up period.

**Seroconversion to anti-HBe in treated and untreated patients.** No correlation was found between seroconversion and age, sex, and baseline ALT level. Median age at seroconversion (10 years; range, 3–22 years) did not differ between treated (median age, 10.3 years; range, 3–21 years) and untreated patients (median age, 9 years; range, 3–22 years; the P value is not statistically significant). The mean ALT serum level measured at the evaluation before seroconversion increased 1.5 times the value detected at the previous control evaluation (P < .001). No other significant predictive factors of seroconversion were identified. In addition, the long-term outcome of CHB was not significantly related to the different route of infection among 74 children with known source of infection (table 2).

At the end of the follow-up period, 86 children (79.6%) had experienced HBeAg seroclearance. This clearance was associated with development of anti-HBe and an HBV DNA level <10\(^5\) copies/mL in 74 patients. Twenty-two patients (20.4%) still had a detectable HBeAg level at the end of the follow-up period. The median duration of follow-up was 13.2 years (range, 5–23 years) for patients with undetectable HBeAg levels and 6.7 years (range, 5.1–20.4 years) for those with persistent detectable HBeAg levels (P = .01).

Among the treated children, throughout the observation period, 9 patients who experienced complete response maintained an undetectable HBeAg level in serum. In addition, 24 (75%) of 32 nonresponders seroconverted to anti-HBe >12 months after treatment withdrawal. Among the untreated children, 43 (69.3%) of 62 patients with a detectable HBeAg level at baseline experienced HBeAg clearance. At year 6 after the start of treatment or observation, the rate of HBeAg clearance in treated and untreated patients overlapped, becoming 63.4% among...
treated patients and 62.7% among untreated children (the P value is not statistically significant); the final clearance rate of HBeAg did not statistically differ between treated and untreated patients (table 3). None of the patients who seroconverted to anti-HBe (either spontaneously or as a result of therapy) developed acute exacerbation or seroreversion to HBeAg during a median postseroconversion period of 7.2 years (range, 2.3–20 years).

**DISCUSSION**

This 24-year retrospective study contributes information regarding the long-term outcome of CHB acquired in childhood. None of the patients in our study became symptomatic or developed decompensated liver disease requiring liver transplantation. None of the patients died of liver-related causes. At the final evaluation, 65.8% of treated patients and 54.8% of untreated patients with detectable HBeAg levels seroconverted to anti-HBe and had serum HBV DNA levels <10^5 copies/mL; ~8% of the patients achieved a detectable anti-HBsAg level. With regard to seroconversion to anti-HBe in adults, several predictors have been identified [18]; in the present study, a significant increase in the serum ALT level was observed during the months preceding seroconversion.

Several studies on CHB acquired during childhood revealed a benign course of disease [7, 21, 22]. In a study including 52 children followed up for 3–22 years, Fujisawa et al. [21] reported a percentage of HBeAg clearance of 83.3% in untreated patients and 87.5% in treated patients; none of the patients achieved an undetectable HBsAg level. The authors explained that this lower rate of HBsAg clearance was a result of perinatal acquisition of HBV infection. In a recent longitudinal study involving 97 white patients with CHB, 91% of untreated patients and all treated patients without cirrhosis became inactive carriers; the HBsAg clearance rate was 17.5% [22].

Inactive carrier state seems to be stable for many years, as previously reported in Italian studies [22, 23]. In our cohort, patients were strictly followed up for a long period after seroconversion to anti-HBe. The serological profiles of our patients were stable for a median period of 7.2 years after seroconversion. It is notable that patients who did not experience seroconversion had a significantly shorter observation period than did patients who experienced seroconversion; therefore,
it is possible that a percentage of these patients will experience seroconversion at a later time. All inactive carriers had serum HBV DNA levels <10^5 copies/mL, but a group of these patients had a detectable HBV DNA level by PCR. Other authors reported that a sizable percentage of patients with an undetectable HBeAg level had a detectable HBV DNA level by PCR [24, 25]. These low levels of viremia probably reflect a low viral replication persisting for several years in patients with CHB, even after remission of liver disease and clearance of HBsAg. In our cohort, 4.6% of anti-HBe–positive patients experienced mild ALT level abnormalities during the follow-up period, in the absence of HBeAg seroreversion and increase in viral load. However, it is unlikely that the low HBV DNA level (<=300 copies/mL) detected in this subgroup could be responsible for the slight ALT level elevation.

Several studies involving adults have shown that the long-term outcome in patients after IFN-related HBeAg seroconversion is favorable in terms of HBV clearance, reduction of HCC, and prolongation of survival [26, 27]. On the other hand, there is evidence that spontaneous seroconversion induces sustained remission in the majority of cases [28]. Although seroconversion seems to also confer favorable outcomes in children with HBV infection, Bortolotti et al. [22] reported 2 cases of HCC, 9 and 16 years after seroconversion. The possibility of spontaneous late reactivation and the occurrence of cirrhosis and HCC in patients with undetectable HBeAg and HBV DNA levels suggest that long-term surveillance is necessary for all patients with CHB, including inactive carriers and patients who do not have cirrhosis [29].

In our study, the development of HBeAg-negative CHB was not frequently observed both in untreated children and in treated children, being present in only 5 of 108 patients. This result is in contrast with results in earlier reports involving adults with CHB, in which the patients experienced HBeAg-negative CHB in a much higher proportion of cases [8]. Although we did not investigate the presence of an e-minus mutant, none of the patients in our study who had HBeAg-negative CHB showed clinical, laboratory, or echographic signs of severe liver damage.

To date, there have been no studies with a sufficiently long follow-up period to calculate the risk of progression towards serious liver disease in children with CHB. Cirrhosis and HCC due to chronic HBV infection have been described to occur rarely during the pediatric age and more frequently during the second decade of life [10–12]. In our series, only 1 patient had histological signs of cirrhosis, and none of the patients developed HCC. Because follow-up biopsies were not systematically performed in our study, it is possible that the progression to compensated cirrhosis could have been missed. However, strict investigation using ultrasonographic examination did not reveal features of severe liver damage in any of the patients.

A careful understanding of the natural history of HBV infection in children is important in making decisions regarding treatment. One of the most debated questions is whether treatment can modify the long-term course of CHB. To date, indications for treatment of children with CHB are still controversial, and current therapy has limited long-term benefits [30]. The goal of antiviral therapy is to reduce liver-related morbidity and mortality and to minimize the risk of transmission. Although it has been reported that IFN-α therapy accelerates HBeAg seroconversion in children [15], it is still not known whether faster seroconversion can modify the natural history of the disease. In our series, no advantage at final evaluation in terms of normalization of ALT levels, clearance of HBV DNA, and seroconversion was observed in patients treated with IFN-α, compared with untreated patients. In addition, the rate of

**Table 2. Outcome of chronic hepatitis B virus infection in 74 children with overt route of infection.**

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Vertical (n = 46)</th>
<th>Horizontal (n = 28)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clearance of HBeAg and acquisition of anti-HBe</td>
<td>35 (76.1)</td>
<td>25 (89.3)</td>
</tr>
<tr>
<td>Persistence of detectable HBeAg levels</td>
<td>9 (19.6)</td>
<td>2 (7.1)</td>
</tr>
<tr>
<td>HBeAg-negative chronic hepatitis B</td>
<td>2 (4.3)</td>
<td>1 (3.6)</td>
</tr>
</tbody>
</table>

**NOTE.** Hepatitis B early antigen (HBeAg)–negative chronic hepatitis B is defined as chronic hepatitis B in the absence of a detectable HBeAg level. Anti-HBe, antibody to HBeAg.

**Table 3. Outcome of chronic hepatitis B virus (HBV) infection in treated and untreated children at the end of the observation period.**

<table>
<thead>
<tr>
<th>Outcome</th>
<th>No (%) of IFN-treated patients (n = 41)</th>
<th>No. (%) of untreated patients (n = 67)a</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clearance of HBeAg and acquisition of anti-HBe</td>
<td>27 (65.9)</td>
<td>38 (56.7)</td>
</tr>
<tr>
<td>Resolved hepatitis B</td>
<td>4 (9.7)</td>
<td>7 (10.4)</td>
</tr>
<tr>
<td>Persistence of detectable HBeAg levels</td>
<td>6 (14.8)</td>
<td>16 (23.9)</td>
</tr>
<tr>
<td>HBeAg-negative chronic hepatitis B</td>
<td>2 (4.8)</td>
<td>3 (4.5)</td>
</tr>
<tr>
<td>Fluctuating ALT levels, HBV DNA level &lt;10^5 copies/mL, and an undetectable HBeAg level</td>
<td>2 (4.8)</td>
<td>3 (4.5)</td>
</tr>
</tbody>
</table>

**NOTE.** Hepatitis B early antigen (HBeAg)–negative chronic hepatitis B is defined as chronic hepatitis B in the absence of a detectable HBeAg level. Anti-HBe, antibody to HBeAg.

* Five of 67 untreated children already had undetectable HBeAg levels at study entry.
HBsAg clearance was similar between the 2 groups, confirming that IFN-α therapy accelerates only seroconversion to anti-HBe. Compared with IFN-α, lamivudine is less expensive and well-tolerated, but the durability of response appears to be lower, and prolonged therapy is associated with increasing risk of lamivudine-resistant mutants, which may compromise the initial benefits and eventually determine a worsening of liver disease. Although viral clearance is important to reduce the risk of transmission, especially in a region where the vaccine is not extensively used, currently, the real impact of antiviral therapy seems to be scarce. However, it is not possible to exclude that, in the upcoming years, the use of new antiviral treatments (e.g., adefovir, entecavir, and telbivudine) that have not yet been approved for use in children could modify the management of CHB during pediatric age.

In conclusion, the overall prognosis for CHB in vertically and horizontally infected patients seems to be favorable. On the basis of our data, IFN-α treatment did not significantly influence the long-term seroconversion rate among patients with chronic HBV infection acquired during childhood. These findings should be considered when selecting candidates for treatment and choosing antiviral agents.

Acknowledgments

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References