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LONG – TERM FOLLOW – UP OF HEPATITIS B VIRUS CARRIERS WITH NORMAL TRANSAMINASES LEVELS*

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There has been very little data recorded on the natural course of chronic hepatitis B virus infection in asymptomatic children. In order to assess the natural course of liver disease in hepatitis B surface antigen (HBsAg) carriers with normal liver tests, 124 such children (81 males, 65.3%) were followed for six to 144 months (mean 36.8 ± 22.8 months). Liver tests and hepatitis B virus (HBV) markers were tested at least every six months.

In the beginning, hepatitis B e antigen (HBeAg) was positive in 61 (53%) of the 115 carriers of HBsAg who were tested. Anti-HBe was positive in 51 (44.3%), and both HBeAg antigen and anti-HBe were negative in three (2.7%) carriers. The prevalence of HBeAg was not affected by the patient's age or sex. During follow-up, 11 patients (18%) lost HBeAg (a mean annual clearance rate of 5.8%), and 12 patients (9.7%) lost HBsAg (a mean annual clearance rate of 3.1%). We found no difference in the clearance of HBsAg and HBeAg by age and sex. The presence of another HBsAg positive person in the family affected HBsAg clearance rate but not HBeAg clearance. Only seven patients (5 HBeAg positive and 2 anti-HBe positive) developed transient elevations in liver transaminases. Three of five HBeAg positive children cleared HBeAg after transaminases elevations. Of the five patients who underwent percutaneous liver biopsies, non-specific changes were found.

It is concluded that hepatitis B carriers with normal liver tests should be followed with liver function tests alone and that long-term prognosis is good. *Key words: hepatitis B virus carrier, transaminases level, long-term follow-up.*

Most carriers of hepatitis B surface antigen (HBsAg), both children and adults, are clinically asymptomatic and serum transaminases levels are normal[1-3]. In most of the patients, HBsAg is detected incidentally or as part of the screening of blood donors and relatives of HBsAg positive individuals. Although the natural course of hepatitis B virus (HBV) infection in adults has been reported from various parts of the world[3, 4], there has been very little data recorded on the natural course of HBV infection in asymptomatic children, particularly in those with normal liver tests. Most of the reports have been based on observations

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of long-term HBsAg carriers with elevated liver enzymes and with chronic liver disease[5-9], whereas the infection in most children is asymptomatic and with normal liver enzyme concentrations[1-2, 10-11].

In order determine the natural course and the frequency of significant liver disease in HBsAg carrier children with normal liver biochemical tests, we followed a series of such patients.

Material and Methods

The patients were enrolled into the study either incidentally, by screening of relatives of HBsAg carriers or by following children with acute hepatitis B infection. HBsAg carrier state was defined in children recruited by screening, as any individual who had two HBsAg positive serum samples obtained six months apart. Only patients with normal tansaminases levels during this period were enrolled into the study; those with abnormal alanine aminotransferase (ALT) values (> 40 IU/L) during the first six months of follow-up were excluded. Patients with acute hepatitis B infection were enrolled into the study after their transaminases levels returned to normal and remained as such for six months. Liver biopsies were performed when fluctuations of transaminases were seen after the first six month period.

Patients were seen regularly at least every six months. Each follow-up visit included both a physical examination and biochemical tests including ALT, bilirubin, albumin and alkaline phosphatase levels. Routine liver functions were studied by sequential multiple autoanalyzer (Hitachi 911 Automatic Analyzer), at 37 °C, with standards supplied by the manufacturer. Markers of HBV, including HBsAg, hepatitis B e antigen (HBeAg), and antibodies against HBsAg (anti-HBs) and HBeAg (anti-HBe), were also determined at each visit by commercially available radioimmunoassay kits. All patients underwent abdominal ultrasonographic examination at least on one occasion. Hepatitis B virus DNA determination was not possible during the follow-up period. Available parents and siblings were also evaluated for HBV markers.

Differences in the proportions were tested with chi-square or Fisher's exact test. Differences between means were tested with Student's t test or Mann-Whitney U test when appropriate[12]. A p value of less than 0.05 was considered as significant. Data were given as mean ± SD.

Results

There were 124 patients (81 males, 65.3%; 102 recruited by screening, 82.2%) who ranged in age from nine months to 17 years (mean, 88.1 ± 45.7 months) at the time of enrollment. They were followed for six months to 144 months (mean,

36.8 ± 22.8 months, 380 patient years). Twenty of them were followed for more than 60 months. The mean age of the patients at the end of follow-up was 124.2 ± 46.6 months (range 18 to 231 months).

One hundred and fifteen patients were tested for HBeAg and anti-HBe. Sixty-one of them (53%) were found to be positive for e antigen and 51 (44%) carried anti-e. The remaining three (2.7%) were negative for both e and anti-e. There was no difference in the prevalence of HBeAg for males and females, 51.3% and 56.4%, respectively (p = 0.6, chi-square test). There was no statistically significant difference in the mean age between HBeAg positive and negative children (89.4 ± 44.1 months vs. 93.7 ± 45.2 months, p = 0.27, Student's t test).

Thirty-one of 66 mothers (46.9%) tested were HBsAg positive. This ratio was 76.7 percent (42/55) and 27.2 percent (15/55) respectively, for siblings and fathers tested. At least one family member was HBsAg positive in 60 out of 84 (71.4%) families tested. The percentages of HBeAg positivity in patients were not different between children whose family members were HBsAg positive and those whose family members were HBsAg negative (52.5% vs. 52.2%, p = 0.98, chi-square test). Table I shows the baseline clinical, serological and epidemiological features of the patients.

Table I: Baseline Clinical, Serological and Epidemiological Features of the Patients (n = 124)

	n (%)
Sex	
Male	81 (65.3)
Female	43 (34.7)
Age (mo)	
at the beginning, range (mean ± SD)	9-204 (88.1 ± 45.7)
at the end, range (mean ± SD)	18-231 (124.2 ± 46.6)
of HBeAg (+) patients (n = 61) (mean ± SD)*	89.4 ± 44.1
of HBeAg (-) patients (n = 54) (mean ± SD)*	93.7 ± 15.2
Duration of follow up	
Range (mo), (mean ± SD)	6-144 (36.8 ± 22.8)
HBeAg/anti-HBe status (n = 115)	
HBeAg (+)†	61 (53.0)
male	39/76 (51.3)
female	22/39 (56.4)
Anti-HBe (+)‡	51 (44.3)
male	35/76 (46.0)
female	16/39 (41.0)
Neither	3 (2.7)
Household HBsAg status (positive/number of studied persons)	
Maternal HBsAg positivity	31/66 (46.9)
Paternal HBsAg positivity	15/55 (27.2)
Sibling HBsAg positivity	42/55 (76.7)
At least one positive family member	60/84 (71.4)

* p = 0.27 † p = 0.6 ‡ p = 0.75

Only 11 of 61 patients (18%) who were HBeAg positive cleared HBeAg from their sera during the study period and all of them had seroconversion to anti-HBe. Since they were followed for a mean of 37 months, the mean annual seroconversion rate was 5.8 percent. The clearance of HBeAg occurred between eight and 60 months (median 12 months). There was no difference between males and females with regard to seroconversion, 17.9 and 9.1 percent, respectively ($p > 0.05$, Fisher's exact test). Although the difference was statistically insignificant, the patients clearing HBeAg were older than those who did not, 97.1 ± 48.2 months vs. 86.9 ± 43.1 months ($p = 0.5$, Mann-Whitney U test). The presence of another HBsAg positive person in the family also did not affect HBeAg clearance rate.

Twelve of 124 patients (9.7%) cleared HBsAg. The mean annual seroconversion rate was 3.1 percent. Clearance was seen more frequently among females than males although it was not statistically significant (13.9% vs. 7.4%, $p = 0.34$, Fisher's exact test). There was no difference in the mean age between the patients who cleared HBsAg and those who did not, 90.8 ± 36.5 months and 90.3 ± 45.5, respectively ($p = 0.97$, Mann-Whitney U test). None of the children (0/60) with a HBsAg positive person in the family seroconverted to anti-HBs, whereas 16.7 percent (4/24) of the patients whose family members were free of HBsAg ($p = 0.005$, Fisher's exact test) seroconverted to anti-HBs. Ten of 12 patients who cleared HBsAg were anti-HBe positive on enrollment. Only two of 11 patients who cleared HBeAg also cleared HBsAg.

During the follow-up, seven individuals (5 HBeAg positive and 2 anti-HBe positive) developed an abnormal ALT on at least one occasion after the first six months of follow-up. None of these patients had antibodies against hepatitis delta or C viruses. Three of five HBeAg positive children cleared HBeAg after ALT elevation. None of the two anti-HBe positive children converted to HBeAg positive state during or after the elevation of ALT. Only five of these patients had unexplained fluctuating ALT concentrations (less than three times normal) on repeat analysis. Liver biopsy was performed on these patients and all biopsies had nonspecific changes, including fatty changes, focal necrosis of hepatocytes, and ground glass hepatocytes.

All the children had normal serum albumin and bilirubin levels. None of the 20 patients who were followed for more than five years had clinical or biochemical indices of chronic hepatitis and/or cirrhosis or had hepatocellular carcinoma.

Discussion

HBsAg positivity indicates that the individual's liver is infected with the HBV. Infection in childhood increases the likelihood of becoming a chronic carrier[13]. In Turkey, the prevalence of HBsAg positivity in children is 5.4 percent, and carriers of HBsAg are usually infected during early childhood[14]. The expenses of treatment and the risks of biopsy in such a large population also require consideration.

The prevalence of HBeAg among HBsAg carriers ranges from zero[15] to > 90 percent[16] and it decreases as the ages of the patients increase[1,7,17]. This prevalence has been reported as higher than 80 percent in studies with children[2,7,8,11]. In our study, however, the prevalence of HBeAg positive children was only 53 percent, lower than the prevalences reported in the above studies. Age did not affect the prevalence of HBeAg positivity in our study, in contrast to some studies[1,7,18]. Similar to other studies[1,18], we could not show any significant effect of sex on the prevalence of HBeAg positivity. The annual clearance rate of HBeAg from HBsAg carrier children ranged from 6.2[17] to 16 percent[19]. Although some factors have been reported to affect the clearance rate of HBeAg in HBsAg carrier children, this has not been confirmed in all studies. Female sex[18], younger age[11,17,18], source of recruitment (whether recruited because the parents seek medical care for their children or by screening)[17], and positive maternal HBsAg status[17,20] have been reported as factors increasing HBeAg clearance rate. In our study, although the patients clearing HBeAg were older than those who did not, the difference was not significant. The presence of a person positive for HBsAg in the family and female sex also did not affect the HBeAg clearance. Similar to our results, sex[1,11,17] and age[1] did not affect the HBeAg clearance rate in some studies. During seroconversion, an increase in ALT activity was observed in most of the children[2,8]. In our study, three of five HBeAg positive children had elevated ALT levels before clearing HBeAg.

The annual clearance rate of HBsAg from carrier children has ranged from zero[1,2] to 1.5 percent[11]. Hsu et al.[19] found that the HBsAg clearance rate was significantly higher in children who had anti-HBe, children who were at an older age on entry, children whose mothers were HBsAg negative, and in children with severe histological changes of the liver detected while they were HBeAg positive. In our study, only children whose family members were HBsAg negative seroconverted to anti-HBs more frequently than those whose family members were HBsAg positive. There was no difference in the mean age and gender between the patients who cleared HBsAg and those who did not. Only two of 11 patients who cleared HBeAg also cleared HBsAg, the remaining 10 children clearing HBsAg were anti-HBe positive on entry. This is also in accordance with Hsu et al.'s[19] study. In their study, the annual clearance rate for HBsAg was 0.6 percent after a follow-up of 4.3 years. This rate was 1.7 percent in children with anti-HBe on entry. This study supports our findings that chronic HBV carrier children rarely lose HBsAg. In our study, the overall annual clearance rate of HBsAg was 3.2 percent and it was 5.7 percent in patients who were anti-HBe positive on entry. The discrepancies in annual HBeAg and HBsAg clearance rates may result from differences in ethnic origin, sources of recruitment, age distribution, or severity of the disease in the liver. In this study, we also observed a familial clustering of HBsAg carrier status that indicates the importance of

horizontal transmission. The prevalence of the presence of another HBsAg carrier in the family was 71.4 percent. This is in accordance with our previous study[21] and other studies in different countries[7, 17].

Long-term follow-up results of HBsAg positive children are controversial. Some reports have indicated occurrence of liver cirrhosis[10, 22, 23] or primary hepatocellular carcinoma[18] in HBV carrier children, whereas in some studies[5, 6] spontaneous complete remission in children with chronic active hepatitis has been reported. Most of these studies are related to children with abnormal transaminases and chronic hepatitis and/or cirrhosis. We could not find any studies with children that included only carrier children with normal liver tests. Koretz et al.[4] followed 54 HBsAg carrier adults with normal liver tests for four to 48 months with monthly liver tests. During follow up, only four patients (7.4%) (all HBeAg negative) developed persisting abnormalities in liver tests. Of the 23 patients who underwent percutaneous liver biopsies, chronic persistent hepatitis histology was found in only three patients. Chronic active hepatitis and/or cirrhosis was not seen in any biopsies. The risk of developing low grade transaminases abnormalities over a three-year period was 16 percent. It was 5.6 percent in our study. Koretz et al.'s[4] literature review showed that the frequency of finding "significant" liver disease (chronic active hepatitis, cirrhosis) in patients with normal transaminases levels was 5/237 (2.1%) compared to 19 percent with abnormal tests. They concluded that HBsAg carriers should be followed with serial liver tests and that those whose tests remain normal should not be considered for liver biopsy. In contrast, Sakuma et al.[3] followed 202 adult carriers for five years. Eighty-three of the carriers had normal liver tests initially and 94.3 percent of the 83 had normal tests continuously. They also found that mortality in carriers with normal liver tests was 44.5 times higher than that in noncarriers with normal tests. They concluded that routine liver function tests appear of limited value in predicting prognosis. There is weak correlation between abnormal tests and subsequent deaths in five years among carriers.

Clinical and biochemical features suggest that the majority of Turkish HBsAg carrier children with normal liver tests have stable liver disease. It is also clear that in Turkish HBsAg carrier children there is little clinical morbidity during childhood. Although this does not imply that chronic HBV infection is always a benign disease in children[18, 34], chronic HBV carriers can be followed with serial ALT levels alone. The incidence of significant liver disease in carrier children with normal transaminases levels is too low to justify the risk and expense of liver biopsy. We agree with those authors who conclude that a biopsy is not warranted in asymptomatic carriers[4, 25].

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