

## Hepatocellular Carcinoma in Children with Hepatitis B Surface Antigen

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TAZAWA, Y., NISHINOMIYA, F., NOGUCHI, H., TSUCHIYA, S., HAYASHI, Y., ABUKAWA, D., WATABE, M., NAKAGAWA, M., IMAIZUMI, M., OHI, R., TADA, K. and KONNO, T. *Hepatocellular Carcinoma in Children with Hepatitis B Surface Antigen*. Tohoku J. Exp. Med., 1992, **167** (1), 47-55 — This study discusses four children of hepatocellular carcinoma (HCC) who were asymptomatic HBsAg carriers or had HBsAg-positive chronic hepatitis for 3 to 11 years before the occurrence of the carcinoma. Three of these four patients were positive for anti-HBe at 3 to 5 years before the diagnosis of hepatocellular carcinoma. Autopsy findings disclosed liver cirrhosis in all the four patients. To the best of our knowledge few reports have documented children in HBsAg carrier status or with HBsAg-positive hepatitis prior to the development of hepatocellular carcinoma. It is emphasized that HBsAg-positive children, with or without detectable hepatic lesions in routine examinations, have a possibility of developing HCC, and should be carefully monitored for long periods. — hepatocellular carcinoma; hepatitis B virus

Hepatoma developing in childhood consists of two major histologic types, hepatoblastoma and hepatocellular carcinoma (HCC). The latter is not histologically different from the hepatomas found in adults (Kasai and Watanabe 1970; Landing 1976), and is associated with various diseases including metabolic, cholestatic and posthepatic diseases (Landing 1976). Although the combination of hepatitis B virus (HBV) infection and HCC has been documented in children (Shimada et al. 1975; Shimoda et al. 1980; Beasley et al. 1982; Ohaki et al. 1983; Chang et al. 1984; Tanaka et al. 1986; De Potter et al. 1987; Wu et al. 1987; Tong and Govindarajan 1988), liver diseases preceding HCC have not

been fully described. In this study, we retrospectively investigated four patients who had HBsAg-positive liver diseases and later HCC during the follow-up, to elucidate the relationship between HBV infection and the occurrence of HCC in Japanese children.

## MATERIALS AND METHODS

From April 1979 to March 1988 twelve Japanese children with hepatoma were admitted to our medical centers in Sendai, including 6 cases of hepatoblastoma and 6 cases of HCC. Excluding 2 cases of secondary HCC, one associated with glycogen storage disease (type I) and the other with giant cell hepatitis in infancy, the other 4 patients of HCC associated with HBsAg were included in this study. There were 3 males and one female, ranging in age from 6 to 13 years. Routine biochemical examinations on admission for liver tumor were unremarkable, except for mild elevation of serum transaminases. Serum alpha-fetoprotein levels were markedly increased in all patients, and serum cholesterol levels were moderately elevated in two patients (Table 1). The hepatitis C and delta antigen were not determined. In spite of intensive treatment, all 4 patients with HCC died within 8 months. After receiving the consent of the parents autopsied materials were obtained from all patients.

The diagnosis of HCC was histologically confirmed by open liver biopsy in 3 cases and autopsy in one. HBsAg was tested by the reverse passive hemagglutination method. HBeAg and anti-HBeAg were determined by micro-Ouchterlony method or radioimmunoassay. Alpha-fetoprotein was measured by radioimmunoassay. For demonstration of HBsAg in the liver specimen, orcein stain was employed as described by Shikata et al. (1974).

## CASE REPORTS

Case 1 (K.A.). This 6-year-old boy was first diagnosed as an HBsAg carrier positive for anti-HBeAg at the age of 3 years (1982), when his brother was hospitalized for chronic active hepatitis associated with HBsAg and HBeAg. His mother was positive for HBsAg and HBeAg. At that time the patient had no hepatic dysfunction but thereafter was followed up once or twice a year. Except for the presence of HBsAg and anti-HBeAg laboratory examinations including serum alpha-fetoprotein had disclosed no abnormalities

TABLE 1. *Serum biochemical data of the patients at the diagnosis of hepatocellular carcinoma*

Case	Total bilirubin (mg/100 ml)	GOT	GPT	LDH	ALP	GGTP	LAP	Total cholesterol (mg/100 ml)	AFP
1. K.A.	1.0	252	63	709	272	107	113	365	1,200
2. A.A.	0.4	114	21	621	292	11	54	180	51
3. H.A.	0.9	92	82	401	N.D.	117	222	376	4,300
4. M.M.	0.5	250	220	300	N.D.	161	N.D.	180	>100
Normal	<1.0	<32	<31	<440	<380	<58	<90	120-250	<0.02

GOT, glutamic oxalacetic transaminase (IU/liter); GPT, glutamic pyruvic transaminase (IU/liter); LDH, lactate dehydrogenase (IU/liter); ALP, alkaline phosphatase (IU/liter); GGTP,  $\gamma$ -glutamyltranspeptidase (IU/liter); LAP, leucine aminotranspeptidase (IU/liter); AFP,  $\alpha$ -fetoprotein ( $\mu$ g/ml); ND, not determined.

before the age of 6 years (1985), when a huge abdominal tumor associated with abdominal pain was found. Laparotomy revealed an enlarged liver and multiple hepatic tumors in both lobes. Histologic findings showed HCC with trabecular appearance. In the noncancerous regions macronodular cirrhosis with moderately broad fibrous septa was found. HBsAg was demonstrated by orcein stain in the noncancerous areas of the liver.

Case 2 (A.A.). This 10-year-old boy was first admitted to a hospital at the age of 5 years (1979) because of acute lymphocytic leukemia (ALL). At that time he was positive for HBsAg and anti-HBeAg without apparent hepatic dysfunction. His mother was an HBsAg carrier. Chemotherapy with vincristine, prednisolone, methotrexate, and 6-mercaptopurine, achieved a complete remission of ALL, but hepatic dysfunction developed. At the age of 9 years (1983), a percutaneous liver biopsy for persistent hepatic dysfunction revealed liver cirrhosis. At that time serum alpha-fetoprotein levels were within normal limits. At the age of 10 years (1984), he was admitted with gross hematemesis. He was still positive for HBsAg and anti-HBeAg. The diagnosis of hepatoma was based on multiple mass lesions of the liver, demonstrated by x-ray computed tomography, and increased levels of serum alpha-fetoprotein. Autopsy findings showed multiple hepatic tumors in both lobes. Histologic examinations revealed hepatocellular carcinoma with trabecular appearance, and micronodular cirrhosis with moderately broad stroma in the noncancerous regions. Orcein staining demonstrated HBsAg in the noncancerous areas of the liver near HCC regions (Fig. 1).

Case 3 (H.A.). This 12-year-old girl was first admitted because of hepatosplenomegaly and liver dysfunction at the age of 19 months (1972). Liver biopsy demonstrated chronic active hepatitis. Small doses of prednisolone were given without apparent improvement, but the liver dysfunction gradually improved during the subsequent 3-year-follow up period. Thereafter she had not received any treatment nor visited any hospital for 7 year until she was admitted because of a huge abdominal tumor at the age of 12 years (1982). At

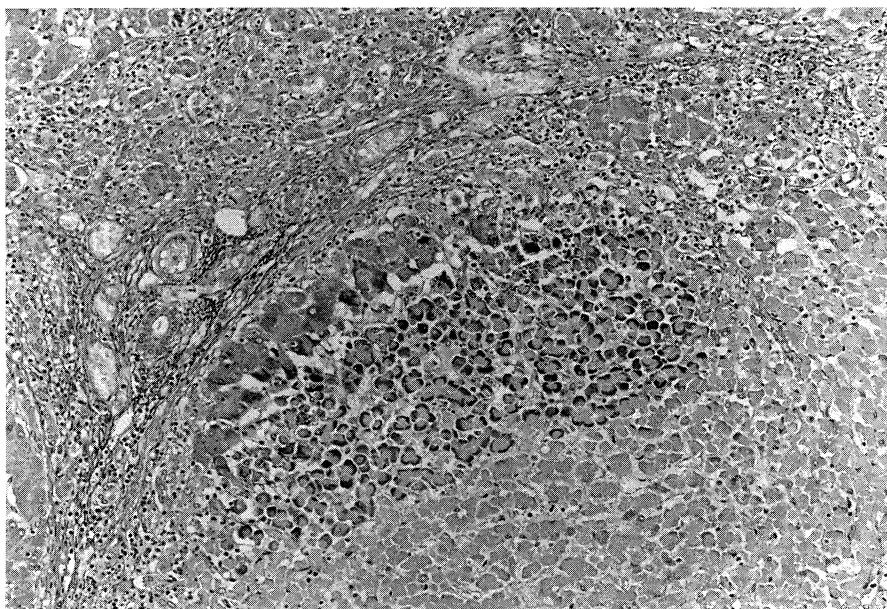


Fig. 1. Distribution of HBsAg in a noncancerous area of the liver near hepatocellular carcinoma regions in case 2 (A.A.). Strongly positive hepatic cells are shown being localized like an islet in a nodule. (Orcein stain,  $\times 100$ )

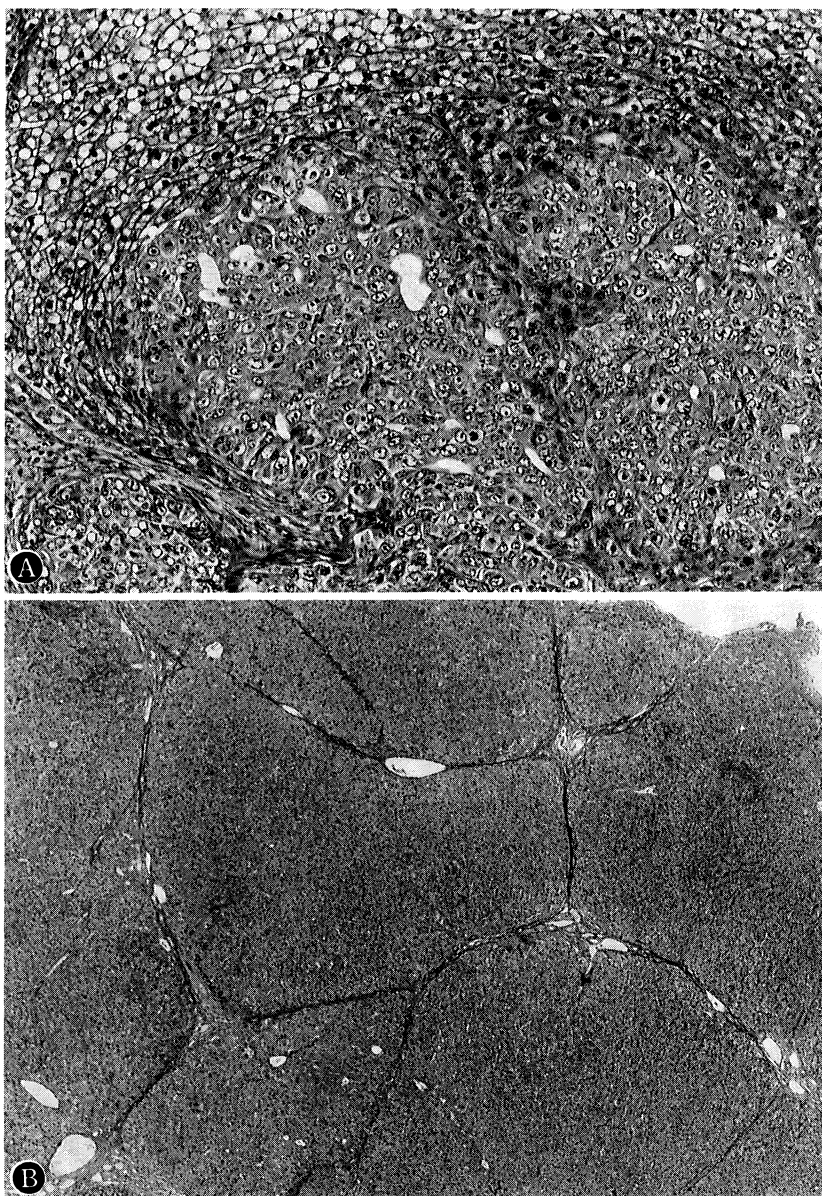


Fig. 2. Histologic features of the liver of case 3 at the age of 12 years.

A: Hepatocellular carcinoma; highly atypical, enlarged cells of neoplasm with trabecular or tubular appearance and mitotic figures, oppressing the noncancerous liver tissue. (Masson trichrome stain,  $\times 100$ ) B: Macro-nodular cirrhosis in the noncancerous portion of the liver. (Masson trichrome stain,  $\times 20$ )

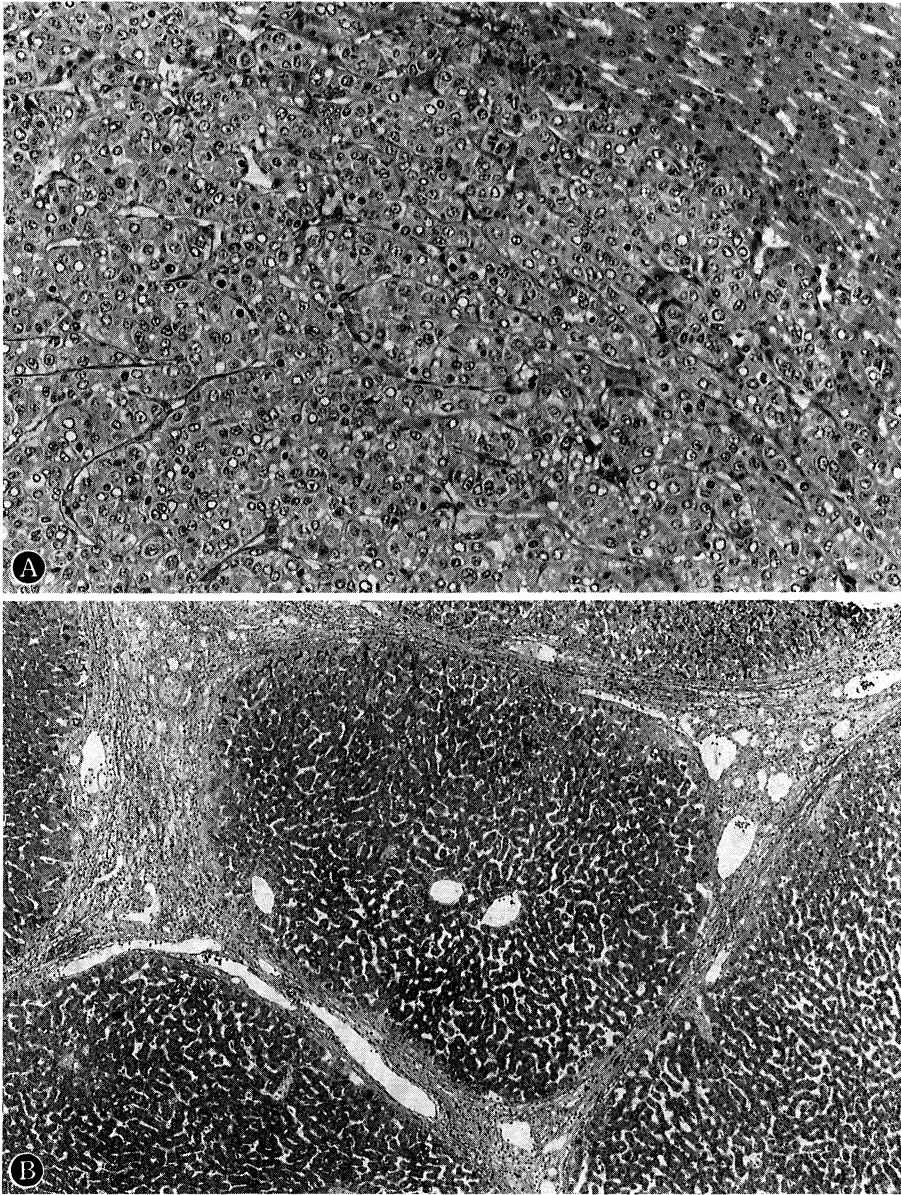


Fig. 3. Histologic features of the liver of case 4 at the age of 13 years.

A : Hepatocellular carcinoma consisting of bizarre, large neoplastic cells with trabecular patterns, and mitotic figure. (HE stain,  $\times 100$ ) B : Macronodular cirrhosis seen in the noncancerous portion of the liver. (Masson trichrome stain,  $\times 40$ )

laparotomy multiple hepatic tumors were found. The biopsied specimens showed HCC with trabecular or tubular appearance (Fig. 2A). In the noncancerous regions, macronodular cirrhosis with narrow fibrous septa was found (Fig. 2B). HBsAg was shown by orcein stain in the noncancerous areas of the liver. She had remained positive for HBsAg since 19 months of age, and she was positive for anti-HBeAg at the age of 12 years. Her mother was positive for HBsAg and HBeAg.

Case 4 (M.M.). This 13-year-old boy was first noticed to have hepatosplenomegaly at the age of 4 years (1971). At that time biochemical tests showed mild elevations of serum transaminases, and serologically he was positive for HBsAg, although his mother was negative for HBsAg. Liver biopsy demonstrated chronic inactive hepatitis with mild inflammatory changes and fibrosis in the portal tracts. He had periodical examinations for hepatitis without any treatment for a several years, but was not examined for the last 5 years before his admission when a huge abdominal tumor was found at the age of 13 years (1980). He was still positive for HBsAg. At laparotomy multiple hepatic tumors were disclosed and the biopsied specimens showed hepatocellular carcinoma with trabecular appearance (Fig. 3A). In the noncancerous regions macronodular cirrhosis with moderately broad stroma was observed (Fig. 3B). No distribution of HBsAg in the noncancerous and cancerous areas of the liver bearing HCC was shown by orcein stain.

## DISCUSSION

Clinical and epidemiological studies have demonstrated a close relationship between HBV infection and HCC (Arthur et al. 1984). Although HCC in HBsAg carriers usually occurs late in adulthood, the combination of HBV infection and HCC is also observed in children (Shimada et al. 1975; Shimoda et al. 1980; Beasley et al. 1982; Ohaki et al. 1983; Chang et al. 1984; Tanaka et al. 1986; De Potter et al. 1987; Wu et al. 1987; Tong and Govindarajan 1988), but the carrier status and liver diseases are rarely documented prior to the development of a tumor. In the present study 3 cases were demonstrated to have chronic liver disease by biopsy 1, 9, and 11 years, respectively, before the diagnosis of HCC. Although the other case had been followed up as an "asymptomatic" carrier for 3 years before the occurrence of HCC, cirrhosis was documented at autopsy.

The incidence of HCC in children is far less than in adults, but the vertical transmission of HBV may be associated with the occurrence of HCC in children as shown in adults (Beasley et al. 1982; Ohaki et al. 1983; Chang et al. 1984; Tanaka et al. 1986; De Potter et al. 1987; Wu et al. 1987; Tong and Govindarajan 1988). In Japan the frequency of HBsAg carriers has been reported to be 2-3%, and the majority of cases are considered to be transmitted from the mothers during the perinatal period (Okada et al. 1976). Three of the 4 patients described in this paper had HBsAg-positive mother, suggesting the vertical infection.

Among triggering factors likely to be involved in the oncogenic potential of chronic HBV infection, HBV-DNA integration seems to be most important (Arthur et al. 1984). However, HBV-DNA integration to the host hepatic cell DNA can not simply explain the development of HCC, since such an integration commonly occurs in patients with HBV infection including asymptomatic carriers (Shafritz et al. 1981; Shafritz 1982). Factors other than HBV may be involved in the

development of HCC (Gerin 1983). Accordingly it is interesting that HCC developed in ALL patients. Pratt et al. also reported an HBsAg-positive child who developed HCC after ALL (Pratt et al. 1988). These cases suggest that chemotherapy for ALL is involved in carcinogenesis by HBV. The occurrence of HCC in those patients could be the result of chemotherapy, especially methotrexate. The occurrence of HCC in a child with methotrexate-induced hepatic fibrosis was reported (Ruymann et al. 1977).

The youngest case reported in this study is 6 year of age. Excluding a case of 8 month-old (Wu et al. 1987), Youngest cases with HCC related to HBV who have reported were 4 year-old (Chang et al. 1984 ; Tanaka et al. 1986). The fact points an early occurrence of HCC related to HBV in children, compared with that in adult. The early occurrence of HCC related to HBV in children also may suggest that other factors are involved in the carcinogenesis by HBV. However the early occurrence of HCC has been also documented in children with other cirrhotic or metabolic diseases, usually developing at the age of 1 to 4 year (Okuyama 1965 ; Ishak and Glunz 1967 ; Weiberg et al. 1976). The explanation for the early occurrence of HCC related to HBV in childhood is difficult, but growing individuals might have a diathesis of developing HCC in liver injury caused by various origins, or superimposed infections or coexist metabolic disarrangement might be involved in the occurrence of HCC in childhood.

In three of our 4 patients HBsAg-positive hepatocytes were demonstrated by orcein staining in the noncancerous area. Although hepatitis B viral DNA integrated in the tumor cell genome (Tanaka et al. 1986), HBsAg-positive cells existed only in the noncancerous area (Ohaki et al. 1983 ; Chang et al. 1984 ; Tanaka et al. 1986 ; De Potter et al. 1987). Blumberg proposed a hypothesis that the tumor develops from the integration of viral DNA in resistant R-cells, which are resistant to destruction as they do not replicate the virus (Blumberg 1976). The R-cell clone forms a growing tumor, and viral antigens are no longer produced. This could explain why HBsAg is only found in the noncancerous area in patients with HCC.

The presence of cirrhosis seems significant to the occurrence of HCC (Kew and Popper 1984), in particular in the population with HBsAg and anti-HBeAg (Eleftheriou et al. 1975 ; Werner et al. 1976 ; Heyward et al. 1982). All 4 patients in this study had cirrhosis at autopsy, and all 3 patients examined had anti-HBeAg. Accordingly, the possibility of HCC developing should be kept in mind in HBsAg-positive children with cirrhosis or anti-HBeAg.

We have neither prospective or retrospective data about how to screen HCC in high risk children at present. In a prospective study of Alaskan Eskimos older than 15 years, significant rises of AFP levels were shown to occur as long as 2 years before the clinical onset of HCC (Heyward et al. 1982). Therefore, periodic checks to estimate serum AFP and ultrasonography should be carried out at least once or twice a year in pediatric patients. In addition, liver biopsy to determine



the presence or absence of cirrhosis is recommended in patients with chronic liver disease, since even clinically silent cirrhosis may heighten the suspicion of HCC.

In view of the close relationship between chronic HBV infection and occurrence of HCC, primary prevention by vaccination against HBV infection is essential. A comprehensive HBV vaccination program targeted against high-risk individuals should reduce the global incidence of the tumor. A world-wide program of immunization against HBV for newborns born to HBsAg-positive mothers would help to decrease the morbidity of HCC as well as that of liver diseases caused by HBV in childhood. Moreover, vaccination of HBsAg-negative children must to be undertaken to prevent HBV infection during childhood. Such vaccination programs will eradicate the chronic HBsAg-positive carrier state and significantly reduce the incidence of HCC.

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