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# Further Histological Study of the Role of Hepatitis B Infection on the Development of Hepatocellular Carcinoma

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**Abstract:** A total of 953 cases of various types of liver diseases were selected for examination from 6339 autopsies performed in Nagasaki University Hospital from 1964 through 1980. The association between HBs-Ag in liver tissue and the histology of the liver was studied using Shikata's orcein staining method. Of the 6339 autopsies performed, 488 cases of atrophic liver cirrhosis (7.7%) and 303 cases of primary hepatocellular carcinoma (4.8%) were identified. These incidences of liver diseases were statistically significantly higher compared with those of national averages of Japan (5.2% and 3.5%, respectively). A histological analysis of 953 cases indicated that 242 out of 488 cases of atrophic liver cirrhosis were associated with hepatocellular carcinoma (49.6%) and that 242 out of 303 cases of hepatocellular carcinoma were combined with atrophic liver cirrhosis (78.9%). No HBs-Ag was detected in the tissues of cases of acute liver atrophy or liver cirrhosis with the exception of atrophic liver cirrhosis. HBs-Ag was present in 6 out of 22 cases of subacute hepatitis (27.2%) and in 6 out of 37 chronic hepatitis (16.2%). The highest rate of detectable HBs-Ag was observed in the liver tissues of the subgroup of atrophic liver cirrhosis with hepatocellular carcinoma (60.6%). Among 196 cases of hepatocellular carcinoma examined, HBs-Ag was detected in 14 of those cases which did not exhibit atrophic liver cirrhosis. These results suggest that hepatocellular carcinoma can arise without setting of hepatitis B chronic liver cirrhosis, and support the hypothesis that HBs-Ag may be directly oncogenic. Liver cell dysplasia was found in 69 out of 154 cases of atrophic liver cirrhosis with hepatocellular carcinoma (44.8%), and in 22 out of 122 cases of atrophic liver cirrhosis without hapatocellular carcinoma (18.0%). Furthermore, there was a strong correlation between the presence of HBs-Ag in the liver tissue of Nagayo's type B atrophic liver cirrhosis and the existence of cellular dysplasia.

*Key words*: Hepatitis B surface antigen (HBs-Ag), Atrophic liver cirrhosis, Hepatocellular carcinoma

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## INTRODUCTION

A strong correlation between the hepatitis B virus and hepatocellular carcinoma has been reported in various countries (Wu, 1978; Atiyeh *et al.*, 1980; Beasley *et al.*, 1981; Keshgegian *et al.*, 1981). The carrier rate for HBs-Ag is much higher in a certain part of Africa and in Southeast Asia, East Africa and the Pacific islands (up to 10%) than in North America, Europe or Australia (up to 1%) (McCollum *et al.*, 1981). The average carrier rate of HBs-Ag among blood donors in Japan is reported to be 1.4% to 1.9%, indicating that HBs-Ag is moderately prevalent in Japan (Halliday *et al.*, 1980). The incidence of hepatocellular carcinoma is roughly concomitant with the incidence of positive serum HBs-Ag and is observed in 150 cases out of 100,000 in Africa and Asia but in only 1 to 3 cases out of 100,000 in North America and West Europe (Maupus *et al.*, 1981). These reports suggest that the hepatitis B virus may be one of the human oncogenic viruses involved in hepatocellular carcinoma. The incidence of the hepatitis B antigenema, however, differs considerably depending upon geographical location. These discrepancies may indicate a true geographical variation in the frequency of HBs-Ag in diseased hepatic tissues rather than technical variations in the mesurements of the hepatitis B antigen. This study was undertaken as an extention of our previous work (Kanda, 1977) with respect to the role of hepatitis B infection on the development of chronic hepatic diseases, such as liver cirrhosis and hepatocellular carcinoma, in Nagasaki Japan.

## MATERIALS AND METHODS

A total of 953 cases of various diseases of the liver were selected for study from 6339 autopsies performed at Nagasaki University from 1964 through 1980. At the same time, a statistical review of the incidences of various hepatic diseases in all autopsies performed in Japan from 1965 through 1980 was conducted. In 652 of the 953 autopsy cases selected for this study from which two to three representative paraffin-blocks were available, liver tissues were histologically examined. Sections 5 $\mu$m thick were cut and stained with hematoxylin and eosin as well as with orcein using Shikata's method (1973; 1974). Other histological staining procedures, such as modified Mallory's collagen staining (Luna, 1968), Gomori's method for iron (Luna, 1968) and silver staining (Senba, 1983) were also conducted when necessary. In each case, the detailed hepatic histology, such as the type of liver diseases, the presence of liver cell dysplasia, stromal inflammation and the grade of malignancy, was recorded. The presence of HBs-Ag in hepatic tissue was expressed according to Kanda's criteria. Anthony's difinition (1973) and Edmondson's criteria (1954) were applied in determining whether or not liver cell dysplasia existed and the grade of malignancy.

## RESULTS

*Statistical review*

A total of 6339 autopsies were performed at Nagasaki University from 1964 through 1980. Of those 6339 cases, 488 (7.7%) were histologically diagnosed as atrophic liver cirrhosis and 303 (4.8%) as hepatocellular carcinoma. The incidences of atrophic liver cirrhosis and hepatocellular carcinoma were much higher than those of the national sverage of Japan, as shown in Table 1. The male to female ratio was 3 to 1 for atrophic liver cirrhosis and 5 to 1 for hepatocellular carcinoma. Table 2 shows the incidences of various liver diseases in a total of 953 autopsy cases examined at Nagasaki University. There were 22 cases of acute liver atrophy and 36 cases of subacute liver atrophy. Various types of liver cirrhosis were diagnosed in 561 of those cases which included 488 cases of atrophic liver cirnhosis, 303 were diagnosed as hepatocellular carcinoma, 17 as cholangiocellular

Table 1. Comparative study of incidence of liver cirrhosis and hepatocellular carcinoma between Nagasaki University and all Japan

| | Total autopsy cases | Atrophic liver cirrhosis | Hepatocellular carcinoma |
|---|---|---|---|
| Nagasaki University 1964−1980 | 6,339 | 488 (7.7%) | 303 (4.8%) |
| All Japan 1965−1989 | 386,731 | 20,072 (5.2%) | 13,418 (3.5%) |

Table 2. Main liver diseases among autopsy cases (Nagasaki University, 1964−1980)

| Liver diseases | M | F | Total | Percentage in total autopsy cases |
|---|---|---|---|---|
| Acute liver atrophy | 10 | 12 | 22 | 0.4 % |
| Subacute liver atrophy | 18 | 18 | 36 | 0.56% |
| Liver cirrhosis | (420) | (141) | (561) | (8.8 %) |
| Atrophic cirrhosis | 367 | 121 | 488*1 | 7.7 % |
| Micronodular cirrhosis | 12 | 6 | 17 | 0.27% |
| Biliary cirrhosis | 24 | 11 | 35 | 0.55% |
| Cardiac cirrhosis | 9 | 3 | 12 | |
| Parasitic cirrhosis | 1 | 0 | 1 | 0.19% |
| Wilson's disease | 7 | 1 | 8 | 0.13% |
| Primary liver carcinoma | (259) | (74) | (333) | (5.25%) |
| Hepatocellular carcinoma | 246 | 57 | 303*2 | 4.78% |
| Cholangiocellular carcinoma | 6 | 11 | 17 | 0.27% |
| Combined type | 6 | 6 | 12 | 0.19% |
| Other malignant tumor | 1 | 0 | 1 | |
| | 708 | 245 | 953 | 100% |

*1 With or without hepatocellular carcinoma
*2 With or without atrophic liver cirrhosis

carcinoma and 12 as a combination of hepatocellular and cholangiocellular carcinoma. The correlation between atrophic liver cirrhosis and hepatocellular carcinoma is shown in Table 3. Of 488 cases examined, 242 (49.6%) were atrophic liver cirrhosis combined with hepatocellular carcinoma, while 242 out of 303 cases of hepatocellular carcinoma (78.9%) were accompanied by atrophic liver cirrhosis.

Table 3. Incidence and correlation between atrophic liver cirrhosis and hepatocellular carcinoma

| Atrophic liver cirrhosis | M | F | Total | % |
|---|---|---|---|---|
| Atrophic liver cirrhosis alone | 159 | 77 | 236 | 48.4% |
| With hepatocellular carcinoma | 203 | 39 | 242 | 49.6% |
| With other primary liver carcinoma | 5 | 5 | 10 | 2.0% |
| | 367 | 121 | 488 | 100.0% |
| **Hepatocellular carcinoma** | **M** | **F** | **Total** | **%** |
| With atrophic cirrhosis | 203 | 39 | 242 | 78.9% |
| Without atrophic cirrhosis | 43 | 18 | 61 | 21.1% |
| | 246 | 57 | 303 | 100.0% |

Table 4. Liver diseases examined histologically in this study

| Liver diseases | M | F | Total |
|---|---|---|---|
| Acute liver atrophy | 7 | 8 | 15 |
| Subacute liver atrophy | 13 | 9 | 22 |
| Chronic hepatitis, active | 17 | 3 | 20 |
| Chronic hepatitis, inactive | 11 | 6 | 17 |
| Fatty liver | 3 | 6 | 9 |
| Liver cirrhosis | (257) | (80) | (377) |
| Atrophic liver cirrhosis | 219 | 67 | 286*1 |
| Micronodular cirrhosis | 13 | 3 | 16 |
| Cardiac cirrhosis | 3 | 1 | 14 |
| Parasitic cirrhosis | 1 | 0 | 1 |
| Wilson's disease | 4 | 1 | 5 |
| Primary liver carcinoma | (172) | (57) | (229) |
| Hepatocellular carcinoma | 157 | 39 | 196*2 |
| Cholangiocellular carcinoma | 11 | 11 | 22 |
| Combined type | 4 | 7 | 11 |
| Kartoffeleber | 0 | 3 | 3 |
| | 480 | 172 | 652 |

*1 With or without hepatocellular carcinoma

*2 With or without atrophic liver cirrhosis

*Histological review*

A histological classification of the types of liver diseases was devised for 652 cases of hepatic diseases (Table 4). Nagayo's difinition (1914; Miyake, 1960) was used to determine the subtypes of atrophic liver cirrhosis. The other types of atrophic liver cirrhosis, with the exception of atrophic liver cirrhosis, were classified according to criteria specified by Gall (1960) and Mori (1964). The classification of chronic hepatitis was accomplished according to criteria specified at the Inuyama Symposium (1975). Of those 652 cases examined, 15 cases were diagnosed as acute liver atrophy, 22 as subacute liver atrophy, 37 as chronic hepatitis, 337 as liver cirrhosis, of which 286 were identified as atrophic liver cirrhosis, and 229 as primary liver carcinoma which included 196 cases of hepatocellular carcinoma. Nagayo's type B liver cirrhosis was observed in most cases of atrophic liver cirrhosis (93.4%). The age distribution of atrophic liver cirrhosis was mostly from 30 to 80 years. The peak incidence of atrophic liver cirrhosis occured in the 6th decade of life (Table 5). Biliary or micronodular cirrhosis occured in a younger population than atrophic liver cirrhosis. The 196 cases of hepatocellular carcinoma that were examined were mainly distributed between the ages of 40 to 60 years. The peak age for hepatocellular carcinoma was the 6th decade of life as was the case with atrophic liver cirrhosis. Cholangiocellular carcinoma was observed in an older age group than hepatocellular carcinoma.

Table 6 presents the histological features of non-cancerous liver tissue observed in cases of hepatocellular carcinoma. Among the 196 cases of hepatocellular carcinoma examined, 155 (79.1%) were associated with atrophic liver cirrhosis. No cases exhibiting Nagayo's type A liver cirrhosis were found to have hepatocellular carcinoma (Fig. 1). In addition, 23 cases (11.7%) of hepatocellular carcinoma were either combined with non-specific fibrosis or displayed no particular histological changes.

HBs-Ag was histologically detected in 6 of 22 cases of subacute liver atrophy (27.2 %) and in 6 of 37 cases of chronic hepatitis (16.2%). The 15 cases diagnosed as acute

Table 5. Age distribution of atrophic liver cirrhosis and primary liver carcinoma

| Diseases | | Age | | | | | | | | Total |
|---|---|---|---|---|---|---|---|---|---|---|
| | | 0–9 | 10–19 | 20–29 | 30–39 | 40–49 | 50–59 | 60–69 | 70–79 | 80– | |
| Atrophic cirrhosis | Nagayo A | 2 | 1 | 3 | 4 | 4 | 2 | 3 | | | 19 |
| | Nagayo B | 1 | 6 | 11 | 75 | 86 | 62 | 23 | 3 | | 267 |
| Primary liver carcinoma | Hepatocellular carcinoma | | 4 | 6 | 53 | 67 | 53 | 13 | | | 196 |
| | Cholangiocellular carcinoma | | | | 2 | 6 | 10 | 1 | 1 | | 22 |
| | Combined type | | | 1 | 2 | 3 | 4 | 3 | | | 11 |

Table 6. Pathological changes of non-cancerous tissue in cases of hepatocelullar carcinoma

| Hepatic pathology | | M | F | Total | Percentage in total cases of hepatocellular cases |
|---|---|---|---|---|---|
| Atrophic cirrhosis | Nagayo A | 0 | 0 | 0 | 0% |
| | Nagayo B | 126 | 29 | 155 | 79.1% |
| Chronic hepatitis, active | | 8 | 0 | 8 | 4.1% |
| inactive | | 9 | 0 | 9 | 4.6% |
| Mild fibrosis or nothing particular | | 13 | 10 | 23 | 11.7% |
| Wilson's disease | | 1 | 0 | 1 | 0.5% |
| Total | | 157 | 39 | 196 | 100 % |

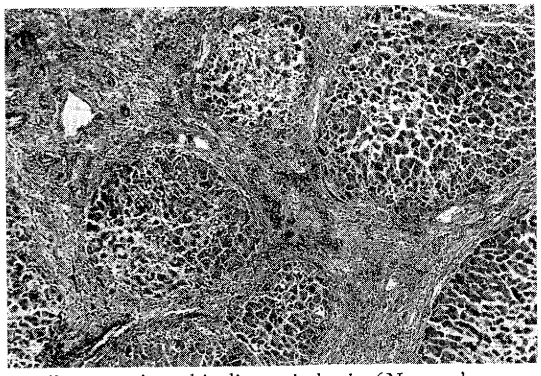

Fig. 1. Atrophic liver cirrhosis (Nagayo's type A) showing wide connective tissue. (H. E. stain, Original magnification, ×40)

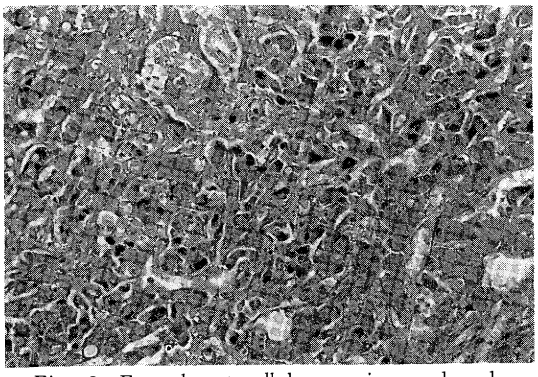

Fig. 2. From hepatocellular carcinoma based on Nagayo's type B liver cirrhosis. Orcein positive liver cells scattered in the cirrhotic nodule. (Orcein stain, Original magnification, ×100)

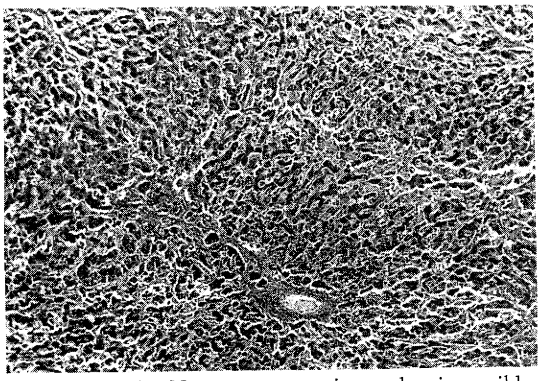

Fig. 3. A: Non-cancerous tissue showing mild fibrosis observed in a case combined with primary hepatocellular carcinoma. (H. E. stain, Original magnification, ×40)

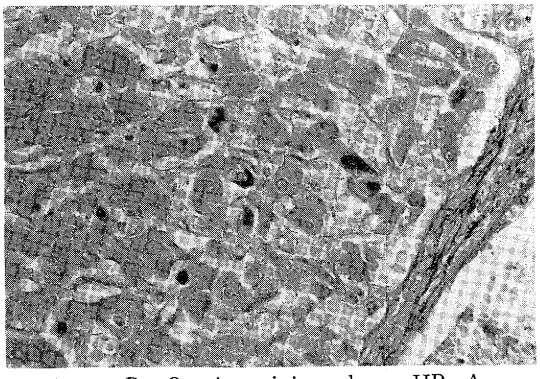

Fig. 3. B: Orcein staining shows HBs-Ag in non-cancerous tissue. (Orcein stain, Original magnification, ×200)

liver atrophy were all HBs-Ag negative. The differences in the rates for detectable HBs-Ag between cases of cirrhosis without hepatocellular carcinoma (30.3%) and cases with hepatocellular carcinoma (60.6%, Fig. 2) were statistically significant ($\chi^2$ test, $P<0.005$) (Table 7). No HBs-Ag was detected in any of the cases of liver cirrhosis with the exception of atrophic liver cirrhosis. Of the 196 cases of hepatocellular carcinoma examined, 108 were positive for HBs-Ag. As illustrated in Table 8, the incidence of HBs-Ag in cases of hepatocellular carcinoma was highly correlated with the presence of histological features typically found in non-cancerous liver tissue. The rate of demonstrable HBs-Ag was high in cases of hepatocellular carcinoma associated with both atrophic liver cirrhosis (60.6%) and chronic active hepatitis (75.0%), but low in cases of chronic inactive hepatitis (22.5%), mild fibrosis (Fig. 3) and those exhibiting no significant histological changes (26.1%). The prevalence of HBs-Ag among 286 cases of atrophic liver cirrhosis as related to age is shown in Figure 4. All groups exhibiting liver cirrhosis were divided into subgroups, namely, those with or without hepatocellular carcinoma. The highest HBs-Ag frequency in liver tissue was observed in patients who were in their third decade of life. After 30 years of age, the presence of HBs-Ag in liver tissue decreased with age in both of the subgroups.

The incidence of HBs-Ag among cases of atrophic liver cirrhosis was investigated in relation to stromal inflammation (Table 9). An entire subgroup of cases of Nagayo's

Table 7. Incidence of HBs-Ag in liver diseases

| Diseases | | HBs-Ag(+) | HBs-Ag(−) | Total cases examined |
|---|---|---|---|---|
| Acute liver atrophy | | 0 | 15 | 15 |
| Subacute liver atrophy | | 6 (27.2) | 16 | 22 |
| Chronic hepatitis | | 6 (16.2) | 31 | 37 |
| Atrophic liver cirrhosis*1 | without hepatocellular carcinoma | 37 (30.3) | 85 | 122 |
| | with hepatocellular carcinoma | 94 (60.6) | 61 | 155 |
| | with cholangiocellular carcinoma | 2 | 0 | 2 |
| | with combined | 4 (57.1) | 3 | 7 |
| Micronodular cirrhosis | | 0 | 16 | 16 |
| Cardiac cirrhosis | | 0 | 4 | 16 |
| Biliary cirrhosis | | 0 | 25 | 25 |
| Parasitic cirrhosis | | 0 | 1 | |
| Wilson's disease | | 0 | 5 | 5 |
| Primary liver carcinoma | Hepatocellular carcinoma | 108 (55.1) | 88 | 196 |
| | Cholangiocellular carcinoma | 2 ( 9.1) | 20 | 22 |
| | Combined | 4 (36.4) | 11 | 15 |

( ) Parenthesis express percentage of positivity of HBs-Ag in liver tissue
*1 Average detectable rate for HBs-Ag in this group is 47.9% (137/286).

Table 8. Incidence of HBs-Ag in non-cancerous tissue in cases of hepatocellular carcinoma

| Hepatic histology of non-cancerous tissue | HBs-Ag(+) | HBs-Ag(−) | Positive ratio% |
|---|---|---|---|
| Atrophic cirrhosis | 94 | 61 | 60.6% |
| Chronic hepatitis, active | 6 | 2 | 75.0% |
| Chronic hepatitis, inactive | 2 | 7 | 22.5% |
| Mild fibrosis or nothing particular | 6 | 17 | 26.1% |
| Wilson's disease | 0 | 1 | 0 % |
| | 108 | 88 | 196(100%) |

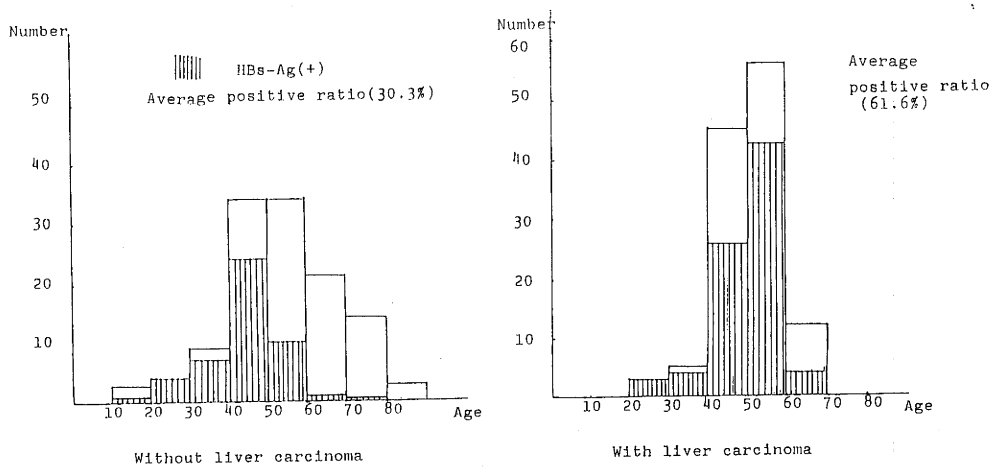


Fig. 4. Aging incidence of HBs-Ag in cases of atrophic liver cirrhosis.

Table 9. Relationship between stromal inflammatory reaction and incidence of HBs-Ag in cases of atrophic liver cirrhosis

| Diseases | | Stromal inflammatory reaction | HBs-Ag{+) | HBs-Ag(−) | Total cases examined |
|---|---|---|---|---|---|
| Without primary liver carcinoma | Nagayo A | active | 8 (42.4) | 11 | 19 |
| | | inactive | 0 | 0 | 0 |
| | | undetermined | 0 | 0 | 0 |
| | Nagayo B | active | 23 (33.8) | 45 | 68 |
| | | inactive | 6 (20.0) | 24 | 30 |
| | | undetermined | 0 | 5 | 5 |
| With primary liver carcinoma | | active | 52 (58.4) | 37 | 89 |
| | | inactive | 35 (60.3) | 23 | 58 |
| | | undetermined | 14 (82.4) | 3 | 17 |

( ) Parenthesis express percentage of positivity of HBs-Ag in liver tissue

Table 10. Incidence of liver cell dysplasia in cases of atrophic liver cirrhosis

| Atrophic liver cirrhosis | | Dysplasia(+) | Dysplasis(−) | Positive ratio% |
|---|---|---|---|---|
| Nagayo A | alone | 2 | 17 | 22/122 (18.0%) |
| Nagayo B | without liver carcinoma | 20 | 83 | |
| Nagayo B | with hepatocellular carcinoma | 67 | 83 | 69/154 (44.8%) |
| | with other primary liver carcinoma | 2 | 2 | |

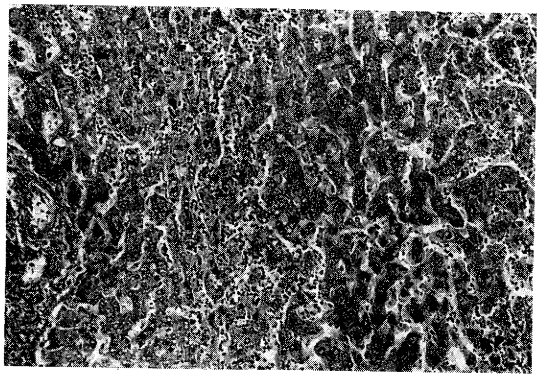

Fig. 5. A case of hepatocellular carcinoma alone. Groups of hepatocytes with nuclear pleomorphism and cytoplastic enlargement are shown (H. E. stain, Original magnification, ×100)

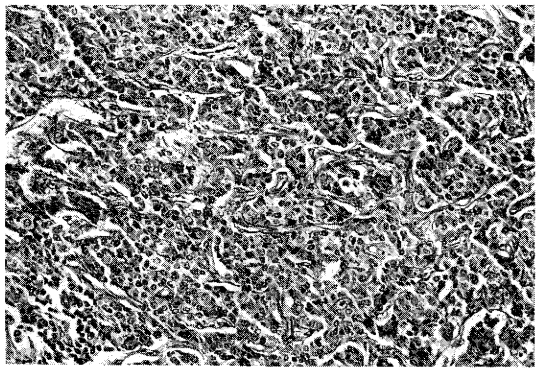

Fig. 6. Hepatocellular carcinoma (Grade III) from a case associated with atrophic liver cirrhosis. (H. E. stain, Original magnification, ×100)

Table 11. Relationship between dysplasia and HBs-Ag in atrophic liver cirrhosis

| | Nagayo A | Nagayo B without hepatocellular carcinoma | Nagayo B with hepatocellular carcinoma | Nagayo B with other primary carcinoma |
|---|---|---|---|---|
| Dysplasia (+) | 1/2 (50 %) | 12/20 (60 %) | 44/66 (66.7%) | 3/4 (75%) |
| Dysplasia (−) | 7/17 (41.2%) | 17/83 (20.5%) | 50/89 (56.2%) | 3/5 (60%) |

Table 12. Relationship between HBs-Ag and grade of malignancy in cases of hepatocellular carcinoma

| | Grade of malignancy | | |
|---|---|---|---|
| | II | III | IV |
| With atrophic liver cirrhosis | 39/69 (56.5%) | 45/68 (66.2%) | 10/18 (55.6%) |
| Without atrophic liver cirrhosis | 5/14 (35.7%) | 5/20 (25.0%) | 3/7 ( 42.9%) |

Grade I : No cases

type A liver cirrhosis was found to be free of hepatocellular carcinoma but exhibited active stromal inflammation. The frequency of HBs-Ag was moderately high in the liver tissues of both Nagayo's A and B liver cirrhosis with active stromal inflammation.

The frequency of dysplasia among cases of atrophic liver cirrhosis was also studied (Table 10). According to Anthony's definition, 2 representative sections were used the evaluation of dysplasia, which included cellular enlargement, nuclear pleomorphism with hyperchromasia and multinucleation (Fig. 5). Dysplasia was most frequently observed in cases of atrophic liver cirrhosis with primary liver carcinoma. Dysplasia was present in 22 cases of atrophic liver cirrhosis without hepatocellular carcinoma (18.0%) and in 69 (44.8%) cases of atrophic liver cirrhosis associated with primary liver carcinoma (Table 10). These differences were highly significant statistically ($\chi^2$ test, $P<0.005$). Table 11 lists the correlations among HBs-Ag in liver tissue, dysplasia and cirrhosis with or without liver cell carcinoma. In Nagayo's type B cirrhosis subgroup alone, there was a difference in the prevalence of HBs-Ag between cases with dysplasia (12 out of 20; 60%) and cases without dysplasia (17 out of 83; 20.5%). In the other subgroups, there was a slightly higher prevalence of HBs-Ag in those with dysplasia than those without dysplasia. However, this difference was not statistically significant. Liver tissue with a high prevalence of HBs-Ag was noted in cases of hepatocellular carcinoma with or without dysplasia.

Malignancies in all cases of hepatocellular carcinoma were graded according to Edmondson's criteria. The results indicated that the incidence of grade III hepatocellular carcinoma was the highest in this group (Fig. 6). No cases of grade I hepatocellular carcinoma were observed. No significant association was observed between the presence of HBs-Ag and the grade of malignancy (Table 12).

## DISCUSSION

The incidence of atrophic liver cirrhosis and hepatocellular carcinoma among the total number of autopsies performed at Nagasaki University were 7.7% and 4.8% respectively. The incidence of these 2 diseases were much higher in Nagasaki than in other districts of Japan. According to statistical data collected from 8 universities (Miyaji, 1965) in Japan, a correlation between atrophic liver cirrhosis and hepatocellular carcinoma was quite evident: 42.2% of the liver cirrhosis cases had developed hepatocellular carcinoma and 84.3% of the cases of hepatocellular carcinoma were associated with atrophic liver cirrhosis. In the present study, 242 out of 488 cases (49.6%) of atrophic liver cirrhosis had also developed hepatocellular carcinoma, whereas 242 out of 303 hepatocellular carcinoma cases were associated with atrophic liver cirrhosis (78.9%). All the liver cirrhosis cases that were combined with hepatocellular carcinoma were identified as Nagayo's type B liver cirrhosis.

Studies on the association of hepatocellular carcinoma and HBs-Ag in the tissue have advantages over serum studies in that they can be used for large scale retrospective

investigation. Shikata *et al.*, (1973, 1974) showed that an orcein staining technique specifically stains HBs-Ag in fixed paraffin embedded tissue. Another researcher (Huang, 1975) has reported that the sensitivity and specificity of the orcein stain are almost equal to those of immunological methods of mesurement. The present study indicated that HBs-Ag was present at a rate of 47.9% in cases of atrophic liver cirrhosis and that the incidence of HBs-Ag in liver tissue was quite high, especially in cases of atrophic liver cirrhosis with hepatocelluler carcinoma. HBs-Ag was detected in 108 of 196 hepatocellular carcinoma cases (55.1%). The HBs-Ag rate in the tissue among cases of hepatocellular carcinoma tended to be highly dependent on the histology of non-cancerous tissue. That is, the incidence of HBs-Ag in liver tissues was higher in hepatocellular carcinoma subgroups combined with atrophic liver cirrhosis or chronic active hepatitis than in hepatocellular carcinoma subgroups associated with mild fibrosis or in those exhibiting no histological significant changes.

These results were almost identical to those obtained in Hong-Kong (Wu, 1978) where the carrier rate for the hepatitis B virus is as high as 8.4%. A similar investigation was carried out on the east coast of the United States (Keshegegian *et al.*, 1981) using the orcein staining technique. Although the incidence of HBs-Ag was as low as 15.1% in cases of hepatocellular carcinoma, it was also highly correlated with the presence of atrophic liver cirrhosis. The highest incidence of HBs-Ag in liver tissue occured in the second decade of life in cases of hepatocellular carcinoma and atrophic liver cirrhosis. The rate then decreased gradually with age. These morphological phenomenon might best be explained by the concept of seroconversion proposed by Kubo *et al.*, (1977) and Szmness (1975). These researchers also indicated that the carrier rate for HBs-Ag among blood donors in New York city was highest at 50 years of age then decreased with increasing age.

It has been reported by Almeida *et al.*, (1969), Shulman (1969), Edinton, *et al.*, (1975) that a hepatitis B virus-induced immune reaction may be responsible for causing the hepatocyte injury which leads to chronic liver diseases such as chronic hepatitis, liver cirrhosis and hepatocellular carcinoma. Our present data suggests that a long term incubation of HBs-Ag may cause a stromal inflammatory reaction. Patients with detectable levels of both serum HBs-Ag and core-antigen reportedly ran the highest risk of developing liver cirrhosis (Gudat *et al.*, 1975). Controversies exists as to whether or not the hepatitis B virus has a direct oncogenic role in the etiology of hepatocellular carcinoma (Atiyeh *et al.*, 1980) and whether or not a hepatitis B infection causes liver cirrhosis predisposing patients to hepatocellular carcinoma (Zukerman, 1977). In 1969, Harris, *et al.*, first reported the following hypothesis concerning the role of the hepatitis B virus on oncogenesis: The hepatitis B virus induces malignant changes in infected liver cells, which then fuse with the neighboring normal liver cells to form large, abnormal dysplastic cells. Some of these dysplastic cells then develop into cancer cells. In 1977, Lutwick *et al.*, found the HBV-DNA complex in a DNA fraction extracted from a chronically infected liver by

means of a DNA-DNA hybridization technique. Their results indicated that the hepatitis B virus in possessed of an essential candidation of oncogen. At almost the same time, Macnab *et al.*, (1976) derived a human cell line (Alexander cell PLC/PRF/5) which produced hepatitis B virus surface antigen in the culture supernatant. They then succeeded in producing neoplasia in nude mouse by transplantation of that cell line. Later, another cell line derived by Aden (Hep 3B) was found to produce $\alpha$-fetoprotein as well as HBs-Ag (Alexander *et al.*, 1978). These results lend support to the theory that the hepatitis B virus plays a direct role in oncogenesis.

In the present study, the incidence of HBs-Ag in cases of atrophic liver cirrhosis associated with hepatocellular carcinoma was found to be remarkably greater than in cases without hepatocellular carcinoma. Furthermore, HBs-Ag was detected in 14 cases of hepatocellular carcinoma which did not exhibit atrophic liver cirrhosis: In 6 of those cases, a histological examination of the non-cancerous tissue revealed only mild fibrosis or no significant changes. These results indicate that hepatocellular carcinoma can arise as a direct result of a hepatitis B virus infection without the development of atrophic liver cirrhosis.

In 1973, Anthony *et al.*, defined liver cell dysplasia using cytological features such as cellular enlargement, nuclear pleomorphysm with hyperchromasia and multinucleation. The present study has shown a strong correlation between the presence of the hepatitis B virus and liver cell dysplasia, indicating a possible carcinogenic role of the long term incubation of the hepatitis B virus in the development of hepatocellular carcinoma.

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## REFERENCES

1) Alexander, J., Macnab, G. and Saunders, R. (1978): Studies on *in vitro* production of hepatitis B surface antigen by a human hepatome cell line. Perspect. Virol., 10, 103−120

2) Almeida, J. D. and Waterson, A. P. (1969): Immune complexes in hepatitis. Lancet, II, 983−986

3) Anthony, P. P., Vogal, C. L. and Baker, L. F. (1973): Liver cell dysplasia: a malignant condition. J. Clin. Pathol., 36, 217−223

4) Atiyeh, M. and Ali, M. A. (1980): Primary hepatocellular carcinoma in Saudi Arabia: A clinicopathological study of 54 cases. Am. J. Gastroenterol., 74 (1), 25−29

5) Beasley, R. P., Hwang, L. Y., Lin, C. C. and Chein, C. S. (1981): Hapatocellular carcinoma

and hepatitis B virus: A prospective study of 22707 men in Taiwan. Lancet, 21, 1129−1133

6) Edginton, T. S. and Chisari, F. V. (1975): Immunological aspects of hepatitis B virus infection. Am. J. Med. Sci., 270, 213−227

7) Edmondson, H. A. and Steiner, P. E. (1954): Primary carcinoma of the liver: A study of 100 cases among 48,900 necropsies. Cancer, 7, 462−503

8) Gall, E. A. (1960): Posthepatitic, postnecrotic and nutritional cirrhosis: a pathological analysis. Am. J. Pathol., 36, 241−259

9) Gudat, F., Bianchi, L., Sonnabend, W., Thiel. G., Aenishaenalin, W. and Stalder, G. A. (1975): Pattern of core and surface expression in liver tissue reflects state of specific immune responce in hepatitis B. Lab. Invest., 32, 1−9

10) Halliday, J. W. and Oda, T. (1980): Report of survey of hepatitis antigens in the Asian-Pacific region. Ann. Acad. Med., 9(2), 133−135

11) Harris, H., Miller, D. J., Klein, G., Worst, P. and Tachibana, T. (1969): Suppression of malignancy by cell fusion. Nature, 223, 363−368

12) Huang, S. N. (1975): Immunohistochemical demonstration of hepatitis B core and surface antigens in paraffin sections. Lab. Invest., 33, 88−95

13) Kanda, T. (1977): Hepatitis B surface antigen in cirrhosis of the liver and primary liver carcinoma in Nagasaki area: A histopathological study. Jpn. J. Trop. Med. Hyg., 5(2), 117−139(Japanese with English abstract)

14) Keshgegian, A. A. and Ochs, R. H. (1981): Orcein positive hepatitis B surface antigen and liver carcinoma: Their association in an eastern US population. Arch. Pathol. Lab. Med., 105, 190−193

15) Kubo, Y. and Okuda, K. (1977): Antibody to hepatitis B core antigen in patients with hepatocellular carcinoma. Gastroenterol., 72, 1217−1220

16) Luna, L. G. (1968): Manual of histologic staining methods of the Armed Forces Institute of Pathology. 3rd ed., McGraw-Hill, New York.

17) Lutwick, L. I. and Robinson, W. S. (1977): DNA synthesized in the hepatitis B Dane particle: DNA polymerase reaction. J. Virol., 21, 96−104

18) Macnab, G. N., Alexander, J. J., Lecatsas, G., Bey, E. M. and Urobanowicz, J. M. (1976): Hepatitis B surface antigen produced by a human hepatoma cell line. Br. J. Cancer, 34, 509−515

19) Maupus, P. and Me[nick, J. L. (1981): Hepatitis B infection and liver cancer. Prog. Med. Virol., 27, 1−5

20) McCollum, R. W. and Zukerman, A. J. (1981): Viral hepatitis: Report on a WHO informal consultation. J. Med. Virol., 8, 1−29

21) Miyaji, T. (1965): Hepatocellular carcinoma in Japan: especially its relation to liver cirrhosis, Tr. Soc. Pathol. Jpn., 54, 23−36 (in Japanese)

22) Miyake, H. (1960): Pathology of the liver. Tr. Soc. Pathol. Jpn., 49, 589−613 (in Japanese)

23) Mori, I. (1964): Classification of liver cirrhosis. Igaku no Ayumi, 48, 371−381 (in Japanese)

24) Nagayo, M. (1914): Liver cirrhosis. Tr. Soc. Pathol. Jpn., 4, 31−72 (in Japanese)

25) Senba, M. (1983): A reliable silver staining method for *Pneumocystitis carinii* and reticulum fibers in histologic sections. Acta. Histochemica. Cytochem., 16, 169−171

26) Shikata, T. (1973): Australia antigen in hepatoma and cirrhosis tissue, Tumor Res., 8, 127−130

27) Shikata, T., Uzawa, T., Yoshiwara, N., Akatsuka, T. and Yamazaki, S. (1974): Staining methods of Australia antigen in paraffin section: Detection of cytoplasmic inclusion bodies. : Jpn. J. Exp. Med., 44(1), 25−36
28) Shulman, N. R. (1969): Virus-like antigen, antibody and antibody complexes in hepatitis mesured by complement fixation. Science, 165, 304−309
29) Szmuness, W. (1975): Recent advances in the study of the epidemiology of the hepatitis B. Am. J. Pathol., 81, 629−649
30) The reports of the 6th Inuyama Symposium (1975): Chronic hepatitis, after that. Chugai Igaku Sha (Tokyo) (in Japanese)
31) Wu, P. L. (1978): Detection of hepatitis B surface antigen in liver biopsies from 655 Chinese patients in Hong-Kong. Asia. J. Infect. Dis., 2, 223−229
32) Zukerman, A. J. (1977): Symposium on liver carcinoma: Hepatocellular carcinoma and hepatitis B. Trans. R. Soc. Trop. Med. Hyg., 71, 459−461

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肝細胞癌発生におけるB型肝炎ウイルスの役割に関する病理組織学的研究

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1964年から1980年における長崎大学での剖検例6339例のうち，肝疾患を主病変とする症例953例について，検討した．全剖検例に占める肝疾患の比率は，萎縮性肝硬変（7.7%），肝細胞癌（4.8%）で，同期間における全日本の平均（日本剖検輯報による）の5.2%及び3.5%と比較し高率であり，統計学的に有意差を示した．又，488例の萎縮性肝硬変のうち242例（49.6%）に，肝細胞癌の合併がみられ，一方303例の肝細胞癌のうち242例（78.9%）に萎縮性肝硬変が認められた．

上記953例のうち，組織学的に詳細な検討が可能であった652例につき，H．E 染色，志方によるオルセイン染色を行い，肝組織型，組織 HBs-Agの有無，間質炎症反応の有無，Dysplasia の有無，肝細胞癌の Grade，及びこれらの相互の関係について検討した．肝組織 HBs-Ag は，萎縮性肝硬変＋肝細胞癌の群では高率で60.6%に陽性であった．196例の肝細胞癌のうち，非癌部の組織が萎縮性肝硬変でないものは41例でこのうちの14例に HBs-Ag が認められた．この14例のうち6例は非癌部の組織が軽度の線維かのみか，又は特に著変を認めないものであり，HBs-Ag は直接に oncogenic 作用を持つという仮説を支技する所見と思われた．萎縮性肝硬変症例について Liver cell dysplasia の有無について検討したが，肝細胞癌の合併の有無により，陽性率に差がみられた．さらに，長与B型の萎縮性肝硬変群では，HBs-Ag の陽性率と Dysplasia の出現との間に有意な関係がみられた．

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