Statistic Analysis of Relationships between Hepatitis B Surface Antigen, Cirrhosis and Hepatocellular Carcinoma

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Abstract: Hepatocellular carcinoma becomes clinically manifest after cirrhosis has been well formed, and the major factor predisposing hepatocellular carcinoma in a population appears to be the presence of cirrhosis caused by chronic hepatitis B virus infection in that population. Cofactors in hepatocarcinogenesis such as foodstuffs contaminated by aflatoxin, probably play a secondary role in the development of hepatoce'lular carcinoma even a country like where Kenya occurs in a high incidence of chronic hepatitis B virus infection. Therefore, we tried statistically to confirm the above hypothesis using iver specimens obtained from Kenya, and verified significant associations between hepatitis B surface antigen, cirrhosis and hepatocellular carcinoma.

Key words: Hepatitis B surface antigen, cirrhosis, hepatocellular carcinoma, statistic analysis.

INTRODUCTION

The discovery of hepatitis B surface antigen (initially called the Australia antigen) by Blumberg triggered an enormous research effort that has providid practical methods for detection of hepatitis B virus (B umberg *et al.*, 1962; Blumberg *et al.*, 1964; Blumberg *et al.*, 1965; Blumberg *et al.*, 1967; Blumberg *et al.*, 1970).

Chronic infection caused by hepatitis B virus may lead to necrosis of liver cells, which can lead to bridge portal triads (bridging necrosis), or involving the adjacent lobules causing wide-spread (multilobular necrosis), resulting in the formation of hepatic fibrosis or cirrhosis. Furthermore, long-standing hepatitis B virus carriers, mostly those with cirrhosis, may develop hepatocellular carcinoma. A possible link between hepatitis B virus infection and hepatocellular carcinoma has been reported (Sherlock *et al.*, 1970; Vogel *et al.*, 1970; Vogel and Linsell, 1972; Vogel *et al.*, 1972; Charinuvati *et al.*, 1975). We

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investigated by statistical analysis relationship between hepatitis caused by hepatitis B virus infection and hepatocellular carcinoma.

MATERIALS AND METHODS

The liver and the spleen specimens from 68 autopsy cases at Rift Valley Provincial General Hospital in Kenya were used (Table 1).

The specimens were fixed in Zenker's formol, or sometimes in 10% formalin, and embedded in paraffin for histopathologic study. Sections were cut at 5 micron and stained with hematoxylin and eosin, periodic acid Schiff (PAS), elastic fibers (Senba, 1982), collagen fibers (Luna, 1960), hepatitis B surface antigen (Senba, 1982) and reticulum fibers (Senba, 1983).

Statistical calculation was performed employing the BMDP (Dixon *et al.*, 1981) on the IBM 4341 system in the Data Center of A-Bomb Disaster in Nagasaki University. The statistical method for this study is the following: Pearson chi-square test for association in contingency table and chi-square test of one degree of freedom for trend of one factor with another. Since these methods are well documented, for instance in Snedecar *et al.* (1967), the detail are omitted here.

RESULTS

Table 1 shows the hepatitis B surface antigen positive results obtained by the orcein staining. Of the 20 hepatitis B surface antigen positive specimens (29%), 13 had cirrhosis (69%), 9 had hepatocellular carcinoma with cirrhosis (82%). In 11 hepatocellular carcinoma with cirrhosis, 7 had hepatitis B surface antigen in only the non-cancerous tissue, 2 showed positivity in the both cancerous and non-cancerous tissue.

Results of the statitistical analysis on hepatitis B surface antigen, cirrhosis, and hepatocellular carcinoma are as follows: Pearson chi-square test and trend test between hepatitis B surface antigen and cirrhosis are 0.0001 and 0.0000, respectively, and those between hepatitis B surface antigen and hepatocellular carcinoma are 0.0000 and 0.0000, and those between cirrhosis and hepatocellular carcinoma are 0.0000 and 0.0000. Thus any two of them are proved highly associated with each other.

DISCUSSION

High correlation of hepatitis B surface antigen, cirrhosis, and hepatocellular carcinoma are disclosed through statistical examination, and these statistic analysis results are acceptable because hepatocellular carcinoma is well developed in cirrhotic patients, hepatic fibrosis and cirrhosis are greatly responsible for many of the sequelae of chronic liver diseases including hepatitis B virus infection.

Table 1. Autopsy cases for statistic analysis of relationships between hepatitis B surface antigen, cirrhosis and hepatocellular carcinoma

CASE NO.	LABEL	AGE	SEX	C	H	HBSAG
1		30	MALE	CIRRHSS	HEPATOMA	2
2		45	MALE	CIRRHSS	HEPATOMA	1
3		45	MALE	CIRRHSS	HEPATOMA	0
4		80	MALE	CIRRHSS	NONH	1
5		35	FEMALE	CIRRHSS	HEPATOMA	3
6		40	MALE	CIRRHSS	HEPATOMA	2
7		70	MALE	CIRRHSS	HEPATOMA	3
8		70	MALE	CIRRHSS	NONH	0
9		13	MALE	CIRRHSS	NONH	0
10		25	MALE	CIRRHSS	HEPATOMA	3
11		45	FEMALE	CIRRHSS	NONH	0
12		36	MALE	CIRRHSS	HEPATOMA	2
13		50	FEMALE	CIRRHSS	NONH	1
14		35	FEMALE	CIRRHSS	NONH	2
15		60	MALE	CIRRHSS	HEPATOMA	2
16		40	MALE	CIRRHSS	HEPATOMA	3
17		40	MALE	CIRRHSS	NONH	2
18		50	MALE	CIRRHSS	HEPATOMA	0
19		35	MALE	CIRRHSS	NONH	0
20		50	MALE	NONC	NONH	0
21		30	FEMALE	NONC	NONH	0
22		30	MALE	NONC	NONH	0
23		32	MALE	NONC	NONH	0
24		35	MALE	NONC	NONH	0
25		35	MALE	NONC	NONH	0
26		25	MALE	NONC	NONH	0
27		35	MALE	NONC	NONH	0
28		30	FEMALE	NONC	NONH	0
29		50	FEMALE	NONC	NONH	0
30		50	MALE	NONC	NONH	0
31		.300	FEMALE	NONC	NONH	0
32		24	FEMALE	NONC	NONH	0
33		13	MALE	NONC	NONH	0
34		7	MALE	NONC	NONH	0
35		8	MALE	NONC	NONH	0
36		62	MALE	NONC	NONH	0
37		10	FEMALE	NONC	NONH	0
38		60	MALE	NONC	NONH	0
39		18	FEMALE	NONC	NONH	0
40		58	FEMALE	NONC	NONH	0
41		70	FEMALE	NONC	NONH	0
42		9	MALE	NONC	NONH	0
43		2	MALE	NONC	NONH	0
44		10	MALE	NONC	NONH	0
45		25	FEMALE	NONC	NONH	3
46		50	FEMALE	NONC	NONH	1
47		35	MALE	NONC	NONH	0
48		60	MALE	NONC	NONH	0
49		60	MALE	NONC	NONH	3
50		25	MALE	NONC	NONH	0
51		10	MALE	NONC	NONH	0
52		20	MALE	NONC	NONH	1
53		65	MALE	NONC	NONH	0
54		50	MALE	NONC	NONH	0
55		4	FEMALE	NONC	NONH	2
56		40	MALE	NONC	NONH	0
57		35	MALE	NONC	NONH	0
58		62	MALE	NONC	NONH	0
59		48	MALE	NONC	NONH	0
60		30	FEMALE	NONC	NONH	0
61		60	FEMALE	NONC	NONH	0
62		40	MALE	NONC	NONH	0
63		40	MALE	NONC	NONH	0
64		50	MALE	NONC	NONH	0
65		40	MALE	NONC	NONH	0
66		6	FEMALE	NONC	NONH	0
67		40	MALE	NONC	NONH	1
68		10	MALE	NONC	NONH	1

CIRRHSS: cirrhosis, HBSAG: hepatitis B surface antigen, NONC: non-cirrhosis, NONH: non-hepatocellular carcinoma, 0: negative, 1: slight positive, 2: moderate positive, 3: marked positive.

Table 2. Incidence of serum hepatitis B surface antigen (HBsAg) in hepatocellular carcinmoa (HCC) patients in different countries

Country	Assay	HBsAg in HCC	HBsAg in Normal population	Author
South Africa	RIA	40%	9%	Kew et al. (1974)
Senegal	RIA	61%	11%	Prince et al. (1974)
Uganda	RIA	79%	7%	Vogel et al. (1970)
Zambia	RIA	63%	8%	Tabor et al. (1976)
Japan	IAHA	37%	3%	Sakurai et al. (1975)
Singapore	IAHA	35%	8%	Simoms et al. (1972)
Taiwan	RIA	80%	15%	Tong et al. (1971)
Vietnam	RIA	80%	25%	Welsh et al. (1976)
New Guinea	RIA	82%	8-20%	Woodfield et al. (1972)
U.S.A.	RIA	26%	0.1%	Tabor et a. (1977)
England	RIA	24%	0.1%	Reed et al. (1973)
Greece	RIA	76%	5%	Hadziyannis et al. (1972)

Studies have shown in Table 2 that, in some regions of Asia and Africa, chronic infection with hepatitis B virus is present in at least as many as 40-80% of patients with hepatocellular carcinoma. Thus, the major factor determining the number of cases of hepatocellular carcinoma in a population appears to be incidence of B virus cirrhosis in that population.

The most plausible explanation for the increased risk of hepatocellular carcinoma is that the acceleration of cellular replication that occurs in cirrhosis enhances effects of many carcinogens including hepatitis B surface antigen. Moreover, the oncogenic potential of hepatitis B virus in the development of hepatocellular carcinoma is well known (Marion *et al.*, 1980; Br chot *et al.*, 1981; Knowles *et al.*, 1980; Aden *et al.*, 1979; Skelly *et al.*, 1979; Sninsky *et al.*, 1979). Marion et al. stated in their manuscript "PLC/PRF/5, a tissue culture cell line isolated from a human hepatocellular carcinoma and producing hepatitis B surface antigen, was studied for the presence of hepatitis B virus (HBV)-specific DNA and RNA. PLC/PRF/5 cell DNA accelerated the rate of reassociation of HBV[^{32}p] DNA, and quantitative experiments indicated that the cells contained approximately four copies of viral DNA per haploid, mammalian cell DNA equivalent. PLC/PRF/5 DNA accelerated the rate of reassociation of all individual resyriction endonuclease *Hinc*II and *Hae*III fragments of HBV [^{32}p] DNA, indicating that DNA from all regions of the viral genome is present in the cells. This suggests that these cells contain at least most, and possibly all, of the viral genome."

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肝炎・肝硬変および肝癌に経る相互関係の統計による分析

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ケニア国の剖検例の肝臓を用いて，肝炎・肝硬変および肝癌とB型肝炎ウイルスの相互関係を統計により分析を試みた．B型肝炎ウイルスの感染が全体に29%，肝硬変に69%，肝癌に82%の割合で存在することが解った．統計による分析の結果，肝炎・肝硬変および肝癌の相互関係は高い相関が認められた．

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