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Epidemiological Study of Hepatitis B Virus in Nagasaki Area of Japan

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The epidemiological studies of Hepatitis B Virus (HBV) in Nagasaki Prefecture well-known for the high incidence of liver cirrhosis, primary hepatoma and other liver diseases have been carried out by means of detecting several viral and related markers, such as the surface antigen of HBV (HBs Ag), the antibody to HBs Ag (anti-HBs), the antibody to the core of HBV (anti-HBc), *e* antigen (*e* Ag) and the antibody to *e* Ag (anti-*e*), in the sera among healthy blood donors, healthy inhabitants and hospitalized patients.

HBs Ag was detected in 3.8% of 15,149 healthy persons (blood donors and inhabitants) and in 4.8% of 1,192 patients by means of immunoelectrosyneresis (IES). The positive rate (3.8%) in healthy persons was apparently higher than that in those of other areas in Japan. 1.1% of healthy persons and 5.9% of hospitalized patients were found to be anti-HBs-positive by IES. The HBs Ag-positive frequency was significantly higher in male than in female, but the anti-HBs-positive frequency was lower in male than in female.

Dividing Nagasaki Prefecture into five areas, i. e., Nagasaki City, Gotoh islands, the North, the East and the West areas, the prevalence of HBs Ag tended to be higher in the West and lower in the East. Especially, the positive occurence of HBs Ag in Gotoh area showed the highest value.

Among subtypes of 359 HBs Ag-positive donor sera, HBs-antigenic determinants *d* (*d*, 99% ; *y*, 1%) and *r* (*r*, 93% ; *w*, 7%) were dominant.

The positive frequencies of *e* Ag and anti-*e* in 297 HBs Ag-positive donor sera were 53 (17.8%) and 141 (47.5%) respectively. Both *e* Ag and anti-*e* (*e* markers) were never detected in HBs Ag-negative sera. No relationship was found between the presence of *e* markers and HBs Ag subtypes.

Although no HBc Ag was detected in any sera tested, anti-HBc was detected in all three classified groups of sera, i. e., negative group for both HBs Ag and anti-HBs, positive group for only anti-HBs, and positive group for only HBs Ag. In the first group, 17.7% of samples was anti-HBc-positive, and 81.3% and 100% of samples in the second

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and the third groups respectively were also positive.

Therefore, about 39% of healthy persons was immersed with HBV in past by calculating from the data described above.

INTRODUCTION

The presence of hepatitis virus has long been believed from the epidemiological observations of hepatitis, and many investigators have made efforts to discover it in vain.[12)43)46)55)]

In 1965, an antigen, which showed a precipitin line in agar against the serum of multiply transfused American haemophiliacs, was found in the serum of an Australian aborigine. This antigen was designated as 'Australia' antigen (Au-antigen, at present called as Hepatitis B surface antigen : HBs Ag) by BLUMBERG.[6)] Subsequent studies on the distribution of the HBs Ag in normal population revealed that this HBs Ag was rarely found in the sera of North American and European people, but was frequently found in the sera of people living in the tropics and Asia. It was also frequently detected in patients with Down's syndrome, leukemia and hepatitis.[2)7)35)] Afterward, PRINCE[42)] and OKOCHI[40)] pointed out independently the intimate relationship between HBs Ag and hepatitis, particularly serum hepatitis. KRUGMANN,[23)] BARKER[4)] and others[11)] directly established that HBs Ag-positive serum contained an infectious agent in infection experiments.

Three different morphological particles, i. e., small spherical particle with approximately 20 nm in diameter, tubular form with 20 x 50-230 nm in length[5)] and large spherical particle with approximately 40 nm in diameter first shown by DANE (so-called "Dane particle")[9)] were found in the HBs Ag-positive serum. Dane particle with inner core became to be regarded as a human type B hepatitis virus candidate by several investigators[13)19)20)44)45)] and the inner core (HBc Ag) was antigenically distinct from the surface of Dane particle.[1)8)10)14)]

On the other hand, in 1972, MAGNIUS et al.[27)] demonstrated a new antigen-antibody system distinct from HBs Ag and HBc Ag in HBs Ag-positive serum and designated the new antigenic determinant as"*e*". NIELSEN et al.,[34)] MAGNIUS et al.[29)] and other workers[39)47)] hypothetically stressed that *e* antigen was associated with the infectivity of HBs Ag-positive sera, because the *e* antigen might be efficiently produced in parallel with Dane particle synthesis.[36)]

It is well known that in Nagasaki Prefecture located in western Japan, patients with primary hepatoma and liver cirrhosis have occurred most frequently in Japan.[16)] At present, Dane particle, i. e., hepatitis B virus (HBV), is registered as the pathogenic agent of type B acute and chronic hepatitis, liver cirrhosis as well as primary hepatoma. Although the pathogenesis of HBV among cirrhosis and hepatoma was not yet directly established, vertical transmission from HBs Ag-positive mother followed by healthy carrier state of HBV would play a significant role.[37)] Therefore, it is important to study the dissemination status of HBV among healthy persons, patients with liver diseases and various

patients without liver diseases, and finally to elucidate the relationship between HBV infection and many kinds of liver diseases including hepatoma.

In this report, the epidemiological studies of HBV among healthy persons and various patients in Nagasaki area are presented. The evidences of HBV infection among healthy blood donors, inhabitants and various patients admitted at the Nagasaki University Hospital were obtained by using three antigen-antibody systems, i. e., HBs Ag : anti-HBs, HBc Ag : anti-HBc and e Ag : anti-e as markers of HBV-infection.

MATERIALS AND METHODS

(1) *Serum samples*

Blood donors' sera : 13,555 donor sera collected at the Nagasaki Red Cross Blood Center from August 1972 to July 1973 were studied.

Inhabitants' sera : 1,594 sera were obtained from the local health centers appropriately located in Nagasaki Prefecture.

Patients' sera : From March to May 1974 and from July to August 1976, 1,192 inpatients' sera which were submitted to the Biochemical Section of the Central Laboratory in the Nagasaki University Hospital for testing liver functions, were randomly collected and used in this study.

(2) *Methods used for detecting HBs Ag and anti-HBs*

Immunoelectrosyneresis (IES) method was mainly used for detecting HBs Ag and anti-HBs. Passive hemagglutination (PHA) method was also used for detecting anti-HBs. PHA cells were kindly supplied from Dr. Mayumi of Jichi Medical University and Eisai Company Ltd. IES was performed on glass slides (25×75 mm) overlayed with 1% agar gel in M/100 barbitalsodium-Barbital buffer (pH 8.6, ionic strength 0.05) under a constant current of 2mA/cm for 45 minutes at room temperature. The final decision was made after the reacted agar gel slide was incubated in a moisture chamber for 24 hours at 4-6°C.

Several appropriate high-titered HBs Ag-positive blood donor sera were pooled and used as reference antigen. They were stored at -20°C and were diluted into 5 or 10 times for use. Three reference antibodies were used for detecting HBs Ag by IES, i. e., one from pooled anti-HBs-positive blood donor sera, another from Dr. Okochi of Kyushu University (designated as Yamakawa) and the other from hyperimmune anti-HBs rabbit serum prepared in this laboratory. PHA was performed by using microtiter hemagglutination technique and antibody-positive sera agglutinated the PHA cells at a dilution of 1 : 16 or more within 1 hour.

(3) *Methods for detecting e Ag or anti-e, and for subtyping HBs Ag*

Immunodiffusion (Micro-Ouchterlony : M. O.) method was performed in 0.9% agarose gel in 0.01M Tris buffer supplemented with 0.1M NaCl and 2% Dextran A, at

pH 7.8 by incubation in the moisture chamber for 24 hours at 37°C. Standard *e* Ag and anti-*e* reagents were at first kindly supplied from Dr. Okochi and thereafter, originally prepared from HBs Ag-positive donor plasma. For subtyping HBs Ag, anti-*ad* and anti-*ay* guinea pig sera were obtained from the United States National Institutes of Health (V801-502-058, V802-501-558) and anti-*ar* rabbit serum was obtained from immunized animals with partially purified HBs Ag (subtype : *adr*) prepared from HBs Ag-positive blood donor sera by using CsCl cushion, buoyant density gradient and sucrose stepwise gradient ultracentrifugation in this laboratory.

(4) *Methods for detecting HBc Ag and anti-HBc*

HBc Ag was assayed by a reversed passive hemagglutination (r-PHA) technique and anti-HBc was assayed by the reversed passive hemagglutination inhibition (r-PHAI) technique developed in this laboratory as described elsewhere (Igarashi, et al., to be published). Essentially, the tannic acid-treated human type O red blood cells were coated with anti-HBc IgG prepared from HBs Ag-positive blood donor sera by sedimentation with saturated ammonium sulfate and by purification using DEAE-cellulose column chromatography, and those were used to detect HBc Ag by hemagglutination. When anti-HBc is added to this system, hemagglutination should be inhibited.

RESULTS

Incidence of HBs Ag and anti-HBs

The results of testing sera of healthy persons (blood donors and inhabitants) for HBs Ag and anti-HBs by IES and PHA are shown in Tables 1 and 2. From these results, the HBs Ag-positive frequency is significantly higher in male than in female. To the contrary, the anti-HBs-positive frequency by IES is significantly lower in male than in female.

In Table 2, the higher anti-HBs-positive rate in blood donors than in inhabitants by both IES and PHA would be responsible for the different composition of sex and age

Table 1. Positive frequencies of HBs Ag anti-HBs in healthy persons (blood donors and inhabitants) tested by IES

	No. positive sera / No. tested (%, $\alpha=0.05$)	
HBs Ag	569/15,149	(3.77±0.3)
	male	451/10,944 (4.14±0.37)
	female	117/ 4,113 (2.89±0.51)
Anti-HBs	166/15,149	(1.11±0.17)
	male	97/10,944 (0.9 ±0.18)
	female	62/ 4,113 (1.55±0.37)

Table 2. Positive frequencies of HBs Ag and anti-HBs in blood donors and inhabitants tested by IES and PHA

	Blood donors	Inhabitants	Total (Healthy persons)
HBs Ag (by IES)	526/13,555[a] (3.9%)[b]	43/1,594 (2.7%)[b]	569/15,149 (3.8%)
Anti-HBs (by IES)	132/13,555 (1.0%)[c]	34/1,594 (2.1%)[c]	166/15,149 (1.1%)
Anti-HBs (by PHA)	667/ 3,787 (17.6%)[d]	200/ 825 (24.2%)[d]	867/ 4,612 (18.8%)

a) No. positive sera/No. tested

b) $X_0^2=5.531$ $p<0.02$

c) $X_0^2=17.13$ $p<0.001$

d) $X_0^2=19.5$ $p<0.001$

between the two groups. Tables 3 and 4, and Fig. 1 show age distributions of HBs Ag and of anti-HBs in healthy persons. In these results, prevalence of HBs Ag tends to

Table 3. Age distribution of HBs Ag-positive frequencies in blood donors and inhabitants tested by IES

Group / Age	Blood donor male	Blood donor female	Inhabitant male	Inhabitant female	Total
≦15	-	-	0/ 3[a]	0/ 2	0/ 5
16-21	129/ 2,720(4.74)[b]	47/1,521(3.09)	5/106(4.72)	1/ 95(1.05)	182/ 4,442(4.10)
22-30	150/ 3,413(4.39)	26/ 867(3.00)	4/152(2.63)	3/159(1.89)	183/ 4,591(3.99)
31-40	92/ 2,364(3.89)	11/ 466(2.36)	10/142(7.04)	3/114(2.63)	116/ 3,086(3.76)
41-50	41/ 1,290(3.18)	11/ 388(2.84)	4/166(2.41)	2/118(1.69)	58/ 1,962(2.96)
51-60	10/ 319(3.13)	9/ 165(5.45)	4/118(3.39)	1/ 94(1.06)	24/ 696(3.45)
61≦	0/ 34	0/ 8	1/ 83(1.20)	1/100(1.00)	2/ 225(0.89)
total	422/10,140(4.16)	104/3,415(3.05)	28/770(3.64)	11/682(1.61)	565/15,007(3.76)

a) No. positive sera/No. tested

b) Number in parentheses : %

Table 4. Age distribution of anti-HBs-positive frequencies in blood donors and inhabitants tested by PHA

Group / Age	Blood donor male	Blood donor female	Inhabitant male	Inhabitant female	Total
≦15	-	-	0/ 2[a]	0/ 1	0/ 3
16-21	111/ 680(16.32)[b]	58/349(16.62)	14/ 75(18.67)	6/ 40(15.00)	189/1,144(16.52)
22-30	179/ 966(18.53)	51/272(18.98)	26/137(18.98)	9/ 55(16.36)	265/1,430(18.53)
31-40	117/ 727(16.09)	23/140(16.43)	24/ 89(26.97)	14/ 40(35.00)	178/ 966(17.87)
41-50	52/ 317(16.40)	29/114(25.44)	11/ 52(21.15)	17/ 58(29.31)	109/ 541(20.15)
51-60	17/ 84(20.24)	9/ 33(27.27)	10/ 26(38.46)	17/ 45(37.78)	53/ 188(28.19)
61≦	0/ 7	2/ 2(100)	5/ 24(20.83)	13/ 62(20.97)	20/ 95(21.05)
total	476/2,781(17.12)	172/910(18.90)	90/405(22.22)	76/301(25.25)	814/4,397(18.51)

a) No. positive sera/No. tested

b) Number in parentheses : %

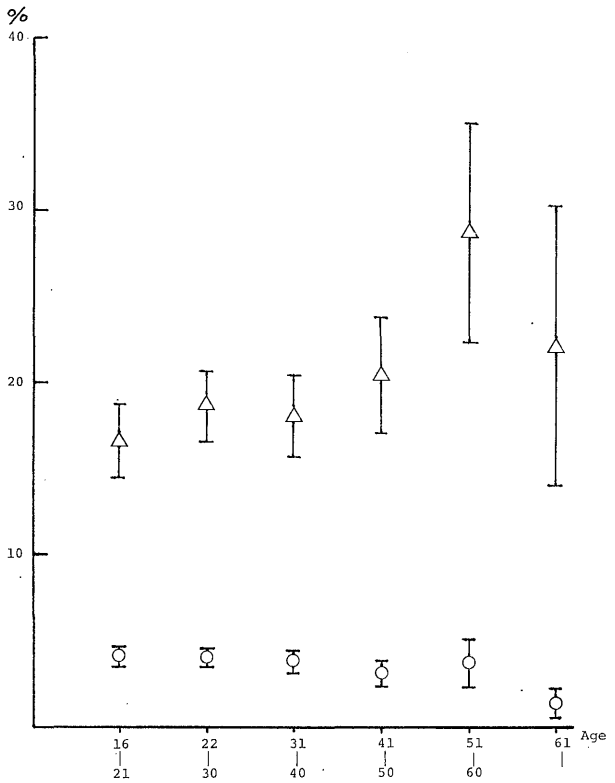

Fig. 1. Age distributions of positive frequencies of HBs Ag tested by IES and of Anti-HBs tested by PHA in healthy persons ($\alpha=0.05$)
(○ HBs Ag △ Anti-HBs)

decrease and that of anti-HBs to increase as the age advances.

In Table 5, HBs Ag- and anti-HBs-positive frequencies in healthy persons in five divided areas of Nagasaki Prefecture, i. e., (1) Nagasaki City, (2) the Western area, (3) the Nothern area, (4) the Eastern area and (5) Gotoh islands in Fig. 2 are presented. HBs Ag-positive rates in Nagasaki City and Gotoh islands are higher than those in other areas, especially significantly higher than those in the Nothern and the Eastern areas,

Table 5. Regional positive frequencies of HBs Ag tested by IES and of anti-HBs tested by PHA in healthy persons

Areas	(1) Nagasaki City	(2) Western area	(3) Northern area	(4) Eastern area	(5) Gotoh Islands	Total
HBs Ag (by IES)	357/8,523 (4.2±0.4)	48/1,376[a] (3.6±1.0)[b]	22/890 (2.7±1.0)	87/3,262 (2.7±0.6)	42/754 (5.8±1.7)	556/14,805 (3.8±0.3)
Anti-HBs (by PHA)	455/2,529 (18.0±1.5)	91/ 478 (19.3±3.5)	62/286 (22.1±4.8)	190/1,025 (18.7±2.4)	69/294 (23.8±4.8)	867/ 4,612 (18.8±1.1)

a) No. positive sera/No. tested
b) Number in parentheses : % ($\alpha=0.05$)

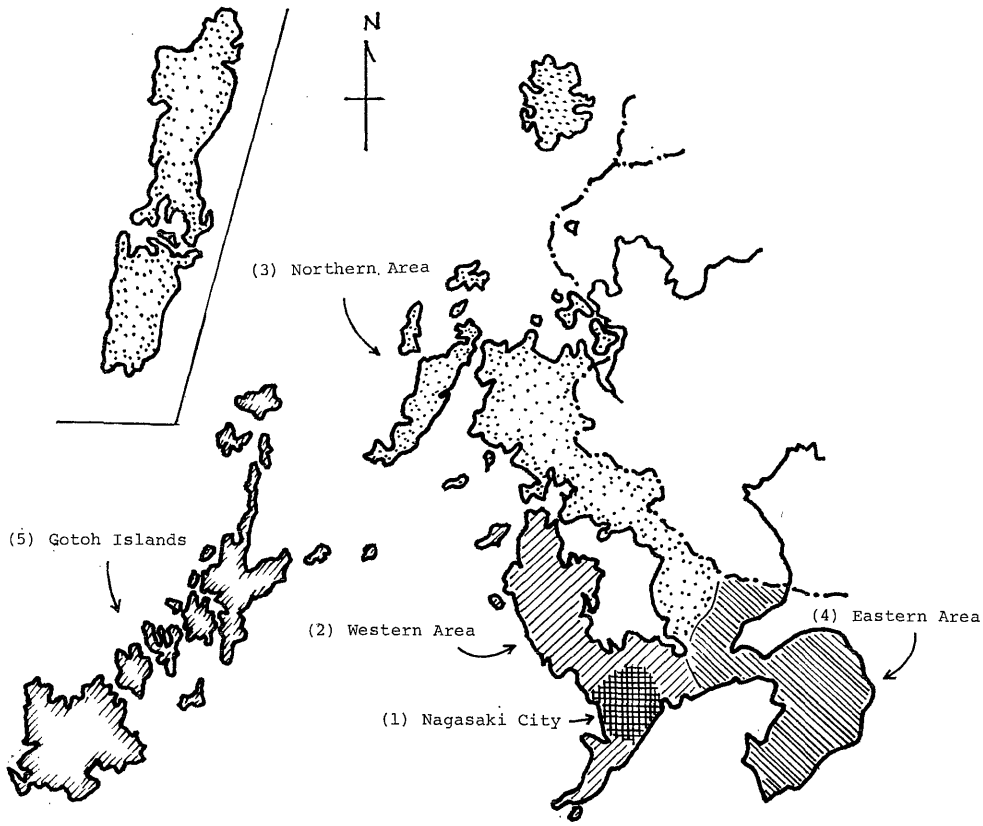

Fig. 2. Map of Nagasaki Prefecture divided into five areas

but in anti-HBs-positive rates, no significant differences are observed as shown in Table 5. The relationships among prevalences of HBs Ag and anti-HBs, occurence rate of hepatitis and death rate of liver cirrhosis and hepatoma in each area are illustrated in Fig. 3 in which death rate of liver disease (liver cirrhosis plus hepatoma), HBs Ag-positive frequency and anti-HBs-positive one in Gotoh islands are markedly high.

In Table 6, positive frequencies of HBs Ag and anti-HBs in hospitalized patients are presented. Comparing the result in Table 6 with that in Table 1, positive frequency of anti-HBs in various patients is significantly higher than in healthy persons as shown in Table 7. The age distribution of HBs Ag-and anti-HBs-positive patients is not shown here, because the number of samples was too small for statistical distribution analyses.

HBs Ag subtype

Subtype analyses of HBs Ag obtained from healthy persons and hospitalized patients in Nagasaki Prefecture are summarized in Table 8. The HBs Ag-subtypes commonly detected are *adr* and *adw*, and *ayr* is present in small size, and *ayw* subtype is absent. There is no significant difference in distribution of HBs Ag-subtypes between the two groups.

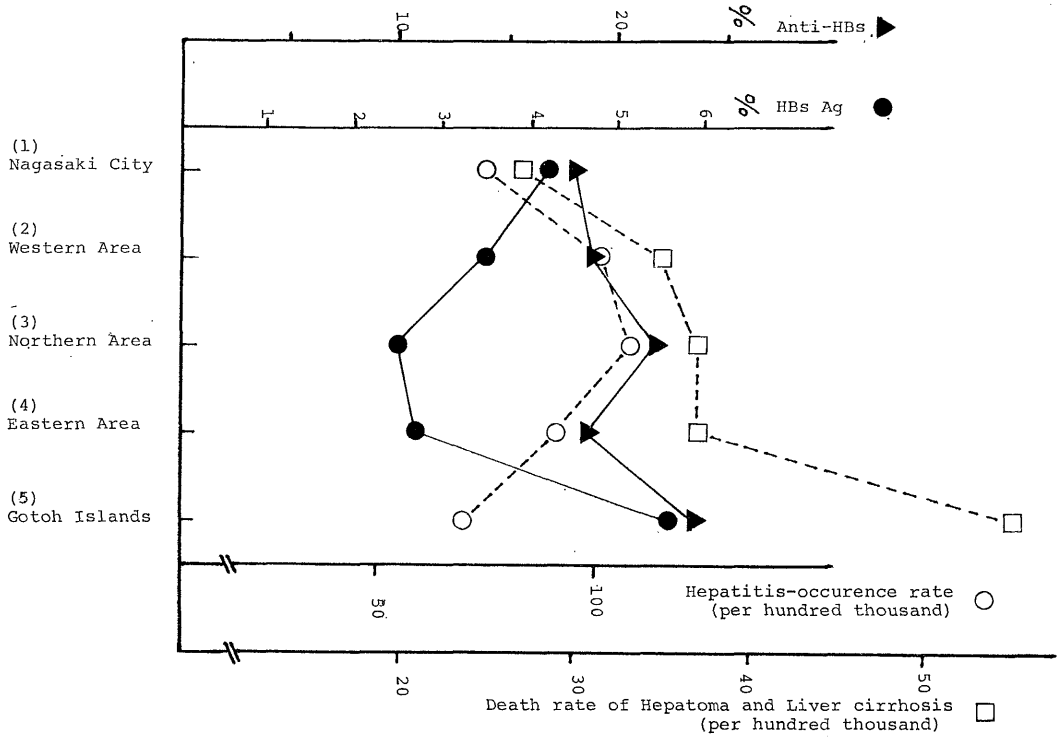

Fig. 3. Correlations among prevalences of HBs Ag and Anti-HBs, hepatitis-occurence rate and death rate of hepatoma and liver cirrhosis in each area of Nagasaki Prefecture

Table 6. Positive frequencies of HBs Ag and anti-HBs in hospitalized patients tested by IES

	No. positive sera / No. tested (%, α=0.05)
HBs Ag	50/1,192 (4.34±1.15)
	male 35/611 (6.0±1.86)
	female 15/581 (2.9±1.32)
Anti-HBs	70/1,192 (6.10±1.34)
	male 35/611 (6.0±1.86)
	female 35/581 (6.3±1.95)

Table 7. Comparisons between HBs Ag-and anti-HBs-positive frequencies in healthy persons and in hospitalized patients tested by IES

	HBs Ag	Anti-HBs
Patients	50/1,192[a)] (4.2%)	70/1,192 (5.9%)
Healthy Persons	569/15,149 (3.8%)	166/15,149 (1.1%)
	X_0^2=0.58 p<0.5	X_0^2=177.14 p<0.001

a) No. positive sear / No. tested

Table 8. Subtypes of HBs Ag in healthy persons and hospitalized patients

Healthy Persons

	d	*y*	total
r	331[a)] (92%)	4 (1%)	335 (93%)
w	24 (7%)	0	24 (7%)
total	355 (99%)	4 (1%)	359 (100%)

Patients

	d	*y*	total
r	16 (89%)	0	16 (89%)
w	2 (11%)	0	2 (11%)
total	18 (100%)	0	18 (100%)

a) No. sear subtyped

e Ag and anti-e

In Table 9, positive frequencies of *e* Ag and anti-*e* in HBs Ag-positive blood donors are presented. *e* Ag is often detected in high-titered HBs Ag-positive sera, and conversely anti-*e* is usually detected in low-titered HBs Ag-samples. *e* Ag and anti-*e* were never detected in HBs Ag-negative sera. No correlation among *e* Ag, anti-*e* and HBs Ag subtype is observed.

Table 9. Positive frequencies of *e* Ag and anti-*e* in HBs Ag-positive blood donors

	No. positive sera / No. tested	HBs Ag high-titer	HBs Ag low-titer
e Ag	53/297 (17.8%)	44 (53.7%)	9 (8.0%)
Anti-*e*	141/297 (47.5%)	38 (46.3%)	103 (92.0%)
	Total	82 (100%)	112 (100%)
		$X_0^2=49.63$	$p<0.001$

HBc Ag and anti-HBc

Positive frequencies of HBc Ag and anti-HBc in blood donors, who were classified into three groups according to the presence or the absence of HBs Ag and of anti-HBs, are shown in Table 10, and those in hospitalized patients are also shown in Table 11.

Comparisons between positive rate and mean titer of anti-HBc in blood donors and in patients are summarized in Table 12. From these results, HBc Ag is not detected at all, but anti-HBc is detected in all three groups, particularly in HBs Ag-positive group (100%). No relationship between HBs Ag subtypes and anti-HBc titer was suggested. And also, no relationship among the presence of *e* Ag or anti-*e* and anti-HBc titer was observed.

Table 10. Positive frequencies of HBc Ag tested by r-PHA and of anti-HBc tested by r-PHA-I in blood donors

HBs Ag Anti-HBs	Negative Negative	Negative Positive	Positive Negative
HBc Ag	0/53 (0)[a)]	0/25 (0)	0/ 25 (0)
Anti-HBc	43/243 (17.7)	78/96(81.8)	107/107 (100)

a) No. positive sera / No. tested (%)

Table 11. Positive frequencies of HBc Ag and anti-HBc in hospitalized patients

HBs Ag Anti-HBs	Negative Negative	Negative Positive	Positive Negative
HBc Ag	0/22 (0)[a)]	0/ 2 (0)	0/ 8 (0)
Anti-HBc	29/99 (29.3)	11/16 (68.8)	22/22 (100)

a) No. positive sera / No. tested (%)

Table 12. Positive frequencies and mean titers of anti-HBc in blood donors and patients

HBs Ag Anti-HBs	Negative Negative		Negative Positive		Positive Negative	
		MT[a)]		MT		MT
Blood donors	43/243 (17.7)[b)]	5.6	78/96 (81.3)	380.5	107/107 (100)	2,611.7
Patients	29/ 99 (29.3)	305.7	11/16 (68.8)	35.5	22/ 22 (100)	17,802.2
P		<0.001		>0.5		<0.001

a) MT : Mean titer, hemagglutination inhibition units
b) No. positive sera / No. tested (%)

DISCUSSION

For the epidemiological survey of hepatitis B virus, HBs Ag and anti-HBs have been investigated about the global distribution.[32)35)50)] Prevalences of HBs Ag and anti-HBs in Asia and Africa are ten times or more higher than those in North America and Western Europe.

In Japan, mean HBs Ag- and anti-HBs- positive rates in blood donors by IES are about 2% and 0.5% respectively.[21)22)] As compared with those values, the results obtained here showed apparently higher values. However, the fact that about 20% of anti-HBs-positive rate by means of PHA is not so high as others may suggest that anti-HBs-positive persons in Nagasaki Prefecture generally possess high-titered antibody.[41)51)] HBs Ag was found more commonly in male than in female and anti-HBs was found more commonly in female, and then the both differences were statistically significant. A clear explanation of these results is not available at present. Occurences of HBs Ag and anti-HBs in age distribution were not statistically significant. It became apparent that high positive frequencies of both HBs Ag and anti-HBs in Nagasaki Prefecture located at the

most western site of Japan conformed to the distribution pattern in Japan except Hokkaido that frequency is high in the West declining toward the North. (Nagasaki Red Cross Blood Center : personal communication)

Dividing the Nagasaki Prefecture into five areas, prevalences of HBs Ag and anti-HBs in Gotoh islands were found to be more frequent than those in other areas, particu$_5$llarly the Eastern area. As this result was consistent with the fact that in Gotoh islands the death rates of liver cirrhosis (24.0 per hundred thousand) and of hepatoma (31.5 per hundred thousand) were the highest in Nagasaki Prefecture, a pathogenesis of these diseases in Gotoh islands might be in response to HBV infection (Annual Report from the Health Dept. of Nagasaki Prefecture Government, 1973). In Fig. 3, the reason why the hepatitis occurence rate in Gotoh islands was low is unknown. However, there might be some biological and/or environmental factors which lead cytocidal HBV infection, that is hepatitis, to noncytocidal latent infection. The long-term latent infection, including vertical transmission, will result in liver fibrosis, liver cirrhosis and hepatoma rather than hepatitis. Therefore, one must be required in future to investigate unknown factors which affect the low hepatitis incidence and high cirrhosis and hepatoma incidences in Gotoh islands.

With regard hospitalized patiants, it is required to examine by using more sensitive method (i. e., PHA) whether surprisingly high positive frequency in anti-HBs (5.9%) may indicate the infection of HBV in hospital or not.

In age distribution, 16 to 21 year-old group had more frequent HBs Ag and anti-HBs positive rates than others, though not significantly.

Since Blumberg et al. had first reported the subspecificities of HBs Ag, many authors have found some determinants. At present, most commonly used combinations of determinants are those of common determinat *a* and two sets of apparantly allelic determinants, *d* : *y* and *w* : *r*. i. e., of *adw*, *adr*, *ayw* and *ayr*.[3)24)26)] A number of other subspecificities including ours have been reported.[17)25)33)48)49)] The author analysed four main subtypes described above. Determinants *d* and *r* prevailed in Nagasaki Prefecture (*d*, 98.9% ; *y*, 1.1%, *r*, 93.1% ; *w*, 6.9%) and this coincides with the results in the southwestern Japan reported by Yamashita et al.[54)]

The *e* Ag, the characteristics and clinical meanings of which are recently reported, is antigenically and biophysically distinct from HBs Ag and seems to be associated with the infectivity of HBs Ag-positive sera and/or liver abnormalities.[18)28)31)38)] Although the *e* Ag-positive rate (17.8%) was almost equal to that elsewhere in Japan, the anti-*e* positive rate (47.5%) seemed to be slightly higher than that in other areas. It remains in future to clarify whether the results described above were due to the problems of assay system or tested sera.[18)37)] The *e* Ag was found more frequently in the sera with high HBs Ag-titer than in those with low one.

HBc Ag was not detected in any sera. This indicated the absence of free HBc Ag or core of HBV in serum. The anti-HBc-positive rates obtained here seemed to be higher than those of other reports, especially in HBs Ag-and anti-HBs-negative group.

This might indicate higher sensitivity of r-PHAI method and/or extensive HBV spread in Nagasaki Prefecture.[15)30)52)] Tsuji et al. reported that extremely high rate of anti-HBc in population abnormal in GPT in Kumayama Area (94.3%) and Bizen City (90.0%), where viral hepatitis prevailed from 1951 to 1953.[53)] The mean anti-HBc titer in hospitalized patients, particularly in HBs Ag-positive group and in HBs Ag-and anti-HBs-negative group, were significantly higher than in blood donors. Although this offers another problem like hospital-acquired infection, more epidemiological data are required.

Since the quantitative assay of the positive rate of anti-HBc provides precise information of HBV infection, one can estimate the spread of HBV among people by calculating the positive frequencies of HBs Ag, anti-HBs and anti-HBc. It became apparant by calculating the positive frequency of anti-HBc in Table 10 and those of the HBs Ag and anti-HBs in Table 2 that about 39% of healthy population was contaminated by HBV.

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