Epidemiological Report

**HEPATITIS B ANTIGEN AND ANTIBODY PREVALENCE
OF JAPANESE SERA SELECTED FROM THE
1972 YEAR'S COLLECTION
AT NATIONAL SERUM BANK, NATIONAL INSTITUTE
OF HEALTH OF JAPAN**

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Since Blumberg et al. (1967) reported that apparently healthy carriers of hepatitis B (HB) virus were the important source of infection with this virus, many reports on the prevalence of HB antigen in healthy populations have appeared (Blumberg et al., 1967; Bank et al., 1971; Szmuness et al., 1973; Grossman et al., 1975; Mazzur and Jones, 1976; Papaevangelou et al., 1976; Sandler et al., 1977). Several surveys for HB antigen and antibody were conducted in Japan with sera prepared from blood for transfusion collected in the Japanese Red Cross Centers or hospitals. Such serum samples did not represent all age groups or localities and there has been a question as to whether those results reflect the general trend in Japan. This report deals with the prevalence of hepatitis B surface antigen (HBsAg) and antibody (HBsAb) among apparently healthy normal populations in Japan. The serum samples included all age groups and localities.

Serum samples were randomly selected from the collection of the National Serum Bank, National Institute of Health of Japan and consisted of those from 923 urban and 976 rural inhabitants of the ages ranging from zero to 98 years. The serum samples collected in 1972 were stored at -70°C . They were heated at 56°C for 30 min before use.

The serum samples were screened for HBsAg by reversed passive hemagglutination (R-PHA) and positive results were confirmed for specificity by the

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TABLE I

Incidence of hepatitis B antigen (HBsAg) by age and sex in urban areas in Japan*

Age (Year)	Male			Female			Total		
	Number tested	positives		Number tested	positives		Number tested	positives	
		Number	%		Number	%		Number	%
0- 4	28	0	0	30	0	0	58	0	0
5- 9	50	2	4	51	1	2	101	3	3
10-14	50	1	2	50	0	0	100	1	1
15-19	50	0	0	50	1	2	100	1	1
20-24	46	2	4.4	50	2	4	96	4	4.2
25-29	45	1	2.2	50	0	0	95	1	1.1
30-39	50	0	0	52	1	1.9	102	1	1
40-49	47	0	0	50	0	0	97	0	0
50-69	50	1	2	50	0	0	100	1	1
70-98	35	0	0	39	0	0	74	0	0
Total	451	7	1.6	472	5	1.1	923	12	1.3

* Titers of 1 : 4 or higher in reversed passive hemagglutination were taken as positive.

TABLE II

Incidence of hepatitis B antigen (HBsAg) by age and sex in rural areas in Japan

Age (Year)	Male			Female			Total		
	Number tested	positives		Number tested	positives		Number tested	positives	
		Number	%		Number	%		Number	%
0- 4	50	0	0	37	0	0	87	0	0
5- 9	50	1	2	49	1	2	99	2	2
10-14	49	3	6.1	50	3	6	99	6	6.1
15-19	50	5	10	49	3	6.1	99	8	8.1
20-24	40	0	0	50	2	4	90	2	2.2
25-29	37	2	5.4	50	0	0	87	2	2.3
30-39	54	0	0	45	1	2.2	99	1	1
40-49	52	2	3.8	50	2	4	102	4	3.9
50-69	51	1	2	50	1	2	101	2	2
70-98	55	1	1.8	58	1	1.7	113	2	1.8
Total	488	15	3.1	488	14	2.9	976	29	3

hemagglutination inhibition test (HAI) (Sekine, 1975). The serum samples showing fourfold or greater reduction in titer in the antibody-inhibited reaction were interpreted as positive. Subtype specificity was determined by HAI (Imai *et al.*, 1974). Monospecific antibody to the a, d, w or r subtype determinant at 2 or 4 units was used. The serum samples were tested also by discontinuous counterimmunoelectrophoresis for HBsAg (Wallis and Melnick, 1971) in which the WHO reference antiserum (caprine WHO-S) and a serum from a hepatitis

patient (SS-2) were used at dilutions of 1:20 and 1:5, respectively.

The HBsAb in the serum samples were detected by PHA using sheep erythrocytes coated with purified antigens and positive results were confirmed for specificity by the HAI test. The serum samples showing fourfold or greater reduction in titer in the antigen-inhibited reaction were interpreted as positive.

The HBsAg prevalence by age and sex among urban and rural healthy populations in Japan is shown in Tables I and II. In both rural and urban areas, Ag positives were first observed in the age group of 5 to 9. In Japan, the rate of the transmission of HB virus from mothers to their infants was reported to be about 1% (Okada, 1975). The absence of HBsAg positives in 145 individuals of the age group of 0 to 4 years in the present study is compatible with the results of his report.

In urban areas, Ag positives were found in all age groups at 1% to 4.2% except two age groups of 40 to 49 and 70 to 98, which lacked Ag positives. There was no significant difference between any two contiguous age groups ($p > 0.05$). In rural areas, 6.1, 8.1 and 3.9% were HBsAg positives at the age groups of 10 to 14, 15 to 19 and 40 to 49, respectively. The rate of HBsAg positives in these three groups were significantly higher than those in other age groups ($p < 0.05$).

The HBsAg prevalence among populations under 24 years of age was apparently higher than that among older populations in both areas (2.0% vs 0.6% in urban and 3.8% vs 2.2% in rural area), but the difference was significant only when the data from both areas were combined and analyzed (2.9% vs

TABLE III

HBs antigen determined by reversed passive hemagglutination and discontinuous counterimmunoelectrophoresis in sera from urban healthy populations

Sample	Age (Year)	Sex	Reversed-PHA		D-CIE against	
			Titer*	Subtype	SS-2**	WHO-S***
1. 020073 (Aomori)	8	F.	12.6	adw	+	+
2. 010122 (Hokkaido)	9	M.	6.0	adw	—	—
3. 040512 (Miyagi)	9	M.	11.6	adr	+	+
4. 010180 (Hokkaido)	14	M.	12.6	adw	+	+
5. 440208 (Ooita)	17	F.	10.6	adr	+	+
6. 240417 (Mie)	20	M.	12.6	adr	+	+
7. C-1294 (Chiba)	21	M.	13.6	adr	+	+
8. 430302 (Kumamoto)	22	F.	4.0	adr	—	—
9. 150095 (Niigata)	22	F.	6.0	adw	—	—
10. C-1272 (Chiba)	25	M.	9.6	adr	+	+
11. 020163 (Aomori)	32	F.	10.6	adw	+	+
12. 040792 (Miyagi)	64	M.	5.0	adw	—	—

* $\times (\log 2)^{-1}$

** Serum from a hepatitis patient 1:5.

*** WHO reference serum 1:20.

TABLE IV

HBs antigen determined by reversed passive hemagglutination and discontinuous counterimmunoelectrophoresis in sera from rural healthy populations

	Sample	Age (Year)	Sex	Reversed-PHA		D-CIE	against
				Titer	Subtype	SS-2	WHO-S
13.	420071 (Nagasaki)	8	F.	11.6	adr	+	+
14.	010043 (Hokkaido)	9	M.	12.6	adr	+	+
15.	010052 (Hokkaido)	10	M.	11.6	adw	+	+
16.	010047 (Hokkaido)	10	F.	5.0	adw	ND*	ND
17.	150685 (Niigata)	11	F.	9.6	adr	+	+
18.	350150 (Yamaguchi)	12	F.	13.6	adr	+	+
19.	420033 (Nagasaki)	13	M.	11.6	adr	+	+
20.	380354 (Ehime)	14	M.	12.6	adw	+	+
21.	340040 (Hiroshima)	15	M.	12.6	adr	+	+
22.	340066 (Hiroshima)	15	M.	12.6	adr	+	+
23.	340082 (Hiroshima)	15	F.	11.6	adr	+	+
24.	150478 (Niigata)	17	M.	10.6	adw	+	+
25.	250028 (Shiga)	17	F.	11.6	adw	+	+
26.	420073 (Nagasaki)	18	F.	7.0	adw	—	—
27.	100094 (Gumma)	19	M.	11.6	adr	+	+
28.	380374 (Ehime)	19	M.	11.6	adr	+	+
29.	040143 (Miyagi)	22	F.	2.0	unidentified	—	—
30.	390100 (Koochi)	23	F.	9.6	adr	+	—
31.	S—349 (Saitama)	29	M.	4.0	unidentified	—	—
32.	350185 (Yamaguchi)	29	M.	9.6	adw	+	+
33.	150492 (Niigata)	39	F.	8.6	adr	ND	+
34.	380396 (Ehime)	40	F.	3.0	adr	—	—
35.	150572 (Niigata)	45	M.	6.0	adw	—	—
36.	150543 (Niigata)	47	F.	7.0	adr	+	+
37.	100216 (Gumma)	48	M.	7.0	adw	+	—
38.	150555 (Niigata)	53	M.	7.0	ND	—	—
39.	150549 (Niigata)	56	F.	4.0	adw	—	—
40.	420138 (Nagasaki)	91	M.	4.0	unidentified	—	—
41.	100806 (Gumma)	95	F.	7.0	adw	ND	ND

* ND: not done.

1.4%, $p < 0.05$). Our results provide only cross-sectional data with HBsAg prevalence, including asymptomatic carriers and abortive infections which temporarily possess HB virus.

The carrier rate appears to be higher in tropical than in temperate settings, and higher in urban than in rural communities. The prevalence of both HB antigen and antibody is high among populations living under overcrowded or unhygienic conditions (WHO, 1975). In the present report, the overall HBsAg prevalence rate among urban healthy population was 1.3% and that among rural healthy population was 3.0%. The latter was significantly higher than the former ($p < 0.02$), with no significant difference between males and females in

both areas ($p > 0.5$, $p > 0.5$). The overall prevalence rate in Japan was 2.2% (41/1899), the value being similar to that (2.7%) reported previously (295/10738, Nishioka et al., 1975).

The HBsAg titers of positive sera were determined by R-PHA. The reference sample containing one μg per ml of adr antigen showed 64 (2^6) HA units under our experimental conditions. Discontinuous counterimmunoelectrophoresis (D-CIE) was performed in parallel against anti-HB reference sera. The results are summarized in Tables III and IV. All the samples having less than 64 (2^6) HA units were negative in D-CIE tests. Two of our samples containing 128 (2^7) HA units in R-PHA were negative and one of them was positive only against SS-2. Therefore, the sensitivity of D-CIE was about one-hundredth that of R-PHA in detecting HBsAg. The HBsAg titers in relation to the ages are also summarized in Tables III and IV. The mean titers of HBsAg positives were 2050 (2^{11}), 294 ($2^{8.2}$) and 49 ($2^{5.6}$) HA units in age groups of 5–19, 20–39 and >40 years, respectively. The difference was significant among these three groups ($t < 0.01$, $t < 0.05$), indicating age-dependent decrease in HBsAg titer in the serum of adults older than 20 years.

Subtypes of HBsAg were identified in 37 of 41 samples, 20 as adr (54.1%) and the other 17 as adw (45.9%). Since the discovery of d, y, w and r subtype determinants of HBsAg (LeBouvier, 1971; Bancroft et al., 1972), data on peculiar geographic distribution of each determinant have been accumulated (Mazzur et al., 1973; Feinman et al., 1973; Dodd et al., 1973). Data in Tables III and IV also reveal the geographic distribution of each subtype in Japan. The south-to-north descending gradient in distribution of the r determinant in Japan is evident from the tables and this is in general agreement with the data obtained by other researchers (Ishida et al., 1973; Mazzur et al., 1974; Yamashita et al., 1975) except that the frequency of r determinant was lowest in Hokkaido in our study but it was higher than that in the northern part of Honshu Island in their studies.

The HBsAb distribution by age and sex among urban and rural populations is shown in Tables V and VI. A few HBsAb positives were found in the youngest age group in both areas. In urban areas, HBsAb prevalence increased with age up to 40 years and then decreased, but in rural areas, it appeared to reach a plateau at the age of 20 years. The overall positive rate of HBsAb was nearly the same in both areas (16.5% in urban and 16.3% in rural areas), but it was significantly higher in females than in males ($p < 0.01$), mainly due to the significant difference in urban areas ($p < 0.02$). An increase in the prevalence of HBsAb with age was shown in the present study, as had been established by previous serosurveys.

The HBsAb prevalence rate in Japan is 16.4% (311/1899), the rate being similar to that (18.4%, 1949/10567) obtained by Nishioka et al. (1975).

The HBsAb titers of positive sera were determined by PHA. The results are shown in Fig. 1. Although in the urban area, there was no significant difference in average Ab titers between every two contiguous age groups, in

TABLE V

Incidence of HBs antibody (HBsAb) by age and sex in urban areas in Japan

Age (Year)	Male			Female			Total		
	Number tested	Positives		Number tested	Positives		Number tested	Positives	
		Number	%		Number	%		Number	%
0-4	28	1	3.6	30	1	3.3	58	2	3.4
5-9	50	0	0	51	3	5.9	101	3	3
10-14	50	1	2	50	6	12	100	7	7
15-19	50	7	14	50	5	10	100	12	12
20-24	46	2	4.3	50	7	14	96	9	9.4
25-29	45	5	11.1	50	10	20	95	15	15.8
30-39	50	15	30	52	12	23.1	102	27	26.5
40-49	47	14	29.8	50	17	34	97	31	32
50-69	50	11	22	50	16	32	100	27	27
70-98	35	6	17.1	39	13	33.3	74	19	25.7
Total	451	62	13.7	472	90	19.1	923	152	16.5

TABLE VI

Incidence of HBs antibody (HBsAb) by age and sex in rural areas in Japan

Age (Year)	Male			Female			Total		
	Number tested	Positives		Number tested	Positives		Number tested	Positives	
		Number	%		Number	%		Number	%
0-4	50	1	2	37	0	0	87	1	1.1
5-9	50	1	2	49	4	8.2	99	5	5.1
10-14	49	4	8.2	50	8	16	99	12	12.1
15-19	50	7	14	49	8	16.3	99	15	15.2
20-24	40	9	22.5	50	9	8	90	18	20
25-29	37	7	18.9	50	8	16	87	15	17.2
30-39	54	11	20.4	45	9	20	99	20	20.2
40-49	52	7	13.5	50	13	26	102	20	19.6
50-69	51	13	25.5	50	10	20	101	23	22.8
70-98	55	11	20	58	19	32.8	113	30	26.5
Total	488	71	14.5	488	88	18.0	976	159	16.3

rural the antibody titer was significantly higher at age 5 to 19 ($F > 0.05$, $F < 0.01$) than that in other age groups.

From our data, the overall average rate of minimum estimate of prior infection in Japan must be 18.5% (Fig. 2). In urban areas, the rate of minimum estimate reached the average at ages of 30 to 39 (30%) in males and 20 to 24 (18%) in females. On the other hand, in rural areas it reached the average at ages of 15 to 19 (24%) in males and 10 to 14 (22%) in females.

In the present study the ratio of the HBsAb frequency to the HBsAg

Fig. 1. Titration for HBs antibody of sera of healthy population in Japan.

Fig. 2. Minimum estimate of prior infection with hepatitis B virus among healthy Japanese.

frequency in inhabitants in urban areas is significantly higher than that in rural areas, without sex difference. This indicates that in rural areas there are more HBsAg positives especially among those younger than 20 years, who will often become chronic carriers.

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