

An epidemiologic study of herpes simplex virus type 1 and 2 infection in Japan based on type-specific serological assays

M. HASHIDO¹*, F. K. LEE², A. J. NAHMIAS², H. TSUGAMI³,
S. ISOMURA⁴, Y. NAGATA⁵, S. SONODA⁶ AND T. KAWANA⁷

¹ *Infectious Diseases Surveillance Center, National Institute of Infectious Diseases, Toyama 1-23-1, Shinjuku-ku, Tokyo 162, Japan*

² *Department of Pediatrics, Emory University School of Medicine, Atlanta, GA, U.S.A.*

³ *Osaka Prefectural Bandai Clinic for STD, Osaka, Japan*

⁴ *Department of Medical Zoology, Nagoya University School of Medicine, Nagoya, Japan*

⁵ *Department of Obstetrics and Gynecology, Faculty of Medicine, Kagoshima University, Kagoshima, Japan*

⁶ *Department of Virology, Kagoshima University School of Medicine, Kagoshima, Japan*

⁷ *Department of Obstetrics & Gynecology, Tokyo University Branch Hospital, Tokyo, Japan*

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SUMMARY

A seroepidemiologic study of herpes simplex virus type 1 (HSV-1) and 2 (HSV-2) was performed on Japanese adults. Serum samples collected between 1985–9 from a total of 536 healthy adults, female prostitutes, males with sexually transmitted diseases (STD), homosexual men, and pregnant women were studied by immunodot assays using HSV type-specific antigens, glycoproteins G (gG1 and gG2). HSV-1 infections correlated mostly with age and was widely prevalent among subjects < 40 years. HSV-2 prevalence varied greatly among subgroups defined by sexual activity and was associated with risk behaviours for prostitution, infection with STD, and homosexual activity. HSV-2 seroprevalence was highest among prostitutes (80%), lowest among pregnant women (7%), and intermediate in STD patients (23%) and homosexuals (24%). Because HSV-1 infection during childhood has been decreasing, primary genital HSV-2 infection, with its higher frequency of clinical manifestations, will become a greater burden to the public health in Japan.

INTRODUCTION

Although herpes simplex viruses (HSV) are among the most common infectious agents in humans, the extent of their pervasiveness and the epidemiological characteristics of HSV infections in Japan have remained unknown so far. HSV infections caused by the two antigenic types, HSV-1 and HSV-2, with HSV-1 being most often thought to be transmitted non-sexually and HSV-2 sexually [1, 2], show a wide variety of

clinical manifestations, from recognizable symptomatic diseases to subclinical, asymptomatic infections. Of the subjects with HSV-specific antibodies, only 20–30% report a positive history of clinical symptoms, while more than 70% of HSV infections may be inapparent and therefore require serological determination [2–6].

However, the serological assessment of the type-specific immune status of HSV infections has been difficult [7, 8]. Many serological assays, including microneutralization, kinetic neutralization, multiplicity analysis, inhibition, passive haemagglutination tests, radioimmunoassay, and enzyme-linked immunosorbent assay have been developed to determine the

* Author for correspondence.

† 'Infectious Diseases Surveillance Center, National Institute of Infectious Diseases' is the former 'Department of Epidemiology, National Institute of Health', Tokyo, Japan. (Changed on April 1, 1997).

HSV antibody type (types 1 and/or 2). These are based on absorption using the heterologous antigen or comparison between the relative reactivities against whole HSV-1 or HSV-2 antigens [7, 9–12]. However, none of these serological methods specifically discriminated between the antibodies to the two HSV types, particularly in individuals infected with both viruses, due to the extensive antigenic cross-reactivity [13, 43].

Recently the antibody response to HSV glycoprotein G (gG) has been shown to be entirely type-specific due primarily to the presence of a 526 amino-acid insertion in gG of HSV-2 (gG2) relative to that of HSV-1 (gG1) [13]. The use of type-specific proteins, gG1 and gG2 as antigens in immunological assays now allows differentiation of prior infection with HSV-1, HSV-2, or both virus types [15–20].

This is the first report from Japan on the type-specific seroepidemiology of HSV-1 and HSV-2 based on gG antigens. It is of interest to compare the prevalence of HSV-1 and HSV-2 in Japanese populations with that in different parts of the world reported previously [3, 4].

MATERIALS AND METHODS

Sera: A total of 536 serum samples were collected in four areas of Japan (Fig. 1) between 1985–9. In the Nagoya area, samples were collected from 41 male blood donors (mean age; 38.8 ± 11.9), 19 female blood donors (38.2 ± 9.8), and 34 male homosexuals (41.4 ± 14.7) who participated in an anonymous confidential HIV testing programme [21]. The homosexual participants were customers at two bath-houses in Nagoya City, representative of places visited only by homo/bisexual men. Information on number of sexual partners, duration of homosexual activity, and history of STD, which was obtained through self-administered questions in the programme, was available. In Osaka, samples were collected from 56 healthy adult women (48.7 ± 8.8) examined for routine health checks, 70 female prostitutes (34.7 ± 11.4), and 26 males with sexually transmitted disease (44.7 ± 14.4), attending a prefectural STD clinic. Records on syphilis in the prostitutes were available. Syphilis had been serologically diagnosed by rapid plasma reagin and *Treponema pallidum* haemagglutinating test.

Samples collected from pregnant women attending university prenatal clinics and screened for human T-lymphotropic virus type I (HTLV-I) antibody were



Fig. 1. Areas in Japan where the serum samples used in this study were collected.

also included. Two hundred serum samples (mean age; 29.1 ± 4.1) were taken in the Kagoshima area [22], the southwestern part of Japan, and 90 (29.9 ± 4.6) in the Tokyo metropolitan area. HTLV-I antibody was detected by a gelatin-particle-agglutination assay (Serodia-ATL; Fujirebio Inc., Tokyo, Japan). Positive results were confirmed by an enzyme immunoassay [23] and by an immunofluorescence assay, as reported previously [24].

Immunodot assays for HSV type-specific antibodies: HSV-1- and HSV-2-specific antibodies were detected by immunodot assays that used HSV glycoprotein gG-1 or gG-2 as antigens [15, 16]. Briefly, the purified gG1 and gG2 antigens were adsorbed onto the centre of nitrocellulose-membrane disks at the appropriate dilutions. After the disks were blocked with 5% bovine serum albumin, serum samples at a dilution of 1:50 were applied in duplicate wells. After the plates were incubated overnight at room temperature, antibody reactions in the test serum with the gG on the disks were detected using horseradish peroxidase-conjugated goat antiserum specific for human IgG (Zymed, San Francisco, CA, USA), and a substrate containing H_2O_2 and 1-chloro-4-naphthol, and visualized as purple-blue dots. Positive and negative controls were included in each assay.

Statistical analysis: The Mantel–Haenszel method was used to evaluate associations of risk factors to HSV-1 or HSV-2 infection while controlling for age. The Fisher's exact probability test was done for comparison of antibody prevalence in each population group.

Table 1. Prevalence of HSV-1 and HSV-2 antibodies in Japan in 1989

Study group	Number of cases	Percent of antibodies to HSV type			
		1+2	1 only	2 only	Negative
Nagoya area					
Male homosexuals	34	21	56	3	21
Female blood donors	19	0	74	0	27
Male blood donors	41	0	63	2	34
Osaka area					
Healthy adult women	56	2	86	13	0
Female prostitutes	70	59	17	21	3
Syphilis-positive	39	51	26	21	3
Syphilis-negative	31	68	7	23	3
Male STD patients	26	19	54	4	23
Tokyo area					
Pregnant women	90	1	49	6	44
HTLV-I-positive	32	0	56	3	41
HTLV-I-negative	58	2	45	7	47
Kagoshima area					
Pregnant women	200	15	58	2	27
HTLV-I-positive	100	15	68	3	14
HTLV-I-negative	100	14	47	0	39

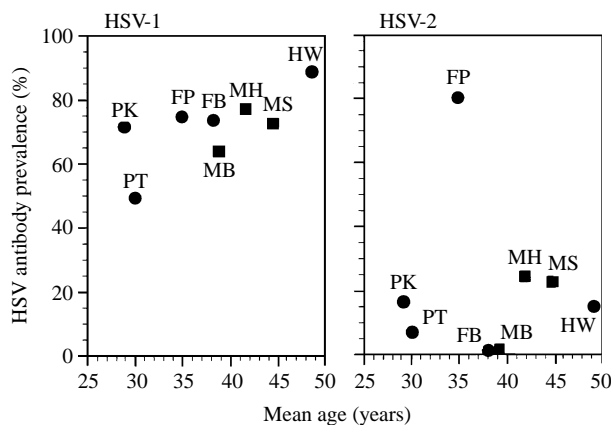


Fig. 2. The association between the prevalence of HSV-1 and HSV-2 antibodies and the mean age in each group. HW; healthy adult women in Osaka. PK; pregnant women in Kagoshima. PT; pregnant women in Tokyo. FP; female prostitutes. FB; female blood donors in Nagoya. MB; male blood donors in Nagoya. MH; male homosexuals. MS; male STD patients.

RESULTS

Prevalence of HSV-1 and HSV-2 antibodies: The prevalence of HSV type-specific antibody is shown in Table 1. For the HSV-1 antibody, generally high prevalence of HSV-1 antibody was observed, being highest in healthy adult women in Osaka (88%), and lowest in pregnant women in Tokyo (50%). The HSV-1 seroprevalence of each population tended to

increase with age (Fig. 2). However, pregnant women in Kagoshima had a higher prevalence (73%) than those in Tokyo (50%) ($P < 0.05$), suggesting the potential presence of a regional difference in HSV-1 epidemiology.

HSV-2 seroprevalence correlated markedly with sexual activity. The highest prevalence was found in female prostitutes. Of interest is that the HSV-2 prevalence (80%) was actually higher than that of HSV-1 (76%). Male homosexuals (24%, $P < 0.01$) and STD patients (23%, $P < 0.05$) also had higher prevalence than male blood donors (2%). Among the general population, the women in Osaka had the highest prevalence (15%). Pregnant women in Kagoshima also tended to have higher HSV-2 prevalence (17%) than those in Tokyo (7%).

Increase of HSV antibody prevalence with age: The relationship between HSV antibody prevalence and age is shown in Table 2 for the general population, male homosexuals, and STD patients. A tendency to increase with age was observed for both types of HSV infection. As expected for a sexually-transmitted infection, the HSV-2 prevalence increased after the age of 30 in the homosexuals and STD patients which are the groups with higher sexual activity, earlier than in the general populations. Among the general population studied, HSV-1 antibody prevalence exceeded 50% at about the age of 30 and reached 90%

Table 2. *HSV seroprevalence in relation to age*

Study group	Age group					
	< 20	20–29	30–39	40–49	50–59	≥ 60
Percent of antibodies to HSV-1						
Male blood donors*	—	33 (4/12)	67 (6/9)	75 (6/8)	92 (11/12)	—
Healthy women†	—	100 (3/3)	59 (10/17)	89 (25/28)	90 (18/20)	100 (7/7)
Male homosexuals	0 (0/1)	38 (3/8)	83 (5/6)	91 (10/11)	100 (3/3)	100 (5/5)
Male STD patients	—	40 (2/5)	80 (4/5)	75 (6/8)	100 (3/3)	80 (4/5)
Percent of antibodies to HSV-2						
Male blood donors	—	0 (0/12)	0 (0/9)	13 (1/8)	0 (0/12)	—
Healthy women	—	0 (0/3)	0 (0/17)	11 (3/28)	20 (4/20)	14 (1/7)
Male homosexuals	0 (0/1)	0 (0/8)	17 (1/6)	36 (4/11)	33 (1/3)	40 (2/5)
Male STD patients	—	0 (0/5)	20 (1/5)	25 (2/8)	33 (1/3)	40 (2/5)

* Male blood donors in Nagoya.

† Female blood donors in Nagoya and healthy adult women in Osaka.

Table 3. *Association of prevalence of HSV antibodies and risk factors, with control for age**

	HSV-1 antibody		HSV-2 antibody	
	Odds ratio (95% CI)	<i>P</i> Value	Odds ratio (95% CI)	<i>P</i> Value
Sex/general population† women vs. men	1.9 (0.73–5.09)	N.s.‡	3.2 (0.38–27.7)	N.s.
Men				
Homosexuals vs. blood donors	1.5 (0.47–4.63)	N.s.	13.0 (1.45–117)	< 0.01
STD patients vs. blood donors	1.2 (0.38–3.72)	N.s.	10.9 (1.18–99.6)	< 0.05
Homosexuals vs. STD patients	1.3 (0.36–4.54)	N.s.	1.1 (0.32–4.04)	N.s.
Male homosexuals				
number of partners, 1 month				
≥ 3 vs. < 3	0.8 (0.04–17.0)	N.s.	∞	< 0.05
years of experience				
≥ 10 vs. < 10	1.5 (0.08–28.9)	N.s.	∞	< 0.05
history of STD				
≥ 1 vs. 0	0.7 (0.04–12.8)	N.s.	1.6 (0.29–8.42)	N.s.
syphilis				
positive vs. negative	3.3 (0.16–64.6)	N.s.	∞	N.s.
Female prostitutes				
syphilis				
positive vs. negative	1.2 (0.39–3.47)	N.s.	0.3 (0.07–1.08)	N.s.
Pregnant women				
area				
Kagoshima vs. Tokyo	2.1 (1.27–3.64)	< 0.05	2.2 (0.91–5.20)	N.s.
HTLV-I antibody				
positive vs. negative	2.5 (1.49–4.24)	< 0.001	1.1 (0.55–2.24)	N.s.

* Adjustment for age was made by the Mantel–Haenszel method.

† Including blood donors in Nagoya and female healthy adults in Osaka.

‡ Not significant.

after the age of 50, whereas HSV-2 infection was not observed in young individuals being highest in women 50–59 years of age (20%).

Association of HSV antibody prevalence and risk factors: After adjustment for age, the association

between several risk factors and HSV infections was explored (Table 3). HSV-1 antibody prevalence was associated with residence in Kagoshima ($P < 0.05$) and a positive serological test for HTLV-I infection ($P < 0.001$) in pregnant women. HSV-2 seroprevalence

was associated with homosexuality ($P < 0.01$) and men with STD ($P < 0.05$). In the male homosexuals, 3 or more partners during the previous 1 month ($P < 0.05$), as well as 10 or more years of homosexual experience ($P < 0.05$) were associated with a higher HSV-2 prevalence. No significant correlation between syphilis and HSV infection was demonstrated. There was also no significant difference noted in HSV seroprevalence between men and women in the general population, although the prevalence of both types tended to be higher in women. The odds ratio for HSV-2 infection between Kagoshima versus Tokyo was > 2 , but the difference was not significant.

DISCUSSION

To our knowledge, this is the first study on HSV-1 and HSV-2 epidemiology in Japan based on type-specific serological tests. The use of type-specific antibody assays for HSV-1 and HSV-2, which detect subclinical as well as clinical infections, enabled us to estimate HSV-1 and HSV-2 seroprevalence among general populations and in several high-risk communities. Our findings are generally consistent with the accepted characterizations of the time course of infections with HSV-1 and HSV-2.

As with previous studies performed in other countries [3, 4], HSV-1 was widely prevalent among individuals age over 40 in Japan. The age at which HSV-1 antibody prevalence exceeded 50% in 1989 is estimated to be 30 among general subjects in the Osaka and Nagoya areas. Toba reported that HSV seroprevalence has been decreasing during these decades [25]. In his report, assays for type-common neutralizing or complement-fixing antibody to HSV revealed that the age at which the HSV antibody prevalence reaches 50% in the general population was 9 years old in 1959–60 and 18 in 1969 in Tokyo, and 15 in 1970–2 and 28 in 1984 in Yokohama, a city neighbouring Tokyo [26–30]. Thus, it appears that HSV-1 prevalence has continuously decreased recently in Japan. As HSV-1 prevalence is known to differ depending upon the socio-economical levels, such as crowding and sanitary conditions [5], these data may reflect the improvement in socio-economical status in modern Japan.

The lower HSV-1 prevalence in the metropolitan Tokyo, than in a rural city Kagoshima, may be explained by better sanitary conditions and fewer family members in Tokyo, the most modernized city in Japan. Especially the change in family composition

in urban cultures, which tend to exclude elderly people, has led the lesser chance of HSV-1 transmission during infancy.

The significant association of HSV-1 with HTLV-I infection might be explained by the higher susceptibility to infection among HTLV-I carriers and is probably due to the immunosuppression resulting from vertical HTLV-I infections [31]. Asymptomatic HTLV-I carriers, as well as patients of HTLV-I associated adult T-cell leukemia, have been known to be immunosuppressed, although mildly and with changes in humoral and cellular immunities [32], depressed delayed-type hypersensitivity [33] and higher rates of opportunistic infections [34]. There may be a subsidiary relationship to a factor accompanying the birth cohort [35], or a possible linkage between the susceptibility to both infections and human leukocyte antigen (HLA) types [36]. In Haiti, HTLV-I infection has been associated with HSV-2 infection, supporting the sexual transmission of HTLV-I in that region [37]. However, we found no significant relationship between HSV-2 and HTLV-I infections in the present study, partly due to the low HSV-2 seroprevalence and the small sample number studied here.

Another interesting finding is that the HSV-1 prevalence in Japanese pregnant women, especially in Tokyo (50%), is among the lowest obtained compared with other countries. According to Nahmias and colleagues [3], HSV-1 antibody prevalence rates among women attending prenatal clinics around the mid 1980s was $> 90\%$ in blacks in Atlanta, whites in Spain and Italy, and in orientals in Taipei. The rate was 70–80% in Reykjavik, Lyon, and Sydney, and about 60% in Sweden. Although sexual transmission seems to have contributed less to the HSV-1 epidemiology, since no distinct differences in HSV-1 antibody prevalence was observed between the general population and more sexually active groups in this study, an increase in number of adults without any antibodies to HSV may increase the incidence of genital HSV-1 infections. This tendency is consistent with the finding by Kawana and colleagues [38] that the rate of HSV-1 as a causal virus of primary infection of female genital herpes is 40% in Tokyo, much higher than 10% or less in the USA [3, 4, 39]. This greater tendency of primary genital infection being due to HSV-1 in Japan is likely to influence the HSV type of neonatal infections, and explain why HSV-1 is commonly associated with newborn infection [40]. Thus, according to a nation-wide clinical

survey on neonatal HSV infections in Japan, the number of cases caused by HSV-1 and HSV-2 was 41 and 21 in 1988.

The HSV-2 prevalence varied greatly among subgroups defined by sexual activity in our study. HSV-2 antibodies were associated with risk behaviour for prostitution, infection with STDs, and homosexual activities with a greater number of partners and longer time of experience. These findings are similar to those obtained in other countries [3, 4, 41]. Comparison of the HSV-2 prevalence in pregnant women in Japan and other countries around the mid 1980s revealed that, the rate of 7% in Tokyo was the lowest as seen in HSV-1 prevalence. However, the strikingly high HSV-2 seroprevalence among the female prostitutes, 80%, found in this study indicates that HSV-2 prevails among Japanese prostitutes to the same extent as in the prostitutes in the USA and other countries [3, 4], and that they are likely to be a major source of HSV-2 infection in Japan.

Also, among the female prostitutes the HSV-2 prevalence (80%) was higher than the HSV-1 prevalence (76%), while 21% of them had only HSV-2 antibodies without those to HSV-1. This seems strange considering that this population has frequent person-to-person contact via mucosa. Nahmias and colleagues explained the same epidemiological phenomenon in a report describing an unusually low HSV-1 seroprevalence and high 'pure' (without concomitant HSV-1 antibodies) HSV-2 seroprevalence in the Congo, where people become sexually active very early in life [3, 4]. It may suggest different degrees of cross-reactivity immunity between HSV-1 and HSV-2 [42], i.e., if infections with HSV-2 occurred in life earlier than those with HSV-1, HSV-2 immunity could offer protection from the subsequent HSV-1 infection, through the reverse is not true. With the indication that HSV-1 seroprevalence has been decreasing during these decades in Japan, the female prostitutes may have been exposed to HSV-2 through sexual experiences before acquiring HSV-1 infection.

Although the present report pertains only to the late 1980s, the trends observed are supported by the recent increase in the number of reported cases of genital herpes in the Infectious Diseases Surveillance Report by the Ministry of Health and Welfare, Japan [43]. This tendency toward increase is unique to genital herpes, as the number of reported cases of gonorrhoea and genital chlamydia has been decreasing since 1992, owing to the mass campaign against AIDS. The tendency toward decrease in HSV-1

infection during infancy and high HSV-2 seroprevalence among the sexually active population may predict an increase in primary HSV infections in Japanese adulthood. Primary infections of genital herpes are likely to demonstrate serious clinical manifestations and are known to relate to more frequent recurrent lesions [5]. They have also been associated with infections of the human immunodeficiency virus as a risk factor (reviewed in [3, 4]). Therefore, genital herpes will become a greater burden to public health and continuous monitoring of the trend remains indispensable.

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