

Antibodies to Human T-cell Lymphotropic Virus Type I (HTLV-I) by Particle Agglutination (PA) Test in Korean Blood Donors

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Abstract

HTLV-I infection is a recently recognized disease entity that is common in some tropical and subtropical areas, including the southwestern district of Japan. Despite the geographical proximity and frequent cultural exchanges between Korea and Japan, it is understood that Korea is not an endemic area and HTLV-I-associated illnesses are very rare in Korea. This study was designed to evaluate the positive rate of anti-HTLV-I antibodies in Korean blood donors and its regional distribution. Sera were obtained from blood donors from various districts around Korea. Anti-HTLV-I antibodies were detected by using the microtiter particle agglutination test employing an indirect agglutination technique. A total of 9,281 donors were tested and 12 donors (0.13%) were positive for anti-HTLV-I antibodies, 10 (0.11%) out of 8,845 males and 2 (0.46%) out of 436 females, with relative female predominance. A relatively high incidence of anti-HTLV-I positive donors was observed in Cheju Island (0.80%), Kyungnam (0.31%), and Chonnam (0.15%). In conclusion, the positive rate of anti-HTLV-I antibodies seemed to be very low in Korea, but the highest positive rate of anti-HTLV-I antibodies was noticed on Cheju Island, warranting further research for confirmation.

Key Words: HTLV-I, antibodies, particle agglutination test, Korea

INTRODUCTION

Human T-cell lymphotropic virus type I (HTLV-I), a type C retrovirus, is known to be etiologically associated with adult T-cell leukemia/lymphoma (ATL)¹⁻⁵ and HTLV-I-associated myelopathy/tropical spastic paresis (HAM/TSP).^{1,5-7} HTLV-I tends to occur within endemic areas, including the southwestern islands of Japan,⁸⁻¹² the Caribbean,^{1,10,13} Central America, and Africa.^{14,15} Recently, in some areas of Taiwan¹⁶ and China¹⁷ neighboring Japan, HTLV-I associated illnesses were identified. However, despite geographical proximity and frequent cultural exchanges between Korea and Japan, it had been accepted that Korea was not an endemic area and that HTLV-I-associated illness was not a health concern. In 1983, one study reported that none of 373 residents in

Korea was positive for the anti-HTLV-I antibody.¹⁸ In 1986, however, a study using ELISA and indirect immuno-fluorescent test reported that the seroprevalence rate of the anti-HTLV-I antibody was 0.25% among 9,603 patients who had visited hospitals in Korea.¹⁹ The first case of HTLV-I-associated ATL and the first case of HAM were reported in 1987²⁰ and in 1990,²¹ respectively. So this study was designed to reevaluate the positive rate of anti-HTLV-I antibodies in Korea.

MATERIALS AND METHODS

Sera were obtained from 9,281 blood donors from various districts of Korea (4,774 in Seoul, 405 in Chungnam, 1,124 in Kyungbuk, 1,377 in Chonnam, 979 in Kyungnam, and 622 donors on Cheju Island). In age group distribution, 903 in 10-19 years, 7,563 in 20-29 years, 564 in 30-39 years, and 251 in over-40 years were tested. Males were 8,845 and females were 436.

We performed anti-HTLV-I antibody test by using the microtiter particle agglutination (PA) method (Fujirebio Inc., Tokyo, Japan). If polyvalent anti-

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HTLV-I antibodies were present in the serum or plasma, they react with antigen coated on the gelatin particles and then cause agglutination. In this assay, serum samples were serially diluted in volumes of 25 μ l per well in a U-bottom microdilution plate. 25 μ l of 1% antigen-sensitized particles and 25 μ l of unsensitized particles were dropped in the wells containing 1 : 4 diluted (final dilution 1 : 8) and 1 : 8 diluted (final dilution 1 : 16) serum samples, respectively. The contents of the wells were mixed with a tray mixer and allowed to stand at room temperature for 2 hours. In the test performance, a sample that showed a negative reaction with unsensitized particles but showed agglutination with sensitized particles was regarded as giving a positive reaction. We analyzed the data by using the Chi-square with Fisher's exact test. A *p*-value of less than 0.05 was considered to be statistically significant.

RESULTS

Overall positive rate of anti-HTLV-I antibodies and positive rate according to sex

Among the 9,281 blood donors, 12 donors (10 males and 2 females) had antibodies against HTLV-I and the overall positive rate was 0.13%. According to sex, the positive rate was 0.11% in males and 0.46% in females, with relative female predominance (Fisher's exact test, *p*<0.05) (Table 1).

Positive rate of anti-HTLV-I antibodies according to age

Among those in the 20–29 age group, 11 of 7,563 donors were positive (0.15%) for anti-HTLV-I anti-

Table 1. Overall Positive Rate of Anti-HTLV-I Antibodies and Positive Rate According to Sex Among Blood Donors in Korea

Sex	No. tested	No. of positive cases	Positive rate (%)
Male	8,845	10	0.11*
Female	436	2	0.46*
Total	9,281	12	0.13

* χ^2 with Fisher's exact test, *p*<0.05

bodies. One of 564 donors in the 30–39 age group was positive (0.18%). None of the 903 donors in the 10–19 age group or of the 251 donors in the over-40 age group was positive.

Positive rate of anti-HTLV-I antibodies according to districts

Two donors in Seoul (0.04%), two in Chonnam (0.15%), three in Kyungnam (0.31%), and five in

Table 2. Positive Rate of Anti-HTLV-I Antibodies According to Districts in Korea

District	No. tested	No. of positive cases	Positive rate (%)
Seoul	4,774	2	0.04
Chungnam	405	0	0.00
Kyungbuk	1,124	0	0.00
Chonnam	1,377	2	0.15*
Kyungnam	979	3	0.31*
Cheju Island	622	5	0.80*
Total	9,281	12	0.13

* χ^2 with Fisher's exact test, *p*<0.05

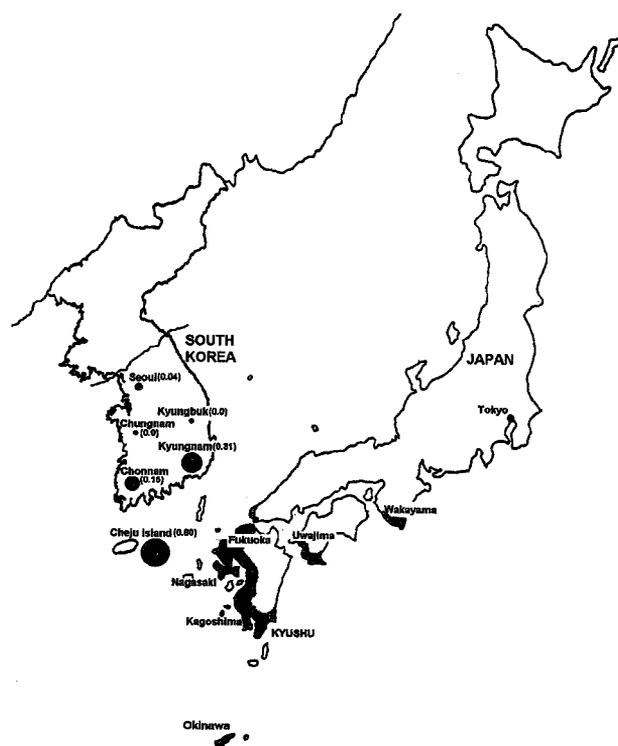


Fig. 1. Geographic distribution of positive rate (%) of anti-HTLV-I antibodies in Korean blood donors. The shaded areas in Japan indicate HTLV-I endemic areas.

Cheju Island (0.80%) were positive, none in Chungnam and Kyungbuk was positive (Table 2 and Fig. 1).

DISCUSSION

As a member of the human retroviruses, HTLV-I has been shown to be associated with adult T-cell leukemia/lymphoma (ATL),¹⁻⁵ HTLV-I-associated myelopathy/tropical spastic paraparesis (HAM/TSP),^{1,5-7} and multiple sclerosis.^{5,7,22} There have been many other non-infectious syndromes associated with latent HTLV-I infection, but none of these syndromes have been definitely linked to HTLV-I. Among the more likely putative correlates are uveitis, polymyositis, lymphocytic interstitial pneumonia, inflammatory arthritis, and mycosis fungoides.¹⁰

HTLV-I is found worldwide, but the prevalence of HTLV-I infection varies widely both over large geographic areas and within areas of endemicity. The two areas with the highest prevalence rates are the Caribbean basin with 4–9% seropositivity and the islands of southwestern Japan with 6–37% seropositivity.⁸⁻¹² The endemic areas in the Caribbean basin include the West Indies,²³ northern South America, and the southeastern United States.^{13,18} In Japan, a high incidence of anti-HTLV-I antibody was observed in southwestern regions.⁸ HTLV-I infection is also described in immigrant populations, including West Indians living in England, natives of southern Japan living in Hawaii, and in blacks from the southern United States living in New York City.¹ In addition to these well-studied populations, serologic surveys indicated the presence of HTLV-I in other areas including Africa,^{14,15} the Middle East, the Arctic, and islands of the Indian Ocean and the south Pacific.^{1,24} In Taiwan, seropositive results for anti-HTLV-I antibodies amounted to 0.9% in 2,545 adult blood donors aged 30 or over.¹⁶ Taiwan had been in close contact with Japan socio-economically and was occupied by Japan for 50 years prior to and during the Second World War. Particularly, the high prevalence (2.9%) of anti-HTLV-I antibody in Keelung in northern Taiwan, which is a harbor close to the HTLV-I endemic southwestern part of Japan, is noteworthy.^{16,19} In China, a survey in Beijing and Tianju detected HTLV-I antibodies in 1984.¹⁷ Another survey in Shanghai and Chongqing identified HTLV-I antibodies in one out of 1,059 sera from blood donors and individuals with an increased risk of HTLV-I infection in 1991,²⁵ while the first HAM patient was

reported in 1994.²⁶

Despite geographical proximity and frequent cultural exchanges between Korea and Japan, Korea has been not an endemic area and HTLV-I-associated illnesses have been very rare. In 1983, the anti-HTLV-I antibodies in Seoul, Pusan, and Cheju Island were studied by indirect immunofluorescent (IF) tests and none of 373 residents was seropositive.¹⁸ In 1986, however, another study using ELISA and indirect immunofluorescent test reported 0.25% of prevalence rate among 9,603 patients who visited hospitals.¹⁹ This was the first study that confirmed the presence of HTLV-I infection in Korea. After that, the first case of HTLV-I-associated ATL and the first case of HAM was reported in 1987²⁰ and in 1990,²¹ respectively. So it was necessary to reevaluate the positive rate of anti-HTLV-I antibodies in Korean blood donors and its regional distribution.

We studied the sera of 9,281 blood donors from various districts of Korea and the results showed a 0.13% positive rate of anti-HTLV-I antibody. This result was lower than the previous results reported in 1986.¹⁹ Since the study subjects were blood donors, the majority of the subjects were in the 20–30 age group and predominantly male. This youthful distribution and male predominance may have tended to decrease the overall positive rate because the rate is known to be higher in females and tends to increase with age.^{1,19,27} This was much lower than the average prevalence rate (0.9%) of Japan, except for the endemic southwestern areas.

HTLV-I and HTLV-II infection can be diagnosed serologically by immunofluorescence (IF), gelatin particle agglutination (PA), enzyme-linked immunoassay (EIA or ELISA), radioimmuno-precipitation assay (RIPA), and Western blotting (WB). Among them, IF, PA, and EIAs are suitable for screening, but RIPA or WB is required to confirm the diagnosis. Data on the sensitivity and specificity of some of the screening assays were summarized with other published independent studies. In that comparison, the PA test using the same kit (Fujirebio Inc., Tokyo, Japan) was most sensitive (100%) and highly specific (98%).²⁸ Especially, the PA test can detect polyvalent antibody. Therefore, the PA test seems to be more suitable for the purpose of screening blood donors, it is necessary to carry out confirmation by using different procedures such as Western blot, RIPA or PCR that can differentiate HTLV-I and II.²⁹⁻³²

It is known that the positive rate of anti-HTLV-I antibodies is higher among older people and in

females.^{1,8,27} This study also showed that the positive rate was 0.11% in males and 0.46% in females, with relative female predominance. As well, none in 10–19 age group, 0.15% in 20–29, and 0.18% in 30–39 were positive for anti-HTLV-I antibodies, with the tendency to increase gradually with age. In the over-40 age group, however, the number of samples was too small to observe the increasing age tendency. Geographically, the positive rate of Chonnam, Kyungnam and Cheju Island in this study was 0.15%, 0.31% and 0.80% respectively, and these were relatively high compared to other areas ($p < 0.05$). These revealed an increasing tendency of incidence as the districts got closer to the endemic southwestern areas of Japan (Fig. 1). Particularly, the positive rate of Cheju Island (0.8%), closest to Japan, was similar to the average positive rate (0.9%) of Japan with the exception of the endemic southwestern areas. However, the positive rate (0.04%) of Seoul, which is relatively distant from Japan, was relatively higher than Chungnam and Kyungbuk. Seoul is the economic and cultural center and brings together many people from other parts of Korea. This is likely the reason why the positive rate of Seoul was relatively high.

In conclusion, the positive rate of anti-HTLV-I antibodies seemed to be very low in Korea, but the positive rate of anti-HTLV-I antibodies tended to be higher in the areas adjacent to the endemic area of southwestern Japan and this may be due to more frequent cultural and personal exchanges. Confirmatory assays such as Western blot, RIPA, or PCR should be performed in the areas with a high positive rate of anti-HTLV-I antibodies.

REFERENCES

- Centers for Disease Control and Prevention and the U.S.P.H.S Working Group. Guidelines for counselling persons infected with human T-lymphotropic virus type I (HTLV-I) and type II (HTLV-II). *Ann Intern Med* 1993; 118:448-54.
- Clark JW, Robert-Guroff M, Ikehara O, Henzan E, Blattner WA. Human T-cell leukemia-lymphoma virus type 1 and adult T-cell leukemia-lymphoma virus in Okinawa. *Cancer Res* 1985;45:2849-52.
- Hinuma Y, Nagata K, Hanaoka M, Nakai M, Matsumoto T, Kinoshita K, et al. Adult T-cell leukemia associated antigen in an ATL cell line and detection of antibodies to the antigen in human sera. *Proc Natl Acad Sci USA* 1981;78:6476-80.
- Poiesz BJ, Ruscetti FW, Gazdar AF, Bunn PA, Minna JD, Gallo RC. Detection and isolation of type C retrovirus particles from fresh and cultured lymphocytes of a patient with cutaneous T-cell lymphoma. *Proc Natl Acad Sci USA* 1980;77:7415-9.
- Spalton DJ, Nicholson F. HTLV-I infection in human disease. *Br J Ophthalmol* 1991;75:174-5.
- Gessain A, Barin F, Vernant JC, Gout O, Maurs L, Calender A, et al. Antibodies to human T-cell lymphotropic virus type-I in patients with tropical spastic paralysis. *Lancet* 1985;11:407-9.
- Sarma PS, Gruber J. Human T-cell lymphotropic viruses in human diseases. *J Natl Cancer Inst* 1990;82:1100-6.
- Hinuma Y, Komoda H, Chosa T, Kondo T, Kohakura M, Takenaka T, et al. Antibodies to adult T-cell leukemia-virus-associated antigen (ATLA) in sera from patients with ATL and controls in Japan: A nationwide seroepidemiologic study. *Int J Cancer* 1982;29:631-5.
- Kazunari Y. Human T-lymphotropic virus type I in Japan. *Lancet* 1994;343:213-6.
- Marsh BJ. Infectious complications of human T cell leukemia/lymphoma virus type I infection. *Clin Infect Dis* 1996;23:138-45.
- Shimoyama M, Minato K, Tobinai K, Horikoshi N, Ibuka T, Deura K, et al. Anti-ATLA (Antibody to the adult T-cell leukemia cell-associated antigen)-positive hematologic malignancies in the Kanto districts. *Jpn J Clin Oncol* 1982;12:109-16.
- Tajima K, Tominaga S, Kuroishi T, Shimizu H, Suchi T. Geographical features and epidemiological approach to endemic T-cell leukemia/lymphoma in Japan. *Jpn J Clin Oncol* 1979;9 Suppl:495-504.
- Blattner WA, Kalyanaraman VS, Robert-Guroff M, Lister TA, Galton DAG, Sarin PS, et al. The human type C retrovirus, HTLV, in Blacks from the Caribbean region, and relationship to adult T-cell leukemia /lymphoma. *Int J Cancer* 1982;30:257-64.
- Fleming AF, Yamamoto N, Bhusnurmath SR, Maharajan R. Antibodies to ATL (HTLV) in Nigerian blood donors and patients with chronic lymphatic leukemia or lymphoma. *Lancet* 1983;2:334-5.
- Saxinger W, Blattner WA, Levine PH, Clark J, Biggar R, Hoh M, et al. Human T-cell leukemia virus (HTLV-I) antibodies in Africa. *Science* 1984;225:1473-6.
- Pan IH, Chung CS, Komoda H, Imai J, Hinuma Y. Seroepidemiology of adult T-cell leukemia virus in Taiwan. *Jpn J Cancer Res* 1985;76:9-11.
- Zeng Y, Lan XY, Fang J, Wang PZ, Wang YR, Sui YF, et al. HTLV antibody in China. *Lancet* 1984;i:799-800.
- Hinuma Y, Chosa T, Komoda H, Mori I, Suzuki M, Tajima K, et al. Sporadic retrovirus (ATLV)-seropositive individuals outside Japan. *Lancet* 1983;i:824-5.
- Lee YY, Lee HB, Seo CW, Kye KC, Kim ES, Park SY, et al. Seroepidemiology of Human T-cell leukemia virus type-I in the Republic of Korea. *Korean J Med* 1986; 31:141-7.
- Lee MH, Kim BK, Lee HB, Park KS, Suh CW, Lee JH, et al. Adult T-cell leukemia-The first case in Republic of Korea. *J Korean Med Assoc* 1987;30:1146-52.
- Park JH, Park SC, Suk SH, Kim WJ, Jung JW, Lee BI. A case of human T lymphotropic virus type I associated

- myelopathy (HAM). *Korean J Neurol* 1990;8:378-83.
22. Koprowski H, DeFreitas EC, Harper ME, Sandberg-Wollheim M, Sheremata WA, Robert-Guroff M, et al. Multiple sclerosis and human T-cell lymphotropic retrovirus. *Nature* 1985;318:154-60.
 23. Greaves MF, Galton DAG, McCluskey DR, Lampert I, Ireland R, Bridges JM, et al. Adult T-cell lymphoma-leukemia in Blacks from the West Indies. *Lancet* 1982; 2:639-43.
 24. Larkin J, Sinnott JT, Weiss J, Holt DA. Human T-cell lymphotropic virus type I. *Infect Control Hosp Epidemiol* 1990;11:314-8.
 25. Pan XZ, Qiu ZD, Chein N, Gold JWM, Hardy D, Zuckerman E Jr., et al. A seroepidemiological survey of HTLV-I infection in Shanghai and Chongqing cities in China. *AIDS* 1991;5:782-3.
 26. Seyfert S, Blum S. HTLV-I-associated myelopathy in China; A disease acquired overseas? *J Neurol* 1994;241: 398-400.
 27. Takezaki T, Tajima K, Komoda H, Imai J. Incidence of human T lymphotropic virus type I seroconversion after 40 among Japanese residents in an area where the virus is endemic. *J Infect Dis* 1995;171:559-65.
 28. The HTLV European Research Network. Seroepidemiology of the human T-cell leukemia/lymphoma viruses in Europe. *J AIDS Hum Retrovirology* 1996;13:68-77.
 29. Chadburn A, Athan E, Wiczorek R, Knowles DM. Detection and characterization of human T-cell lymphotropic virus type I (HTLV-I) associated T-cell neoplasm in an HTLV-I nonendemic region by polymerase chain reaction. *Blood* 1991;77:2419-30.
 30. Inaba S, Sato H, Okochi K, Fukada K, Takakura F, Tokunaga K, et al. Prevention of transmission of human T-lymphotropic virus type I (HTLV-I) through transfusion, by donor screening with antibody to the virus; One-year experience. *Transfusion* 1989;29:7-11.
 31. Kline RL, Brothers T, Halsey N, Boulos R, Lairmore MD, Quinn TC. Evaluation of enzyme immunoassays for antibody to human T-lymphotropic viruses type I/II. *Lancet* 1991;337:30-3.
 32. Kobayashi S, Yoshida T, Hiroshige Y, Matsui T, Yamamoto N. Comparative studies of commercially available particle agglutination assay and enzyme-linked immunosorbent assay for screening of human T-cell leukemia virus type I antibodies in blood donors. *J Clin Microbiol* 1988; 26:308-12.
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