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Original Article

Seroprevalence and associated factors of *Toxoplasma gondii* among HIV-infected patients in Tokyo: A cross sectional study☆

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ABSTRACT

HIV infection, in particular in patients with developing AIDS, carries a risk of causing toxoplasmosis with encephalitis, which is mostly caused by a form (bradyzoite) of the protozoan parasite *Toxoplasma gondii*. HIV/AIDS in Japan has been recognized as a serious health issue in recent years. In this study, to elucidate *T. gondii* seroprevalence in HIV-positive patients in Japan and associated characteristics with *Toxoplasma* parasite infection, the titer of *T. gondii* IgG (Tg-IgG) was measured in 399 HIV-positive patients who visited a hospital in Tokyo, Japan, between 2015 and 2017. A questionnaire survey was also conducted to investigate associations between lifestyle and customs. As a result, the overall prevalence of Tg-IgG-positive serum was 8.27% (33 cases of 399). All the cases positive for Tg-IgG were confirmed using the Sabin-Feldman dye test; the titers between each examination correlated robustly ($p < 0.001$, $r = 0.6$). A correlation between *Toxoplasma* infection rate and age was determined ($p < 0.001$), whereas there was no significant correlation with lifestyle customs such as consuming undercooked meat or owning a cat. An association between *Toxoplasma* infection and experience of dwelling in the Hokkaido area, the northern part of Japan, was observed ($p = 0.001$). These results suggested that the proportion of those who were previously exposed to *Toxoplasma* parasites in the HIV-positive population has been maintained at a similar level as that of the HIV-negative population in Japan, providing clear information about the potential risk of toxoplasmic encephalitis.

1. Introduction

Toxoplasmic encephalitis caused by infection with a protozoan *Toxoplasma gondii* is a well-known complication in HIV-positive patients [1–3]. Toxoplasmic encephalitis includes neurological symptoms, such as hemiparalysis, impaired consciousness, and death in progressive cases. The number of cases of mortality from *Toxoplasma* encephalitis has decreased in recent years due to the establishment of effective treatments, including antiretroviral therapy, and to well-managed infection prophylaxis [4]. However, toxoplasmic encephalitis still remains a risk for patients who have developed AIDS, in particular, in patients with lower numbers of $CD4^+$ T-cells (under 200/μl).

It has been reported recently that HIV/AIDS cases in Japan have reached approximately 29,000 in 2018, and about 30% of these cases have been diagnosed with AIDS [5]. Whereas most public health centers nationwide offer free and anonymous HIV tests in Japan, a considerable number of HIV-positive patients are diagnosed after contracting AIDS. Assessing the seroprevalence of *T. gondii* in the HIV-positive population in Japan would be of importance for estimating the risk of toxoplasmosis in Japanese

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medical settings. A small sized retrospective study reported that 5.4% of HIV-positive patients (n = 56) were also positive for anti-*T. gondii* IgG [6]. However, the associated factors for *Toxoplasma* infection in HIV-positive patients have not been examined in Japan. In this study, we investigated *Toxoplasma* seroprevalence in HIV-positive patients in the Tokyo area and the possible associated factors that may contribute to *Toxoplasma* infection on these patients.

2. Materials and methods

2.1. Study design

This study was a single-center, cross-sectional study carried out between June 2015 and April 2017 at Jikei University Hospital, which is located in the center of Tokyo, Japan. Adult patients aged over 20 years-old with laboratory-confirmed HIV infection were registered in the study (Fig. 1). Each participant received a single blood examination to check for anti-*Toxoplasma gondii* IgG (Tg-IgG), CD4-positive lymphocyte count, and amount of HIV-RNA. The samples positive for Tg-IgG were additionally examined using the Sabin-Feldman dye test (SFDT) for confirmation of active infection of *Toxoplasma* parasites [7]. Patient information from medical records was collected at the time of registration. The following patients were excluded from the study: patients who declined to provide informed consent, those under the age of 18 years, lack of understanding of the study, and people with insufficient Japanese ability.

2.2. Serological diagnosis

Blood samples were drawn from each participant at Jikei University Hospital and stored for two examinations. Each sample was divided into two; one was stored at 4 °C for an enzyme-linked immunosorbent assay (ELISA) and the other at −80 °C for SFDT. The refrigerated samples were transferred to a commercial reference laboratory (SRL Inc., Tokyo, Japan) for the detection of Tg-IgG within 2 days after collection of blood. An assay kit (PLATELIA™ TOXO IgG; Bio-Rad) was used to measure the amount of Tg-IgG. The cut-off value of Tg-IgG was set at <3 IU/ml to increase in sensitivity of the examination. Samples showing positive Tg-IgG titers were then subjected to the SFDT for specific confirmation. The method for the SFDT was described in a previous report [8].

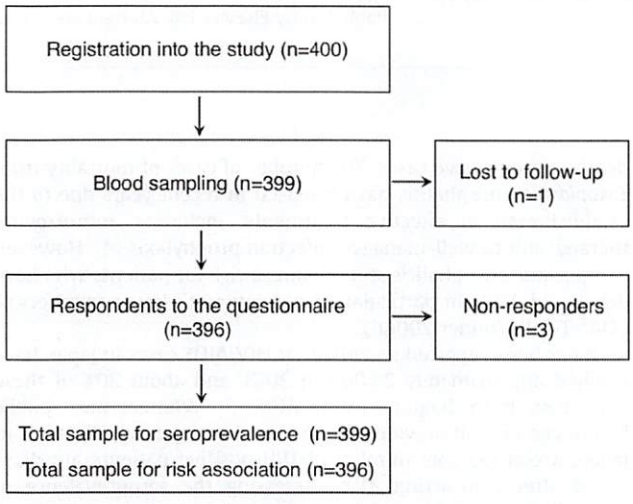

Fig. 1. Flow chart describing the number of HIV-positive patients and samples enrolled in the study at Jikei University Hospital between June 2015 and April 2017. Questionnaire sheets were provided at the time of enrollment and collected by the practitioners. Blood samples were drawn at the first routine blood examination at the clinic after enrollment.

2.3. HIV/AIDS profiles

CD4-positive lymphocyte count and plasma HIV-RNA load were determined by the commercial reference laboratory (SRL Inc., Tokyo, Japan). CD4-positive lymphocytes were counted using FACS Canto2/T4 FITC (BD Biosciences, NJ and Beckman Coulter, CA). HIV-RNA was detected using the cobas8800 system/cobas6800/8800 system HIV-1 (Roche Molecular Systems, NJ, USA), which is based on real-time PCR in accordance with the manufacturer's instructions.

2.4. Questionnaire

Participants were requested to fill out a self-administered questionnaire sheet once at registration for the study. The questionnaire contained the following questions: Q1) history of pet-owning, Q2) dietary habit of consuming raw or undercooked meat, Q3) medical history of food-poisoning, Q4) frequency of washing hands, Q5) history of residence, and Q6) other habits of daily life. Multiple-choice questions were used in Q1, Q2, Q4, and Q6. The multiple-choice questions contained the following options: Q1 (none, dogs, cats, birds, and others), Q2 (beef, pork, chicken, mutton, game meat [venison and wild boar meat], horse meat, bivalves, goat meat, and others), Q4 (every time, sometimes, and never) and Q6 (contact with animals, farming or gardening, sports in dart field or stadium, handling seafood, alfresco dinning [open-air dinning], chef or cook, and healthcare provider as occupation). Q5 was an open question of residence experience: both in Japan and countries abroad.

2.5. Ethical statement

This study was performed in accordance with the Declaration of Helsinki. The study protocol was approved by the ethics committee at the Jikei University School of Medicine (permission number 27-023 [7907]) and the clinical study committee at Jikei University Hospital (permission number 28-373 [8616]). All participants provided their written, informed consent form. No patients were involved in the design or conception of this study.

2.6. Statistical analysis

R version 3.3.3 was used for the statistical analyses. Continuous variables were compared using the Mann-Whitney *U* test, and categorical variables were compared using the Chi-squared test or Fisher's exact test. A two-sided p value < 0.05 was considered statistically significant. For the correlation analysis of titers of Tg-IgG and SFDT, Spearman's rank correlation coefficient was used for the calculation. Twoby2 in package "Epi" version 2.30 was used to analyze the results of the questionnaire. Binary logistic regression analysis was conducted to classify an individual as seropositive or seronegative based on associated factors.

3. Results

3.1. Characteristics

During the study, total 400 HIV-positive patients were enrolled. Eventually, 399 blood samples (99.8%) and 396 answers for the questionnaire (99.0%) were collected (Fig. 1). Most of the

participants were Japanese males (92.0%, n = 367). Whereas Japanese females, non-Japanese males, and non-Japanese females were 4.0% (n = 16), 3.5% (n = 14), and 0.5% (n = 2), respectively (Table 1).

3.2. Seroprevalence of T. gondii

Among the 399 patients, 33 cases (8.3%) were found to be *Toxoplasma* seropositive based on Tg-IgG (29 Japanese males, 2 Japanese females, and 2 non-Japanese males). The median age of the Tg-IgG-positive patients (44 years old, range 20–75, SD ± 9.52) was significantly higher than that of the Tg-IgG-negative patients (41 years old, range 28–77, SD ± 12.9) ($p < 0.001$) (Table 1). No correlation was observed between *Toxoplasma* seropositivity and either CD4-positive lymphocyte count ($p = 0.88$) or HIV-RNA copies ($p = 0.96$). In total, 85.2% of the participants maintained HIV infection under control (plasma HIV-RNA <20 copies/ml). No toxoplasmosis cases were diagnosed during the study period. Each Tg-IgG-positive sample was then examined using the SFDT, an alternative serological test to detect *Toxoplasma* antibodies, and all 33 cases were also determined as seropositive (Fig. 2). Antibody titers of Tg-IgG robustly correlated with the results of the SFDT ($p < 0.001$, Spearman's rho = 0.6). No significant correlations were observed between age and Tg-IgG titer ($p = 0.09$, Spearman's rho = 0.299), or between age and SFDT titer ($p = 0.249$, $r = 0.299$).

3.3. Associated factors of T. gondii infection

The results of the questionnaire indicated no significant differences between the two serological groups in terms of factors such as custom of eating raw meat, consuming raw bivalves (oysters, clams, and mussels), history of owing a pet, experience of food poisoning, or frequency of washing hands (Table 2). On the other hand, HIV-positive participants with experience of residence in Hokkaido, the northern-most prefecture of Japan, was significantly associated with seropositivity of Tg-IgG ($p < 0.001$) (Table 2). Binary logistic regression analysis showed that either age ($p < 0.05$) or a history of dwelling in Hokkaido ($p < 0.001$) was the independent factor associated with seropositivity.

4. Discussion

In this study, total 400 HIV-positive patients were enrolled and titers of anti-*T. gondii* antibodies in the sera of these patients were first measured using ELISA, which was then further confirmed using the SFDT. The combination of two serodiagnostic tests identified overall seroprevalence as 8.3% (33 in 399 patients), suggesting

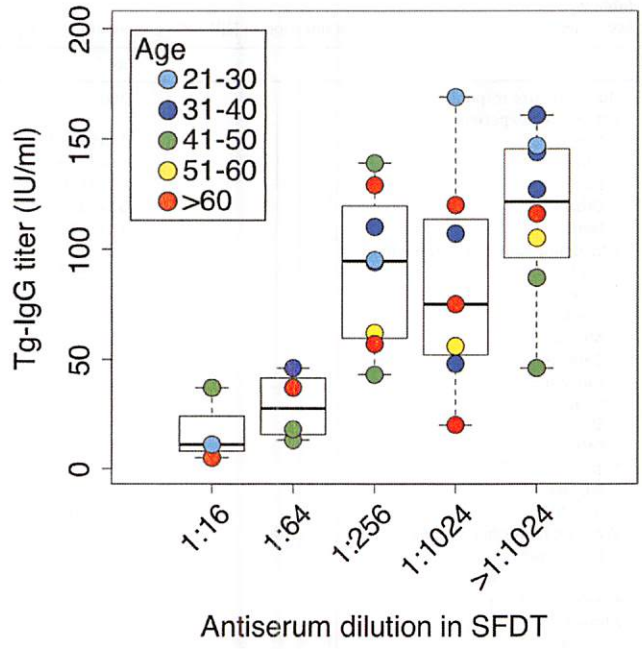

Fig. 2. Box and whisker plot showing the distribution of Tg-IgG titers across different SFDT titers. The band inside the boxes indicates the median. The boxes indicate the interquartile range and the whiskers indicate the values between (1st quartile − step) and (3rd quartile + step) where a step = 1.5 × interquartile range. Positive correlations were observed between two validations ($p < 0.001$, $r = 0.6$) using Spearman's rank correlation coefficient. Three samples with extremely high Tg-IgG titers were excluded as outliers with their values two steps above the 3rd quartile and requiring 10-fold serum dilution for measurements.

that *Toxoplasma* seroprevalence among HIV-positive patients may be maintained at quite low levels in Japan, whereas it has previously been reported that variation in seroprevalence in these patients fluctuated between 26% and 60% in other countries [9]. The results obtained from this study were equivalent to or less than those of general populations such as Japanese pregnant women, among which 10.3% are seropositive for *Toxoplasma* parasites [10]. It seems to be generally consistent with the fact that there were no notable changes in the prevalence of *Toxoplasma* infection in the HIV-positive population in Japan during the last decade [6].

One of the associated factors of *Toxoplasma* infection in HIV-positive patients that was indicated from this study was aging. The association between age and *Toxoplasma* infection was

Table 1
Characteristics and seroprevalence of *Toxoplasma gondii* among HIV/AIDS patients in Tokyo, Japan.

	All	Tg-IgG positive	Tg-IgG negative	*p*
Patients enrolled	399	33 (8.3)	366 (91.7)	
Age, years				<0.001
Median [Range]	42 [20–77]	44 [20–75]	41 [28–77]	
Sex				0.65
Male	381 (95.5)	31 (93.9)	350 (95.6)	
Female	18 (4.5)	2 (6.1)	16 (4.4)	
Race				0.63
Non-Japanese	16 (4.0)	2 (6.1)	14 (3.8)	
Japanese	383 (96.0)	31 (93.9)	352 (96.2)	
CD4 count/μl				0.88
Median [Range]	577 [26–1871]	536 [221–1055]	560 [26–1871]	
HIV-RNA copies/ml				0.96
<20	340 (85.2)	29 (87.9)	311 (85.0)	
<1000	26 (6.5)	1 (3.0)	25 (6.8)	
≥1000	33 (8.3)	3 (9.1)	30 (8.2)	

Values are *n (%)* unless otherwise indicated.

Table 2
Associated factors of *Toxoplasma gondii* infection in HIV/AIDS patients in Tokyo, Japan.

	All	Tg-IgG positive	Tg-IgG negative	*p*
Questionnaire respondents	396	33 (8.3)	363 (91.7)	
Pet-owning experience				
Dog(s)	174 (43.9)	15 (45.5)	159 (43.8)	1
Cat(s)	114 (36.4)	14 (42.2)	100 (27.5)	0.11
Bird(s)	83 (21.0)	6 (18.2)	77 (21.2)	0.81
Others	51 (12.9)	4 (12.1)	47 (12.9)	1
None	133 (33.6)	7 (21.2)	126 (34.7)	0.16
Consuming undercooked meat				
Beef	222 (56.1)	18 (54.5)	204 (56.2)	0.85
Pork	154 (38.9)	16 (48.5)	138 (38.0)	0.33
Chicken	149 (37.6)	9 (27.2)	140 (38.6)	0.32
Mutton	43 (10.9)	5 (15.1)	38 (10.5)	0.53
Game meat	17 (4.3)	1 (3.0)	16 (4.4)	1
Horse meat	98 (24.7)	10 (30.3)	88 (24.2)	0.5
Goat meat	7 (1.8)	1 (3.0)	6 (1.7)	0.39
Shellfish	238 (60.1)	20 (60.6)	218 (60.0)	1
Others	3 (0.8)	0 (0)	3 (0.8)	–
Experience of food poisoning				0.85
Positive	132 (33.3)	10 (7.6)	122 (92.4)	
Negative	262 (66.2)	23 (8.8)	239 (91.2)	
Washing hands before meals				0.20
Every time	171 (43.2)	18 (10.5)	153 (89.5)	
Sometimes + never	224 (56.6)	15 (6.7)	209 (93.3)	
Residence history				
Abroad		8 (24.2)	103 (28.4)	0.69
Africa		1 (3.0)	3 (0.8)	0.29
North America		4 (12.1)	45 (12.4)	1
South America		2 (6.0)	5 (1.4)	0.11
Asia		3 (9.0)	45 (12.4)	0.78
Europe		0 (0)	32 (8.8)	–
Oceania		1 (3.0)	9 (2.5)	0.59
Japan		33 (100.0)	363 (100.0)	–
Hokkaido (Northernmost prefecture)		7 (21.2)	15 (4.1)	0.001
Tohoku (Northeast region)		3 (9.0)	36 (9.9)	1
Kanto (Greater Tokyo area)		31 (93.9)	340 (93.7)	1
Chubu (Central region)		5 (15.1)	59 (16.3)	1
Kansai (Southern-central region)		7 (21.2)	73 (20.1)	0.37
Chugoku (Western region)		4 (12.1)	20 (5.5)	0.13
Shikoku (Island located south of mainland)		2 (6.0)	13 (3.6)	0.36
Kyushu-Okinawa (Southwesternmost region)		4 (12.1)	50 (13.8)	1

Values are *n* (%) or *n* unless otherwise indicated.

reported in several studies previously [10–12], suggesting that multiple opportunities for recurring infection of the *Toxoplasma* parasite throughout life may maintain seropositivity for years also in the HIV-positive population, given the long half-life of IgG antibodies. An association of *Toxoplasma* seroprevalence with dwelling in Hokkaido, the northern-most prefecture in Japan, was a novel finding demonstrated by this study. A possible explanation for this is the local eating habits often seen in Hokkaido, barbecued lamb and mutton, might be a cause of *Toxoplasma* infection. A report has described that 28.78% of sheep bred in Hokkaido prefecture showed a positive antibody titer for *T. gondii* [13]. Additionally, surveillance of cats, a definitive host for *Toxoplasma* parasites, which are excreted as unsporulated oocysts in cat feces, demonstrated high seroprevalence up to 16.1% in a sub-prefecture in Hokkaido [14], suggesting that considerable numbers of *Toxoplasma* parasites might be circulating among certain livestock such as sheep in the Hokkaido area. Further study would be needed to clarify the association between specific eating habits in the past and *Toxoplasma* seropositivity in HIV-positive patients.

In this study, consuming undercooked meat did show an association with *Toxoplasma* infection in HIV-positive patients. It has often been noted that the opportunity for *Toxoplasma* infection from domestic animals in Japan has decreased through the improvement of food safety in the Japanese farming industry. The number of toxoplasmosis cases in Japanese domestic pigs has decreased to approximately one-seventh in the last two decades [15]. Seroprevalence in other domestic animals such as cattle, chickens, and horses, which are also capable of harboring *Toxoplasma* bradyzoites are maintained at quite low level due to well-managed breeding programs [16].

In addition, no significant difference was observed between *Toxoplasma* seropositivity and history of keeping domestic cats, which has frequently been considered as one of the most probable associated factors by presumably increasing the opportunities to ingest *Toxoplasma* parasites. Despite the large population of domestic cats in Japan (approximately 9.5 million), *Toxoplasma* seroprevalence in cat shelters set in urban areas has decreased from 2.5% to 1.7% in a decade [17]. According to a previous report [18], approximately 75% of domestic cats in Japan did not go outdoors throughout their life-span. Contact with cats no longer seems to be an important risk factor for acquiring *Toxoplasma* infection in city areas.

A limitation of this study is that all participants were recruited at a single center in Tokyo. Although most of the enrolled patients lived in the Tokyo metropolitan area where approximately one-third of the HIV/AIDS cases in Japan have been recorded, this study presented only the preliminary findings from a selected area, not from a nationwide survey. It is known that *Toxoplasma* seroprevalence in pregnant Japanese women varies depending on the region [11]. Local differences in *Toxoplasma* seroprevalence might be affected by indigenous eating habits and the number of infected cats carrying oocysts. Full assessment of the population of *Toxoplasma*-infected HIV-positive patients and possible risk factors

associated with infection requires further surveillance that covers a wider area of Japan.

Conflicts of interest

The authors declare that they have no conflict of interest.

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