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Human T-Lymphotropic Virus Type 1 and 2 Seroprevalence Among First-Time Blood Donors in Chile, 2011–2013

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Infection with human T-lymphotropic virus type 1/2 (HTLV-1/2) is a major health problem. HTLV-1/2 infection is endemic in Chile but representative donor prevalence data are lacking. Data on all blood donors in a large network of Chilean blood centers were examined during 2011–2013. Screening of HTLV-1/2 antibodies were measured by enzyme immunoassay (EIA) at all blood banks. Blood samples with anticoagulants from initially reactive blood donors were analyzed by serological confirmation tests (immunofluorescence or recombinant immunoblot) at the HTLV National Reference Laboratory of the Public Health Institute of Chile. Additionally, detection of HTLV-1 and HTLV-2 provirus in peripheral blood mononuclear cells (PBMCs) was performed in all blood donors as confirmatory test. Prevalence rates were calculated. Among 694,016 donors, 706 were seropositive for HTLV-1 (prevalence, 1.02 cases per 1,000; 95% confidence interval [CI], 0.94–1.09), and 97 were seropositive for HTLV-2 (prevalence, 0.14 cases per 1,000; 95% CI, 0.11–0.17). Prevalence of HTLV-1 differed considerably by region, from 0.51 to 1.69 per 1,000. Prevalence of HTLV-2 was similar across the country (0.12–0.16). HTLV-1 prevalence was associated with female sex, older age, and residence in the north of Chile. HTVL-2 prevalence was associated with older age. The HTLV-1 prevalence among Chilean blood donors was relatively high and could be reduced by improving donor recruitment and selection in high prevalence areas. Blood center data may contribute to surveillance for HTLV-1 and HTLV-2 infections. ***J. Med. Virol. 88:1067–1075, 2016***.

KEY WORDS: HTLV-1; HTLV-2; prevalence; Chilean blood donors

INTRODUCTION

Human T-cell lymphotropic virus type one is the etiologic agent of a serious chronic neuroinflammatory disease called HTLV-Associated Myelopathy/Tropical Spastic Paraparesis (HAM/TSP) and the aggressive cancer in T cells called Adult T-cell leukemia/lymphoma (ATL) [Yoshida et al., 1984; Gessain et al., 1985]. Furthermore, the viral infection is associated with peripheral neuropathies, infectious dermatitis, uveitis, myositis, arthritis, Sjogren's syndrome and autoimmune disorders. It is estimated 5–10 million people HTLV-1- infected worldwide [Gessain and Cassar, 2012]. This infection is endemic in Japan, Central Africa, the Caribbean, and South America [Murphy and Biswas, 2009]. Most infected individuals remain as asymptomatic carriers, although 0.3–3.8% and 3–5% develop HAM/TSP or ATL, respectively [Lairmore et al., 2011; Saito and Bangham, 2012; Satake et al., 2012]. In addition, it is estimated that between 5 and 10% of people at risk of developing a disease associated with HTLV-1 [de Thé and Bomford, 1997; Gessain and Cassar, 2012]. Blood donors have seroprevalence of HTLV-1 up to 2% in Brazil, Colombia, Venezuela,

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Argentina, and Peru [Galvão-Castro et al., 1997; Sanchez-Palacios et al., 2003; Gastaldello et al., 2004]. Serological screening at blood banks has been mandatory in Chile since 2009. All repeatedly positive sample of blood donors have to be sent to confirmatory tests at the National Reference Laboratory of the Public Health Institute.

HTLV-1 is an ancestral virus in South America, present for thousands of years between the Andean amerindian populations [Li et al., 1999]. HTLV-1 seroprevalence among Chilean indigenous people from isolated regions is higher than general Chilean population. The Atacameños, Andean people from Chile with ethnic relationship with HTLV-1 carrier mummy, have 6.5% HTLV-1 seroprevalence [Cartier et al., 1993]. The Mapuches and the Huilliches from Southern of Chile have 1.0 and 1.8% HTLV-1 seroprevalence, respectively [Cartier and Cartier, 1996; Li et al., 1999]. Surprisingly, Chile has one of the highest prevalence of HAM/TSP cases in the world [Cartier and Cartier, 1996]. Despite the clinical-epidemiological importance of HTLV-1/2 infection in Chile, there are very few studies on Chilean blood banks from Metropolitan Area and none in national blood bank network. Our preliminary results suggest that the prevalence of HTLV-1/2 would be very different among blood donors from different geographical areas in Chile. A very few or older epidemiological studies reported prevalence between 0.24 and 0.7% in some blood centers from central region [Vasquez et al., 1991; Vasquez, 2003; Chandía et al., 2010]. These results suggest HTLV-1/2 is more prevalent than those reported for other blood-transmitted viruses, such as HIV, Hepatitis B and Hepatitis C. Nevertheless, it has not been reported any study on the national HTLV-1 and HTLV-2 seroprevalence in Chilean blood banks. Besides, this is the first report of HTLV-2 seroprevalence in Chile. The accurate knowledge of seroprevalence rates in different population groups may be helpful in establishing prophylactic measures to reduce the rates of viral transmission from infected individuals. The aim of this study was to evaluate the prevalence of HTLV-1 and HTLV-2 among the first-time Chilean blood donor population.

MATERIALS AND METHODS

Study Design

The study was conducted between January 2011 and December 2013. We assessed HTLV-1 and HTLV-2 infection and gathered demographic data routinely collected from blood donors at a large network of Public Blood Systems blood centers, located throughout Chile. The national network of Public Blood Services in Chile is responsible for the collection, processing and distribution of blood components in 12 administrative regions and the Metropolitan Area, which correspond to a total of 17.7 million inhabitants.

The procedures for blood donor selection (clinical examination and interview, blood collection, laboratory testing) are uniform throughout the network, following official written guidelines issued by the Chilean Ministry of Health. Blood donor candidates are submitted to a routine predonation questionnaire and clinical examination. Individuals considered eligible for blood donation are 18–65 years of age, in good health, and report no risk behavior for retrovirus infection (use of illegal injecting drugs, unsafe sexual practices, etc.).

The administrative regions were grouped in three health regions: north, central, and south. The north region included I, II, III, IV, and V administrative regions, which were conducted at the Regional Blood Center of Valparaiso city. The central region included XI and XII regions, and Metropolitan Area were conducted at the Metropolitan Blood Center of Santiago city. The south region included VI, VII, VIII, IX, and X administrative regions, which were conducted at the Regional Blood Center of Concepcion city. Regional data were grouped into geographic regions on blood centers location. National data were gathered at the Ministry of Health.

The ethical issues of this study were regulated by Resolutions N° 2344 (July 23, 2009) and N° 2190 (August 6, 2010) of Ministry of Health of Chile. All the procedures were performed in compliance with relevant laws and the University of Chile Ethics Committee guidelines in accordance with the ethical standards of the Declaration of Helsinki.

Serological Screening Testing

HTLV-1 and HTLV-2 antibody screening was established by enzyme immunoassay (EIA) using serum/plasma samples from all blood donors. During the study period, three different kits were used. The EIA test was selected according to convenience and to the supply available each blood center: HTLV-I/II ELISA 4.0 (MP Biomedicals, Strasbourg, France), Murex HTLV I+II (DiaSorin S.p.A., Dartford, UK), Architect HTLV I/II i2000 SR (Abbott Laboratories, Wiesbaden, Germany). All HTLV-1/2 EIA samples that were reactive in the primary test were retested.

Serological Confirmatory Testing

For serological confirmation of HTLV-1/2 infections, a blood sample with heparin (5.0–8.0 ml) was obtained from all blood donors with repeatedly reactive on serological screening. Blood samples were sent for confirmatory tests at the National Reference Laboratory of the Public Health Institute. Plasma and PBMC fraction from each blood sample was obtained by centrifugation with Ficoll-Hypaque gradient. Plasma was stored at −20°C until serological confirmation assays were performed. PBMC were stored at −20°C until molecular assays (PCR) were carried out.

All repeatedly reactive samples on serological screening were tested further by HTLV-I/II ELISA

4.0 (MP Diagnostics), indirect immunofluorescence assay with MT-2 [Matsumoto et al., 1990] or a line immunoassay (INNO-LIA™ HTLV I/II Score, Innogenetics N.V., Gent, Belgium). Both tests were performed according to the manufacturer's instructions.

Real Time PCR Confirmatory Assay

All repeatedly reactive samples on serological screening were additionally confirmed by real time polymerase chain reaction (PCR) of proviral DNA in PBMCs. The HTLV-1/2 proviral DNA load was measured using Brilliant II SYBR® Green QPCR Master Mix (Agilent Technologies, cat 600828, La Jolla, CA). DNA was extracted from 2 to 3×10^6 PBMC according to a commercial and automated method (NucliSENS® easyMAG®, bioMérieux SA, Lyon, France). Three extraction controls used identical procedures: without cells, negative control (H9 HTLV-1/2 non-infected cells), and positive control (MT-2 HTLV-1 infected cells). We amplified the following genomic regions: 159 bp of HTLV-1 *tax* with primers SK43-1 (position 7358-7377) and SK44-1 (position 7516-7496), 159 bp of HTLV-2 *tax* with primers SK43-2 (position 7248-7267) and SK44-2 (position 7406-7386), and 268 bp of β-globin gene with primers PC04 and GH20 for internal calibration [Ehrlich et al., 1990]. PCR conditions were as follows: 100 ng DNA was added to 20 μl reaction mixture containing 1x Brilliant II SYBR® Green QPCR Master Mix, 0.5 mM each primer. Thermocycling was performed in a Stratagene Mx3000P (Agilent Technologies) under the following conditions: 4 min at 95°C (hot start), and then 45 cycles of 10 sec at 95°C (denaturation) followed by 5 sec at 55°C (annealing) and 7 sec at 72°C (extension). HTLV typing and purity of amplified products were checked by observation of a single melting peak in HTLV-1 and HTLV-2 PCR. The HTLV-1 proviral DNA load was calculated by the following formula: copy number of HTLV-1 *tax* per 100 cells = (copy number of tax/[copy number of β-globin/2)] × 100.

Statistical Analysis

We computed the prevalence of HTLV-1 and HTLV-2 infection per 1,000 donations by dividing the number of confirmed positive HTLV infection in donors screened in 2011–2013 by the number of donor-years. Repeatedly positive donors were excluded because they overestimate the seroprevalence of HTLV-1/2. The number of donors, age, and gender by year and region were obtained from the DEIS database (Department of Statistical and Health Information) of the Ministry of Health.

Arc GIS 9.3 software was used to construct the HTLV prevalence map.

Data regarding donor gender, age and different blood donor's types were analyzed by the χ^2 test. The statistical analyses were performed using Prism 6.04 software (GraphPad Software, Inc., San Diego, CA). Values of P lower than 0.05 were considered to be statistically significant.

RESULTS

Between January 2011 and December 2013, 694,016 blood donors were obtained from the National Blood Centers of Chile. All samples were screened with serological tests for anti-HTLV-1/2 (Fig. 1). During this period, 1,527 (0.22%) donations were found initially reactive, and 692,482 (99.78%) resulted negative by screening tests (Fig. 1). Blood samples of all screening reactive donations were analyzed by confirmatory tests (serologic and molecular biology assays). Both EIA and IFI or LIA were performed in all samples with repeatedly positive HTLV-1/2 screening tests (n = 1,527). A total of 858 donations were positive by a second EIA and IFI or LIA tests. All of these 858 samples were also positive for HTLV-1/2 provirus in PBMCs by PCR: 803 samples were HTLV-1 or HTLV-2, and 55 were untypable HTLV. Additionally, 669 reactive samples by screening tests were found negative by confirmatory assays (Fig. 1).

The rate of national HTLV-1/2 prevalence was 1.24 per 1,000 Chilean blood donors (95%CI, 1.15–1.32) during 3-year period (Table I). The mean age of blood donors with HTLV-1/2 reactive samples was 42 years throughout the 3 years of the study. It was not detected any difference in mean age of subjects between males and females (Table I). During this period of time, there were confirmed 706 and 97 donors with HTLV-1 and HTLV-2 provirus, respectively. Therefore, the HTLV-1 prevalence was 1.02 per 1,000 Chilean blood donors (95%CI, 0.94–1.09) and the HTLV-2 prevalence was 0.14 per 1,000 Chilean blood donors (95%CI, 0.11–0.17). Significantly different rates of HTLV-1 and HTLV-2 prevalence were found in donors by donor age (Table I). HTLV-1 prevalence increased with age, ranging from a prevalence of 0.55 per 1,000 in the 18–34-year-old group to 2.52 per 1,000 in the 45–64-year-old group ($P < 0.0001$). HTLV-2 prevalence similarly increased with age, ranging from a prevalence of 0.07 per 1,000 in the 18–34-year-old group to 0.35 per 1,000 in the 45–64-year-old group ($P < 0.0001$). During the analyzed period, the majority of HTLV-1-positive individuals were females (53.7%) and HTLV-2 were males (57.7%) (Table I). Therefore, HTLV-1 prevalence was significantly higher among women (1.22 per 1,000) versus men (0.85 per 1,000) donors. Nevertheless, HTLV-2 prevalence was similar among women (0.13 per 1,000) and men (0.15 per 1,000) donors.

Analysis by age revealed increasing HTLV-1 prevalence by age in every years, raising from 0.42–0.66 per 10^3 (age 18–34) to 1.80–3.16 per 10^3 (age 45–64) (χ^2 test for trend $P < 0.0001$) but with a higher slope in 2011 than in 2012 and 2013 (Fig. 2A). Similarly, analysis by age revealed increasing HTLV-2

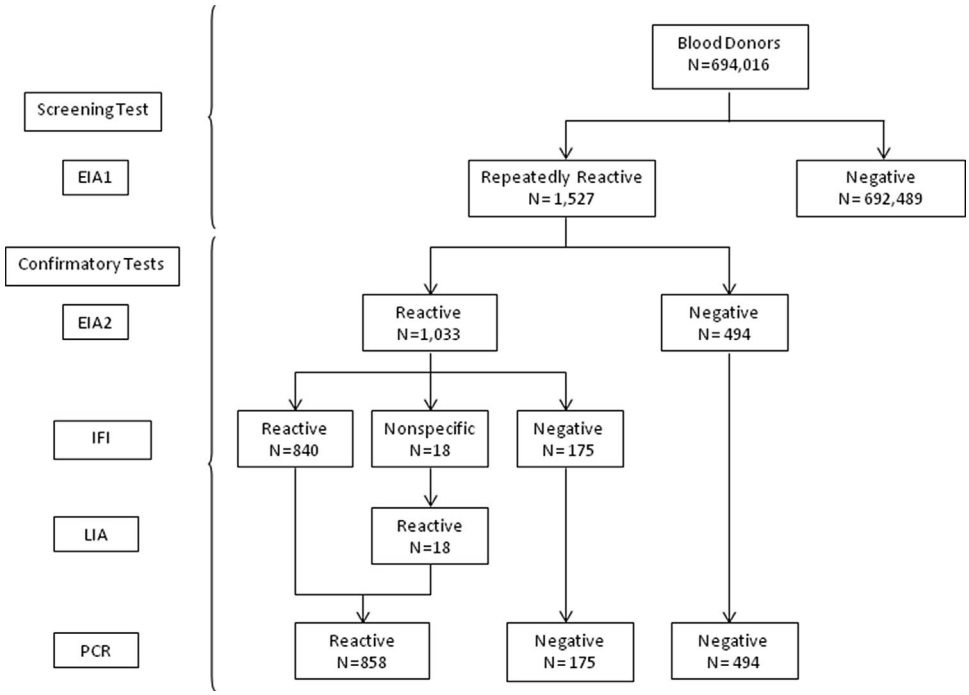

Fig. 1. Confirmatory algorithm of infection with human T-lymphotropic virus Type 1/2 (HTLV-1/2) among Chilean blood donors, 2011–2013. Enzyme immunoassay (EIA), indirect immunofluorescence assay (IFA), line immunoassay (LIA), and Polymerase Chain Reaction (PCR).

prevalence by age in 2011 and 2013, raising from 0.06–0.10 per 10^3 (age 18–34) to 0.39–0.48 per 10^3 (age 45–64) (χ^2 test for trend $P < 0.0001$) but with a higher slope in 2011 than in 2012 and 2013 (Fig. 2A). However, HTLV-2 prevalence by age in 2012 increased from 0.04 per 10^3 in 18–34-year-old donors to 0.26 per 10^3 in35–44-year-old donors, and there was decline among donors aged 45–64 years (Fig. 2A). Analysis by gender revealed higher HTLV-1 prevalence among females (0.92–1.42 per 10^3) than in males (0.57–1.04 per 10^3) in every year. Rates were higher in 2011 than in 2012 and 2013 (Fig. 2B). Nevertheless, trend of HTLV-2 prevalence among females (0.10–0.15 per 10^3) was slightly lower than males (0.13–0.18 per 10^3) during this period of time.

The average of HTLV-1 (15.09–15.27 copy number of HTLV-1 *tax* per 100 cells) and HTLV-2 (9.51–9.80) proviral load was similar among blood donors from three geographic regions (Table II). However, the range of HTLV-1 proviral load (0.03–1809.85 copies of tax gene/100 PBMC) was higher than HTLV-2 (0.03–99.53 copies of tax gene/100 PBMC).

HTLV-1 prevalence rates differed by region (Table II), ranging from 0.51 per 10^3 in Concepción in the VIII Region (southern) to 1.69 per 10^3 in Valparaíso in the V region (central), with little variation during the analyzed period (Fig. 3A). Nevertheless, HTLV-2 prevalence rates was similar in the three geographical regions of Chile (Table II), ranging from 0.12 to 0.16 per 10^3 (Fig. 3B).

DISCUSSION

This study, the largest to date among blood donors in Chile, found HTLV-1 seroprevalence rates in the order of one–two per thousand donors, which are higher than in the United States and Europe but lower than in the Brazilian general population [Schreiber et al., 1996; Taylor, 1996; Murphy et al., 1999; Tseliou et al., 2003; Pinto et al., 2012; Chang et al., 2014]. Our study found HTLV-2 seroprevalence rates was almost an order of magnitude lower than HTLV-1 seroprevalence. Therefore, HTLV-2 seroprevalence in Chile is lower than in the United States. We recognized HTLV-1 associations with age and female gender. Nevertheless, we only found HTLV-2 association with age. Broad scale geographic data such as this will allow better monitoring of trends in HTLV-1and HTLV-2 prevalence by Chilean blood bankers and public health authorities.

Prevalence found for HTLV-1 and HTLV-2 confirm previous studies that Chile is an endemic country for these viruses [Vasquez et al., 1991; Chandía et al., 2010]. The overall HTLV-1 prevalence found in blood donors from the three centers in Chile (1.02/1,000) is comparable to donors in countries in South America [Gotuzzo et al., 2000; Proietti et al., 2005; Carneiro-Proietti et al., 2006] and lower than among blood donors in countries in Central America, Caribbean, Africa, Iran, and Melanesia [Murphy et al., 1991; Gotuzzo et al., 2000; Proietti et al., 2005; Carneiro-Proietti et al., 2006; Gonçalves et al., 2010]. However,

TABLE I. Human T-Lymphotropic Virus Prevalence Among First-Time Chilean Blood Donors, 2011–2013

	Donations (N)	Confirmed Positive for HTLV-1 or HTLV-2[a] (N)	Prevalence/ 1,000 donations	(95%CI)	*P*-value	Confirmed Positive for HTLV-1 (N)	Prevalence/ 1,000 donations	(95% CI)	*P*-value	Confirmed Positive for HTLV-2 (N)	Prevalence/ 1,000 donations	(95%CI)	*P*-value
Overall	694,016	858	1.24	(1.15–1.32)		706	1.02	(0.94–1.09)		97	0.14	(0.11–0.17	
Donation year													
2011	230,308	332	1.44	(1.29–1.60)	<0.0001	278	1.21	(1.07–1.35)	<0.0001	31	0.13	(0.09–0.18)	<0.0001
2012	234,161	229	0.98	(0.85–1.10)		171	0.73	(0.62–0.84)		28	0.12	(0.08–0.16)	
2013	229,547	297	1.29	(1.15–1.44)		257	1.12	(0.98–1.26)		38	0.17	(0.11–0.22)	
Age, y													
18–34	409,887	266	0.65	(0.57–0.73)	<0.0001	226	0.55	(0.48–0.62)	<0.0001	27	0.07	(0.04–0.09)	<0.0001
35–44	156,911	201	1.28	(1.10–1.46)		159	1.01	(0.86–1.17)		25	0.16	(0.10–0.22)	
45–64	127,218	391	3.07	(2.77–3.38)		321	2.52	(2.25–2.80)		45	0.35	(0.25–0.46)	
Gender													
Female	311,462	446	1.43	(1.30–1.56)	<0.0001	379	1.22	(1.09–1.34)	<0.0001	41	0.13	(0.09–0.17)	
Male	382,554	412	1.08	(0.97–1.18)		327	0.85	(0.76–0.95)		56	0.15	(0.11–0.18)	

[a]Data include untypable HTLV. Therefore, the number of infected subjects is greater than sum of the number of subjects infected with HTLV-1 and HTLV-2.

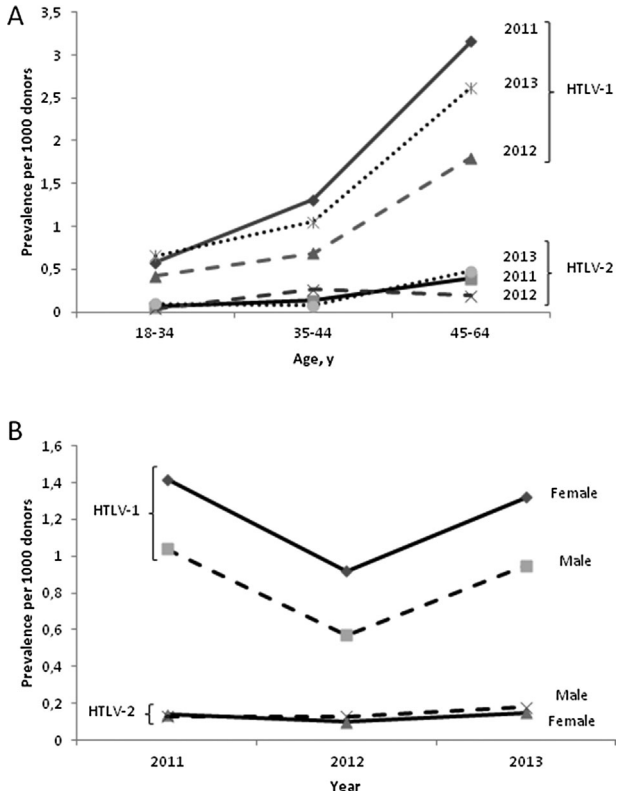

Fig. 2. Age- and gender-specific prevalence of human T-lymphotropic virus Type 1 (HTLV-1) and 2 (HTLV-2) among first-time Chilean blood donors, 2011–2013. **A**: Age-specific prevalence per 1,000 donors during 2011 (◇), 2012 (□), and 2013 (Δ). **B**: Gender-specific prevalence per 1,000 donors for males (□) and females (◇) in 2011–2013.

it is much higher than those found in blood donors in Europe (0.02–0.05/1,000), and the United States and Canada (0.1–0.3/1,000) [Proietti et al., 2005]. In Japan, after introduction of HTLV-1/2 screening in blood banks, the prevalence in blood donors in the endemic areas dropped considerably [Proietti et al., 2005]. It is noteworthy that in many endemic countries in South and Central America, the Caribbean region, and Africa, the screening for HTLV-1/2 is not currently performed in all blood banks, although it is transmitted in a very effective way through cellular components of the blood [Manns et al., 1999]. Furthermore, these regions do not perform leukoreduction (removal of leukocytes from donated blood by filtration) routinely, a procedure that could significantly reduce the risk of HTLV-1/2 transmission through cellular blood components (red blood cell and platelet concentrates).

The associations of HTLV-1 seroprevalence with age and gender seen in this study are similar to those reported by other studies in endemic countries. Higher prevalence and increasing slope with age in women compared to men have been attributed to the increased role of sexual transmission in the infection of women [Manns et al., 1999; Roucoux and Murphy, 2004;

TABLE II. Human T-Lymphotropic Virus (HTLV) Prevalence and Mean of HTLV-1 and HLTV-2 Proviral Load in PBMC Among First-Time Chilean Blood Donors in Three Regional Blood Centers, 2011–2013

Regional blood center	Donations (N)	Confirmed positive for HTLV (N)	Prevalence/ 1,000 donations	(95%CI)	*P*-value	Confirmed positive for HTLV-1 (N)	Mean PVL[a] HTLV-1 (±δ)	Prevalence/ 1,000 donations	(95%CI)	*P*-value	Confirmed positive for HTLV-2 (N)	Mean PVL[a] HTLV-2 (±δ)	Prevalence/1,000 donations	(95%CI)	*P*-value
Valparaiso	139,237	277	1.99	(1.76–2.22)	<0.0001	236	15.27 (±101.70)	1.69	(1.48–1.91)	<0.0001	20	9.51 (±32,68)	0.14	(0.08–0.21)	0.1000
Santiago	309,752	402	1.30	(1.17–1.42)		346	15.09 (±101.06)	1.12	(1.00–1.23)		37	9.68 (±33.47)	0.12	(0.08–0.16)	
Concepcion	245,027	179	0.73	(0.62–0.84)		124	15.21 (±101.51)	0.51	(0.42–0.60)		40	9.80 (±35.76)	0.16	(0.11–0.21)	
Total	694,016	858	1.24	(1.15–1.32)		706	15.09 (±100.98)	1.02	(0.94–1.09)		97	9.44 (±32.06)	0.14	(0.11–0.17)	

[a]PVL, provirus load.

Proietti et al., 2005]. However, increasing prevalence with age has also been attributed to a birth cohort effect in Jamaica, Japan, and the United States, namely that younger generations had lower HTLV infection rates than older generations [Murphy et al., 1991, 1999; Manns et al., 1999; Proietti et al., 2005].

The differences in HTLV-1 prevalence among the three centers were expected, and probably reflect population origins beyond those reflected in the demographic variables. However, our study could not analyze the donors according the skin color, race, or socioeconomic status. Northern region of Chile, where one of the centers (Valparaíso) is located, has the most notable history among our three centers of imported slave labor from tropical South American countries or Africa. Also, this region has a high percentage of descendant individuals from Andean people. On the other hand, Central region and Metropolitan Area have immigration both from the North and South regions of Chile and from Europe. Therefore, those regions showed a medium prevalence of HTLV-1 among blood donors. Finally, South region of Chile has mainly had immigration from others South regions of Chile and Europe showed a low prevalence of HTLV-1 among donors. The finding of different prevalence of HTLV-1 in these blood centers could be associated with immigrants, or their descendants, from endemic areas. Therefore, it would be interesting to evaluate in further studies the HTLV prevalence in blood donors among Chilean indigenous and non-indigenous origen.

We only found HTLV-2 association with age. Additionally, HTLV-2 prevalence was about 10 times lower than HTLV-1 prevalence. These findings show a HTLV-2 epidemiological pattern very different from HTLV-1 in Chile. This is the first report of HTLV-2 prevalence in Chile, and there are not previous data to compare. Although we can not discard endemic circulation of HTLV-2 in Chile, our data would suggest a more recent entry of HTLV-2 compared with HTLV-1. The HTLV-1 was detected in an Andean mummy [Li et al., 1999]. Therefore, HTLV-1 carriers have been present since pre-Hispanic times in Chile. Nothing is known about HTLV-2 circulation in Chilean ancient populations. Additional studies will be required to understand better the HTLV-2 epidemiology in Chile.

The average of both HTLV-1 and HTLV-2 proviral load was similar among blood donors from three blood centers. Nevertheless, we detected that range of proviral load of HTLV-1 was broader than HTLV-2 in PBMC. The significance of this variation in blood donors is still uncertain, although the infectious dose and route of infection may affect the proviral load. The infectious dose could influence the level of proviral load because it may affect the initial number of infected lymphocytes in a carrier. Consequently, these infected cells could undergo the clonal expansion predominantly detected in HTLV-infected individuals [Wattel et al., 1995]. Alternatively, it has

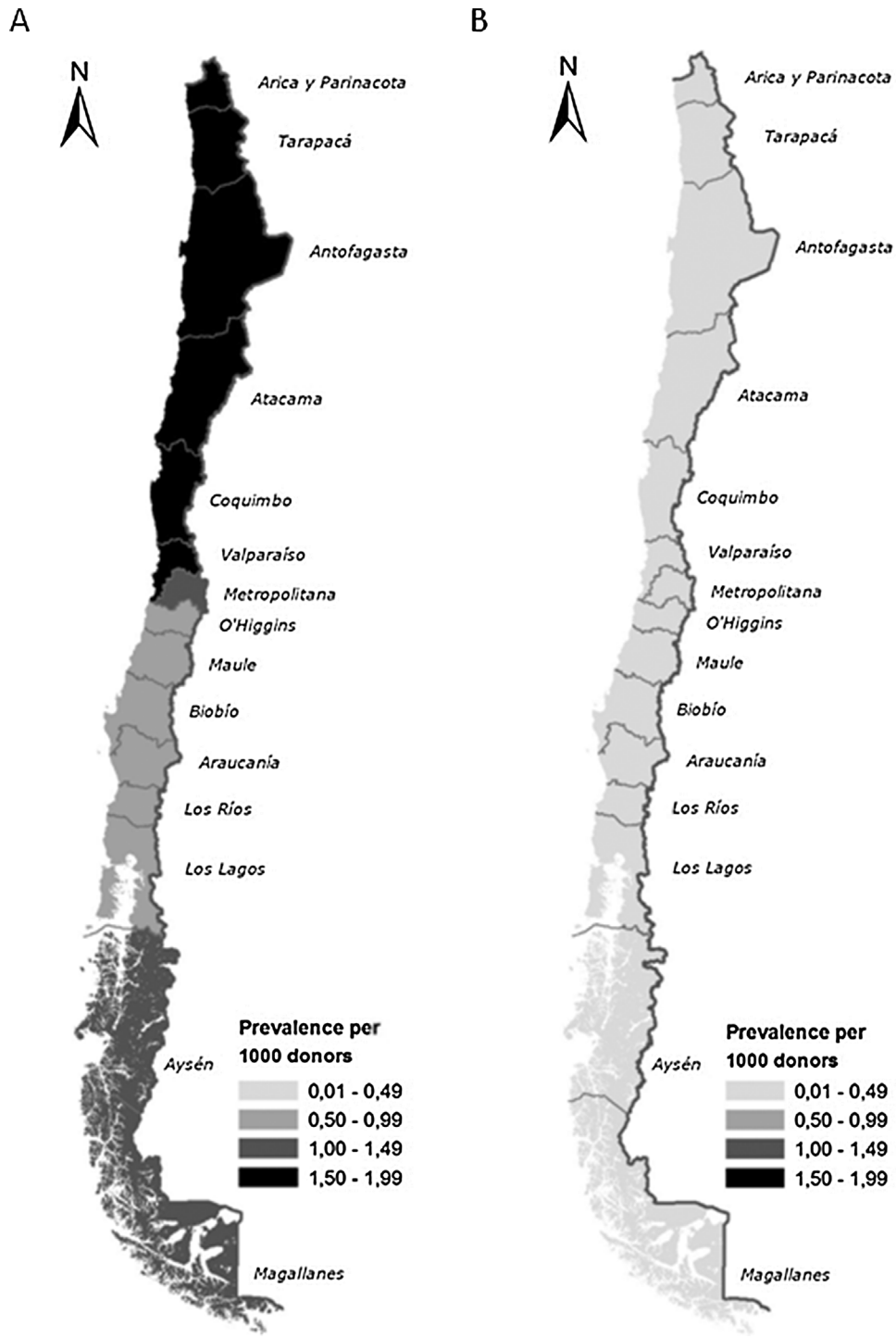

Fig. 3. Human T-lymphotropic virus Type 1 (HTLV-1; A) and 2 (HTLV-2; B) prevalence in first-time blood donors by Chilean region of donor residence, 2011–2013.

been reported that people infected in childhood produce higher proviral load than infection acquired later in life [Gabet et al., 2006]. This situation could occur in children due to a weaker initial immune response. HTLV-2 is a less pathogenic virus than HTLV-1. It has been shown that proviral loads in the PBMC from HTLV-2-infected individuals were lower than in HTLV-1-infected individuals; however, these individuals were chronically infected for an unknown period of time [Murphy et al., 2004]. The increased

proviral load has been associated with disease progression in patients with TSP/ HAM [Nagai et al., 1998]. We previously found a wide temporal variation in the proviral load in TSP/HAM patients, which showed to be independent of the functional damage [Ramirez et al., 2007]. Follow-up studies with HTLV infected blood donors are necessary to analyze the temporal variation of proviral load in PBMC. These studies would be very important to understand infection progression and pathogeny of HTLV.

Data from blood donors can be extrapolated to other groups, but with caution, since blood donors are selected to be at low risk of parenterally transmissible infections. Data from metropolitan areas ofEurope suggest that the HTLV-1 seroprevalence in pregnant women may be up to 100 times higher than in blood donors [Taylor, 1996]. It would be necessary to assess the HTLV prevalence in Chilean pregnant women. This population should have higher prevalence than blood donors. Therefore, this finding could be very important to decide the national HTLV screening in pregnant women. Moreover, a residence duration of more than 10 year and individuals aged over 30 years in communities in the eastern Brazilian Amazon were associated with a virus prevalence higher than Brazilian blood donors [Falcão et al., 2013]. Since blood donors come from a relatively healthier stratum of the population, and in the studied centers tend to be younger and males, we estimate that the HTLV-1 and HTLV-2 prevalences in the Chilean general population is several times higher. This hypothesis would be support by a preliminary study of national surveillance of HTLV-1/2 recently showed a seroprevalence of 0.5% in the general population in Chile [Ministry of Health of Chile, 2010]. Nevertheless, these results must be confirmed by additional studies.

Since 2009 is mandatory in Chile the serological screening and confirmation of HTLV-1/2 in all blood banks and at the Public Health Institute, respectively. The National Reference Laboratory of the Public Health Institute has confirmed almost 290 new infected donors every year. It was previously reported HTLV-1/2 prevalences between 0.24 and 0.7% in Chilean blood donors. However, these studies were conducted in very small populations and they did not have a national representation[Vasquez et al., 1991; Chandía et al., 2010, 2003]. The strengths of the current study include the inclusion of large numbers of blood donors tested for HTLV-1/2 antibodies using consistent laboratory procedures. Data on demographic characteristics were also available from a research database formulated from operational blood bank computer systems. In conclusion, we have presented a national and large-scale HTLV-1 and HTLV-2 seroprevalence analysis based on screening and confirmation of Chilean blood donors. We hope to continue to collect similar data in order to monitor main trends, and to increase the size of the research database by including private blood centers from other Chilean geographic regions.

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