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SHORT REPORT

HTLV-1 cosmopolitan and HTLV-2 subtype b among pregnant women of non-endemic areas of Argentina

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ABSTRACT

Objectives The objective of this study was to estimate the prevalence of human T cell lymphotropic virus (HTLV)-1/2, HIV-1, hepatitis B virus (HBV), *Trypanosoma cruzi*, *Treponema pallidum* and *Toxoplasma gondii* infections and to identify the subtypes/subgroups of HTLV-1/2 among pregnant women (PW) from non-endemic provinces of Argentina.

Methods Methods A total of 2403 samples were screened for HTLV-1/2 and confirmed by western blot and PCR. The long terminal repeat (LTR) of HTLV-1 and HTLV-2 were amplified. Phylogenetic analysis was performed by Neighbour Joining by using molecular evolutionary genetics analysis (MEGA) 4.0.

Results Among a total of 2403 PW studied, 6 (0.25%) tested positive for HTLV-1/2 (3 HTLV-1 (0.12%) and 3 HTLV-2 (0.12%)). The total prevalence when distributed by province was 0.3% (3/804) for Buenos Aires (BA), 0.4% (1/241) for BA surroundings, 0.1% (1/707) for Neuquen and 1.0% (1/95) for Ushuaia. In San Juan, no PW were HTLV-1/2 positive. The prevalence was similar when compared with rates among blood donors of the same areas and years. The phylogenetic analysis classified one sequence as HTLV-1 aA and one as HTLV-2b. The prevalence of HIV-1, HBV, T cruzi, T pallidum and T gondii was 0.6%, 0.2%, 1.4%, 1.2% and 20.9%, respectively. One case of HTLV-1/HIV-1 and one of HTLV-2/HIV-1 co-infection were detected.

Conclusions HTLV-1/2, which have been associated with different diseases, are circulating among PW of Argentina, even in non-endemic areas. Therefore, testing should be recommended in women who have risk factors for these infections given that the majority of HTLV-1/2 mother to child transmission can be prevented by the avoidance of breast feeding.

INTRODUCTION

Several human T cell lymphotropic viruses (HTLVs) have been described and are designated HTLV-1 to HTLV-4.[1] HTLV-1 is associated with adult T cell leukaemia and HTLV-1 associated myelopathy/tropical spastic paraparesis.

HTLV-2 causes no specific disease but has been associated with higher mortality, infections and neurological disorders.[2] HTLV-1/2 infection is endemic in well-defined geographic regions, and studies conducted in pregnant women (PW) reported prevalence in agreement with the different endemic regions.[3] HTLV-1/2 transmission occurs through sexual, parenteral and vertical route, mainly through breast feeding.

HTLV-1 is classified in seven subtypes (a–g), being the Cosmopolitan (a) subtype disseminated worldwide and composed of five subgroups (A–E). For HTLV-2, three subtypes have been described (a, b and d).[1]

In Argentina, similar to other South American countries, HTLV-1/2 infection is characterised by extreme ethnic/geographic restriction with HTLV-1 being naturally endemic among Amerindians in the Northwest and HTLV-2 in those of the North.[4] The rest of the country is considered as non-endemic based on the prevalence (0.03%–0.09%) reported among blood donors (BD).[4] HTLV-1 Transcontinental subgroup A and HTLV-2 (a and b) have been detected among aborigines and urban populations of Buenos Aires (BA) city.[5 6]

The objective of this study was to estimate the prevalence of HTLV-1/2, HIV-1, Hepatitis B virus (HBV), *Trypanosoma cruzi*, *Treponema pallidum* and *Toxoplasma gondii* and to identify the circulating subtypes/subgroups of HTLV-1/2 among PW from non-endemic areas of Argentina.

MATERIALS AND METHODS

Study population

This study took place in Argentina between 2005 and 2009. All PW older than 18 years of age who consecutively attended the antenatal clinic in five public hospitals were asked to participate in the study. The recruitment was carried out at Maternidad Sardá (BA city: Central region), Hospital Fiorito (BA surroundings: Central region), Hospital Rawson (San Juan: West region), Hospital Castro Rendón (Neuquén: Patagonia region) and Hospital Campos (Tierra del Fuego: Southern region). A total of 2403 PW who provided written informed consent were eligible for the study. Of them, 1045 PW were from BA, 556 PW from San Juan, 707 PW from Neuquen and 95 PW from Tierra del Fuego. Socio-demographic data were collected using a standardised questionnaire which was administrated during a confidential face to face interview. All participants received precounselling and postcounselling, and were referred to appropriate medical and social services when necessary. All the results were given within 20 days of blood drawing or, if needed, prior to delivery. All HIV-1 and HTLV-1/2 positive PW were advised not to breast feed. Institutional review board and scientific ethical

committees at the University of Buenos Aires and at each public hospital approved the study protocols.

Diagnosis of HTLV-1/2

HTLV-1/2 antibodies were evaluated by ELISA (Platelia HTLV-I New, BioRad, France). All reactive samples were confirmed by western blot (HTLV Blot 2.4, Genelabs Diagnostics, Singapore) and nested-PCR was performed when necessary as described by Tuke *et al*.[7] DNA was extracted using QIAamp DNA extraction kit (QIAGEN, Hilden, Germany).

Diagnosis of HIV-1, HBV, *T cruzi*, *T pallidum* and *T gondii*

Screening for HIV-1, *T gondii* and HBV surface antigen and/or anticore antibodies (anti-HBc) antibodies was analysed by commercial ELISA. HIV-1 reactive samples were confirmed by western blot assay (Novapath HIV-1 Immunoblot, BioRad, France). For Chagas, HAI and ELISAs were performed. *T pallidum* infection was screened by venereal disease research laboratory test and confirmed by *Treponema Pallidum* haemagglutination and by indirect immunofluorescence test.

Statistical analysis

χ^2 Test and Fisher's exact test were used to compare proportions. All statistical analyses were performed using STATA V.12 (StataCorp. 2011. Stata Statistical Software: Release 12, StataCorp LP, Texas, USA).

Phylogenetic analysis

The 3' long terminal repeat region for HTLV-1 and 2 was amplified by a hemi-nested-PCR as previously described.[8 9] Direct sequencing reactions were done by using an ABI Prism Big Dye Terminator Cycle Sequencing Ready Reaction V.3.0 mixture (Applied Biosystems). Sequences were generated on an ABI Prism 3100 Genetic Analyser and aligned with Clustal W (BioEdit 7.0.4.1 sequence alignment editor). The phylogenetic analysis was performed by Neighbour Joining by using MEGA 4.0. The substitution model was chosen using MODELTEST 3.0. The tree topology was visualised with TreeView (http://taxonomy.zoology.gla.ac.uk/rod/treeview.html). Three of the sequences belonging to PW (PW1, PW2, PWAR4) were previously reported by our group.[5 6] All sequences used are named on the online supplementary web appendix.

RESULTS

The overall prevalence of HTLV-1/2 was 0.25% (6/2403, 95% CI 0.029 to 0.470) being 0.12% (3/2403, 95% CI 0.026 to 0.364) for HTLV-1 and 0.12% (3/2403, 95% CI 0.026 to 0.364) for HTLV-2, similar when compared with BD of the same regions and years. The prevalence by province was 0.3% (3/804, 95% CI 0.077 to 1.087) for BA, 0.4% (1/241, 95% CI 0.011 to 2.290) for BA surroundings, 0.1% (1/707, 95% CI 0.004 to 0.786) for Neuquen and 1.0% (1/95, 95% CI 0.027 to 5.726) for Ushuaia. In San Juan, no PW were HTLV-1/2 positive. Demographic characteristics and risk factors for HTLV-1/2 infected PW are shown in table 1. The prevalence for HIV-1, HBV, *T cruzi*, *T pallidum* and *T gondii* was 0.6% (95% CI 0.258 to 0.908), 0.2%, (95%: CI 0.068 to 0.638) 1.4% (95% CI 0.922 to 1.908), 1.2% (95% CI 0.802 to 1.780) and 20.9% (95% CI 19.244 to 22.537), respectively. One case of HTLV-1/HIV-1 and one HTLV-1/HIV-2 co-infection were detected. The HTLV-1 sequence (PW3) was classified within the Cosmopolitan subtype Transcontinental subgroup (aA) and the HTLV-2 sequences (PWAR5 and PWAR6) within HTLV-2b (data not shown).

DISCUSSION

This study demonstrated that HTLV-1/2 are circulating among PW of different non-endemic areas of Argentina with a similar prevalence to that of BD of the same regions and years.[10 11] The only report on HTLV-1/2 in PW in Argentina was reported in Cordoba in 2007, showing a prevalence 10 times higher than in BD.[12] The difference with our study could be due to the number of PW in each province or due to the different epidemiological characteristics of the population attending the antenatal clinics in that province.

The associated risk factors for HTLV-1/2 infection were being born or having a sexual partner born in endemic areas and having an HTLV-1/2 positive or at risk sexual partner. Concerning phylogeny, an HTLV-1a (PW3) clustered with references belonging to the big Latin American cluster, which is the major reported subtype in different populations of Argentina. On the other hand, two HTLV-2b sequences (PW5 and PW6) clustered with references from Argentine urban populations and Amerindians.[5] These data show that these subtypes/subgroups could have been introduced in the studied areas due to a high internal migration rate from endemic areas, as well as due to an increasing rate of immigration from HTLV-1/2 endemic countries to Argentina.

In summary, HTLV-1/2 is nowadays circulating among PW countrywide. It is known that vertically acquired HTLV-1 leads to adult T cell leukaemia or HTLV-1 associated myelopathy/tropical spastic paraparesis in 1%–5% of children born to HTLV-1 positive mothers. On the other hand, HTLV-2 has been associated with an increased number of other infections and neurological disorders.[2] Moreover, HTLV-1 and 2 positive individuals will not be eligible as blood and organ donors.

Although there are known endemic areas for both viruses in our country, public antenatal care in Argentina does not include HTLV-1/2 detection. Regarding these data, HTLV-1/2 testing should be considered in women who have risk factors for these infections, even in non-endemic areas, given that the majority of mother-to-child transmission can be prevented by the avoidance of breast feeding.

Table 1 Demographic characteristics of HTLV-1/2 infected PW

Sample|ID	Infection	Age band	Region of birth	Region of residency, type of location	Risk factors for HTLV-1/2 infection
PW1	HTLV-1	Mid-20s	Bolivia	Central Argentina, large city	Bolivian partner
PW2	HTLV-1	Early 40s	Central Argentina	Central Argentina, large city	HIV-1+/HCV+, partner IDU HIV+/ HTLV-1+/ HCV+
PW3	HTLV-1	Late 20s	Northern Argentina	Southern Argentina, small city	Bolivian parents, partner HTLV-1+
PWAR4	HTLV-2	Early 40s	Central Argentina	Central Argentina, large city	HIV-1+, partner HIV-1+/ HTLV-2+
PWAR5	HTLV-2	Mid-30s	Central Argentina	Central Argentina, large city	Partner and mother-in-law HTLV-2+
PWAR6	HTLV-2	Early 40s	Chile	Southern Argentina, large city	Chilean parents

HCV+, hepatitis C virus positive; HTLV, human T cell lymphotropic virus; IDU, injecting drug user; PW, pregnant women; PWAR, pregnant woman from Argentina.

Key messages

- Human T cell lymphotropic virus (HTLV)-1 and 2 are circulating among pregnant women of Argentina in the presence or not of co-infection with other pathogens.
- Cosmopolitan aA of HTLV-1 and subtype b of HTLV have been detected consistent with Argentinean molecular epidemiology.
- Antenatal testing in women who have risk factors would prevent HTLV-1/2 vertical transmission.

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Contributors CAB has written the manuscript, followed the start-up at the different settings, gathered the results from all the centres, created the databases and did the data analysis. CD helped with the writing of the manuscript and the data entry. OT was in charge of the study at Hospital 'Ramon Sarda', Buenos Aires, Argentina. GG was in charge of the study and performed the laboratory tests at Hospital 'Pedro Fiorito', Buenos Aires, Argentina. RE was in charge of the study at Hospital 'Guillermo Rawson', San Juan, Argentina. LP was in charge of the study at Hospital 'Horacio Heller', Neuquen, Argentina. MJ was in charge of the study at Hospital 'Eduardo Castro Rendon', Neuquen, Argentina. GA was in charge of the study at Hospital 'Ernesto Campos', Ushuaia, Argentina. MN performed the laboratory tests at Hospital 'Ramon Sarda', Buenos Aires, Argentina. MEE helped with the phylogenetic analysis of HTLV-1 positive samples. MMB was the principal investigator of the whole multicentric study and followed the start-up in each setting.

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Competing interests None.

Patient consent Obtained.

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Notes