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Prevalence of HTLV infection in pregnant women in Spain

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Objective: To estimate the prevalence of HTLV infection among pregnant women in Spain.

Methods: A commercial ELISA incorporating HTLV-I and HTLV-II antigens was used for HTLV antibody screening. Repeatedly reactive samples were further examined by western blot. Moreover, confirmation with PCR was performed when cells were available.

Results: 20 366 pregnant women in 12 different Spanish cities were tested in a 3 year period (July 1996 to August 1999). 32 samples were repeatedly reactive by ELISA, and 10 of them were confirmed as positive by western blot (eight for HTLV-II and two for HTLV-I). In addition, three of 13 women who had an indeterminate western blot pattern yielded positive results for HTLV-II by PCR. All 11 HTLV-II infected women had been born in Spain, and all but one were former drug users. Seven of them were coinfecting with HIV-1. One HTLV-I infected woman was from Peru, where HTLV is endemic and where she most probably was infected during sexual intercourse.

Conclusion: The overall prevalence of HTLV infection among pregnant women in Spain is 0.064% (13/20 366), and HTLV-II instead of HTLV-I is the most commonly found variant. A strong relation was found among HTLV-II infection and specific epidemiological features, such as Spanish nationality and injecting drug use. Although HTLV-II can be vertically transmitted, mainly through breast feeding, both the low prevalence of infection and its lack of pathogenicity would not support the introduction of HTLV antenatal screening in Spain.

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Keywords: HTLV; pregnant women; adult T cell leukaemia/lymphoma; tropical spastic paraparesis; epidemiology

Introduction

Human T lymphotropic virus types I and II (HTLV-I and HTLV-II) are two old human retroviruses with a close genetic sequence. HTLV-I, the first human retrovirus identified, is endemic in Japan, west Africa, the Caribbean, some areas of South America, and Melanesia.^{1,2} In contrast, HTLV-II infection is common among several Amerindian tribes of North, Central, and South America,^{3,4} pygmies in Central Africa,⁵ and among injecting drug users (IDUs) in North America and Europe.^{6–8} In Spain, HTLV-I/II infection is rare. Up to December 1999, 240 individuals with HTLV-II infection and 40 with HTLV-I had been identified.^{8–10} Most HTLV-I infected people were immigrants from endemic areas, or natives who had travelled or had had sexual partners from those areas, whereas HTLV-II infection was mainly found in Spanish IDUs, often coinfecting with HIV-1.^{8–10}

HTLV-I/II infection is transmitted during sexual intercourse (more efficiently from male to female),¹¹ blood transfusion,¹² sharing of contaminated needles,¹³ and from mother to child, mainly through breast feeding,¹⁴ although transplacental transmission can also occur.¹⁵ In endemic areas, HTLV is mainly transmitted heterosexually and from mother to child. Up to 25% of babies born to HTLV-I infected mothers become infected if they are breast fed, and this rate declines to 5% if they are bottle fed.^{16,17}

HTLV-I infected individuals are prone to develop lymphoproliferative and neurological disorders as adults, classically adult T cell leukaemia/lymphoma (ATL),¹ and tropical spastic paraparesis or HTLV-I associated myelopathy (TSP/HAM),¹⁸ although other inflammatory conditions have also been linked to HTLV-I infection. Overall, HTLV-I carriage is associated with a 2–5% lifetime risk of developing disease.¹⁹ HTLV-II has not yet been definitively associated with any disease, although several reports of neurological diseases mimicking TSP/HAM have appeared in the literature.^{20,21}

HTLV-I infection in Europe is thought to be unusual except among immigrants from regions where HTLV is endemic. The screening of blood donors, which is currently mandatory in some European countries, has revealed a very low prevalence of infection across Europe.²² However, recent serosurveillance studies have pointed out that the rate of infection in pregnant women can be 50–100-fold higher than in blood donors.^{23–25} Of note, HTLV-I rather than HTLV-II is the virus implicated in most instances. These data might support the cost effectiveness of the antenatal HTLV screening.

The aim of our study was to estimate the prevalence of HTLV-I/II infection among pregnant women in Spain in the past 3 years, and therefore to provide information for considering whether HTLV antenatal screening should be recommended.

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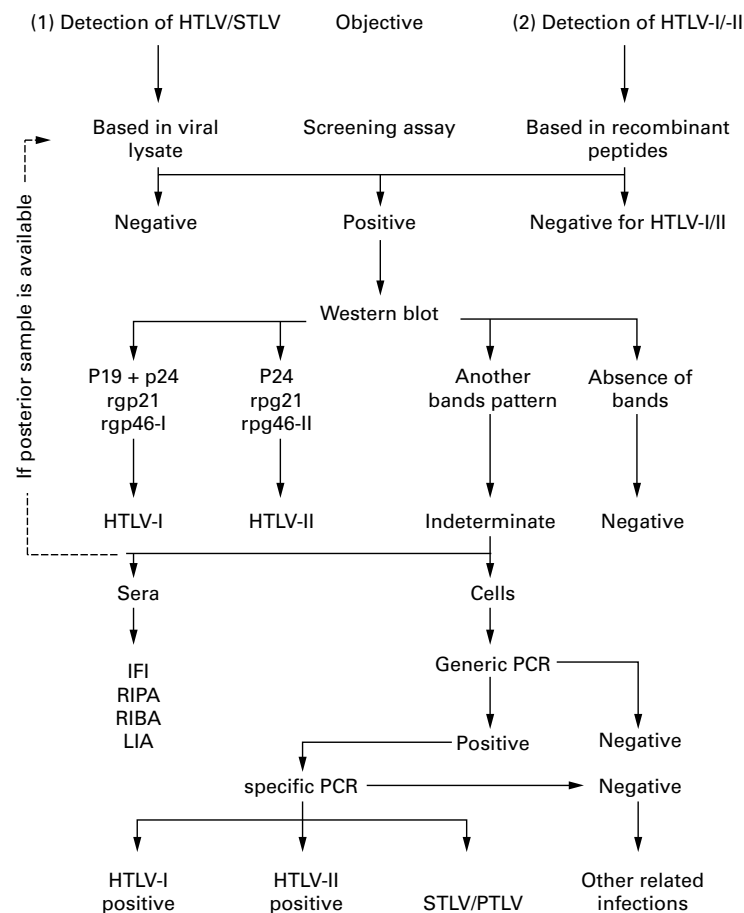


Figure 1 Algorithm for the diagnosis of HTLV infection.

Material and methods

STUDY POPULATION

The prevalence of antibodies to HTLV in pregnant women was examined using sera collected in 12 different hospitals, distributed throughout Spain. Women had been referred for routine rubella and toxoplasma antibody testing. HTLV screening was performed by anonymous testing. In some institutions, the technicians were able to analyse those sera which were reactive to HTLV antibodies. In these cases, and after patient's signed consent, new blood was drawn, and peripheral blood mononuclear cells (PBMCs) were available for further polymerase chain reaction (PCR) analyses.

Between July 1996 and August 1999, a total of 20 366 samples were analysed. Their origin was Valencia (n=7523), Barcelona (n=3725), Santiago (n=3238), Valladolid (n=2686), Madrid (n=1786), Cadiz (n=754), Seville (n=479), and Orense (n=128).

SEROLOGY

Antibodies to HTLV-I/II were screened in pools of five sera, using a commercial enzyme linked immunosorbent assay (ELISA), which incorporates both HTLV-I and HTLV-II antigens (Murex HTLV-I+II; Dartford, Kent). Each reactive pool was further analysed by testing the five corresponding sera separately.

Repeatedly reactive ELISA samples were confirmed by western blot (WB HTLV plus; Genelabs, Singapore), which incorporates on a single nitrocellulose strip HTLV-I and HTLV-II antigens. The HTLV European Research Network (HERN) criteria were used for interpreting western blot patterns.²² Figure 1 summarises the diagnostic algorithm proposed by the HERN for HTLV and other related infections.

The presence of HIV antibodies was examined in specimens showing reactivity against HTLV. A commercial ELISA (AxSYM HIV 1+2, Abbott, Chicago, IL, USA), and western blot (HIV blot, Sanofi-Pasteur, Paris, France) were used for screening and confirmatory purposes, respectively. All assays were performed according to the manufacturer's instructions.

HTLV POLYMERASE CHAIN REACTION

Frozen PBMCs were stored from a few HTLV seroreactive subjects, from whom new blood could be drawn, in order to perform genetic analysis by PCR (Amplicor HTLV-I/II PCR kit, Roche, Switzerland).²⁶

Results

Thirty two (0.16%) of the 20 366 tested sera were repeatedly reactive using the screening ELISA HTLV-I+II. However, only 10 samples were confirmed as positive by western blot (eight HTLV-II and two HTLV-I), the remaining samples were either indeterminate (13 sera) or negative (nine sera).

HTLV-I/II PCR analysis could be performed in specimens belonging to 11 women, six of whom had indeterminate western blot patterns, four a positive western blot for HTLV-II, and one for HTLV-I. Three women with HTLV indeterminate western blot were confirmed as HTLV-II positive by PCR. All of them showed a characteristic pattern on western blot, with reactivity against the HTLV-II recombinant protein rgp46-II along with any other isolated band (see Fig 2, cases 1, 4, and 8). Of note, all three were coinfecting with HIV-1.

PCR results obtained in the four women with positive HTLV-II western blot and another positive for HTLV-I western blot yielded concordant results, respectively.

In summary, of 20 366 pregnant women tested in Spain since 1997, 11 (0.054%) were found to be infected with HTLV-II and two (0.01%) with HTLV-I. All 11 HTLV-II carriers were Spanish, and all but one had been IDUs. Another was the sex partner of a male IDU. Seven of the 11 were coinfecting with HIV-1.

One woman who was positive for HTLV-I was an immigrant from Peru, and was HIV-1 negative. She denied drug addiction practices or blood transfusions, but admitted multiple heterosexual contacts in her country of origin. The other HTLV-I infected woman was HIV-1 negative, and no epidemiological data were available for her. Table 1 summarises the main features of all HTLV infected women identified in this study.

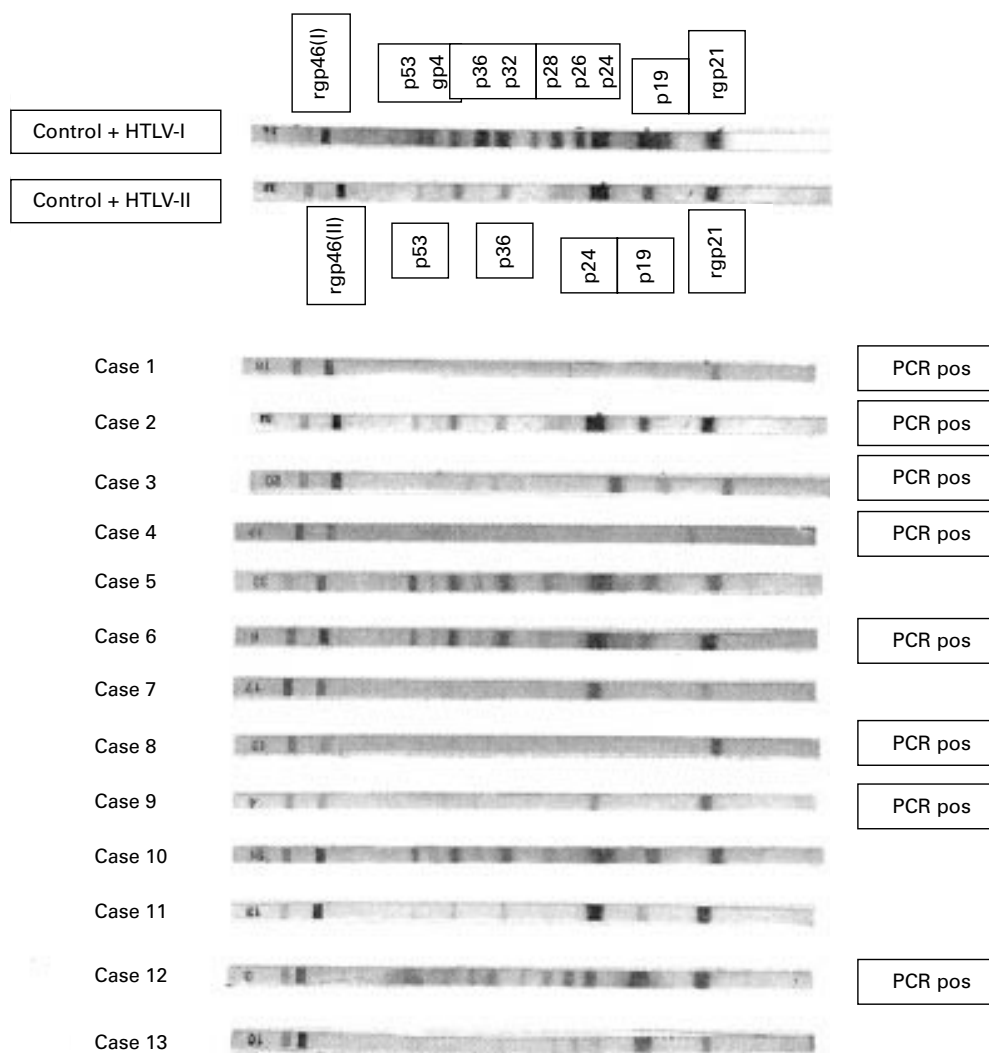


Figure 2 Western blot pattern in 11 infected pregnant Spanish women.

Discussion

HTLV-I infection has been associated with neurological disease or lymphoproliferative disorders in adults. The virus can be transmitted by sexual contact,¹¹ parenteral exposure,^{12, 13} or from mother to child.^{14, 15} Recent studies have suggested that breast feeding accounts for most cases of vertical transmission of HTLV-I infection,^{16, 17} thus providing an opportunity to avoid most infec-

tions in children born of seropositive mothers. However, data from blood donors underline that HTLV infections are rare in Spain, like in other European countries, in the range of 0.001%.^{10, 22} Antenatal screening is therefore not offered to pregnant women. However, recent reports have pointed out that HTLV prevalence in blood donors might be misleading, since rates of HTLV infection 50–100-fold higher have been noticed in studies

Table 1 Main features of HTLV-III infected pregnant women in Spain

No	Place of diagnosis	Year of diagnosis	Country of birth	Risk practice	HIV-1	HTLV WB pattern	PCR
1	Valencia	1996	Spain	IDU	Positive	rgp46-II+rgp21	HTLV-II+
2	Valencia	1996	Spain	IDU	Positive	rgp46-II+rgp21+p24+p19	HTLV-II+
3	Valencia	1996	Spain	IDU	Negative	rgp46-II+rgp21+p24+p19	HTLV-II+
4	Valencia	1997	Spain	IDU	Positive	rgp46-II+rgp21	HTLV-II+
5	Barcelona	1997	Spain	IDU	Positive	rgp46-II+rgp21+p24+p19	ND
6	Cádiz	1998	Spain	IDU	Negative	rgp46-II+rgp21+p24+p19	HTLV-II+
7	Barcelona	1998	Spain	IDU	Positive	Rgp46-II+rgp21+p24	ND
8	Valencia	1998	Spain	IDU	Positive	rgp46-II+rgp21	HTLV-II+
9	Valencia	1998	Spain	IDU	Negative	Rgp46-II+rgp21+p24	HTLV-II+
10	Valencia	1998	Spain	IDU partner	Negative	rgp46-II+rgp21+p24+p19	ND
11	Madrid	1999	Spain	IDU	Positive	rgp46-II+rgp21+p24+p19	ND
12	Barcelona	1998	Peru	Sexual (?)	Negative	rgp46-I+rgp21+p24+p19	HTLV-I+
13	Barcelona	1999	unknown	unknown	Negative	rgp46-I+rgp21+p24+p19	ND

ND = not done.

directly targeting pregnant women in Europe.²³⁻²⁵ In our study the prevalence of HTLV infection in pregnant women from several Spanish cities was 0.064%, more than 50 times higher than that found in Spanish blood donors.^{10, 22} In some studies carried out in England and France most HTLV positive pregnant women were infected with HTLV-I.²³⁻²⁵ This could be explained mainly by the fact that these studies have focused on metropolitan areas (London, Paris, etc), which have large African and/or Afro-Caribbean immigrant communities. In contrast, in Spain HTLV-II instead of HTLV-I was the most frequent variant found in pregnant women. Moreover, only one of the two women found to be HTLV-I infected was an immigrant from an endemic region (Peru).

Most HTLV-II infections described so far in Spain have been reported in IDUs, 90% of them coinfecting with HIV-1.⁸ In our study, a strong relation was also found between HTLV-II infection and previous drug addiction practices. All the pregnant women infected with HTLV-II were Spanish, and seven of the 11 were coinfecting with HIV-1. Avoiding breast feeding would reduce the risk of both HIV-1 and/or HTLV-II infection in newborns.

Several reports have shown that reactivity to HTLV-II can be diminished in HIV-1 coinfecting people suffering a profound immune impairment, leading to a characteristic indeterminate pattern on western blot strips (reactivity to rgp46-I or rgp46-II plus any other isolated band).^{27, 28} We found three HTLV-II infected women who had an indeterminate pattern on western blot. All three were Spanish natives, IDUs, and coinfecting with HIV-1.

In conclusion, our results show that the prevalence of HTLV infection among pregnant women in Spain is low, HTLV-II being (with no proved clear pathogenicity) more common than HTLV-I. Moreover, most HTLV-II positive pregnant women were coinfecting with HIV-1. Since stopping breast feeding is already advised in all HIV positive women, additional measures to protect their newborns from HTLV-II does not seem to be required. The low prevalence of HTLV-I infection and the lack of pathogenicity of the predominant HTLV-II would not support the introduction of HTLV antenatal screening in Spain. Selective antenatal screening should to be considered in former IDUs or immigrants from endemic regions. Further cost-benefit studies are needed to determine whether HTLV antenatal screening should be recommended in Spain.

Appendix

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Contributors: AM, VS, CT, EC, AA, and ROL recruited women, took samples, and performed testing. Other members of the HTLV Spanish study group provided specimens, and participated actively in the final discussion of results, and the conclusions of the manuscript.

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