

Co-infections associated with human immunodeficiency virus type 1 in pregnant women from southern Brazil: high rate of intraepithelial cervical lesions

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Human immunodeficiency virus type 1 (HIV-positive) pregnant women require specific prophylactic and therapeutic approaches. The efficacy of established approaches is further challenged by co-infection with other sexually transmitted diseases (STDs). The objective of this study was to determine the prevalence of co-infections in pregnant women infected with different HIV-1 subtypes and to relate these findings, together with additional demographic and clinical parameters, to maternal and infant outcomes. Blood samples from pregnant women were collected and tested for syphilis, hepatitis B virus (HBV) and hepatitis C virus (HCV). Human papillomavirus (HPV) diagnosis was evaluated by the presence of alterations in the cervical epithelium detected through a cytopathological exam. Medical charts provided patient data for the mothers and children. Statistical analyses were conducted with STATA 9.0. We found a prevalence of 10.8% for HCV, 2.3% for chronic HBV, 3.1% for syphilis and 40.8% for HPV. Of those co-infected with HPV, 52.9% presented high-grade intraepithelial lesions or in situ carcinoma. Prematurity, birth weight, Apgar 1' and 5' and Capurro scores were similar between co-infected and non-co-infected women. The presence of other STDs did not impact maternal and concept outcomes. More than half of the patients presenting cervical cytology abnormalities suggestive of HPV had high-grade squamous intraepithelial lesions or cervical cancer, evidencing an alarming rate of these lesions.

Key words: pregnancy - HIV-1 - co-infections - STDs - cervical carcinoma

According to the latest UNAIDS epidemiological report, 33 million people are infected with the human immunodeficiency virus (HIV) worldwide. Among them, 18 million are women and two million are children between zero-14 years of age (WHO 2010). In Latin America, Brazil is one of the countries most affected by the acquired immunodeficiency syndrome (AIDS), harbouring over 500,000 registered cases at the end of 2009. Early in the epidemic, the man-to-woman infection ratio was 40:1; by June 2008, this difference had been reduced to 1.3:1. This proportion is inverted for cases between ages of 13-19, where 10 girls are infected with HIV for every six boys (MS 2010a).

By 2010, in Rio Grande, a city driven by harbour and university activities and with a population of approximately 200,000 inhabitants, 1,382 cases of AIDS have been reported, making it one of the top 10 most-affected cities in the state of Rio Grande do Sul (RS), the south-

ernmost state of Brazil (MS 2010a). Among the reported cases, 551 were in women and 61 were in children younger than 14 years of age.

Pregnant women infected with HIV-1 receive tailored prophylactic and therapeutic approaches, as established by international and country-based guidelines. However, the efficacy of those approaches can be compromised when the patient is co-infected with other viruses, such as with hepatitis B virus (HBV), hepatitis C virus (HCV), human papillomavirus (HPV) or with bacteria such as *Treponema pallidum*, the etiological agent of syphilis. As HIV-1 and other pathogens share some or all transmission routes, the occurrence of co-infections is frequent. Among all those infected with HIV in the world, it is estimated that approximately 10% are co-infected with HBV and approximately 15% with HCV (Alberti et al. 2005, Soriano et al. 2006). It has been suggested that HIV-1/HCV co-infection of pregnant women increases the rate of mother-child transmission of HCV to their infants (Polis et al. 2007). Syphilis also displays increased prevalence among HIV-1-infected pregnant women. The disease may result in miscarriage/abortion, intrauterine and neonatal death or severe sequelae to the newborn. HIV-1-positive pregnant women co-infected with syphilis have a higher risk of vertically transmitting HIV-1 and the course of syphilis may also be altered during co-infection (Mwapasa et al. 2006, Thorne et al. 2008). In co-infected patients, the recommended therapeutic

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schemes for both syphilis and HIV might not be as effective as in mono-infected patients (MS 2010b). Lastly, HPV is another relevant infection concerning women. HPV is a virus associated with cervical cancer that is sexually transmitted and studies indicate that women infected by HIV-1 have a higher prevalence of HPV infection, as well as a greater risk of developing cervical cancer (Franceschi & Jaffe 2007).

It has been suggested that the genetic diversity of HIV-1 leads to different clinical manifestations of the infection, such as different times of progression to disease (Kiwauka et al. 2008), susceptibility to antiretrovirals (Poveda et al. 2008) and transmissibility (Renjifo et al. 2004). In Rio Grande, HIV-1 subtypes B and C are equally predominant (Soares et al. 2005). Therefore, the objective of this study was to determine the prevalence of co-infections associated with infections by different HIV-1 subtypes among pregnant women followed-up at University Hospital of Rio Grande, RS. We also describe demographic factors, antiretroviral responses and maternal and neonatal outcomes in this HIV-positive pregnant women cohort.

PATIENTS, MATERIALS AND METHODS

This was a cross-sectional study carried out with HIV-1-infected pregnant women followed between July 2006-December 2008 at the HIV/AIDS service of Hospital Universitário Dr Miguel Riet Corrêa Jr, Federal University of Rio Grande (FURG).

The presence of antibodies for HBV and HCV was evaluated with commercial enzyme-linked immune sorbent assay methods. A diagnosis for HBV was considered positive if the patient was HBsAg-reactive (confirmed with anti-HBc reactivity). Patients with a positive anti-HCV test were considered to be infected with HCV. For the diagnosis of HPV infection, colposcopic exam results were collected during antenatal care. For the diagnosis of syphilis, the results of VDRL exams were used. A positive diagnosis was considered for patients with a titre $\geq 1:1$.

The maternal HIV-1 subtype was determined through proviral DNA detection by polymerase chain reaction (PCR) in DNA samples extracted from total blood with an Illustra® Blood Genomic Prep Mini Spin Kit (GE Healthcare). A nested PCR was performed to amplify the immunodominant region of the viral *gp41* gene. The primers used in this reaction were as follows: JH38 (5'CAGCAGGAAGCACTATGGG3') and JH41 (5'GGTGAGTATCCCTGCCTAAC3') for the first amplification round and Menv27 (5'AAGCCTCCTACTATCATTATGA3') and Env19 (5'CTGGTATAGTGCAACAG CA3') for the second round. Sequencing was performed in an ABI Prism 3100 system (Applied Biosystems, Foster City, USA) and the reactions were performed according to the manufacturer's suggested protocol. Sequences were edited with the LaserGene software package (DNASar Inc, Madison, USA) and were aligned with the CLUSTALW program. Additional reference sequences obtained from the Los Alamos HIV sequence database (hiv-web.lanl.gov) were included in the alignment. A phylogenetic analysis using the neighbour-

joining method and the Kimura two-parameter correction was carried out with all sequences to determine their HIV-1 subtype by grouping with reference sequences. The phylogenetic inference was conducted using MEGA 4.0 software (Tamura et al. 2007). The robustness of the different clades in the tree was evaluated by bootstrapping with 2,000 replications in MEGA.

The CD4⁺ T cell counts of patients were determined by flow cytometry in a BD FACSCount® System and HIV-1 viral load quantification was performed using a Versant® test (Bayer Diagnostics, EUA) using blood samples of the mothers collected at week 34 of gestation. Some demographical factors were also analysed, such as age, race and level of education. For the maternal outcomes, prematurity (< 37 weeks) and time of membrane rupture (\leq or $>$ 4 h) were evaluated. The following variables were evaluated from the infants: birth weight, Apgar score for the first and fifth minutes and Capurro score. Data were analysed using the chi-squared test for categorical variables and the Student's *t* test for continuous variables.

Ethics - Patients signed an informed consent form to participate in the study and the study was submitted and approved by the FURG's Ethical Committee (protocol 23116001368/2003-44). All procedures were carried out in accordance with the guidelines of the Helsinki Declaration.

RESULTS

One-hundred and thirty women infected by HIV-1 were followed-up at the antenatal service at FURG's specialised ambulatory clinic between July 2003-December 2008. The mean age of these patients was 26.2 (± 5.69) years. Ninety-eight (75.4%) of the patients were white and 32 (24.6%) were afro-descendants or mestizos. Ninety-six patients (73.8%) had completed eight years of education.

Three patients (2.3%) presented a positive HBsAg reactive test and 14 (10.8%) had a positive anti-HCV test. All three HBV-positive patients were simultaneously infected by HCV. Four patients (3.1%) presented a VDRL-reactive test during follow-up. With respect to cytopathological exams, 53 (40.8%) presented cellular alteration, indicating the presence of HPV. Of these, eight (15.1%) presented koilocytosis, 17 (32%) had low-grade lesions and 28 (52.8%) presented high-grade cervical lesions (HSILs) or *in situ* carcinoma (Table I).

HIV-1 viral subtype was determined in 105 cases. Seventy-five women (71.4%) carried HIV-1C infections and 30 (28.6%) had HIV-1B. Data on co-infections were available for 98 patients in this group: 39 (78%) of co-infected women harboured HIV-1C, whereas 11 (22%) had HIV-1B. This difference was not statistically significant ($p = 0.14$).

The average CD4⁺ T-cell lymphocyte count among the co-infected pregnant women was 563.7 ± 323.6 cells and among those non-co-infected it was 632.5 ± 372.2 cells ($p = 0.25$). Among those infected by different HIV-1 subtypes, the average count was 700 ± 335 cells among those with HIV-1B and 547 ± 340 cells among those with HIV-1C ($p = 0.04$). With respect to the HIV-1 viral load, the mean log₁₀ was 1.54 among those co-infected

and 0.97 among those without a co-infection ($p = 0.04$). There was no statistically significant difference in the viral loads between subtype B and C-infected subjects.

We did not find any differences in gestational outcomes between the co-infected and non-co-infected patients or between those infected by different HIV-1 subtypes (Tables II, III). Vertical transmission of HIV was observed in only three children (2.3%).

TABLE I

Prevalence of co-infections and cervical lesions in human immunodeficiency virus+ pregnant women in southern Brazil

Co-infection/cervical lesion	Prevalence n (%)
Hepatitis B	3 (2.3)
Syphilis	4 (3.1)
Hepatitis C	14 (10.8)
Human papillomavirus	53 (40.8)
Koilocytosis	8 (15.1)
Low grade intraepithelial lesion	17 (32)
High grade intraepithelial lesion/ <i>in situ</i> carcinoma	28 (52.8)

DISCUSSION

A 10% HIV-1/HCV co-infection prevalence was observed in our study, which is higher than the HCV prevalence among general pregnant women (0.1%-2.7%) reported in other regions of Brazil (Costa et al. 2009, Lima & Viana 2009). There are very few reports of HIV/HCV co-infection in Brazil. A 5.5% prevalence of this co-infection was reported among HIV-infected women in Rio de Janeiro (Joao et al. 2006) and a 20% prevalence was found in another study in Porto Alegre (Kreitchmann et al. 2004), another city from southern Brazil. These studies, along with our results, provide evidence of a higher rate of HCV infection among HIV-positive subjects. One of the most important risk factors for HIV/HCV co-infection is intravenous drug use (IDU) (Bollepalli et al. 2007), but this variable does not appear to influence the rates observed here, as the reported IDU was low (data not shown). On the other hand, HBsAg reactivity is also associated with HCV seropositivity (Landes et al. 2008), corroborating our finding that all HBsAg+ patients in this study (2.5%) were also infected with HCV. Vertical transmission of HCV in Brazil was reported to be between 2.5-5.6% (Lima et al. 2004), but higher rates have been reported elsewhere (Marine-Barjoan et al. 2007). HCV viremia is an important risk factor for HCV vertical

TABLE II

Maternal and concept variables according to the presence of co-infections in human immunodeficiency virus (HIV)-1-positive pregnant women in southern Brazil

Characteristic	Co-infected	Non-co-infected	p
Race [n (%)]			
Afro-descendant/mestizo	17 (29.2)	15 (22.4)	-
White	46 (70.8)	52 (77.6)	0.54
Age [mean (SD)]	26.4 (5.4)	26.3 (5.6)	0.94
HPV+	25.3 (4.8)	27.0 (5.9)	0.09
Anti-HCV+	30.6 (5.8)	25.8 (5.4)	0.002
HBsAg+	31.3 (1.1)	26.2 (5.7)	0.12
VDRL+	28 (3.5)	26.3 (5.7)	0.55
CD4+ T-cell counts [mean (SD)]	563.7 (323.6)	632.5 (372.8)	0.25
HIV-1 viral load [mean log (SD)]	1.54 (1.71)	0.97 (1.55)	0.04
HIV-1 subtype [n (%)]			
B	11 (22)	17 (35.4)	-
C	39 (78)	31 (64.6)	0.14
Rupture of membranes [n (%)]			
≤ 4 h	63 (96.9)	59 (93.7)	-
> 4 h	2 (3.1)	4 (6.3)	0.60
Prematurity [n (%)]			
Yes	6 (10.2)	2 (3.3)	-
No	53 (89.8)	58 (96.7)	0.16
Mean birth weight [g (SD)]	2863 (547)	2981 (411)	0.17
Apgar 1' [mean (SD)]	7.7 (1.49)	8.1 (1.1)	0.11
Apgar 5' [mean (SD)]	8.9 (0.61)	8.9 (0.6)	0.65
Capurro (weeks) [mean (SD)]	38.7 (1.5)	38.9 (1.3)	0.55

HBsAg: test for hepatitis B virus; HCV: hepatitis C virus; HPV: human papillomavirus; SD: standard deviation; VDRL: test for syphilis.

transmission and HIV infection is an additional risk factor (Polis et al. 2007). Although we have shown that prolonged vaginal delivery and emergency C-section may increase the risk for vertical transmission of HCV, elective caesarean delivery is not yet generally indicated in cases of maternal HCV infection (McIntyre et al. 2006). Therefore, these results should be evaluated and additional studies should be performed to confirm our observation. Furthermore, co-infected patients have a higher risk for both hepatotoxicity and long-term liver damage upon antiretroviral treatment (Moodie et al. 2009).

We also found a high prevalence (40.1%) of HIV-1/HPV co-infection among pregnant women based on cervical smear cytology. Other studies in Brazil using more sensitive techniques, such as PCR, have found an HPV prevalence of 80-96% in HIV-infected pregnant women, which is higher than the rate observed in HIV-non-infected pregnant women (Jalil et al. 2009). A striking observation of our study was that over half of the patients presenting cervical cytology abnormalities had HSILs or cervical cancer, comprising an overall prevalence of HSILs or cervical cancer of 21%. As far as we know, this is twice the rates described in different geographic populations of HIV-infected women (Dames et al. 2009). We do not know the reasons for this discrepancy in relation to other studies. High-risk HPV types in HIV-infected women have been correlated with lower CD4⁺ T-cell

counts (Dames et al. 2009), but such a correlation was not feasible in our study because the CD4 measurements in our study were performed at 34 weeks of gestation, when most patients were already being treated with antiretrovirals. Further studies are ongoing to determine the risk factors for such rapid evolution of HPV infection in our study setting. Although the vertical transmission of HIV was low in this cohort, we could not assess the consequences of foetal exposure to HPV. The maternal-foetal transmission of HPV can occur not only through the genital tract during delivery, but also through the placenta (Rombaldi et al. 2008). One study suggests that HPV infection is associated with a slightly higher risk of foetal chromosome aberration (Eppel et al. 2000).

We found a reactive VDRL rate of 2.8% in our study population, which is lower than that described for HIV-infected women in Porto Alegre (8%) (Kreitchmann et al. 2004). Maternal syphilis was associated with a 2.7-fold increased risk of in utero HIV transmission (Thorne et al. 2008) and implementation of antenatal screening and treatment for syphilis will likely impact the rates of HIV vertical transmission. In 2007, the Brazilian Ministry of Health implemented an operational plan aimed at decreasing syphilis transmission in the country by 30% (MS 2007). For the southern region, where our study city is located, the expected incidence of syphilis per 1,000 live births was 1.7% at the end of 2001.

TABLE III
Maternal and concept variables according to infecting HIV-1 subtype in human immunodeficiency virus (HIV)-1-positive pregnant women in southern Brazil

Characteristics	Subtype B	Subtype C	p
Race [n (%)]			
Afro-descendant/mestizo	10 (33.3)	15 (20.8)	-
White	20 (66.7)	57 (79.2)	0.18
Age [mean (SD)]	25.5 (5.9)	26.2 (5.3)	0.56
CD4 ⁺ T-cell count [mean (SD)]	700 (335)	547 (340)	0.04
HIV-1 viral load [mean log (SD)]	1.03 (1.5)	1.5 (1.7)	0.18
Co-infection [n (%)]	11 (22)	39 (78)	0.14
HPV ⁺	10 (24.3)	31 (75.7)	0.40
Anti-HCV ⁺	1 (11)	8 (89)	0.22
HBsAg ⁺	0 (0)	3 (100)	-
VDRL ⁺	2 (66.7)	1 (33.3)	0.13
Rupture of membranes [n (%)]			
≤ 4 h	26 (92.9)	70 (95.9)	-
> 4 h	2 (7.1)	3 (4.1)	0.82
Prematurity [n (%)]			
Yes	2 (8.9)	6 (8)	-
No	27 (93.1)	69 (92)	0.85
Mean birth weight [g (SD)]	2981 (492)	2914 (542)	0.57
Apgar 1' [mean (SD)]	8 (1.55)	7.85 (1.37)	0.57
Apgar 5' [mean (SD)]	8.9 (0.67)	8.9 (0.56)	0.90
Capurro (weeks) [mean (SD)]	39.1 (1.3)	38.7 (1.4)	0.26

HBsAg: test for hepatitis B virus; HCV: hepatitis C virus; HPV: human papillomavirus; SD: standard deviation; VDRL: test for syphilis.

Significant differences were not observed between the distinct HIV-1 viral subtypes and the presence of a co-infection ($p = 0.12$), but 78% of the co-infected pregnant women were infected by HIV-1 subtype C and 22% were infected by subtype B. It is likely that the high prevalence of co-infected pregnant women carrying subtype C merely reflects the expansion of this variant in southern Brazil (Soares et al. 2005). Therefore, we conclude that the risk of co-infection is more dependent on high-risk sexual behaviour than HIV characteristics. In any case, an accumulation of cases in the next few years or the elaboration of multicentre studies in the southern region of Brazil will allow a more precise evaluation of the influence of HIV-1 subtypes on co-infections.

It is noteworthy to mention that patients carrying HIV-1 subtype C presented a lower mean CD4⁺ T-cell count when compared to those infected by subtype B. This finding is in opposition to the findings of other studies, such as the work of Abraha et al. (2009) that stated a slower progression to AIDS in subtype C-infected subjects based on CD4⁺ T-cell decline. Due to the low number of subtype B-infected patients in addition to the unknown time of HIV-1 infection of patients analysed, further studies are required to evaluate the influences of viral subtype on progression. We observed, however, that the presence of a co-infection did not alter the mean CD4⁺ T-cell counts.

The plasma HIV-1 viral load of co-infected patients was greater than that of non-co-infected. Considering that most co-infected patients were HPV carriers, these results agree with the results of a study by Jalil et al. (2009), that associated the presence of HPV to high HIV loads.

No significant changes were observed in maternal and concepts outcomes despite the presence of co-infections or infection with different HIV-1 subtypes. This could be due to the follow-up, which includes support in improving adherence to treatment, antenatal care, monitoring of the appointments and a multidisciplinary team approach that these patients received at our reference HIV/AIDS hospital.

It was possible to observe that pregnant women, regardless of their serological status or infecting HIV-1 subtype, are still a vulnerable population to sexually transmitted diseases and possibly illicit drug use. Therefore, health policies as well as the intervention strategies carried out by the professionals in this area should focus on incentivising and educating patients about planned pregnancies and seeking antenatal services as early as possible. Moreover, patient access to antenatal services should be made easier and the quality of this service should be monitored. In this fashion, triage, diagnosis and follow-up of patients, as well as the treatment of infections, will be carried out more efficiently, which seems to be the best and simplest way to reduce paediatric infections and to assure better quality of life for both the mothers and their children.

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