CMV AND HCV INFECTIONS IN HIV/NON HIV MOTHERS AND NEWBORNS: PREVALENCE, FREQUENCY AND RISK FACTORS

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INTRODUCTION

The number of new HIV cases in women has increased substantially over the last decade, as has HIV prevalence among women giving birth (1,2,3). The incidence of HIV infections in gestational age is an important Public Health issue as are concerns about coinfection with opportunistic viruses.

In the era that preceded the use of potent combined antiret-roviral therapy (ART), opportunistic infections (OIs), which have been defined as infections that are more frequent or more severe because of immunosuppression in HIV-infected persons, were the leading cause of morbidity and mortality in adults and children infected with HIV. Although deaths have decreased since the implementation of ART, OIs remain a lead-ing cause of morbidity and mortality in HIV-infected persons (4,5). The relationship between OIs and HIV infec-tion is bidirectional: HIV leads to immunosup-pression that allows opportunistic pathogens to cause disease in HIV-infected persons while certain OIs are associated with reversible increases in circulating viral load and could lead to accelerated HIV progression or increased transmission of HIV (4).

HIV coinfection with CMV and HCV during pregnancy is a growing problem, as both are increasingly common OI. The effects of infection caused by CMV in the progression of HIV disease remains controversial; several authors refer higher ratios of CMV cervical shedding in HIV-infected women than in uninfected and congenital CMV infection more frequently in children infected with HIV when compared to non HIV infected (6). Other authors conclude the absence of reciprocal influence of CMV and HIV in the maternal-foetal transmission. However, most studies show the seriousness of the association, as reported by Kovacs (7).

Concerning HCV, vertical transmission probably occurs during delivery and mother's viral load can influence maternal-foetal transmission of HCV (8). Several authors reported that perinatal transmission increases in cases of mothers co-infected with HIV (9,10). The influence of HCV on HIV disease progression is not well defined, but it is known that HIV influences the disease progression caused by HCV(8,11).

AIMS

- To study and characterize CMV and HCV infection and coinfection in HIV-infected women and their newborns between 2006-2009, according to epidemiological, laboratory and clinical data.
- To study and characterize CMV and HCV infection and coinfection in uninfected HIV women and their newborns between 2009-2010, according to epidemiological, laboratory and clinical data.
- To evaluate the frequency of intrauterine and/or perinatal infection of HIV, CMV and HCV in both populations.
- To analyse risk factors for infections and coinfections for HIV, CMV and HCV in both populations.
- To assess clinical and virological outcomes in children infected and not infected by HIV, born to HIV-infected mothers.

METHODOLOGY

Study Population:

Population of HIV-infected women and their newborns - Plasma from 137 HIV-infected women and their 140 newborns (from whom plasma and urine were collected), obtained from an obstetrics and paediatric department of a Lisbon Hospital, were analysed at National Institute of Health (NIH), between January 2006 and May 2009.

Population of uninfected HIV women and their newborns - Plasma from 140 uninfected HIV women and their 140 newborns (from whom urine was collected), obtained from an obstetrics and paediatric department of a Lisbon Hospital, were analysed at NIH, between July 2009 and January 2010.

Laboratory Methods:

HIV-1 and/or HIV-2 proviral DNA nested-PCR (in-house) was performed on HIV-infected mothers and their newborn's plasma. Maternal plasma was screened for CMV IgG and IgM, using a commercial Elisa kit and for HCV antibodies, using commercial reagents based on MEIA technology, in both populations of HIV-infected and uninfected women; RNA determination, genotyping and viral load were performed on women with HCV antibodies, by commercial LIPA HCV Genotype assay and a nucleic acid amplification test for the quantitation of HCV RNA genotypes; their newborn's plasma was also screened for HCV.

Newborn's urine was inoculated for CMV detection in both populations, using the *shell-vial* procedure and staining by immunofluorescence technique with commercial monoclonal antibodies. In cases where urine was insufficient to inoculate, a commercial CMV real-time PCR was performed (Table 1).

Data analysis was performed using SPSS 17.0 and Fisher's exact test. In addition to the main variables of interest – age, sex, race/ethnicity – other socio-demographic risk factors were selected for analysis: country of birth, profession, assessment of economic conditions, previous pregnancies, number of children, informations concerning HIV infection and monitoring during pregnancy.

Table 1 – Laboratory methods applied in viral diagnosis

Virus	Case	Sample	Method
HIV	Mother Newborn	Plasma	Proviral DNA nested-PCR for HIV-1 and/or HIV-2
CMV	Mother Newborn	Plasma	Elisa IgG Elisa IgM
	Newborn		Imunoblot when Elisa IgM positive IgG avidity when Elisa IgM positive
		Urine	*Shell-vial culture* Real-time PCR when urine was insufficient
HCV	Mother	Plasma	MEIA test for antibodies anti-HCV
	Mother Newborn		RNA RT-PCR
			RT-PCR Viral load quantification
			Imunoblot genotyping

RESULTS

Population of HIV-infected women and their newborns

The distribution of cases (mothers) according to HIV type, nationality/geographical origin and age group is described in tables 2, 3 and 4:

Table 3 - Distribution of cases (mothers) according nationality or geographical origin

Geographical origin	n	%	CI 95%
Guinea-Bissau	43	31.4	(27.41 ; 35.39)
Portugal	35	25.6	(21.75 ; 29.25)
Angola	22	16.1	(12.94 ; 19.26)
Cape Verde	22	16.1	(12.94 ; 19.26)
Brazil	4	2.9	(1.46 ; 4.34)
São Tomé and Príncipe	3	2.2	(0.94 ; 3.46)
Senegal	3	2.2	(0.94 ; 3.46)
Malawi	2	1.5	(0.45 ; 2.55)
Mozambique	1	0.7	(0 ; 1.42)
Unknown	2	1.5	
Total	137		

Table 2 – Distribution of cases (mothers) according to HIV type

HIV type	n	%	CI 95%
HIV-1	118	86.1	(83.14 ; 89.06)
HIV-2	15	11.0	(7.61 ; 12.79)
HIV-1 and HIV-2	4	2.9	(2.01 ; 5.19)
Total	137		

Table 4 - Distribution of cases (mothers) according to age group

Age group (years)	n	%	CI 95%
15-19	6	4.4	(2.63 ; 6.13)
20-24	21	15.3	(12.25 ; 18.41)
25-29	40	29.2	(25.31 ; 33.08)
30-34	36	26.3	(22.52 ; 30.04)
35-39	24	17.5	(14.27 ; 20.77)
40-44	9	6.6	(4.45 ; 8.69)
45-49	1	0.7	(0.00 ; 1.46)
Total	137		

Table 5 – HIV1 vertical transmission

HIV1 proviral DNA	n	%	CI 95%
Negative	137	97.9	(96.63 ; 99.08)
Positive	3	2.1	(0.92 ; 3.37)
Total	140		

HIV1 vertical transmission was diagnosed in 3 cases (2.1%) (Table 5). Due to the low number of positive cases in this study, we didn't find associations between the risk factors analyzed and vertical transmission of this virus.

Two HIV-infected women were seronegative for CMV (1.5%). Eleven women were IgG and IgM CMV positive (8.0%) with high IgG avidity (Table 6). A considerable percentage difference was found in "Job classification" variable, with high proportion of IgG/IgM CMV positive cases in unskilled workers, however this was not statistical significant (p=0.193).

Table 6 – Mothers - Laboratory results for CMV

CMV antibodies	n	%	CI 95%
Negative IgG / Negative IgM	2	1.5	(0.46 ; 2.54)
Positive IgG / Negative IgM	124	90.5	(87.99 ; 93.01)
Positive IgG / Positive IgM	11	8.0	(5.68 ; 10.32)
Total	137		

CMV congenital infection was diagnosed in 4 cases (2.9%)(Table 7): 2 cases (1.5%) in 137 HIV negative newborns and 2 cases in the 3 HIV-positive newborns. The proportion of HIV-CMV coinfection in 140 newborns was 1.4% (2/140).

The risk factor significantly associated with this population group was the variable "Ancestry" (p=0.040); children without African ancestry presented higher percentages of CMV congenital infection than the children with African ancestry.

Table 7 – CMV vertical transmission

shell-vials culture / PCR	n	%	CI 95%
Positive	4	2.9	(1.48 ; 4.32)
Negative	136	97.1	(95.68 ; 98.52)
Total	140		

HCV infection was detected in six (4.4%) HIV-infected women and all had HCV positive viral load: 1 case had a HCV viral load at less than 10^6, and the remaining 5 cases a had viral load greater than 10^6. Genotypes 4 and 1were predominant in this study group (Table 8). There was 1 case (16.7%) of maternal-fetal transmission of HCV, with a viral load greater than 10^6 (9 740.000 IU/ml) and with the same genotype as the mother (2a/2c).
No children were HIV/HCV or CMV/HCV coinfected.
The risk factor significantly associated with this population group was the variable "Weeks of pregnancy" (p=0.037), with a higher proportion of cases of HCV viremia, in mothers who had their children before 38 weeks, compared to mothers who had children between 38 and 42 weeks of gestation. A considerable percentage difference was found in variables "Age" (p=0.209), "Social class" (p=0.106) and "Monitoring pregnancy" (p=0.161), with high proportion of viral load positive cases in mothers >= 30 years old / from lower socioeconomic groups / without follow-ups during pregnancy respectively, however these variables were not statistical significant.

Table 8 – Mothers – Laboratory results for HCV

HVC antibodies	n	RNA detection	Viral Load (IU/ml)	Genotype	%	CI 95%
Negative	131	Negative	-	-	95.6	(93.87 ; 97.37)
Positive	6	Positive	1 440.000	1a	4.4	(2.63 ; 6.13)
			323.728	4		
			4 620.000	4a/4c/4d		
			9 670.000	2a/2c		
			5 020.000	4a/4c/4d		
			36 700.000	1b		
Total	137					

The follow-up of children infected by HIV, CMV and/or HCV is considered normal, except for one case of HIV/CMV coinfection that has a slight development delay.

Population of uninfected HIV women and their newborns

The distribution of cases (mothers) according to nationality/geographical origin and age group is described in tables 9 and 10:

Table 9 - Distribution of cases (mothers) according nationality or geographical origin

Geographical origin	n	%	CI 95%
Portugal	79	56.4	(52.03 ; 60.83)
Brazil	14	10.0	(7.34 ; 12.66)
Cape Verde	11	7.9	(5.47 ; 10.24)
Angola	10	7.1	(4.86 ; 9.43)
Guinea-Bissau	4	2.9	(1.38 ; 4.34)
São Tomé and Príncipe	2	1.4	(0.38 ; 2.48)
Eastern Europe	5	3.6	(1.92 ; 5.22)
India	1	0.7	(0 ; 1.46)
England	1	0.7	(0 ; 1.46)
Unknown	13	9.3	
Total	140		

Table 10 - Distribution of cases (mothers) according to age group

Age group (years)	n	%	CI 95%
15-19	6	4.3	(2.57 ; 5.10)
20-24	28	20.0	(16.62 ; 23.38)
25-29	32	22.9	(19.31 ; 26.41)
30-34	51	36.4	(32.36 ; 40.50)
35-39	18	12.8	(10.03 ; 15.69)
40-45	5	3.6	(2.00 ; 5.14)
Total	140		

Table 11 – Mothers - Laboratory results for CMV

CMV antibodies	n	%	CI 95%
Negative IgG / Negative IgM	14	10.0	(7,46 ; 12.54)
Positive IgG / Negative IgM	120	85.7	(82.76 ; 88.67)
Positive IgG / Positive IgM	6	4.3	(2.57 ; 6.00)
Total	140		

Fourteen uninfected HIV women were seronegative for CMV (10.0%) and 6 women were IgG and IgM positive (4.3%) with high avidity IgG (Table 11).
No congenital CMV infection was diagnosed in newborns from uninfected HIV women.
No HCV antibodies were found in uninfected HIV women, so there was no need to test for maternal-fetal transmission.

There were no statistically significant associations between results and the analysed parameters.

COMMENTS

- The proportion of HIV infection in newborns was 2.1% (3 cases), consistent with that described in literature for cases where there are prophylactic attitudes and practices that lead to decreased risk of HIV transmission (12, 13).
  - In one case there was premature membrane rupture and the newborn had contact with maternal blood or secretions; this infant probably wouldn't be infected as the mother had carried out ATR prophylaxis during pregnancy.
  - One case (mother) of the other 2 infected newborns did not comply with ART prophylaxis during pregnancy; the other case (mother) is associated with drug addiction, and, even in the presence of ART prophylaxis, a high value of HIV viral load (595.980 copies/ml), a high value of HCV viral load (36700000 UI/ml) and positive VDRL were diagnosed.

  These conditions increased the risk of HIV transmission.
- In this study HIV-infected women had higher CMV and HCV antibody prevalence and frequency of maternal-fetal transmission than uninfected HIV women, which is consist with national and international reports (7,11,14,15).
- The results show that HCV genotypes 4 and 1 are predominant in this study group. The presence of residents from African origin in hospital catchment population may influence the distribution found in this study once the genotype distribution in Portugal is influenced by the presence of African residents where genotype 4 is the most common. The presence of genotype 1 is consistent with the pattern observed in studies conducted in Portugal and Europe.
- 2/137 HIV seronegative newborns and 2/3 HIV newborns were CMV congenitally infected; this difference should be further studied, as the consequences of CMV/HCV infections may become increasingly serious and complex in the presence of HIV.
  - The risk factor more significantly associated with this population group was the variable "Ancestry"; children without African ancestry presented higher percentages of CMV congenital infection than the children with African ancestry, which leads to the assumption that African ancestry in HIV-infected mothers may be associated with lower frequency of congenital CMV infection or, on the other hand, the characteristics of mothers with no African ancestry, belonging to this group, were associated with some risk factors for vertical CMV transmission.
- Both specific groups do not reflect the Portuguese HIV-infected and uninfected women, nevertheless the significant risk factors found and the other risk factors studied without strong associations ("Weeks of pregnancy", "Age", "Social class" and "Monitoring pregnancy") should be considered in larger studies.

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