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HIV-1 INFECTION AND PREGNANCY IN ADOLESCENT AND YOUNG WOMEN IN BRAZIL: DRUG RESISTANCE, GENETIC DIVERSITY AND CLINICAL OUTCOMES

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Key words: HIV-1, pregnancy, mother-to-child-transmission, antiretroviral resistance.

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ABSTRACT

Objectives: To assess antiretroviral (ARV) drug resistance, HIV-1 diversity and clinical outcomes among young pregnant women aged 15-24 years old.

Setting: A public health antenatal program responsible for screening ~90,000 pregnant women per year for nine different infectious diseases in central western Brazil.

Participants: 96 young pregnant women (35%: 15-19 years; 65%: 20-24 years).

Primary and secondary outcome measures: Clinical/ laboratory data were retrieved from medical files. HIV-1 *pol* gene sequences (entire protease/PR, partial reverse transcriptase/RT) were obtained from plasma RNA. ARV resistance mutations (CPR/Stanford HIV-1; International AIDS Society-USA databases) were identified.

Results: The median age was 21 years, most reported <8 years education, 73% was recently diagnosed. Late presentation (after 26 gestational weeks) was observed (19%, 19/96), 49% reported 2 ≥ previous pregnancies. Possible heterosexual transmission by HIV-1 infected partner (17%), commercial-sex-work (2%) were reported. The median of $CD4^{+}$T cell count was 526 cells/mm^3, the median viral load was: 10,056 copies/mL in ARV-naïve (48/96), 5,881 copies/mL in ARV-exposed (48/96) patients. Two probable seroconversion cases during pregnancy were identified in adolescents. One mother-to-child-transmission (1.0%) was observed. Transmitted-drug-resistance in ARV-naive was 9.3% (CI95%:3.3%-19.6%); secondary drug resistance in ARV-exposed was 12.5% (CI95%: 4.7%-25.6%).

Conclusions: Despite high access to antenatal care, the low socio-economic-educational profiles seen in these young HIV- infected women highlight the necessity of improved public health educational and preventive strategies regarding sexually transmitted infections and early unplanned pregnancy.

STRENGTHS AND LIMITATIONS OF THE STUDY

- Representative sample of HIV-1-infected pregnant young women attending an antenatal care program that screens ~90,000 women per year;
- Assessment of HIV-1 infection and mother-to-child-transmission risk among adolescents and young pregnant women;
- Other studies on groups of HIV-1 infected pregnant adolescent/young women from other settings in Brazil and abroad are important to better define this vulnerable population.

INTRODUCTION

The Brazilian AIDS epidemic is considered stable, however in the last decade a significant increase of AIDS cases in younger population (15-19 years) was observed both among males (53.2%) and females (10.5%)[1]. Adolescence is characterized by a series of physical, emotional and social modifications that influence health, especially factors associated with the start of sexual activities. Young females may also be vulnerable to sexual violence and commercial sex with older men[2]. Adolescents (10-19 years) and young adults (20-24 years) are considered highly susceptible to sexually transmitted infections (STIs), including HIV infection[2]. In Brazil, during the last decade 40% of HIV-1 infected pregnant women were in the age range of 15-24 years [1]. In central western Brazil, adolescents represented 26% of 54,139 pregnant women screened for HIV-1 infection during antenatal care[3].

HIV infection and pregnancy in young women raise concern as long term exposure to highly active antiretroviral therapy (HAART) started at early age, and low adherence may lead to increased selection of drug resistant viruses. Drug resistance mutations may compromise future therapeutic options and mother-to-child-transmission (MTCT) drug prophylaxis. In this context, this study describes transmitted and secondary antiretroviral (ARV) resistance mutation profiles in ARV-naïve and ARV-exposed young pregnant Brazilian women infected with HIV-1.

METHOD

The study population included young pregnant diagnosed women with HIV infection (≤ 24 years) by a comprehensive public health antenatal care program including recent and former diagnosed cases (2008-2013). The Program for the Protection of Pregnant Women-PPPW/Goiás State, central western Brazil, offers "opt out" serological screening (~90,000 women/year) for HIV-1/2, syphilis, hepatitis B and C, cytomegalovirus, toxoplasmosis, HTLV-1/2, rubella, and Chagas disease, regardless of previous diagnosis. Prevalence of HIV-1 infection in this population has been recently estimated as 1.59/1000 (95% CI 1.27‰ – 1.96‰)[3].

Socio-demographic information was obtained using a standardized questionnaire. Clinical and laboratory data were retrieved from medical files at the main local public HIV-1/AIDS reference hospital (Dr. Anuar Auad Tropical Diseases Hospital, Goiânia, Goiás, Brazil). This research protocol was approved by local Ethical Committees (University Hospital, Federal University of Goias, #protocol 163/2010; Dr. Anuar Auad Tropical Diseases Hospital, #protocol 003/2011). Written informed consents were obtained from all participants except for participants younger than 18 years which had informed consents signed by one of the parents/legal guardian.

Plasma RNA was used for genetic analyses in *pol* gene as previously described[4]. Transmitted drug resistance (TDR) rate among ARV-naïve patients was determined by the Calibrated Population Resistance tool (Stanford Surveillance Drug Resistance Mutations-SDRM). Secondary drug resistance mutations and resistance profiles were defined by the Stanford HIV Drug Resistance Database and by the International AIDS Society-USA major mutation lists. GenBank accession numbers of sequences analyzed in this study are: JN114114-JN114220, JN114222-JN114227, JN114235, JN662426-JN662437, KC249749-KC249766, and KJ658974-KJ659016. Descriptive analyses of the study variables were performed (Epi Info™ vs.7, CDC, Atlanta, GA, USA). Chi-square or Fisher´s exact test were used for differences among categorical variables with a significance level of 5%.

RESULTS

During the study period, 96 young pregnant women infected with HIV-1 were enrolled: 35% (34/96) adolescents (15-19 years), 65% (62/96) within 20-24 years of age. Most patients was diagnosed during antenatal screening (69/96, 72%). For women with former diagnosis (28.1%, 27/96), the median time since diagnosis was 3 years (1-12 years range). The median gestational age at diagnosis/enrollment was 18 weeks (range: 8-37 weeks) and 19% (19/96) presented after 26 gestational weeks.

Among these patients, low socio-economic profile predominated: 28% (27/96) did not have a formal job, only 2% (2/96) self-reported as students, 18% (17/96) had less than 8 years of formal education, 4% (4/96) were illiterate. Regarding HIV-1 acquisition risk, 17% (16/96) reported a known HIV-1 infected sexual partner, 2% (2/96) stated commercial sex work. A 23 years old widow referred having had a regular partner who died of AIDS-related conditions. Half of participants (48/96) reported a stable sexual partner. Despite their young age, almost half of participants (47/96) had at least two previous pregnancies (median number of previous pregnancies=2; range: 0-7).

The median of CD4+ T cell counts in this group was 526 cells/mm3 (range: 82-1324 cells/mm3), 30% (28/96) were in the CDC stages 2 and 3 (CD4+ T cell count<500 cells/mm3). Half of patients (48/96) were ARV-naïve. The ARV-exposed group included temporary exposure to MTCT prophylaxis or continuous HAART. The median of plasma viral loads was 10,056 copies/mL (range: 299-750,000 copies/mL) among ARV-naïve participants and 5,881 copies/mL (range: 157-507,108 copies/mL) in ARV-exposed patients. Viral loads higher than 100,000 copies/mL were detected in around 8% of naïve patients (4/48) and in 4% of ARV-exposed patients (2/48).

Two cases of probable seroconversion during pregnancy were identified in adolescents that were negative at the first trimester and became seropositive upon retesting late in pregnancy. One case of HIV-1 MTCT was observed (1/96; 1.0%). The 23 years old transmitting mother was a late presenter to antenatal care (32 gestational weeks). Two days before delivery, CD4+ T cell count was 714 cells/mm^3 and viral load was 5,688 RNA copies/mL.

In ARV-naïve patients, TDR was identified in 9.3% (CI95%: 3.3%-19.6%) and among ARV-exposed participants, secondary drug resistance was 12.5% (CI95%: 4.7%-25.6%). Single-class and dual-class resistance mutations were observed in both ARV-naïve and ARV-exposed groups. Individual ARV transmitted and secondary resistance profiles in young HIV-1 infected pregnant women are depicted in Table 1.

Table 1: Drug resistance mutation profiles of HIV-1 isolates obtained from Brazilian young pregnant women infected with HIV-1

GenBank nº	Age (years)	ARV status	CD4 count (cells/mm³)	Viral load (copies/mL)	Subtype PR/RT	Resistance mutations			Resistance level		
						PI	NRTI	NNRTI	Low	Intermediate	High
KC249761	22	Naïve	415	6,227	B/B	L90M	M41L, T215C	-	FPV, LPV, ABC, DDI, TDF, AZT, D4T	ATV, IDV, SQV	NFV
KC249760	23	Naïve	532	21,458	B/B	L90M	M41L, T215D	-	FPV, LPV, ABC, DDI, TDF, AZT, D4T	ATV, IDV, SQV	NFV
JN114172	23	Naïve	524	<399	B/B	-	-	K103N	-	-	EFV, NVP
JN114216	24	Naïve	NA	2,630	C/C	-	-	K103N, P225H	-	-	EFV, NVP
JN114197	15	Exposed	NA	44,378	B/F1	-	-	M230L	-	EFV, ETR, RPV	NVP
JN114174	20	Exposed	1,082	5,577	C/C	T74S	-	-	NFV	-	-
KR559754	22	Exposed	225	19,076	B/B	-	M184V	E138Q	ABC, RPV	-	3TC, FTC
JN114142	22	Exposed	NA	NA	B/F1	T74S	-	-	NFV	-	-
JN114145	22	Exposed	567	3,629	C/C	-	M184V	K103N	ABC	-	EFV, NVP, 3TC, FTC

PR: Protease; RT: Reverse transcriptase; NA: Not available; FPV: Fosamprenavir; LPV: Lopinavir; ABC: Abacavir; DDI: Didanosine; ATV: Atazanavir; IDV: Indinavir; SQV: Saquinavir; NFV: Nelfinavir; 3TC: Lamivudine; AZT: Zidovudine; D4T: Stavudine; EFV: Efavirenz; NVP: Nevirapine; TDF: Tenofovir disoproxil fumarate; ETR: Etravirine; RPV: Rilpivirine; FTC: Emtricitabine

DISCUSSION

This group of young pregnant Brazilian infected with HIV-1 including adolescents presented a moderate rate of both TDR and secondary drug resistance. This highly vulnerable group of young women is characterized by low socio-economic and educational level, risky sexual behaviors, including known HIV-1-infected sexual partners, commercial sex work and early exposure to antiretroviral drugs. This unfavorable combination of features in such a young population highlights failures in educational and preventive public health interventions for STIs, including HIV-1. Multiparity in young women indicates that improved policies for unplanned pregnancies at early age are highly needed.

Late presentation for antenatal care reported can also increase the risk of MTCT and should be reduced by new public health approaches. Late presentation at antenatal care can jeopardize re-testing during pregnancy which is important to identify recent seroconversion cases. HIV infection during pregnancy is associated with increased risk of MTCT and higher transmission risk to sexual partners[5]. HIV-1 testing programs should be expanded to include at risk, non-pregnant young women. Ideally, HIV-1 diagnosis should precede pregnancy since virological failure at delivery and MTCT are more likely in women diagnosed during pregnancy[6].

Exposure to ARV drugs early in life was observed: more than half of adolescents (20/34) had been previously exposed to ARV drugs. However, patients with high viral loads (500,000-750,000 copies/mL) were observed both among ARV-naïve and ARV-exposed groups, indicating high risk for both MTCT and sexual transmission. High viral loads in ARV exposed young women may reflect low adherence to ARV treatment, as previously reported in adolescents, possibly due to rebellious behavior, collateral effects of ARV drugs and psychosocial factors such as depression[7]. Adolescents have shown higher virological failure after six months of HAART presenting higher viral loads[8]. In our study, viral loads were highly variable and no data on adherence to ARV was available.

The moderate rate of TDR observed in this young group raises concern regarding the efficacy of future HIV-1 MTCT prophylaxis and maternal treatment options. Dual-class resistance for the first line combination drugs used for treatment (tenofovir-TDF + lamivudine-3TC + zidovudine-AZT) and for MTCT prophylaxis (lopinavir/ritonavir-LPV/r+3TC+AZT) was observed. Previous studies among ARV-naïve pregnant women from this setting reported absence of TDR in 2003 and the 9.3% TDR rate reported here suggests a rising trend[9]. Continuous TDR monitoring should be emphasized, especially with the new policy adopted by the Brazilian Ministry of Health of universal distribution of ARV drugs for all diagnosed cases of HIV-1 infection, regardless of CD4+ T cell counts.

Regarding ARV-exposed patients, a moderate rate of secondary drug resistance was observed, as previously demonstrated in this setting[9]. Temporary exposures to ARV prophylaxis for MTCT during pregnancy was recommended in Brazil until 2013, a strategy that could have favored the selection of ARV resistant isolates. In Brazil secondary resistance rates over 20% were previously reported in HIV-1 pregnant women exposed to ARV prophylaxis for MTCT[10].

CONCLUSION

This study highlights the low socio-educational and economic profiles of highly vulnerable young HIV-1-infected pregnant women in Brazil. High viral loads and late presentation for

antenatal care indicate higher risk for MTCT and sexual transmission. Furthermore, moderate levels of drug resistance in ARV-naïve and ARV-exposed pregnant women emphasize the need for continued drug resistance surveillance studies to assure effective MTCT measures and future treatment options. The profile of vulnerable HIV-1-infected young pregnant women can help delineate better public health strategies to promote improved educational and preventive measures for STIs and unplanned pregnancies as well as early diagnosis of asymptomatic cases and MTCT prophylaxis.

Contributors

Lima, YAR contributed to the study design, data collection, analysis of data and preparation of the final document. Reis, MNG contributed to the analysis of data. Cardoso, LPV contributed to the analysis of data and preparation of the final document. Stefani, MMA contributed to the study design and preparation of the final document. All authors read and approved the final document.

Competing interests

None declared.

Data sharing statement

No additional data available.

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Key words: HIV-1, pregnancy, mother-to-child-transmission, antiretroviral resistance.

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ABSTRACT

Objectives: To describe socio-economic and antiretroviral (ARV) drug resistance profiles among young pregnant women infected with HIV-1.

Setting: A public health antenatal program responsible for screening ~90,000 pregnant women per year for nine different infectious diseases in central western Brazil.

Participants: 96 young pregnant women (15-24 years) infected with HIV-1.

Primary and secondary outcome measures: Primary and secondary outcome measures: Standard interviews and blood samples were taken at the time of recruitment, at the first medical appointment after confirmation of diagnosis of HIV-1 infection, before ARV prophylaxis initiation. Clinical and laboratory data were retrieved from medical files. HIV-1 *pol* gene sequences (entire protease/PR, partial reverse transcriptase/RT) were obtained from plasma RNA. ARV resistance mutations (CPR/Stanford HIV-1; International AIDS Society-USA databases) were identified.

Results: The median age was 21 years, most reported <8 years education, 73% were recently diagnosed. Late presentation (after 26 gestational weeks) was observed (19%, 19/96), 49% reported ≥2 previous pregnancies. Possible heterosexual transmission by HIV-1 infected partner (17%) and commercial-sex-work (2%) were reported. The median of CD4 cell count was 526 cells/mm^3, the median viral load was: 10,056 copies/mL in ARV-naïve (48/96), 5,881 copies/mL in ARV-exposed (48/96) patients. Two probable seroconversion cases during pregnancy were identified in adolescents. One mother-to-child-transmission case (1.0%) was observed. Transmitted-drug-resistance among ARV-naïve was 9.3% (CI95%:3.3%-19.6%); secondary drug resistance among ARV-exposed was 12.5% (CI95%: 4.7%-25.6%).

Conclusions: Despite high access to antenatal care, the low socio-economic-educational profiles seen in these young HIV-1-infected women highlight the necessity of improved public health educational and preventive strategies regarding HIV infection and early unplanned pregnancy.

STRENGTHS AND LIMITATIONS OF THE STUDY

- Representative sample of HIV-1-infected pregnant young women attending an antenatal care program that screens ~90,000 women per year;
- Assessment of drug resistance and mother-to-child-transmission potential risk among adolescents and young pregnant women;
- Other studies on larger groups of HIV-1 infected pregnant adolescent/young women from other settings in Brazil and abroad are important to better define this vulnerable population.

INTRODUCTION

The Brazilian AIDS epidemic is considered stable, however in the last decade a significant increase of AIDS cases in younger population (15-19 years) was observed both among males (53.2%) and females (10.5%)[1]. Adolescence is characterized by a series of physical, emotional and social modifications that influence health, especially factors associated with the start of sexual activities. Adolescents (10-19 years) and young adults (20-24 years) are considered highly susceptible to sexually transmitted infections (STIs), including HIV infection[2]. This vulnerability in younger age is certainly associated with engagement in risky sexual behaviors such as early sexual debut, multiple sexual partners and lack of consistent use of preservatives[3]. Young females may also be vulnerable to sexual violence and commercial sex with older men[2].

In Brazil, during the last decade 40% of HIV-1 infected pregnant women were in the age range of 15-24 years[1]. In the central western region, adolescents represented 26% of 54,139 pregnant women screened for HIV-1 infection during antenatal care[4]. Compared to adults, younger HIV-1 infected patients had lower attendance in specialized clinical care, higher HIV-1 sequelae and difficulties in initiation and adherence to HAART[5 6]. Therefore, HIV infection and pregnancy association in young women raises concern as long-term exposure to highly active antiretroviral therapy (HAART) started at early age, and low adherence may lead to increased selection of drug resistant viruses. First line combination drugs used for treatment in Brazil at the time of study includes: tenofovir-TDF + lamivudine-3TC + zidovudine-AZT. MTCT prophylaxis includes: lopinavir/ritonavir-LPV/r + AZT + 3TC [7]. Drug resistance mutations may compromise future therapeutic options and MTCT drug prophylaxis.

In Central Western Brazil, antenatal care is provided free of charge by a comprehensive public health program (Program for the Protection of Pregnant Women-PPPW/Goiás State, central western Brazil). PPPW offers "opt out" serological screening (~90,000 women/year) for HIV-1/2, syphilis, hepatitis B and C, cytomegalovirus, toxoplasmosis, HTLV-1/2, rubella, and Chagas disease, regardless of previous diagnosis for women from 246 municipalities at Goiás State. This is a feasible setting for cross-sectional studies among pregnant women. Prevalence of HIV-1 infection in this population has been recently estimated as 1.59/1000 (95% CI 1.27‰ – 1.96‰)[4]. In this context, this study aimed to describe in ARV-naïve and ARV-exposed young pregnant Brazilian women infected with HIV-1: socio-economic variables, transmitted and secondary ARV resistance mutation profiles and pregnancy outcomes, such as MTCT.

METHODS

This study was delineated as a cross-sectional survey to describe socio-economic, resistance mutation profiles and pregnancy-related outcomes among young pregnant women from Brazil. From 2008 to 2013, young pregnant women (<25 years) attending a local antenatal care program with a positive result for HIV-1 infection were recruited at the time of first medical visit after the diagnosis. Inclusion criteria were: pregnant women with 24 years old or less, ARV-naïve or exposed.

Socio-demographic information was obtained using a standardized questionnaire at the time of recruitment. Clinical and laboratory data were retrieved from medical files at the main local public HIV-1/AIDS reference hospital (Dr. Anuar Auad Tropical Diseases Hospital, Goiânia, Goiás, Brazil). This research protocol was approved by local Ethical Committees (University Hospital, Federal University of Goias, protocol #163/2010; Dr. Anuar Auad Tropical Diseases

Hospital, protocol #003/2011). Written informed consents to participate in the study and to provide blood samples were obtained from all patients except for those younger than 18 years which had informed consents signed by one of the parents/legal guardian.

Blood samples were obtained at the time of recruitment. ARV-naïve patients had blood samples drawn before ARV-prophylaxis initiation. Plasma RNA (QIAamp® Viral RNA Mini Kit, Qiagen, Hilden, Germany) was used for genetic analyses in *pol* gene as previously described[8]. Transmitted drug resistance (TDR) rate among ARV-naïve patients was determined by the Calibrated Population Resistance tool (Stanford Surveillance Drug Resistance Mutations-SDRM). Secondary drug resistance mutations and resistance profiles were defined by the Stanford HIV Drug Resistance Database and by the International AIDS Society-USA major mutation lists. All genotyping profiles obtained in this study were made available to the responsible clinician.

Descriptive analyses of the study variables were performed (Epi Info™ vs.7, CDC, Atlanta, GA, USA). Categorical variables were presented as percentage values and continuous variables were analyzed by the median value. Missing data were not included in the analysis. Chi-square or Fisher´s exact test were used for differences among categorical variables with a significance level of 5%.

RESULTS

During the study period, 96 young pregnant women infected with HIV-1 were enrolled: 35% (34/96) adolescents (15-19 years), 65% (62/96) within 20-24 years of age. Most patients were diagnosed during antenatal screening (69/96, 72%). For women with former diagnosis (28.1%, 27/96), the median time since previous diagnosis was 3 years (1-12 years range). The median gestational age at diagnosis or at enrollment was 18 weeks (range: 8-37 weeks) and 19% (19/96) started antenatal care after 26 weeks of gestation.

Among these patients, low socio-economic profile predominated: 28% (27/96) did not have a formal job, only 2% (2/96) self-reported as students, 18% (17/96) had less than 8 years of formal education, 4% (4/96) were illiterate. Regarding HIV-1 acquisition risk, 17% (16/96) reported a known HIV-1 infected sexual partner, 2% (2/96) stated commercial sex work. Half of participants (48/96) reported a stable sexual partner. Despite their young age, almost half of participants (47/96) had at least two previous pregnancies (median number of previous pregnancies=2; range: 0-7).

The median of CD4+ T cell counts in this group was 526 cells/mm3 (range: 82-1324 cells/mm3), 30% (28/96) were in the CDC stages 2 and 3 (CD4+ T cell count<500 cells/mm3). Half of patients (48/96) were ARV-naïve. The ARV-exposed group included women who were previously exposed to a MTCT ARV prophylaxis and then discontinued it after delivery or women on continuous HAART. The median of plasma viral loads was 10,056 copies/mL (range: 299-750,000 copies/mL) among ARV-naïve participants and 5,881 copies/mL (range: 157-507,108 copies/mL) in ARV-exposed patients. Viral loads higher than 100,000 copies/mL were detected in around 8% of naïve patients (4/48) and in 4% of ARV-exposed patients (2/48).

Two cases of probable seroconversion during pregnancy were identified in adolescents who were negative at the first trimester and became seropositive upon retesting late in pregnancy. In our study group one case of HIV-1 MTCT was observed (1/96; 1.0%): the infected infant was born from a 23 years old mother who had a late presentation to the antenatal program, at 32 weeks

gestation. Two days before delivery, maternal CD4 cell count was 714 cells/mm^3 and viral load was 5 688 RNA copies/ml.

In ARV-naïve patients, TDR was identified in 9.3% (CI95%: 3.3%-19.6%) and among ARV-exposed participants, secondary drug resistance was 12.5% (CI95%: 4.7%-25.6%). Single-class and dual-class resistance mutations were observed in both ARV-naïve and ARV-exposed groups. Individual ARV transmitted and secondary resistance profiles among patients are depicted in Table 1. GenBank accession numbers of sequences analyzed in this study are: JN114114-JN114220, JN114222-JN114227, JN114235, JN662426-JN662437, KC249749-KC249766, and KJ658974-KJ659016.

Table 1: Drug resistance mutation profiles of HIV-1 isolates obtained from Brazilian young pregnant women infected with HIV-1

GenBank nº	Age (years)	ARV status	CD4 count (cells/mm³)	Viral load (copies/mL)	Resistance mutations			Resistance level		
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JN114174	20	Exposed	1,082	5,577	T74S	-	-	NFV	-	-
KR559754	22	Exposed	225	19,076	-	M184V	E138Q	ABC, RPV	-	3TC, FTC
JN114142	22	Exposed	NA	NA	T74S	-	-	NFV	-	-
JN114145	22	Exposed	567	3,629	-	M184V	K103N	ABC	-	EFV, NVP, 3TC, FTC

PR: Protease; RT: Reverse transcriptase; NA: Not available; FPV: Fosamprenavir; LPV: Lopinavir; ABC: Abacavir; DDI: Didanosine; ATV: Atazanavir; IDV: Indinavir; SQV: Saquinavir; NFV: Nelfinavir; 3TC: Lamivudine; AZT: Zidovudine; D4T: Stavudine; EFV: Efavirenz; NVP: Nevirapine; TDF: Tenofovir disoproxil fumarate; ETR: Etravirine; RPV: Rilpivirine; FTC: Emtricitabine

DISCUSSION

This study population comprises a representative sample of young HIV-1-infected pregnant women attending a major Brazilian public health antenatal program that screens ~90,000 women per year. Assessment of HIV-1 infection and mother-to-child-transmission potential risk was performed. Main characteristics included a moderate rate of both TDR and secondary drug resistance, low socio-economic and educational level, risky sexual behaviors, including known HIV-1-infected sexual partners, commercial sex work and early exposure to antiretroviral (ARV) drugs. High risk behaviors such as unprotected intercourse with multiple partners has been shown among adolescents, including those perinatally or behaviorally infected[9 10]. This unfavorable combination of features in such a young population highlights failures in educational and preventive public health interventions for HIV infection.

Multiparity in young women indicates that improved policies for unplanned pregnancies at early age are highly needed. Previous studies demonstrated that most pregnancies in young HIV-infected women are unplanned and seropositivity is associated with higher previous pregnancies rates[10 11].

Late presentation to antenatal care reported among this population is also associated with increased risk for MTCT since it jeopardizes re-testing during pregnancy which is important to identify recent seroconversion cases. Previous reports demonstrated that late presentation to antenatal care represents an important barrier for MTCT prevention and is associated with variables such as unplanned pregnancies and fear of HIV testing[12 13]. HIV infection during pregnancy is associated with increased risk of MTCT and higher transmission risk to sexual partners [14]. HIV-1 testing programs should be expanded to include at risk, non-pregnant young women as suggested by a previous study among high risk female adolescents seeking for HIV testing in Brazil[11]. Ideally, HIV-1 diagnosis should precede pregnancy since virological failure at delivery and MTCT are more likely in women diagnosed during pregnancy[15].

Exposure to ARV drugs early in life was observed: more than half of adolescents (20/34) had been previously exposed to ARV drugs. However, patients with high viral loads (500,000-750,000 copies/mL) were observed both among ARV-naïve and ARV-exposed groups, indicating high risk for both MTCT and sexual transmission. A previous study carried out in Haiti among adolescents and young patients receiving ARV treatment demonstrated that after 12 months more than half of the patients presented a detectable viral load, probably associated with low adherence and drug resistance[16]. High viral loads in ARV exposed young women may reflect low adherence to ARV treatment, as previously reported in adolescents, possibly due to rebellious behavior, collateral effects of ARV drugs and psychosocial factors such as depression[6]. Adolescents have shown higher virological failure after six months of HAART presenting higher viral loads[17]. However, in our study viral loads were highly variable and no data on adherence to ARV was available.

HIV-1 infection in adolescents and young adults may include both perinatal and sexual transmission cases since significant advances and broader pediatric access to antiretroviral treatment have declined mortality rates in infected children[2]. Therefore a growing number of perinatally infected children is now reaching adolescence and becoming sexually active[18 19]. Although in this study most patients reported heterosexual risk behavior, perinatal HIV-1 infection, especially among adolescents, could not be excluded, even in recent diagnosed ARV-naïve patients, since maternal health status was not available and these could represent slow-progressor cases.

The moderate rate of TDR observed in this young group raises concern regarding the efficacy of future HIV-1 MTCT prophylaxis and maternal treatment options. Dual-class resistance for the first line combination drugs used for treatment and for MTCT prophylaxis was observed. Previous studies among ARV-naïve pregnant women from this setting reported absence of TDR in 2003 and the 9.3% TDR rate reported here suggests a rising trend[20]. Continuous TDR monitoring should be emphasized, especially with the new policy adopted by the Brazilian Ministry of Health of universal distribution of ARV drugs for all diagnosed cases of HIV-1 infection, regardless of CD4+ T cell counts.

Regarding ARV-exposed patients, a moderate rate of secondary drug resistance was observed, as previously demonstrated in this setting[20]. Temporary exposure to ARV prophylaxis for MTCT (from 14th gestational week until delivery) was recommended in Brazil until 2013, a strategy that could have favored the selection of ARV resistant isolates. In Brazil, secondary resistance rates over 20% were previously reported in HIV-1 pregnant women exposed to ARV prophylaxis for MTCT[21].

CONCLUSION

This study highlights the low socio-educational and economic profiles of highly vulnerable young HIV-1-infected pregnant women in Brazil. High viral loads and late presentation for antenatal care indicate higher risk for MTCT and sexual transmission. Furthermore, moderate levels of drug resistance in ARV-naïve and ARV-exposed pregnant women emphasize the need for continued drug resistance surveillance studies to assure effective MTCT measures and future treatment options. These findings observed among an expressive population of pregnant women in a large country such as Brazil, represent important challenges to achieve the initiatives of the elimination of vertical transmission of HIV/AIDS in Latin America and the Caribbean. The profile of vulnerable HIV-1-infected young pregnant women can help delineate better public health strategies to promote improved educational and preventive measures for HIV infection and unplanned pregnancies as well as early diagnosis of asymptomatic cases and MTCT prophylaxis. Extended studies on larger groups of young HIV-1 infected pregnant women are needed to better define this vulnerable population.

Contributors
Lima, YAR contributed to the study design, data collection, analysis of data and preparation of the final document. Reis, MNG contributed to the analysis of data. Cardoso, LPV contributed to the analysis of data and preparation of the final document. Stefani, MMA contributed to the study design, data analysis and interpretation and for the preparation of the final document. All authors read and approved the final document.

Competing interests
None declared.

Data sharing statement
No additional data available.

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HIV-1 INFECTION AND PREGNANCY IN YOUNG WOMEN IN BRAZIL: SOCIO-ECONOMIC AND DRUG RESISTANCE PROFILES IN A CROSS-SECTIONAL STUDY

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Key words: HIV-1, pregnancy, mother-to-child-transmission, antiretroviral resistance.

Word Count: 2002

ABSTRACT

Objectives: To describe socio-economic and antiretroviral (ARV) drug resistance profiles among young pregnant women infected with HIV-1.
Setting: A public health antenatal program responsible for screening ~90,000 pregnant women per year for nine different infectious diseases in central western Brazil.
Participants: 96 young pregnant women (15-24 years) infected with HIV-1.
Primary and secondary outcome measures: Primary and secondary outcome measures: Standard interviews and blood samples were taken at the time of recruitment, at the first medical appointment after confirmation of diagnosis of HIV-1 infection, before ARV prophylaxis initiation. Clinical and laboratory data were retrieved from medical files. HIV-1 *pol* gene sequences (entire protease/PR, partial reverse transcriptase/RT) were obtained from plasma RNA. ARV resistance mutations (CPR/Stanford HIV-1; International AIDS Society-USA databases) were identified.
Results: The median age was 21 years, most reported <8 years education, 73% were recently diagnosed. Late presentation (after 26 gestational weeks) was observed (19%, 19/96), 49% reported ≥2 previous pregnancies. Possible heterosexual transmission by HIV-1 infected partner (17%) and commercial-sex-work (2%) were reported. The median of CD4 cell count was 526 cells/mm^3, the median viral load was: 10,056 copies/mL in ARV-naïve (48/96), 5,881 copies/mL in ARV-exposed (48/96) patients. Two probable seroconversion cases during pregnancy were identified in adolescents. One mother-to-child-transmission case (1.0%) was observed. Transmitted-drug-resistance among ARV-naïve was 9.3% (CI95%:3.3%-19.6%); secondary drug resistance among ARV-exposed was 12.5% (CI95%: 4.7%-25.6%).
Conclusions: Despite high access to antenatal care, the low socio-economic-educational profiles seen in these young HIV-1-infected women highlight the necessity of improved public health educational and preventive strategies regarding HIV infection and early unplanned pregnancy.

STRENGTHS AND LIMITATIONS OF THE STUDY

- Representative sample of HIV-1-infected pregnant young women attending an antenatal care program that screens ~90,000 women per year;
- Assessment of drug resistance and mother-to-child-transmission potential risk among adolescents and young pregnant women;
- Other studies on larger groups of HIV-1 infected pregnant adolescent/young women from other settings in Brazil and abroad are important to better define this vulnerable population.

INTRODUCTION

The Brazilian AIDS epidemic is considered stable, however in the last decade a significant increase of AIDS cases in younger population (15-19 years) was observed both among males (53.2%) and females (10.5%)[1]. Adolescence is characterized by a series of physical, emotional and social modifications that influence health, especially factors associated with the start of sexual activities. Adolescents (10-19 years) and young adults (20-24 years) are considered highly susceptible to sexually transmitted infections (STIs), including HIV infection[2]. This vulnerability in younger age is certainly associated with engagement in risky sexual behaviors such as early sexual debut, multiple sexual partners and lack of consistent use of preservatives[3]. Young females may also be vulnerable to sexual violence and commercial sex with older men[2].

In Brazil, during the last decade 40% of HIV-1 infected pregnant women were in the age range of 15-24 years[1]. In the central western region, adolescents represented 26% of 54,139 pregnant women screened for HIV-1 infection during antenatal care[4]. Compared to adults, younger HIV-1 infected patients had lower attendance in specialized clinical care, higher HIV-1 sequelae and difficulties in initiation and adherence to HAART[5 6]. Therefore, HIV infection and pregnancy association in young women raises concern as long-term exposure to highly active antiretroviral therapy (HAART) started at early age, and low adherence may lead to increased selection of drug resistant viruses. First line combination drugs used for treatment in Brazil at the time of study includes: tenofovir-TDF + lamivudine-3TC + zidovudine-AZT. MTCT prophylaxis includes: lopinavir/ritonavir-LPV/r + AZT + 3TC [7]. Drug resistance mutations may compromise future therapeutic options and MTCT drug prophylaxis.

In Central Western Brazil, antenatal care is provided free of charge by a comprehensive public health program (Program for the Protection of Pregnant Women-PPPW/Goiás State, central western Brazil). PPPW offers “opt out” serological screening (~90,000 women/year) for HIV-1/2, syphilis, hepatitis B and C, cytomegalovirus, toxoplasmosis, HTLV-1/2, rubella, and Chagas disease, regardless of previous diagnosis for women from 246 municipalities at Goiás State. This is a feasible setting for cross-sectional studies among pregnant women. Prevalence of HIV-1 infection in this population has been recently estimated as 1.59/1000 (95% CI 1.27‰ – 1.96‰)[4]. In this context, this study aimed to describe in ARV-naïve and ARV-exposed young pregnant Brazilian women infected with HIV-1: socio-economic variables, transmitted and secondary ARV resistance mutation profiles and pregnancy outcomes, such as MTCT.

METHODS

This study was delineated as a cross-sectional survey to describe socio-economic, resistance mutation profiles and pregnancy-related outcomes among young pregnant women from Brazil. From 2008 to 2013, young pregnant women (<25 years) attending a local antenatal care program with a positive result for HIV-1 infection were recruited at the time of first medical visit after the diagnosis. Inclusion criteria were: pregnant women with 24 years old or less, ARV-naïve or exposed.

Socio-demographic information was obtained using a standardized questionnaire at the time of recruitment. Clinical and laboratory data were retrieved from medical files at the main local public HIV-1/AIDS reference hospital (Dr. Anuar Auad Tropical Diseases Hospital, Goiânia, Goiás, Brazil). This research protocol was approved by local Ethical Committees (University Hospital, Federal University of Goias, protocol #163/2010; Dr. Anuar Auad Tropical Diseases

Hospital, protocol #003/2011). Written informed consents to participate in the study and to provide blood samples were obtained from all patients except for those younger than 18 years which had informed consents signed by one of the parents/legal guardian.
Blood samples were obtained at the time of recruitment. ARV-naïve patients had blood samples drawn before ARV-prophylaxis initiation. Plasma RNA (QIAamp® Viral RNA Mini Kit, Qiagen, Hilden, Germany) was used for genetic analyses in *pol* gene as previously described[8]. Transmitted drug resistance (TDR) rate among ARV-naïve patients was determined by the Calibrated Population Resistance tool (Stanford Surveillance Drug Resistance Mutations-SDRM). Secondary drug resistance mutations and resistance profiles were defined by the Stanford HIV Drug Resistance Database and by the International AIDS Society-USA major mutation lists. All genotyping profiles obtained in this study were made available to the responsible clinician.
Descriptive analyses of the study variables were performed (Epi Info™ vs.7, CDC, Atlanta, GA, USA). Categorical variables were presented as percentage values and continuous variables were analyzed by the median value. Missing data were not included in the analysis.

RESULTS

During the study period, 96 young pregnant women infected with HIV-1 were enrolled: 35% (34/96) adolescents (15-19 years), 65% (62/96) within 20-24 years of age. Most patients were diagnosed during antenatal screening (69/96, 72%). For women with former diagnosis (28.1%, 27/96), the median time since previous diagnosis was 3 years (1-12 years range). The median gestational age at diagnosis or at enrollment was 18 weeks (range: 8-37 weeks) and 19% (19/96) started antenatal care after 26 weeks of gestation.
Among these patients, low socio-economic profile predominated: 28% (27/96) did not have a formal job, only 2% (2/96) self-reported as students, 18% (17/96) had less than 8 years of formal education, 4% (4/96) were illiterate. Regarding HIV-1 acquisition risk, 17% (16/96) reported a known HIV-1 infected sexual partner, 2% (2/96) stated commercial sex work. Half of participants (48/96) reported a stable sexual partner. Despite their young age, almost half of participants (47/96) had at least two previous pregnancies (median number of previous pregnancies=2; range: 0-7).
The median of CD4+ T cell counts in this group was 526 cells/mm3 (range: 82-1324 cells/mm3), 30% (28/96) were in the CDC stages 2 and 3 (CD4+ T cell count<500 cells/mm3). Half of patients (48/96) were ARV-naïve. The ARV-exposed group included women who were previously exposed to a MTCT ARV prophylaxis and then discontinued it after delivery or women on continuous HAART. The median of plasma viral loads was 10,056 copies/mL (range: 299-750,000 copies/mL) among ARV-naïve participants and 5,881 copies/mL (range: 157-507,108 copies/mL) in ARV-exposed patients. Viral loads higher than 100,000 copies/mL were detected in around 8% of naïve patients (4/48) and in 4% of ARV-exposed patients (2/48).
Two cases of probable seroconversion during pregnancy were identified in adolescents who were negative at the first trimester and became seropositive upon retesting late in pregnancy. In our study group one case of HIV-1 MTCT was observed (1/96; 1.0%): the infected infant was born from a 23 years old mother who had a late presentation to the antenatal program, at 32 weeks gestation. Two days before delivery, maternal CD4 cell count was 714 cells/mm^3 and viral load was 5 688 RNA copies/ml.

In ARV-naïve patients, TDR was identified in 9.3% (CI95%: 3.3%-19.6%) and among ARV-exposed participants, secondary drug resistance was 12.5% (CI95%: 4.7%-25.6%). Single-class and dual-class resistance mutations were observed in both ARV-naïve and ARV-exposed groups. Individual ARV transmitted and secondary resistance profiles among patients are depicted in Table 1. GenBank accession numbers of sequences analyzed in this study are: JN114114-JN114220, JN114222-JN114227, JN114235, JN662426-JN662437, KC249749-KC249766, and KJ658974-KJ659016.

Table 1: Drug resistance mutation profiles of HIV-1 isolates obtained from Brazilian young pregnant women infected with HIV-1

GenBank nº	Age (years)	ARV status	CD4 count (cells/mm³)	Viral load (copies/mL)	Resistance mutations			Resistance level		
					PI	NRTI	NNRTI	Low	Intermediate	High
KC249761	22	Naïve	415	6,227	L90M	M41L, T215C	-	FPV, LPV, ABC, DDI, TDF, AZT, D4T	ATV, IDV, SQV	NFV
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KR559754	22	Exposed	225	19,076	-	M184V	E138Q	ABC, RPV	-	3TC, FTC
JN114142	22	Exposed	NA	NA	T74S	-	-	NFV	-	-
JN114145	22	Exposed	567	3,629	-	M184V	K103N	ABC	-	EFV, NVP, 3TC, FTC

PR: Protease; RT: Reverse transcriptase; NA: Not available; FPV: Fosamprenavir; LPV: Lopinavir; ABC: Abacavir; DDI: Didanosine; ATV: Atazanavir; IDV: Indinavir; SQV: Saquinavir; NFV: Nelfinavir; 3TC: Lamivudine; AZT: Zidovudine; D4T: Stavudine; EFV: Efavirenz; NVP: Nevirapine; TDF: Tenofovir disoproxil fumarate; ETR: Etravirine; RPV: Rilpivirine; FTC: Emtricitabine

DISCUSSION

This study population comprises a representative sample of young HIV-1-infected pregnant women attending a major Brazilian public health antenatal program that screens ~90,000 women per year. Assessment of HIV-1 infection and mother-to-child-transmission potential risk was performed. Main characteristics included a moderate rate of both TDR and secondary drug resistance, low socio-economic and educational level, risky sexual behaviors, including known HIV-1-infected sexual partners, commercial sex work and early exposure to antiretroviral (ARV) drugs. High risk behaviors such as unprotected intercourse with multiple partners has been shown among adolescents, including those perinatally or behaviorally infected[9 10]. This unfavorable combination of features in such a young population highlights failures in educational and preventive public health interventions for HIV infection.

Multiparity in young women indicates that improved policies for unplanned pregnancies at early age are highly needed. Previous studies demonstrated that most pregnancies in young HIV-infected women are unplanned and seropositivity is associated with higher previous pregnancies rates[10 11].

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Exposure to ARV drugs early in life was observed: more than half of adolescents (20/34) had been previously exposed to ARV drugs. However, patients with high viral loads (500,000-750,000 copies/mL) were observed both among ARV-naïve and ARV-exposed groups, indicating high risk for both MTCT and sexual transmission. A previous study carried out in Haiti among adolescents and young patients receiving ARV treatment demonstrated that after 12 months more than half of the patients presented a detectable viral load, probably associated with low adherence and drug resistance[16]. High viral loads in ARV exposed young women may reflect low adherence to ARV treatment, as previously reported in adolescents, possibly due to rebellious behavior, collateral effects of ARV drugs and psychosocial factors such as depression[6]. Adolescents have shown higher virological failure after six months of HAART presenting higher viral loads[17]. However, in our study viral loads were highly variable and no data on adherence to ARV was available.

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Regarding ARV-exposed patients, a moderate rate of secondary drug resistance was observed, as previously demonstrated in this setting[20]. Temporary exposure to ARV prophylaxis for MTCT (from 14th gestational week until delivery) was recommended in Brazil until 2013, a strategy that could have favored the selection of ARV resistant isolates. In Brazil, secondary resistance rates over 20% were previously reported in HIV-1 pregnant women exposed to ARV prophylaxis for MTCT[21].

CONCLUSION

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