Official journal of the Spanish Society of Chemotherapy

Brief report

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Reverse transcriptase and protease inhibitors mutational viral load in HIV infected pregnant women with transmitted drug resistance in Argentina

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ABSTRACT

Objective. Argentina has reported high levels of transmitted drug resistance (TDR), in HIV-infected pregnant women by population sequencing. We aimed to describe, in patients with TDR, the percentage of quasispecies harboring resistance mutations (RAMs) and mutational load (ML).

Patients and Methods. Retrospective study in a cohort of 40 naïve HIV-infected pregnant women, whose pretreatment samples had been genotyped by TRUGENE (period 2008-2014). Samples were re-sequenced with Ultra-deep Sequencing and ML was calculated considering baseline HIV-1 RNA load multiplied by the frequency of quasispecies harboring RAMs.

Results. TDR for NNRTIs, NRTIs and PIs was 17.5% (n=7 patients), 10% (n=4), 12.5% (n=5) respectively. Predominant NNRTI RAMs were K103N (n=4; 10%) and G190A/E/S (n=3; 7.5%). For NNRTIs, 78% of RAMs were present in >93.5% of viral population and ML was >1000 copies/mL (c/mL) for 89%, with a median (IQR) of 8330 c/ml (7738-29796). The following NRTI RAMs were described (per patient: % of quasispecies, ML): T215I (99.7%, 11014 c/ml); D67G (1.28%, 502 c/mL); M41L (79.8%, 88578 c/mL) and M184I (1.02%, 173 c/mL). Most frequent PI-RAMs were I85V, M46I, I50V and L90M (n=2, 5% each). For PIs, quasispecies with RAMs were <2.3% of viral population and ML was <350 c/mL for 77.8% of them.

Conclusion. NNRTI-RAMs are predominant within the viral population, usually exceeding the threshold of 1000 c/mL, indicating potential higher risk of perinatal transmission. Conversely, PI mutations appear mostly as minority variants, with potential lower risk of transmission. Among NRTI, quasispecies harboring RAMs and ML values were variable.

Keywords: drug resistance, HIV, pregnancy,

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Carga viral mutacional para inhibidores de la transcriptasa reversa y proteasa en embarazadas infectadas por VIH con resistencia transmitida en Argentina

RESUMEN

Objetivos. Argentina ha informado de altos niveles de farmacorresistencia transmitida (TDR), en mujeres embarazadas infectadas por el VIH por secuenciación poblacional. Nuestro objetivo fue describir, en pacientes con TDR, el porcentaje de cuasiespecies que albergan mutaciones de resistencia (RAM) y la carga mutacional (ML).

Pacientes y métodos. Estudio retrospectivo en una cohorte de 40 mujeres embarazadas VIH positivas naïve, cuyas muestras pretratamiento habían sido genotipificadas por TRUGENE (período 2008-2014). Las muestras se genotipificaron por secuenciación ultraprofunda y se calculó la ML considerando la carga viral multiplicada por la frecuencia de las cuasiespecies que albergan RAMs.

Resultados. La TDR para ITINN, ITIAN e IP fue del 17,5% (n=7 pacientes), 10% (n=4), 12,5% (n=5) respectivamente. Las RAMs ITINN predominantes fueron K103N (n=4; 10%) y G190A/E/ S (n=3; 7,5%). Para los ITINN, el 78% de los RAM estaban presentes en >93,5% de la población viral y la ML fue >1000 copias/ml (c/mL) para el 89%, con una mediana (IQR) de 8330 c/ml (7738-29796). Se describieron los siguientes RAM de ITIAN (% de cuasiespecies, ML): T215I (99,7%, 11014 c/ml); D67G (1,28%, 502 c/mL); M41L (79,8%, 88578 c/mL) y M184I (1,02%, 173 c/mL). Las IP-RAMs más frecuentes fueron I85V, M46I, I50V y L90M (n=2, 5% cada una). Para los IP, las cuasiespecies con RAMs fueron <2,3% de la población viral y la ML fue <350 c/mL para el 77,8% de ellas.

Conclusión. Los ITINN-RAMs son predominantes dentro de la población viral, por lo general superan el umbral de 1000 c/mL, que indicaría un riesgo potencial más alto de transmisión

D. Cecchini, et al.

Reverse transcriptase and protease inhibitors mutational viral load in HIV infected pregnant women with transmitted drug resistance in Argentina

perinatal. Por el contrario, las mutaciones de IP aparecen principalmente como variantes minoritarias, con un riesgo menor de transmisión. Para ITIAN, las cuasiespecies que albergan RAM y los valores de ML fueron variables.

Palabras clave: resistencia a antirretrovirales, VIH, embarazo

INTRODUCTION

HIV mother-to-child transmission (MTCT) remains high (>4%) in Argentina and several countries of Latin America. Despite massive public health efforts, perinatal HIV transmission occurs often among women who present late in pregnancy, those with non-adherence to prescribed ART (antiretroviral therapy), antiretroviral drug resistance issues, or late entry into care. In Argentina, the provision of antiretroviral drugs is ensured by the state, free of charge, to all HIV-positive patients. ART is mandatory for pregnant women to suppress HIV replication to prevent MTCT [1-5].

The emergence of primary mutations in the viral genome is a major cause of drug resistance, which in turn can lead to treatment failure. Thus, the presence of drug resistance–associated mutations (RAMs) in HIV-infected pregnant women (HPW) may increase the risk of perinatal transmission and impact the women's future treatment options. According to most recent epidemiological surveys, levels of transmitted drug resistance (TDR) in Argentina are high, including pregnant women (>10%, especially to non-nucleoside reverse transcriptase inhibitors, NNRTIs) [6-9]. For such reason, national guidelines currently recommend performing resistance testing to every HPW and with detectable viremia. The high levels of HIV drug resistance observed in HPW might compromise the efficacy of maternal ART and neonatal prophylaxis [2-4].

In Argentina pregnant women´s plasma samples are routinely processed by population sequencing, which only detects mutations present in >20% of viral population [4]. Ultra-deep Sequencing (UDS) technology is not routinely available in our country and allows readings applying different filters for detection within viral population. Applying this technology among HPW´s resistance samples allows not only a more sensible detection of RAMs (1% of viral population), but also can provide with accuracy the proportion of quasispecies harboring each resistance mutation [10-12]. In this context, it would be of major impact to describe prevalence of RAMs among HPW by UDS, contributing to better quality resistance surveillance statistics and providing a quantification of the mutational viral load (ML) of each RAM.

PATIENTS AND METHODS

This retrospective cross-sectional study was performed in plasma samples from a cohort of HPW. Women in this cohort were enrolled in a reference public Hospital in Buenos Aires city from 2008 until 2014 as part an epidemiological pilot survey [7]. All women enrolled had their blood drawn, processed and stored for determination of HIV resistance (baseline pregnancy simple). Of 89 stored samples (one per patient), 40 (45%) samples belong to naïve patients, which are the focus of this analysis. All samples have been processed during the original study by population sequencing (TRUGENE) and plasma aliquots have been stored for future processing. For this study, naïve pregnant women samples were resequenced (reverse transcriptase and protease genes) by UDS, using a protocol provided by WHO and with a MiSeq Illumina equipment. Bioinformatic analysis were performed with HyDRA software applying 1% threshold of sensitivity [13]. The impact of the mutations found on antiretroviral activity were interpreted using the Stanford algorithm [14]. ML was estimated considering viral load value and the obtained frequency of each mutation. The baseline sample per patient was the starting point to calculate the ML: it was calculated in each patient considering HIV-1 RNA load multiplied by the frequency of quasispecies harboring RAMs. For example, a ML will be 100 copies/mL (c/mL) if a patient has a proportion of 10% corresponding to a specific mutation over a baseline viral load of 1000 c/mL [15].
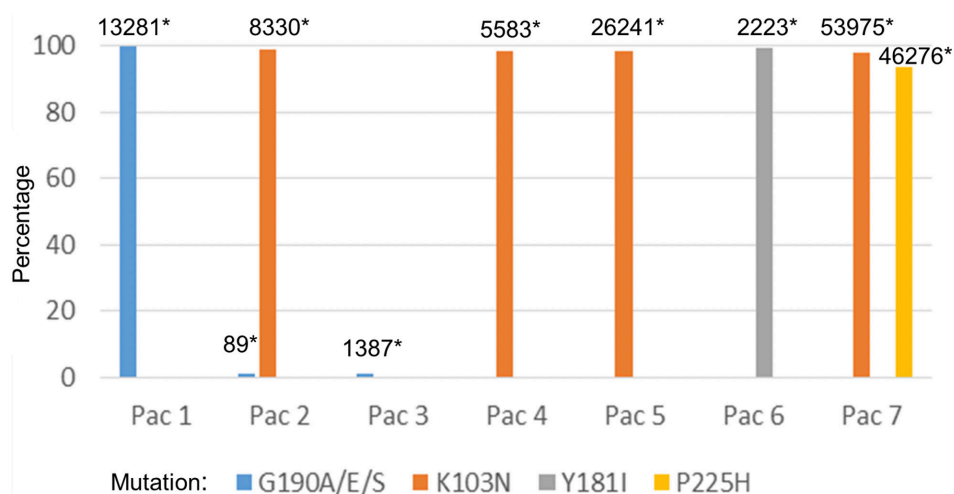
RESULTS

By UDS, TDR for NNRTIs, NRTIs and PIs was 17.5% (n=7 patients), 10% (n=4), 12.5% (n=5) respectively. Predominant NNRTI RAMs were K103N (n=4; 10%) and G190A/E/S (n=3; 7.5%), which confer high level resistance to efavirenz and nevirapine and intermediate to rilpivirine. For NNRTIs, 78% of RAMs were present in >93.5% of viral population (being the exception mutations in G190 position) and ML was >1000 c/mL for 89% of them, with a median (IQR) of 8330 c/ml (7738-29796) as shown in figure 1.

The NRTI RAMs corresponded mostly to thymidine-analog associated mutations (7.5%), which confer variable levels of resistance to zidovudine, stavudine with potential cross resistance with other NRTIs. A low prevalence of mutations in codon 184 (resistance to lamivudine, 2.5%) was observed. The following NRTI RAMs were described (per patient: % of quasispecies, ML): T215I (99.7%, 11014 c/ml); D67G (1.28%, 502 c/mL); M41L (79.8%, 88578 c/mL) and M184I (1.02%, 173 c/mL).

Most frequent PI-RAMS were I85V, M46I, I50V and L90M (n=2, 5% each), all of them with modest impact in the activity of currently available PIs. For PIs, quasispecies with RAMS corresponded to <2.3% of viral population and ML was <350 c/mL for 77.8% of them, with a median (IQR) of 191 c/ml (54-1274).

DISCUSSION

Argentina has unique challenges in achieving HIV mother-to-child transmission rates <2% according to its programmatic objectives [1,16]. Despite late maternal diagnosis and delayed ART initiation remain major causes of perinatal transmission, the high level of transmitted drug resistance constitutes an additional challenge jeopardizing effectiveness of maternal ART and neonatal prophylaxis. In this context, indication of

D. Cecchini, et al.

Reverse transcriptase and protease inhibitors mutational viral load in HIV infected pregnant women with transmitted drug resistance in Argentina

Figure 1 | **Percentage and mutational viral load of viral quasiespecies harboring NNRTI resistance mutations in baseline samples of HIV-infected naïve pregnant women in Buenos Aires, Argentina (2008-2014).**

Pac: patient sample; * mutational viral load (copies/mL)

genotype is mandatory in the adequate management of HPW according to national guidelines [4].

In Argentina, access to genotype during pregnancy may vary according to health coverage and jurisdiction, with population sequencing available only. Despite its utility for epidemiological drug resistance surveys and routine clinical management of HIV-infected patients, it only detects mutations present in >20% of viral population [4].

In this context, our study is the first one in evaluate by UDS the transmitted drug resistance in HIV-infected naive pregnant women in Argentina, with a focus on evaluating mutational viral load of each RAM. This was done based on stored samples (subject to aliquot availability) of our original pilot survey, reported elsewhere [7].

Several aspects of this should be highlighted. Regarding overall prevalence of transmitted drug resistance, RAMs to NN-RTI were predominant (mostly K103N) as in our original study and most Latin American reports [6-9]. Within evaluation of NNRTI-RAMs quasispecies prevalence, our research shows that such mutations are highly predominant in each clinical sample, being frequently above 90% of viral population. This has two important clinical correlations. First, ML for NNRTIs is high, being in almost all cases >1000 c/mL, which is the reference viral load for indication of cesarean section due to high risk MTCT scenario [2-4]. Second, this has an automatic correlation with higher potential of transmissibility and provides empirical evidence of why these mutations are the most frequently transmitted when perinatal infection occurs. This can be attributed not only to their absolute frequency within general HIV population and pregnant women themselves, but also to a high relative frequency within the quasispecies population in each individual patient. This high representation within viral pop-

ulation is explained by the intrinsic nature of this mutations. The NNRTIs are also called allosteric inhibitors, which bind to a hydrophobic pocket distal to the active site within reverse transcriptase (RT) enzyme. The allosteric binding site is not crucial to RT function and is not directly involved in substrate binding or viral DNA synthesis. As a consequence, these mutations do not compromise viral replication capacity. K103N, most frequent NNRTI RAM, has a fitness similar to wild-type virus, a property that justifies its predominance within viral quasispecies population [17]. A notable exception to this are mutations located in G190 position: G190S and G190A both have reduced replication efficiency relative to wild-type and K103N-mutants. Wang et al described that reductions in the rates of RNase H cleavage by these mutants were consistent with their relative reductions in replication efficiency [18]. Our research shows a clinical correlation for this, as in two cases mutations in G190 position had minimal representation within viral population (patient 2 and 3 samples, figure 1). Despite this, in patient 3 sample, mutational viral load was >1000 c/mL due to high maternal viral load value. Considering NNRTI-RAMs were observed in 17.5% of naïve patients in this cohort, these drugs should not be recommended in pregnant women in Argentina and other Latin-American countries. This also should be taken in consideration in other settings (eg. African women), were efavirenz is still widely prescribed.

Conversely, PI mutations had low representation within quasispecies population with low mutational viral load, and potential transmissibility. This is of importance considering these drugs as alternative option for naïve pregnant women. Considering NRTI-RAMS, we found high representation and mutational viral load for M41L and T215I, associated to resistance to thymidine analogs. M184I/V was infrequent in naïve pregnant

D. Cecchini, et al.

Reverse transcriptase and protease inhibitors mutational viral load in HIV infected pregnant women with transmitted drug resistance in Argentina

women. This low prevalence is of importance regarding a potential of starting dual therapy with lamivudine + dolutegravir in naïve non-fertile women in our country. Therefore, updated local studies evaluating prevalence of M184I/V mutations in general population are needed.

Our study has limitations considering its retrospective nature. First, it was undertaken in stored samples from a prior epidemiological survey, therefore, does not reflect an updated prevalence of transmitted drug resistance in this population. In this context, development of epidemiological surveys of transmitted drug resistance with either population or UDS are necessary in our setting [19-20]. Second, the number of samples analyzed and mutations described was limited, penalizing extrapolation to other mutations and other populations.

In conclusion, this research describes that NNRTI-RAMs are predominant within the viral population of pregnant women samples, usually exceeding the threshold of 1000 c/mL, indicating potential higher risk of perinatal transmission. This information is potentially relevant for the clinical management of maternal ART and neonatal prophylaxis.

ACKNOWLEDGEMENTS

CONFLICT OF INTERESTS

The authors declare that they have no conflict of interest.

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D. Cecchini, et al.

Reverse transcriptase and protease inhibitors mutational viral load in HIV infected pregnant women with transmitted drug resistance in Argentina

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