HIV-1 Antiretroviral Drug Resistance in Pregnant Women in Jamaica

A Preliminary Report

II Amarakoon[1, 2], A Ramkissoon[1], R Pierre[3], LM Eyzaguirre[4], JK Carr[4, 5], WA Blattner[3], ME Roye[1]

ABSTRACT

This preliminary report sought to provide insight into the genetic diversity of human immunodeficiency virus drug resistance (HIVDR) in Jamaica. This was done by investigating the genetic diversity associated with drug resistance in pregnant women living with HIV attending antenatal clinics in Kingston, Jamaica. Blood samples were collected and viral RNA were extracted and analysed. The protease and reverse transcriptase (Pro-RT) genes were amplified using the nested polymerase chain reaction (PCR) method. Polymerase chain reaction amplicons were obtained for nine (56%) of 16 patients, of which five (55%) were antiretroviral (ARV) drug naïve and four (45%) were treatment experienced. Three minor protease inhibitor resistant-conferring mutations (A71AT, A71V, A71T) and five mutations conferring high to low-level resistance (K219EK, T69S, K103S, G190A and K103N) were detected in the RT region. More than 50% of the resistance mutations found were detected in ARV drug naïve individuals, implying that viruses are being transmitted with the ARV resistance. These preliminary results will inform the health practitioners of the level of drug resistance that is being transmitted as well as strengthen the need to initiate a national baseline survey on HIVDR in Jamaica.

Keywords: Antiretroviral, drug resistance, HIV, HIV drug resistance, Jamaica, protease, reverse transcriptase

Resistencia a los Medicamentos Antirretrovirales de VIH-1 en Mujeres Embarazadas en Jamaica: Reporte Preliminar

II Amarakoon[1, 2], A Ramkissoon[1], R Pierre[3], LM Eyzaguirre[4], JK Carr[4, 5], WA Blattner[3], ME Roye[1]

RESUMEN

Este reporte preliminar tuvo por objetivo ofrecer una visión de la diversidad genética de la farmacorresistencia del virus de la inmunodeficiencia humana (FRVIH) en Jamaica. El mismo se realizó investigando la diversidad genética asociada con la resistencia a los medicamentos en las mujeres embarazadas que viven con el VIH y que asisten a clínicas prenatales en Kingston, Jamaica. Se recogieron muestras de sangre, y se extrajo y analizó el ARN viral. Los genes de proteasa y transcriptasa inversa (Pro-RT, siglas en inglés) fueron amplificados mediante el método de reacción en cadena (PCR) de la polimerasa anidada. Se obtuvieron amplicones de reacción en cadena de la polimerasa para nueve (56%) de 16 pacientes, de los cuales cinco (55%) no habían sido anteriormente tratados con fármacos antirretrovirales (ARV), mientras que cuatro (45%) tenían experiencia con el tratamiento. Tres mutaciones menores que confieren resistencia a los inhibidores de la proteasa (A71AT, A71V, A71T), y cinco mutaciones que confieren resistencia de nivel alto a nivel bajo (K219EK, T69S, K103S, G190A y K103N) fueron detectadas en la región de RT. Más del 50% de las mutaciones de resistencia encontradas fueron detectadas en individuos sin tratamiento previo de fármacos ARV, lo que implica que los virus se están transmitiendo con resistencia a los medicamentos ARV. Estos resultados preliminares informarán a los profesionales de la salud acerca del nivel de resistencia a los medicamentos que se está transmitiendo, e igualmente reforzará la necesidad de iniciar un estudio

From: [1]Biotechnology Centre, [2]Department of Basic Medical Sciences and [3]Department of Child and Adolescent Health, The University of the West Indies, Kingston 7, Jamaica, [4]Institute of Human Virology, University of Maryland School of Medicine, Baltimore, Maryland, USA and [5]Alabama College of Osteopathic Medicine, Dothan, Alabama, USA.

Correspondence: Dr I Amarakoon, Department of Basic Medical Sciences, The University of the West Indies, Kingston 7, Jamaica, West Indies. E-mail: icolyn.amarakoon@uwimona.edu.jm

DOI: 10.7727/wimj.2014.016

nacional de referencia sobre la farmacorresistencia del virus de la inmunodeficiencia humana (HIVDR) en Jamaica.

Palabras claves: Antirretroviral, farmacorresistencia, VIH, farmacorresistencia del VIH, proteasa, Jamaica, transcriptasa inversa

West Indian Med J 2014; 63 (6): 597

INTRODUCTION

Most Caribbean countries estimated their adult human immunodeficiency virus Type 1 (HIV-1) prevalence based on surveillance of pregnant women (1, 2). In the case of Jamaica, the prevalence rate of 1.7% of the adult population was based on surveillance among pregnant women attending public antenatal clinics (3). For those pregnant women who used private healthcare (approximately 25% of total antenatal visits), the HIV rates were likely to be significantly lower.

Public access to treatment of HIV in Jamaica did not exist before 2002. In the absence of treatment intervention, approximately 25–30% of the babies born to HIV-positive mothers might have been infected, either *in utero*, during delivery or through breastfeeding (4). The partnership with Global Fund grants have enabled many Caribbean countries such as Jamaica to establish public access programmes to antiretroviral therapy (ART). Thus, in Jamaica, programmes were established to prevent HIV transmission from mother-to-child through HIV testing of pregnant women, provide the antiretroviral drugs needed for therapy and chemoprophylaxis as well as encouraging replacement feeds for infants. Since the start of the programme, mother-to-child-transmission (MTCT) rates between 2002 and 2004 showed significant reduction, from over 30% to less than 2%, with the introduction of highly active antiretroviral treatment (HAART) based regimens (5). The introduction of the HAART programme in the pregnant women population has been proven to give the best outcome in enhancing HIV-free survival of infants and improved morbidity and survival of the mother (6). HIV infection does not radically alter the course of pregnancy; however, studies have shown that women living with HIV tended to have a slightly higher risk of adverse pregnancy outcomes such as spontaneous abortions, stillbirths and intrauterine growth retardation. It is therefore imperative that a low and undetectable viral load be maintained to significantly reduce the risk of MTCT (7). Testing for HIV is now widely available and encouraged throughout the country. Despite this, Figueroa (2) postulated that there were still individuals who are reluctant to access services out of fear of testing positive, in addition to the strong stigma associated with HIV infection.

In developed countries, HIV-1 genotypic resistance testing is routinely done to identify mutations associated with drug resistance and since one of the main goals of HIV-1 therapy is to achieve sustainable viral suppression below the limit of detection (less than 50 copies/mL), it is essential to investigate its effectiveness in developing and underdeveloped countries (8). The International AIDS Society-USA panel in 2008 recommended that resistance testing be done whenever possible at the time of HIV infection diagnosis as part of an initial comprehensive patient assessment. In addition, this testing was found to be especially important in cases of virologic failure (9).

The antiretroviral (ARV) drugs that have been made available for first-line treatment of adults are a combination of zidovudine (AZT) and lamivudine (3TC) with efavirenz (EFV) or nevirapine (NVP) while second-line treatment involves the use of a combination of tenofovir (TDF) and emtricitabine (FTC) with lopinavir (LPV/r)/ritonavir (RTV/r). This study therefore sought to provide insight into the genetic diversity of HIV drug resistance (HIVDR). This was investigated by analysing the genome of HIV viruses found in pregnant women living with HIV who were tested for HIV in their antenatal clinics in Kingston, Jamaica. It is anticipated that these preliminary results will inform the Ministry of Health and clinicians of the level of drug resistance that is being transmitted as well as facilitate an expanded baseline survey on HIVDR in Jamaica.

SUBJECTS AND METHODS

Blood samples were collected from pregnant mothers attending the public antenatal HIV clinic of the Ministry of Health for genotypic testing after informed consent. Ethical approval for the study was obtained from the University Hospital of the West Indies/University of the West Indies/Faculty of Medical Sciences Ethics Committee.

Plasma was separated by centrifugation for 10 minutes at 1800 g and stored in 1 mL duplicates at -80 °C until needed for analysis (10). The viral RNA was extracted from 140 µL of plasma using the QIAamp® Viral Mini RNA Assay (QIAGEN) and stored at -80 °C or used immediately for analysis. Reverse transcription was carried out using the SuperScript™ III Reverse Transcriptase kit (Invitrogen) to produce the complementary DNA (cDNA). The protease and reverse transcriptase (Pro-RT) genes were amplified using the nested polymerase chain reaction (PCR) method (11). Using varying concentrations of cDNA as template, the first round of PCR used primer pair Pro5F/RT3474R and the second round of PCR used primer pair Pro3F/ProRT. The PCR amplicons were resolved and visualized on agarose gel with ethidium bromide. The samples which produced bands at the lowest concentration of samples were assumed to

contain the major circulating HIV variant. These samples were further purified and concentrated on a Microcon® Ultracel YM-50 purification column (Millipore) and sequenced. The template DNA was sequenced bidirectionally using BigDye terminator kit (Applied Biosystems Inc) according to the manufacturer's instructions. Eight overlapping primers spanning the entire Pro-RT region of the HIV genome were used during sequencing. The sequences were assembled and edited into the final consensus sequence of the protease and reverse transcriptase region of HIV-1 genome using the Sequencher v 4.7 software (Gene Codes Co).

Sequence searches were done using the HIV database sequence programme (http://www.hiv.lanl.gov/). The Pro-RT genes were analysed for identification of mutations associated with resistance to specific antiretroviral medications. This analysis was carried out using the Stanford University Database (http://hivdb.stanford.edu/hiv).

RESULTS

Polymerase chain reaction amplicons were obtained for nine of 16 patients (56%). The age range for the expectant mothers was 19–43 years with an average CD4 lymphocyte count of 426 cells/μL. All samples were confirmed to be of the HIV-1 B subtype. Among the subjects for which positive PCR amplicons were obtained, five (55%) were ARV drug naïve and four (45%) were treatment experienced. The HIV genome of the drug naïve individuals had 75% of the mutations which conferred drug resistance when compared to the drug experienced individuals.

Analysis of the amplified Pro-RT region of the HIV-1 viral genomes showed three minor protease inhibitor (PI) drug resistance-conferring mutations, A71AT and A71V in two drug naïve subjects and A71T in one treated subject (Table). Other common PI mutations such as L63P were detected in all subjects. Also found common were I13V, M36I and I93L mutations which are normally consensus amino acids found in most non-B subtype.

There were five drug resistance-conferring mutations detected in the reverse transcriptase (RT) region of the genome (Table). Two nucleoside reverse transcriptase inhibitor (NRTI) drug resistance-conferring mutations, K219EK and T69S, were found separately in two individuals. Three non-nucleoside reverse transcriptase inhibitor (NNRTI) drug resistance-conferring mutations, K103S, G190A and K103N, were detected. K103S and G190A were detected in the same subject; K103S conferred high-level resistance to NVP and intermediate to high-level resistance to delavirdine (DLV) and EFV. G190A also conferred high-level resistance to NVP, intermediate-level resistance to EFV and increased DLV susceptibility. It had no effect on etravirine (ETR) susceptibility but was associated with a decreased response to ETR in the DUET studies (12). The K103N mutation was detected in one subject and is associated with high-level resistance to NVP, DLV and EFV; it has been found to have no effect on ETR by itself but may have synergistic effect with L100I and K101P on ETR susceptibility (13).

It was noted that there were between nine and 20 other minor common polymorphisms detected in the Pro-RT regions of the viral genome of each individual. These polymorphisms have been found to be common depending on the treatment therapy; for example, I62V and L63P are common polymorphisms found in PI-treated compared to untreated persons (14).

DISCUSSION

Amplification and sequencing rates were just over 50% for the plasma samples obtained. This suggested that routine handling and storage of specimens could be greatly improved to optimize and preserve the viral RNA and ensure higher rates of amplification. It is also possible that low viral levels in the samples could contribute to the poor amplification rate. Three minor protease resistant-conferring mutations (A71AT, A71V, A71T), and five mutations conferring high to low-level resistance were detected in the RT region (K219EK, T69S, K103S, G190A and K103N). The K103N mutation is one of the most common mutations to occur in patients who fail an NNRTI-based regime (15). Viruses with the K103N mutation are highly resistant but also highly fit and, as such, experts rarely recommend continuing NNRTI medication with this mutation present (15).

Table: Genotypic resistance profile of the protease and reverse transcriptase (Pro-RT) region of the HIV genome illustrating major and minor mutations in addition to other mutations that were detected

Sample ID	NRTI mutations	NNRTI mutations	PI mutations	Other mutations Pro-RT	CD4 count	Antiretroviral treatment
PM1005VJ0040NA	**K103S, G190A**			I13V, L63P, K70R, V179I	209	Drug naive
PM1005VJ0050				I15V, M36I, L63P, L210M	497	Experienced
PM1005VJ0051NA			**A71AT**	M36V, I62V, L63P	260	Drug naive
PM1005VJ0054NA				I13V, L63P	762	Drug naive
PM1005VJ0056				I15V, M36I, L63P, K70R	ND	Experienced
PM1005VJ0066NA	**K219EK**		**A71V**	L63PS, I93L	701	Drug naive
PM1005VJ0055		**K103N**		I13V, L63P, I64L, V77I	403	Experienced
PM1005VJ0067NA	**T69S**			I15V, M36V, K70R	19	Drug naive
PM1005VJ0068			**A71T**	I13V, I62V, L63P, V77I	603	Experienced

NRTI – nucleoside reverse transcriptase inhibitor; NNRTI – non-nucleoside reverse transcriptase inhibitor; PI – protease inhibitor; ND – no data

Protease inhibitor drug-resistant mutations such as the A71T/V mutations are polymorphisms which usually occur in 2–3% of untreated persons and tend to increase in frequency in persons receiving PIs (16). The L63P mutation is a common polymorphism which becomes even more common in persons receiving PIs (14). The NRTI mutations such as the K219EK mutation that was detected causes low-level resistance to AZT as well as a potential low-level resistance to stavudine (d4T). The K219E is believed to decrease AZT and probably d4T susceptibility when present with the K70R or T215Y/F mutations. The T69S mutation has been shown to reduce susceptibility to NRTI such as didanosine (ddI) and d4T (13).

More than 50% of the resistance mutations found were from drug naïve individuals. Drug resistance rarely appears without drug exposure and this kind of primary drug resistance exposure implies that the viruses are being transmitted with ARV resistance (16). The data also showed other minor mutations which may have clinical implications later in life for the patients (17). It is therefore essential to have a drug resistance baseline testing done to have adequate treatment resolutions. However, this type of clinical baseline testing is rarely done in resource limiting settings such as Jamaica. Here in Jamaica, HIVDR mutation detection to inform treatment is not a routine practice.

Antiretroviral drug resistance produced by drug associated mutations in specific regions of the HIV genome has been recognized as one of the main problems during the treatment of HIV-1 infected patients (9). However, it has been shown that ART used in the presence of drug-resistant viruses may increase the risk of HIV-1 mutagenesis which can compromise the efficacy of ARV therapy (18). With the scaling-up of the treatment programmes in Jamaica, the emergence of HIVDR becomes increasingly more apparent (19). Thus, baseline testing should be seen as standard part of treatment.

Scientists have long believed that the genotypic resistance testing in HIV-1-infected individuals is very important since it can effectively guide treatment therapies (20). In resource limiting environments such as Jamaica, there is also the need to determine the long-term cost effectiveness and health consequences in clinical interventions such as genotypic resistance testing in order to effectively and efficiently allocate resources. Genotypic antiretroviral resistance testing has been shown to be effective in guiding the choice of ART and was also shown to be a cost-effective use of the HIV clinical care resources (21).

According to the US AIDS society panel, the testing for drug resistance before initiating any form of therapy may be useful not only for the treatment of experienced patients but also for treatment-naïve patients. Studies have shown that the novel resistance mutations that confer resistance to the older drugs such as AZT are being identified and thus the newer-generation PIs and reverse transcriptase inhibitors have been developed to counteract mutations that confer resistance to these older agents (9). Other studies have proposed that in the absence of viral load testing in resource limiting settings such as Jamaica, patients are likely to stay on failing regimens for prolonged period, because of unavailability of second-line treatment. This factor could contribute to the transmission of drug resistant strains for longer periods than in the higher income countries. Several countries and regions report varying and conflicting reports on the stability of rate of transmission of multidrug-resistance virus (22). This variation in data demonstrates that specific countries and regions need to have localized surveillance systems to monitor transmitted HIV drug resistance, because extrapolation from other countries' data may be misleading. This preliminary data confirms that an expansion of this surveillance system to monitor HIV drug resistance is integral for Jamaica and the Caribbean region.

According to the World Health Organization (WHO), it is not only important to know what the transmission resistant rates are, but it is also important, especially in a resource limiting setting, that a baseline resistance survey as well as a previous ARV experience survey be done before the start of first-line ART at the main treatment sites. The World Health Organization also recommended that a systematic evaluation of HIVDR be done to assess the ART programme at treatment sites (23). The result of all these assessments would provide the evidence needed to strengthen appropriate prescribing, drug supply continuity adherence and HIV transmission prevention and possibly guide the ART policy here in Jamaica. These data are limited by sample methodology (convenience sampling) and a small sample size; however, the data provided evidence that prompts further expansion to a surveillance system as prescribed by the WHO.

This report highlighted the evolving issue of transmitted drug resistance and also the possible role of minor drug resistance mutations in the ART naïve which could compromise the efficacy of the ART regimes in Jamaica.

ACKNOWLEDGEMENT

This work was funded in part by a Fulbright fellowship to II Amarakoon and the Institute of Human Virology, University of Maryland, Baltimore, Maryland, USA. The project described was supported by Grant Number NIH D43-TW001041 from the Fogarty International Center. The content is solely the responsibility of the authors and does not necessarily represent the official views of the Fogarty International Center of the National Institutes of Health.

REFERENCES

1. UNAIDS/WHO. Guidelines for conducting HIV sentinel serosurveys among pregnant women and other groups. Geneva: WHO/UNAIDS; 2003 [cited 2013 Jun 19]. Available from: http://www.who.int/hiv/pub/surveillance/anc_guidelines/en
2. Figueroa JP. The HIV epidemic in the Caribbean: meeting the challenges of achieving universal access to prevention, treatment and care. West Indian Med J 2008; **57:** 195–203.

3. UNAIDS. UNGASS country progress report – Jamaica. UNAIDS 2012 [cited 2013 Apr 12]. Available from: http://www.unaids.org/en/dataanalysis/knowyourresponse/countryprogressreports/2012countries/ce_JM_Narrative_Report [1].pdf
4. Connor EM, Sperling RS, Gelber R, Kiselev P, Scott G, O'Sullivan MJ et al. Reduction of maternal-infant transmission of human immunodeficient virus type 1 with zidovudine treatment. N Engl J Med 1994; **331:** 1173–80.
5. Christie CDC, Steel-Duncan J, Palmer P, Pierre R, Harvey K, Johnson N et al. Paediatric and perinatal HIV/AIDS in Jamaica: an international leadership initiative, 2002–2007, West Indian Med J 2008; **57:** 203–15.
6. Johnson N, Palmer P, Samuels LA, Morgan O, Onyonyor A, Anderson AM et al. Evolving care of HIV-infected pregnant women in Jamaica from nevirapine to HAART. West Indian Med J 2008; **57:** 216–22.
7. Coyne KM, Jones R, Hawkins D. Treating pregnant women with highly active antiretroviral therapy. Future HIV Therapy 2007; **1:** 215–22.
8. Hammer SM, Saag MS, Schechter M, Montaner JSG, Schooley RT, Jacobsen DM et al. Treatment for adult HIV infection: 2006 recommendations of the International AIDS Society-USA panel. JAMA 2006; **296:** 827–43.
9. Hirsch MS, Gunthard HF, Schapiro JM, Brun-Vezinet F, Clotet B, Hammer SM et al. Antiretroviral drug resistance testing in adult HIV-1 infection: 2008 recommendations of an International AIDS Society – USA Panel. Clin Infect Dis 2008; **47:** 266–85.
10. Caron M, Makuwa M, Souquière S, Descamps D, Brun-Vézinet F, Kazanji1 M. Human immunodeficiency virus type 1 seroprevalence and antiretroviral drug resistance-associated mutations in miners in Gabon, central Africa. AIDS Res Hum Retroviruses 2008; **24:** 1225–8.
11. Nadai Y, Eyzaguirre LM, Sill A, Cleghorn F, Nolte C, Carr JK et al. HIV-1 epidemic in the Caribbean is dominated by subtype B. PLoS One 2009; **4:** e4814. doi: 10.1371/journal.pone.0004814.
12. Xu H, Quan Y, Brenner BG, Bar-Magen T, Oliveira M, Schader SM et al. Human immunodeficiency virus type 1 recombinant reverse transcriptase enzymes containing the G190A and Y181C resistance mutations remain sensitive to etravirine. Antimicrob Agents Chemother 2009; **53:** 4667–72.
13. Shafer RW, Schapiro JM. HIV-1 drug resistance mutations: an updated framework for the second decade of HAART. AIDS Rev 2008; **10:** 67–84.
14. Scherrer AU, Ledergerber B, von Wyl V, Böni J, Yerly S, Klimkait T et al. Minor protease inhibitor mutations at baseline do not increase the risk for a virological failure in HIV-1 subtype B infected patients. PLoS One 2012; **7:** e37983. doi: 10.1371/journal.pone.0037983.
15. Deeks SG. International perspectives on antiretroviral resistance. Non-nucleoside reverse transcriptase inhibitor resistance. J Acquir Immune Defic Syndr 2001; **26 (Suppl 1):** S25–33.
16. Shafer RW, Rhee S-Y, Pillay D, Miller V, Sandstrom P, Schapiro JM et al. Human immunodeficiency virus type 1 protease and reverse transcriptase mutations for drug resistance surveillance. AIDS 2007; **21:** 215–23.
17. Johnson JA, Li J-F, Wei X, Lipscomb J, Irlbeck D, Craig C et al. Minority HIV-1 drug resistance mutations are present in antiretroviral treatment-naïve populations and associate with reduced treatment efficacy. PLoS Med 2008; **5:** e158. doi: 10.1371/journal.pmed.0050158.
18. Mansky LM, Pearl DK, Gajary LC. Combination of drugs and drug-resistant reverse transcriptase results in a multiplicative increase of human immunodeficiency virus type 1 mutant frequencies. J Virol 2002; **76:** 9253–9.
19. Hamilton C-LC, Eyzaguirre LM, Amarakoon II, Figueroa P, Duncan J, Carr JK et al. Analysis of protease and reverse transcriptase genes of HIV for antiretroviral drug resistance in Jamaican adults. AIDS Res Hum Retroviruses 2012; **28:** 923–7.
20. Kan VL, Gale HB, Shinol RC. Response to Waters et al. "Successful use of genotypic resistance testing in HIV-1-infected individuals with detectable viraemia". AIDS 2006; **20:** 1991–2.
21. Weinstein MC, Goldie SJ, Losina E, Cohen CJ, Baxter JD, Zhang H et al. Use of genotypic resistance testing to guide HIV therapy: clinical impact and cost-effectiveness. Ann Intern Med 2001; **134:** 440–50.
22. Bennett D, Myatt M, Bertagnolio S, Sutherland D, Gilks C. Recommendations for surveillance of transmitted HIV drug resistance in countries scaling up antiretroviral treatment. Antivir Ther 2008; **13 (Suppl 2):** 25–36.
23. Jordan MR, Bennett DE, Bertagnolio S, Gilks CF, Sutherland D. World Health Organisation surveys to monitor HIV drug resistance prevention and associated factors in sentinel antiretroviral treatment sites. Antivir Ther 2008; **13 (Suppl 2):** 15–23.