Presence of HIV drug resistance in antiretroviral therapy-naive and -experienced patients from Papua New Guinea

Janet Gare1–3*, Claire E. Ryan1, Matthew David2, Diana Timbi2, Petronia Kaima4, Zure Kombati5, Ulato Imara6, Angela Kelly-Hanku2,7, Peter M. Siba2, Suzanne M. Crowe1,3 and Anna C. Hearps1,3

1Centre for Biomedical Research, Burnet Institute, Melbourne, Victoria, Australia; 2Sexual and Reproductive Health Unit, Papua New Guinea Institute of Medical Research, Goroka, Eastern Highlands Province, Papua New Guinea; 3Central Clinical School, Monash University, Melbourne, Victoria, Australia; 4HIV/STI Highlands Region, National Department of Health, Mt Hagen, Western Highlands Province, Papua New Guinea; 5Pathology Department, Mt Hagen General Hospital, Mt Hagen, Western Highlands Province, Papua New Guinea; 6Michael Alpers Clinic, Goroka General Hospital, Goroka, Eastern Highlands Province, Papua New Guinea; 7School of Public Health and Community Medicine, University of New South Wales, Sydney, New South Wales, Australia

*Corresponding author. Centre for Biomedical Research, Burnet Institute, Melbourne, Victoria, Australia. Tel: +613-9282-2298; Fax: +613-9282-2100; E-mail: jgare@burnet.edu.au

Received 3 January 2014; returned 21 January 2014; revised 28 February 2014; accepted 7 March 2014

Objectives: The optimal benefits of antiretroviral therapy (ART) can be compromised by the emergence of HIV drug resistance (HIVDR) resulting in treatment failure. ART was introduced in Papua New Guinea (PNG) in 2004, yet biological data on HIVDR are lacking. The aim of the study was to investigate levels of HIVDR in ART-naive and -experienced patients in PNG.

Methods: We recruited, interviewed and collected blood from 108 ART-naive and 102 ART-experienced patients from two Highlands provinces of PNG. Dried blood spots were tested for HIVDR from all patients with detectable plasma viral load of ≥200 copies/mL using established in-house assays.

Results: The PCR amplification success was 90.6% (n=96) and 66.7% (n=12) using dried blood spots from ART-naive and -experienced patients, respectively. Transmitted drug resistance was detected in 2.1% (n=2) of samples from ART-naive patients; acquired drug resistance was detected in 50% (n=6) of samples from ART-experienced individuals.

Conclusions: Our data showed that transmitted drug resistance in PNG is low and acquired drug resistance is higher with 12.7% of the ART-experienced patients failing treatment. As ART access is rapidly expanding in PNG, monitoring of drug resistance is paramount for early detection of treatment failure.

Keywords: dried blood spots, transmitted drug resistance, acquired drug resistance, treatment failure

Introduction

As of 2012, the estimated HIV prevalence in Papua New Guinea (PNG) is 0.52%1 with higher HIV prevalence (2.3%–19%) in at-risk populations2–3 and major urban sexual health/tuberculosis clinics.3–4 The PNG epidemic is largely driven by heterosexual transmission, with 84% of infections occurring in individuals aged 15–44 years; two-thirds in females aged 15–34 years.5 Antiretroviral therapy (ART) was introduced in PNG in 2004. By late 2012, treatment coverage was estimated at 74% (n=11 764).6 First-line therapy in PNG is in accordance with WHO guidelines. Second-line therapy is uncommon in PNG.

Although ART roll-out in PNG has been rapid and successful, ART monitoring services remain non-existent in most of the country. Flow cytometric CD4 testing is only sporadically available, although point-of-care CD4 testing is currently being expanded. Viral load (VL) testing is being piloted in the capital, Port Moresby. Currently, ART monitoring largely relies on clinical assessments and basic biochemistry/haematology tests.

Long-term ART is associated with the emergence of both transmitted drug resistance (TDR), increasing the risk of early virological failure in newly infected individuals,6–8 and acquired drug resistance (ADR), necessitating a change in the ART regimen. Prevention of HIV drug resistance (HIVDR) is vital in countries where there is limited choice of drug combinations. Although reported TDR in low- to middle-income countries (LMICs) is currently <5% in most regions, effective surveillance is of critical public health importance to ensure sustained effectiveness of first-line treatment. Routine HIVDR testing is not feasible in many LMICs, thus WHO has created early warning indicators (EWI) to capture...
Patients and methods

Patient recruitment

With institutional ethics approvals, consenting HIV+ patients (16–60 years) were recruited at the Michael Alpers and Tininga clinics in Goroka (EHP) and Mt Hagen (WHP), respectively, during July 2010 to January 2011. ART-experienced participants were recruited irrespective of their time on ART. Venous blood (10 mL) was collected.

Specimen preparation

Dried blood spots (DBS) were prepared by spotting five 50 μL aliquots of blood onto Whatman 903 protein-saver cards and drying overnight at room temperature. Plasma was separated from remaining blood by centrifugation. DBS were stored in zip-lock bags containing moisture absorbers at −20°C until transportation to the PNG Institute of Medical Research in Goroka, EHP, where both plasma and DBS were stored at −80°C until analysed.

HIV VL testing

HIV VL was determined using ExaVir™ Load Version 3 (Cavidi AB, Uppsala, Sweden)11 according to the manufacturer’s instructions. The assay has a lower detection limit of 200 HIV RNA copies/mL equivalents.

HIVDR testing

HIVDR testing was performed on all DBS with a plasma VL of ≥200 copies/mL at the WHO Regional Laboratory for HIV Drug Resistance, Burnet Institute, Australia as previously described12 except this study amplified a shorter region of reverse transcriptase (RT) (amino acids 1–250), with the same forward primers for RT–PCR and nested PCR, but different reverse primers (3402rev, 5′-CTGTTAGTGCTTTGGYTCC-3′; and 3346rev, 5′-CTGSGATAAATCGACTTGCCC-3′) in the RT–PCR and nested PCR, respectively). The 1.2 kb nested PCR amplicon was sequenced as previously described,12 except 3346rev was used as the RT reverse sequencing primer. In samples that failed to amplify after two attempts, amplification of the RT regions was attempted using a version of the French National Agency for AIDS Research (ANRS) assay13 modified to improve compatibility of primers with the HIV strains in PNG. The sequences of the modified primers were MU4_a (5′-CTGTTAGTGCTTTGGYTCC-3′) for RT–PCR and A(35)_b (5′-TTGTTACCTTTAATTCCATTTG-3′) for nested PCR. Sequencing was performed in-house using Big Dye Terminator Version 3.1 followed by electrophoretic separation at the Gandel Charitable Trust Sequencing Centre, Monash Institute of Medical Research, Australia.

Drug resistance interpretation

Drug resistance interpretations for ADR and TDR were performed using the Stanford University online tools Sequence Analysis14 and Calibrated Population Resistance Version 6.0, respectively.15 The protease region was excluded from analysis as protease inhibitor use in PNG was absent from our study cohort.

Results

Study population

A total of 210 HIV+ patients were recruited from two sites (Tininga clinic, n = 100; Michael Alpers clinic, n = 110), consisting of 102 ART-experienced patients and 108 treatment-naive patients. There were no significant differences between the two clinics in terms of treatment regimens, sex and age of the study participants; therefore, the data were combined for analysis. Table 1 summarizes cohort demographic and clinical characteristics.

Patient clinical information

The majority of ART-experienced participants (72.5%, n = 74) were diagnosed with HIV ≥1 year prior to recruitment; the majority (90.7%, n = 98) of ART-naive patients were diagnosed within 1 year post diagnosis. There were no significant differences between the two clinics in regards to the ART-naive patients who were ≥18 years of age who were recruited, the data were therefore combined for analysis. There were no significant differences between the two clinics in terms of treatment regimens, sex and age of the study participants; therefore, the data were combined for analysis. Therefore, the data were combined for analysis. Table 1 summarizes cohort demographic and clinical characteristics.
By WHO clinical staging, 50% (n = 51) of ART-experienced patients had disease stages III/IV on ART initiation and on recruitment had been receiving ART for a median of 19 months (range: 0.5–63 months). Participants receiving ART for <6 months were excluded from subsequent analysis. More than three-quarters (78.0%, n = 64) of ART-experienced participants had an undetectable VL (<200 copies/mL). Of the 22.0% (n = 18) with detectable VL (≥200 copies/mL), 13 were failing treatment according to the WHO definition of virological failure (on ART >6 months, VL >1000 copies/mL). Of the ART-naive participants with available VL data, 97.9% had a detectable VL (Table 1). The regimens used for treatment-experienced patients are shown in Table 1.

**ADR in ART-experienced participants**

HIV RT–PCR was attempted on 18 DBS from patients with detectable VL. The median time on ART was 23.5 months (range: 6–63) and the median VL was 4,370 copies/mL (range: 210–263,260). Of these 18 DBS, 66.7% (n = 12) were amplified, of which 50.0% (n = 6) had major mutations to reverse transcriptase inhibitors (RTIs) (Table 2). The median time on ART for the patients with ADR was 35.5 months (range: 19–63) and the median VL was 20,133 copies/mL (range: 287–246,237). All had one or more HIVDR mutations relevant to both classes of RTIs. The most common nucleotide reverse transcriptase inhibitor (NRTI) drug resistance mutations (DRMs) were M184V (83.3%, n = 5) and D67N (50.0%, n = 3). K103N was the most common non-nucleotide reverse transcriptase inhibitor (NNRTI) mutation, occurring in 50.0% (n = 3) of samples harbouring HIVDR, followed by Y181C and G190A each with 33.3% (n = 2).

**TDR in ART-naive participants**

We genotyped 106 DBS from ART-naive participants including 95 with detectable VL and without VL results and successfully genotyped 90.6% (n = 96). Of these, 2.1% (n = 2) had major DRMs conferring resistance to RTIs (G190A in one patient; Y181C in another patient).

### Discussion

This is the first study on HIVDR in PNG. We found 2.1% TDR in 96 ART-naive patients, whilst 6 out of 12 (50%) ART-experienced patients for whom genotyped data were generated had ADR mutations relating to one or more first-line therapy drug.

The majority (78.0%) of ART-experienced patients had undetectable plasma VL, suggesting participants are adhering to treatment with maintained virological suppression. Eighteen ART-experienced patients had detectable VL, of which 66.7% were successfully genotyped and half of these (50%) had HIVDR mutations to both NNRTIs and NRTIs. The most frequently occurring NRTI mutation, M184V, confers high-level resistance to lamivudine, emtricitabine and abacavir. Only lamivudine is in common use in PNG. The detection of NNRTI mutation K103N, conferring high-level resistance to nevirapine and efavirenz, indicates there are HIV-infected patients in PNG requiring second-line ART regimens. Thirteen (12.7%) ART-experienced patients were failing therapy according to the WHO criteria.

The prevalence of TDR in ART-naive patients was low (2.1%). In countries where ART has been available for longer, the TDR frequency ranges from 1% to 14%. TDR in Asia is reported to be <5%. The low TDR rate in PNG is reassuring, providing a useful baseline to monitor TDR in the future.

A minority of DBS from patients with detectable VL failed to amplify, most likely due to low VL, but possibly due to poor DBS quality or primer mismatch.

There are limitations in the design of our study. WHO TDR survey guidelines include targeted recruitment of recently infected individuals; these were not used in this study for practical reasons. Although the median age for treatment-naive female participants in our study was 25 years (fulfilling WHO criteria), males were older (median age 40 years), reflective of the PNG HIV population. Eighty-one percent of the ART-naive participants were diagnosed with HIV within 4 months of recruitment, but many presented with advanced disease suggesting longer infection. As drug-resistant viruses in patients with TDR commonly revert to wild-type when not under drug pressure, our data may underestimate the prevalence of TDR in PNG.

---

**Table 2. Clinical characteristics and drug resistance mutations detected in treated patients**

<table>
<thead>
<tr>
<th>Study ID</th>
<th>Viral load (copies/mL)</th>
<th>Time since diagnosis (months)</th>
<th>Time on ART (months)</th>
<th>WHO disease clinical stage</th>
<th>ART regimen (d4T/3TC/NVP)</th>
<th>Subtype</th>
<th>RTI drug resistance mutations</th>
</tr>
</thead>
<tbody>
<tr>
<td>YL016</td>
<td>16482</td>
<td>84</td>
<td>44</td>
<td>II</td>
<td>ZDV/3TC/NVP</td>
<td>C</td>
<td>D67N, M184V</td>
</tr>
<tr>
<td>YL049</td>
<td>246237</td>
<td>NA</td>
<td>63</td>
<td>III</td>
<td>ZDV/3TC/NVP</td>
<td>B</td>
<td>M41L, D67N, K70R, M184V, K219E</td>
</tr>
<tr>
<td>YL050</td>
<td>NA</td>
<td>NA</td>
<td>19</td>
<td>III</td>
<td>d4T/3TC/NVP</td>
<td>C</td>
<td>M41L, D67N, M184V, T215FY</td>
</tr>
<tr>
<td>YL141</td>
<td>287</td>
<td>156</td>
<td>21</td>
<td>II</td>
<td>ZDV/3TC/NVP</td>
<td>C</td>
<td>K65R, Y188C</td>
</tr>
<tr>
<td>YL154</td>
<td>97570</td>
<td>48</td>
<td>32</td>
<td>I</td>
<td>ZDV/3TC/NVP</td>
<td>C</td>
<td>M184V</td>
</tr>
<tr>
<td>YL172</td>
<td>20133</td>
<td>111</td>
<td>39</td>
<td>IV</td>
<td>d4T/3TC/NVP</td>
<td>C</td>
<td>M184V, K103N, Y181C, G190A</td>
</tr>
</tbody>
</table>

NA, not available; ZDV, zidovudine; d4T, stavudine; 3TC, lamivudine; NVP, nevirapine.

*Previous drug combination that was administered to the patient prior to recruitment.
We found that whilst the majority of PNG patients are responding well to first-line treatment, a significant number of patients are failing therapy. This emphasizes the need for improved access to VL testing for early detection of treatment failure in ART-experienced patients and for continued education on treatment adherence to minimize the development of ADR.

Acknowledgements
These data have been presented at the Australasian HIV/AIDS Conference, Darwin, Australia, 2013 (Poster #31).

We wish to thank our study participants for their involvement in the study. Our gratitude goes to the field assistants: Mrs Zofike Kombati, Mrs Yvonne Minemba and Mr Jeremy Songovare. We also wish to extend our appreciation to the clinical staff at the Tininga clinic and Michael Alpers clinic for their assistance in retrieving patient clinical notes. We gratefully acknowledge the contribution to this work of the Victorian Operational Infrastructure Support Program.

Funding
This work was supported by the National AIDS Council Secretariat (grant number RES090012). J. G. is a recipient of the PNG-Australian Development Scholarship. S. M. C. is a recipient of an NHMRC Principal Research Fellowship.

Transparency declarations
None to declare.

References