Trends of transmitted drug resistance of HIV-1 and its impact on treatment response to first-line antiretroviral therapy in Taiwan

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Objectives: To determine the impact of transmitted drug resistance (TDR) of HIV-1 on treatment outcome in areas where routine testing for drug resistance mutations may not be available before combination antiretroviral therapy (cART) is initiated.

Methods: Genotypic resistance assays were performed on HIV isolates from archived blood samples obtained from 1349 antiretroviral-naive HIV-1-infected patients in Taiwan from 2000 to 2010. Resistance mutations were interpreted with the use of the HIVdb program of the Stanford University HIV Drug Resistance Database. The genotypic sensitivity score (GSS) of the regimens prescribed was calculated. A matched case–control study was conducted to assess the impact of TDR on treatment outcomes.

Results: The overall prevalence of TDR to any antiretroviral agent was 8.0%, declining from 12.3% in 2003–06 to 5.1% in 2007–10. In the matched case–control study, 31 patients with high- or intermediate-level resistance, 16 with low-level resistance and 89 controls were enrolled. Compared with regimens with GSS >2.5, initiation of regimens with GSS ≤2.5 was associated with a higher treatment failure rate (39.3% versus 15.7%, P=0.02) and shorter time to treatment failure (log-rank P<0.001). In patients receiving regimens with GSS ≤2.5, protease inhibitor-based regimens were less likely to result in treatment failure, compared with non-nucleoside reverse-transcriptase inhibitor-based regimens (hazard ratio 0.26, 95% CI 0.06–1.12, P=0.07).

Conclusions: In Taiwan the prevalence of TDR of HIV-1 strains declined and stabilized between 2007 and 2010. Receipt of antiretroviral regimens with GSS ≤2.5 was associated with poorer treatment responses than regimens with GSS >2.5.

Keywords: genotypic resistance mutations, highly active antiretroviral therapy, HIV infection

Introduction

Drug-resistant strains of HIV-1 emerged soon after antiretroviral therapy (ART) was introduced.1 With the widespread use of combination antiretroviral therapy (cART), the rate of resistance has increased rapidly, especially to nucleoside/nucleotide and non-nucleoside reverse-transcriptase inhibitors (NRTIs and NNRTIs, respectively).2,3 Transmission of the resistance mutations has been observed in 6%–16% of antiretroviral-naive patients in Europe and North America,4–8 and has been shown to compromise the effectiveness of first-line ART.9–12 Current antiretroviral treatment guidelines published by the Department of Health and Human Services (USA) recommend that genotypic resistance testing be performed in all newly diagnosed HIV-infected individuals.13,14 Based on drug-resistance testing results, patients with transmitted drug resistance (TDR) have been shown to achieve...
similar immunological and virological responses to initial cART, compared with those without resistance.15,16

However, data on the effect of TDR on treatment outcome remain scarce, and some studies have yielded inconsistent results, partly because of the different thresholds for the effect of TDR, either the level of resistance or the absolute numbers of drug-resistant mutations.4,5,17–19 Furthermore, the clinical impact of TDR has never been described in Asia, Africa or developing countries where the largest proportion of HIV-infected individuals of the world live. With increasing access to ART and increased implementation of short-term peripartum prophylaxis in recent years, primary drug resistance has become a growing concern in developing countries, especially resistance to NNRTIs.20,21 NNRTI-based regimens have been reported to be particularly vulnerable to treatment failure in patients with TDR, compared with regimens containing boosted protease inhibitors (PIs).12

In Taiwan, cART has been provided free of charge since its introduction in April 1997, but routine drug-resistance testing has not been available to clinicians. At the end of June 2011, there were 17,823 people living with HIV in Taiwan, and about 40% of them received cART.22 The prevalence of TDR in Taiwan increased between 1999 and 2006, and the overall prevalence was 9.4%.23 Recently, a decline in antiretroviral resistance mutations to NRTIs and PIs has been reported, following increasing use of more potent NRTIs and ritonavir-boosted PI combinations.24,25 In the present study, we aimed to update the data on the prevalence of TDR of HIV-1 and assess the treatment responses in the first 2 years of first-line cART in patients with TDR in Taiwan.

Patients and methods

Study population and setting

HIV-infected Taiwanese patients receive HIV care according to local treatment guidelines at designated hospitals around Taiwan. Medical costs related to HIV care, including ART and management for opportunistic illnesses (OIs), are totally reimbursed by the government. Drug-resistance testing, however, was only available in two medical centres and restricted to patients experiencing treatment failure and to pregnant women. Since 2000, blood samples have been collected and stored from all antiretroviral-naive HIV-1-infected patients at the National Taiwan University Hospital (NTUH), the largest designated hospital in Taiwan and the only site where surveillance of TDR has been systematically conducted.23,26 This study was approved by the Research Ethics Committee of the hospital, and subjects gave written informed consent.

Genotypic resistance testing and genotypic sensitivity score (GSS)

In this study, genotypic resistance assays were performed retrospectively on isolates from the archived blood samples taken before initiation of ART. The genotypic resistance assays were performed as described previously.23,26 Antiretroviral resistance mutations were identified using the HIVdb program of the Stanford University HIV Drug Resistance Database (http://hivdb.stanford.edu), according to the drug-resistance mutation list of the International AIDS Society-USA Consensus Guidelines.27 Multidrug resistance (MDR) was defined as genotypic resistance to more than one class of antiretroviral agent.

In this study, the Stanford HIVdb algorithm, which assigns GSSs of 1.00, 0.75, 0.50, 0.25 and 0.00 to the five levels of resistance (susceptible, potential low-level, low-level, intermediate-level and high-level resistance, respectively), was used to interpret the genotypic resistance data. The GSS of each regimen was calculated as the sum of the individual scores for the specific agents prescribed, and these have been shown to be valuable in predicting virological treatment outcome.28,29

Case–control study

The case group included antiretroviral-naive patients who had mutations resulting in designation as low-, intermediate- or high-level resistance to any drug that was contained in the regimen prescribed. The control group was a subgroup from antiretroviral-naive patients who had wild-type strains by genotypic testing; these were matched (2:1) to each case patient according to age, sex, baseline plasma HIV RNA load (PVL) and CD4 cell count. Both case and control groups had consecutive measurements of PVL and CD4 cell counts while on cART. All the patients were followed for 96 weeks after initiation of cART or until April 2011.

Similarly to what has been observed in a cohort study30 switch of regimen after initial cART is very common. Therapy changes involving substitutions within NRTI class or within 30 days of treatment due to toxicities or simplification were assumed inconsequential and ignored with respect to resistant cases. Antiretroviral adherence was assessed by patient self-report and by case managers. Missed doses >5% in the past week were classified dichotomously as poor adherence.31

Treatment outcomes

Virological failure was defined as detectable viral load (≥200 copies/mL) after 6 months of treatment or regimen modifications due to inadequate viral response after 4 weeks of treatment (<1 log10 decrease), with the date of first viral load ≥200 copies/mL or the date of regimen modifications as the failure date.12,13,19,31,33 Viral suppression was defined as viral load <200 copies/mL.5,34 The primary outcome was treatment failure, including virological failure and AIDS-related death. The secondary outcomes were time to treatment failure, time to viral suppression and new onset or recurrence of AIDS-related OIs during treatment. Patients were censored if they died from non-AIDS events, were lost to follow-up or had a regimen switch between an NNRTI and a PI beyond 30 days after cART was initiated.

Statistical analysis

Data were analysed using SPSS software (IBM Corporation. NY, USA). Categorical data were analysed using \( \chi^2 \) test or Fisher’s exact tests, as appropriate, and continuous variables were compared using the Wilcoxon test. Time to treatment failure and time to viral suppression were assessed with Kaplan–Meier plots. All variables with \( P < 0.2 \) by univariate analysis were selected for subsequent multivariate analysis. Multivariate analysis was performed using logistic regression analysis and the Cox proportional hazard model. All tests were two-tailed and a \( P \) value <0.05 was considered significant.

Results

Population characteristics and prevalence of resistance

From 2000 to 2010, blood specimens from 1349 HIV-infected antiretroviral-naive patients were subjected to genotypic resistance tests. A total of 108 patients (8.0%) were infected with HIV-1 strains that harboured resistant mutations to any drug. The mutations detected in these individuals are summarized in Table S1 (available as Supplementary data at JAC Online). The prevalence of TDR to any antiretroviral agent increased from
6.1% in the period 2000–02 to 12.3% in 2003–06, and declined significantly (P<0.05) to 5.1% in 2007–10 (Figure 1). The trends remained unchanged after excluding injecting drug users (IDUs) from analysis in the three study periods (data not shown).

Case–control study

Of the 108 patients infected with resistant HIV-1, 31 with high- or intermediate-level resistance and 16 with low-level resistance who initiated cART during the 11 year study period were identified as the case group (Figure 2). Of the patients whose HIV-1 strains did not harbour any resistance-related mutations, 89 who initiated cART were selected as the control group, and were matched with case patients (Table 1). Most patients were men who have sex with men (MSM), infected with subtype B and diagnosed as having HIV infection at late stage with a median CD4 cell count of 105 cells/mm³ (range, 1–972). No patients received lamivudine monotherapy for hepatitis B infection prior to initiation of cART.

Over the 11 year study period, results of genotypic resistance tests were not available to the treating physicians. In this study, a GSS of 2.5 was selected as the cut-off for further assessment of the impact of TDR on the effectiveness of cART (Table S2, available as Supplementary data at JAC Online). Of the 47 case patients, 19 had no resistance mutations to the regimens prescribed. Patients on a regimen with GSS ≤ 2.5 had similar baseline characteristics as patients with GSS > 2.5. The resistance-mutation patterns for 28 patients receiving regimens with GSS ≤ 2.5 are listed in Table S3 (available as Supplementary data at JAC Online).

Responses to treatment

Kaplan–Meier plots of the proportion of patients with treatment failure according to patient groups showed significantly shorter time to treatment failure in the case group (log-rank P=0.05) (Figure 3a). The difference was even more significant by Kaplan–Meier estimates of the proportion of treatment failure according to GSS (log-rank P<0.001) (Figure 3b). Patients receiving regimens with GSS ≤ 2.5 were further divided into two groups: those receiving PI-based and those receiving NNRTI-based cART. A better treatment response was seen in patients receiving PI-based cART, although without achieving statistical significance (Figure 3c). The results of the analysis of virological failure alone (excluding AIDS-related deaths) were similar.

The treatment outcomes are summarized in Table 2. First, the percentages of treatment failure were similar between case and control groups. There were 79 regimen changes in 65 patients during the follow-up period. Among these changes, 53 (67.1%) were due to adverse effects of cART, 19 (24.0%) were due to regimen simplification and 7 (8.9%) were due to treatment failure. Most of the regimen changes (68.4%) were within-class substitutions. A total of 16 patients had across-the-class substitutions of their regimens after 30 days of treatment and were censored at the date of regimen changes. Of 7 across-the-class substitutions of initial regimens within 30 days, 5 (71.4%) occurred within the first 2 weeks. Adherence was generally comparable between the groups.

The overall percentage of treatment failure in patients receiving regimens with GSS ≤ 2.5 was 39.3%, compared with 15.7% in patients receiving regimens with GSS > 2.5 (P=0.02) (Table 2). Patients receiving regimens with GSS ≤ 2.5 had a significantly lower CD4 cell count increase after 1 year of cART, compared with patients receiving regimens with GSS > 2.5 (P=0.05). Fifteen patients (55.6%; 4 receiving regimens with GSS ≤ 2.5 and 11 with GSS > 2.5) had virological failure after initial achievement of viral suppression. Changes in regimens were more frequent in patients receiving regimens with GSS ≤ 2.5 than in patients receiving regimens with GSS > 2.5 (64.3% versus 43.5%, P=0.06), although without reaching statistical significance.

Effects of TDR, regimen types and other factors on treatment outcome

In the multivariate Cox model, we found patients receiving regimens with GSS ≤ 2.5 had a 5.31 times higher risk of treatment failure, compared with regimens with GSS > 2.5 (95% CI, 2.28–12.36; P<0.001). Older age and entry into HIV care after 2003 were protective factors against treatment failure (Table S4, available as Supplementary data at JAC Online). The results of multivariate logistic regression for treatment failure were similar (OR 5.02 for GSS ≤ 2.5 versus GSS > 2.5; 95% CI, 2.28–12.36; P<0.001). In patients receiving regimens with GSS ≤ 2.5, PI-based regimens were associated with better treatment responses compared with NNRTI-based regimens, which was of borderline significance in a Cox proportional hazards model [hazard ratio (HR), 0.26; 95% CI, 0.06–1.12; P=0.07] (Table S5, available as Supplementary data at JAC Online). Further multivariate analysis was performed to evaluate the adjusted HR for treatment failure.

In patients receiving NNRTI-based therapy, regimens with a GSS ≤ 2.5 were associated with a more than 20 times higher risk of treatment failure, compared with regimens with a GSS > 2.5 (HR 23.02; 95% CI, 4.54–116.81; P<0.001). Nevertheless, in patients taking PI-based regimens, the risk of treatment failure
was not statistically significantly increased in patients with GSS ≤ 2.5 (HR 2.48; 95% CI, 0.81–7.57; \( P = 0.11 \)).

**Discussion**

In this 11 year surveillance of TDR of HIV-1 strains, we found that the prevalence of antiretroviral resistance mutations appears to have declined and stabilized in Taiwan. In the assessment of the effect of TDR on clinical outcomes, we found that even low-level TDR (GSS ≤ 2.5) to one antiretroviral agent contained in the regimens could lead to a > 5-fold higher risk of treatment failure in the first 2 years of cART, compared with those without TDR. This effect was particularly significant in patients receiving NNRTI-based cART. Our findings are consistent with previous studies, and further support the current recommendation of routine drug-resistance testing before initiation of ART.9,10,12

In Taiwan, after an initial increase in TDR prevalence from the period 1999–2002 to 2003–06, a decreasing trend of TDR was observed in 2007–10. Because of an outbreak of HIV infection that occurred among IDUs in Taiwan between 2003 and 2007, the lower prevalence of antiretroviral resistance could be attributed to lower exposure to ART among IDUs.23 However, this trend did not change after excluding IDUs from our cohort (data not shown). Therefore, the stabilization of TDR prevalence could be attributed to the introduction of antiretroviral drugs that are more potent and tolerable,5,24,25,35 implementation of a case management programme and increased experience of clinicians.

Our study is unique because very few data addressing the clinical impacts of TDR are available in Asia, Africa or developing countries where the burden of HIV/AIDS is particularly high. Unlike previous studies from many Western countries, the CD4 cell counts in our cohort were much lower. This may be more similar to the situation in resource-limited settings, where most HIV infections are diagnosed at relatively late stages.20

NNRTI-based regimens are the most popular first-line HIV-1 treatment regimens worldwide, and they are recommended by WHO as the preferred first-line cART.12,30,36,37 Many regimens for prevention of mother-to-child transmission also contain an NNRTI. Given the low genetic barrier of NNRTIs to development of resistance, it is not surprising to find that a recent large-scale surveillance by WHO revealed a moderate (5%–15%) prevalence of TDR in 17% of surveys in resource-limited areas.36 Even a low
frequency of NNRTI-resistance mutations has been shown to be associated with more than twice the risk of virological failure.\textsuperscript{38} In the largest study investigating the effects of TDR on response to first-line cART, Wittkop \textit{et al.}\textsuperscript{12} found that ritonavir-boosted PI-based regimens had better virological outcomes than NNRTI-based regimens in patients with susceptible or potentially low-level resistance. They suggested that in regions where genotypic testing is not routinely available, but TDR exists, first-line cART containing a boosted PI should be considered; this is supported by the findings of our study, although randomized controlled trials are needed to confirm this suggestion. It will be valuable to evaluate the cost-effectiveness of this strategy using model assumptions other than those used for Western countries.\textsuperscript{39}

No differences were found in time to viral suppression and PVL reduction during the first 8 weeks of cART in our study, which is in accordance with previous studies.\textsuperscript{4–6,17,19} This may imply that TDR is more likely to jeopardize durability rather than the short-term potency of cART. Based on this finding, regimen changes within the first 30 days of treatment probably had negligible effect, and could be ignored in the comparisons of treatment responses during the first 2 years of cART. In our patients

<table>
<thead>
<tr>
<th>Characteristics\textsuperscript{a}</th>
<th>Case group (N=47)</th>
<th>Control group (N=89)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex, male, n (%)</td>
<td>46 (97.9)</td>
<td>87 (97.8)</td>
</tr>
<tr>
<td>Age, years</td>
<td>33 (26–40)</td>
<td>32 (26.5–38.5)</td>
</tr>
<tr>
<td>CD4 cell count, cells/mm(^3)</td>
<td>142 (43–340)</td>
<td>94 (34–300)</td>
</tr>
<tr>
<td>PVL, log(_{10}) copies/mL</td>
<td>5.29 (4.48–5.86)</td>
<td>5.11 (4.87–5.57)</td>
</tr>
<tr>
<td>Subtype, n (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>41 (87.2)</td>
<td>83 (93.3)</td>
</tr>
<tr>
<td>non-B</td>
<td>6 (12.8)</td>
<td>6 (6.7)</td>
</tr>
<tr>
<td>Risk factor, n (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MSM</td>
<td>38 (80.9)</td>
<td>76 (85.4)</td>
</tr>
<tr>
<td>heterosexual</td>
<td>6 (12.8)</td>
<td>10 (11.2)</td>
</tr>
<tr>
<td>IDU</td>
<td>1 (2.1)</td>
<td>1 (1.1)</td>
</tr>
<tr>
<td>unknown</td>
<td>2 (4.3)</td>
<td>2 (2.2)</td>
</tr>
<tr>
<td>HBsAg (+), n (%)</td>
<td>9/46 (19.6)</td>
<td>16/85 (18.8)</td>
</tr>
<tr>
<td>Anti-HCV (+), n (%)</td>
<td>5/44 (11.4)</td>
<td>5/81 (6.2)</td>
</tr>
<tr>
<td>Initial cART, n (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PI-based</td>
<td>24 (51.1)</td>
<td>37 (41.6)</td>
</tr>
<tr>
<td>NNRTI-based</td>
<td>23 (48.9)</td>
<td>50 (56.2)</td>
</tr>
<tr>
<td>3 NRTIs</td>
<td>0 (0)</td>
<td>2 (2.2)</td>
</tr>
<tr>
<td>Year of entry into care, n (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2000–02</td>
<td>6 (12.8)</td>
<td>26 (29.2)</td>
</tr>
<tr>
<td>2003–06</td>
<td>25 (53.2)</td>
<td>40 (44.9)</td>
</tr>
<tr>
<td>2007–10</td>
<td>16 (34.0)</td>
<td>23 (25.8)</td>
</tr>
</tbody>
</table>

HBSAg, hepatitis B surface antigen; HCV, hepatitis C virus.
\textsuperscript{a}Data are median (IQR) for continuous variables.

Figure 3. Kaplan–Meier estimates of (a) time to treatment failure in patients with (case group) and without (control group) genotypic resistance, (b) time to treatment failure according to GSS (GSS >2.5 versus GSS \(\leq 2.5\)) and (c) time to treatment failure according to the first-line regimen (NNRTI- versus PI-based cART) in patients receiving regimens with GSS \(\leq 2.5\).
transmitted with a regimen of GSS $\leq 2.5$, increases in CD4 cell counts were lower after 12 months of therapy, but this difference decreased after 2 years of cART, partly related to regimen modifications during the course of cART. Our findings are consistent with those of other studies that demonstrated minimal effects of TDR on immunological outcomes.\textsuperscript{4,6,12,18}

As a retrospective cohort study with a small sample size, there are several limitations to our study. First, the date of HIV infection was not available for most of the patients; given the relatively low CD4 cell counts at baseline, most of our patients were probably chronically infected, and the prevalence of TDR we observed may be underestimated because most mutant viruses will be replaced by wild-type strains over time, in the absence of ART. Secondly, although NTUH is the largest designated hospital in Taiwan, this study was based on data from a single centre, and our patients may not be representative of the HIV-infected population in Taiwan. Thirdly, 28% of the patients with baseline resistance were lost to follow-up and were not included in the case–control study. They might experience treatment failure if they receive care in our hospital; furthermore, the records of adherence may not be accurate or complete, so we may have underestimated the treatment failure rate. Fourthly, in patients with treatment failure, we did not perform follow-up genotypic resistance testing; whether the failure was caused by emergence of drug-resistant strains or prior strains with TDR could not be determined. Fifthly, population sequencing was used for genotypic testing in our study, and resistance-associated mutations $<15\%$ of the viral population could not be detected; using ultrasensitive assays to detect drug-resistant minor variants may further improve our analyses. Finally, low-level resistance was chosen as a cut-off for selection of the case group;\textsuperscript{3,18,19,36} patients harboring mutations categorized as susceptible or potential low-level resistance may have been missed or misclassified.

In summary, the prevalence of antiretroviral resistance mutations has decreased and stabilized in Taiwan in 2007–10. HIV-1 strains harbouring TDR may lead to poor virological responses to first-line cART, especially NNRTI-based regimens. Routine genotypic testing before initiation of cART is recommended; however, if this is not available, first-line PI-based therapy would result in better treatment responses than NNRTI-based regimens.

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### Transparency declarations

None to declare.

### Supplementary data

Tables S1–S5 are available as Supplementary data at JAC Online (http://jac.oxfordjournals.org).

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