Can We Predict Neuropathy Risk before Stavudine Prescription in a Resource-Limited Setting?

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Abstract

A toxic sensory neuropathy associated with exposure to inexpensive nucleoside analogue reverse transcriptase inhibitors (NRTIs) [particularly stavudine (d4T)] causes dilemmas in the management of patients with HIV, especially in resource-poor settings. Here patients (n = 96) attending Pokdisus AIDS Clinic at the Cipto Mangunkusumo Hospital, Jakarta who had been treated with d4T were screened for symptomatic neuropathy. Clinical, demographic, and genetic factors were considered as possible neuropathy risk factors. DNA from saliva was used to examine alleles of TNFA-308, BAT1 (intron 10), TNFA-1031, IL1A +4845, and IL12B (3’ UTR). The prevalence of neuropathy (symptoms and signs) was 34%. On multivariate analysis, neuropathy following d4T exposure was associated with increasing age, increasing height, and TNFA-1031*2 (model p = 0.0009). Isoniazid exposure (present in 56% of patients) was not associated with neuropathy in this cohort, where all patients had received pyridoxine coadministration. These data suggest that a simple algorithm based on patient age, height, and TNF genotype could be used to predict the individual’s risk of symptomatic neuropathy prior to prescription of d4T.

Introduction

SENSORY NEUROPATHY (SN) is a common and disabling complication of HIV disease and some HIV treatments. Exposure to stavudine (d4T), a potentially neurotoxic nucleoside analogue reverse transcriptase inhibitor (NRTI), is an independent risk factor for SN among HIV patients in Australia and the United States, and a similar association is reported from resource-limited settings. However, d4T is an effective antiretroviral agent that is widely available in relatively inexpensive generic fixed dose combinations. It is also associated with a lower risk of severe anemia than is seen with zidovudine. It is therefore likely that d4T use will remain common in first-line HIV treatment in countries where access to alternative regimens is limited by cost, despite high rates of toxicities including SN.

Not all patients exposed to d4T develop neuropathy, suggesting that host factors may play a role in the individual’s risk. For example, d4T-associated neuropathy is thought to be caused by the mitochondrial toxicity of this drug and mitochondrial haplogroup T has been associated with neuropathy in white patients exposed to NRTIs. Genetic markers of host inflammatory responses may also be an important determinant of d4T neuropathy risk. Rates and severity of other complications of HIV and HIV treatments are associated with cytokine genotype, notably alleles of TNFA. Further, d4T neuropathy is clinically similar to neuropathy caused by HIV itself, where disordered inflammation and altered cytokine levels are well described.

We have previously documented that demographic features and host cytokine genotype are associated with neuropathy risk following d4T (or didanosine) exposure in Australian whites with HIV. Confirmation of these findings in patients from other ethnic groups would improve our ability to predict the individual’s risk of SN prior to d4T prescription, allowing those at highest risk to be prioritized for access to alternative agents. The aim of the current study was to determine the clinical, demographic, and genetic factors...
associated with risk of neuropathy among Indonesian HIV patients exposed to d4T

**Materials and Methods**

This study was undertaken over 5 weeks in August 2006 in the Pokdisus AIDS Clinic at Cipto Mangunkusumo Hospital, Jakarta, Indonesia. All adult (age ≥18 years) HIV-infected clinic patients who had ever used d4T were invited to be screened for neuropathy and give a sample of saliva as a source of genomic DNA. The study was approved by the local Human Research and Ethics Committee and all subjects gave written, informed consent to participate.

Patients were assessed for neuropathy using the AIDS Clinical Trials Group Brief Peripheral Neuropathy Screen (ACTG BPNS). Neuropathy was defined as present if the individual had one or more of the lower limb neuropathic symptoms elicited using this tool (pain, aching or burning, pins and needles, or numbness) together with at least one of the following: absent ankle reflexes or reduced vibration sense at the great toe (vibration of a 128-Hz tuning fork felt for 10 s or less). All patients who described neuropathic symptoms were questioned regarding the timing of symptom onset relative to stavudine use. Data on possible laboratory, clinical, and demographic risk factors for neuropathy were collected from detailed medical records maintained on all patients attending this clinic. Plasma HIV viral loads are not routinely performed in this clinic and were therefore not included in this study.

DNA was extracted from saliva using a QIAamp DNA mini Kit (QIAGEN, USA) and stored at −80°C. Genomic DNA was screened using established PCR-RFLP assays to determine the alleles carried at BAT1 (intron 10) (rs9281523), TNFA-308 (rs1800629), and TNFA-1031 (rs1799964). Other assays were based on FAM and VIC-labeled probes and Universal PCR Master Mix (Taquin, Applied Biosystems) in 5 μl reactions. Assay IDs were C_9546471_10 for IL1A (rs17561) and C_2084293_10 for IL12B (rs3212227).

Statistical analyses were performed using Stata 9.2 (StataCorp, USA). Demographic details of patients with and without SN were compared using χ² tests (dichotomous variables), Wilcoxon rank-sum tests [nonnormally distributed continuous variables, described using median and interquartile range (IQR)], or unpaired t-tests [normally distributed continuous variables, described using mean ± standard deviation (SD)]. Associations between genotype and SN status were assessed individually using χ² tests [with genotypes grouped as (1,1) versus (1,2 or 2,2) in all analyses to accommodate small numbers with the (2,2) genotype at these loci]. Multivariate analyses were undertaken using multiple case–control logistic regression (including all factors with p < 0.3 on univariate analyses) with a reverse selection procedure.

**Results**

Ninety-six patients participated in this study. Of these, 33 patients (34%) had SN (defined as both symptoms and signs

| Table 1: Univariate Analyses of Demographic and Genetic Factors by Patient SN Status |
|----------------------------------------|----------|-----------------|----------------|-------|
| **Demographic factors**                | **SN (n = 33)** | **SN-free (n = 66)** | **p**          |
| Height (cm)¹                      | 170 ± 8  | 166 ± 7          | 0.02²          |
| Body mass index²                  | 21.3 ± 2.7 | 20.7 ± 2.9       | 0.41²          |
| Initial CD4 T cells/μl³            | 34 (9–98) | 50 (20–130)      | 0.2³           |
| Months HIV⁴                        | 20 (14–34) | 22 (15–32)       | 0.71²          |
| Age (years)⁵                      | 32 ± 7.6 | 29 ± 6.7         | 0.12²          |
| Female gender³                    | 3%       | 19%              | 0.03²          |
| Isoniazid/pyridoxine⁶              | 64%      | 52%              | 0.29²          |
| Stavudine ever⁷                    | 100%     | 100%             | 1.0²           |
| Zidovudine ever⁷                   | 52%      | 54%              | 0.82²          |
| Lamivudine ever⁷                   | 100%     | 98%              | 0.47²          |
| Efavirenz ever⁷                    | 55%      | 41%              | 0.22²          |
| Nevirapine ever⁷                   | 76%      | 81%              | 0.55²          |
| Protease inhibitor ever⁷           | 15%      | 13%              | 0.74²          |
| IVDU⁸                              | 73%      | 67%              | 0.54²          |
| HepC⁹                             | 58%      | 48%              | 0.03²          |
| **Genetic factors⁸**              |           |                  |                |
| TNFA-308*2                         | 10%      | 8%               | 0.8²           |
| BAT1 (intron10)*2                  | 7%       | 9%               | 0.7³           |
| TNFA-1031*2                        | 48%      | 27%              | 0.04²          |
| ILLA + 4845*2                      | 7%       | 21%              | 0.12²          |
| IL12B(3’ UTR)*2                    | 74%      | 63%              | 0.3³           |

¹Parametric data: shown as mean ± standard deviation.
²Unpaired t test (parametric data).
³Nonparametric data: shown as median (interquartile range).
⁴Wilcoxon rank-sum test (nonparametric data).
⁵χ² test (dichotomous data).
⁶This was lopinavir/ritonavir in all cases, with one patient also having used atazanavir.
⁷Shown as percentage of individuals carrying allele 2.
on the ACTG-BPNS. Thirty-one of 33 neuropathy patients stated that their symptoms probably or definitely began after their first exposure to stavudine. Among the 63 patients classified as “SN free,” a further seven (7%) patients had neuropathic symptoms but no signs, and 14 (15%) asymptomatic patients had neurophilic signs.

This cohort was relatively young (mean age 30 years, SD 7 years) and immune deficient at HIV diagnosis (median CD4 T cell count at diagnosis 40 cells/μl, IQR 17–116 cells/μl). Most (86%) were male and 54 (56%) had a history of isoniazid use (all with pyridoxine). Patients had used d4T for 2–42 (17 ± 9) months.

Univariate analyses of demographic parameters established that increasing height, female gender, and hepatitis C seropositivity were associated with SN status. Weaker associations were evident with age and initial CD4 T cell count (Table 1). On multivariable analysis, height and age were the only demographic features independently associated with SN status (model \( p = 0.005 \)).

Univariate analyses of the genotypes studied showed an association between TNFA-1031 and SN status (Table 1). On logistic regression modeling, increasing age and height combined with TNFA-1031*2 to form the best model of SN risk (model \( p = 0.0009 \)) (Table 2).

Discussion

This study found a neuropathy prevalence of 34% among HIV patients in Jakarta exposed to d4T. The independent associations with neuropathy in this cohort were increasing patient age, increasing patient height, and TNF genotype, factors that could readily be measured prior to d4T prescription. The prevalence of SN in this cohort was lower than we have observed in Australian HIV patients using the same definition, despite the fact that all patients in the current study had used d4T. This may be explained by the relative youth of the patients studied here, with 6 of 11 (55%) patients aged at least 40 years having SN, compared with only 27 of 85 (32%) younger patients. The association between neuropathy and height is consistent with our previous description in Australians with HIV, all but one of whom were male. In the current cohort the rate of neuropathy was 56% in patients taller than 170 cm exposed to d4T, but only 27% in shorter individuals. Although isoniazid exposure has been independently associated with neuropathy risk in other HIV treatment centers no such association was observed here. This may relate to the universal coadministration of pyridoxine with isoniazid in this clinic.

Saliva was used in this work as a noninvasive source of genomic DNA requiring minimal processing prior to DNA extraction. Sufficient DNA was obtained for the testing described in all patients, consistent with previous reports of saliva as a reliable and cost-effective alternative to blood as a source of genomic DNA.

The limitations of this study include the modest sample size. In addition, two factors may have resulted in some misclassification of patients. First, our study definition of SN was chosen based on previous work validating the ACTG-BPNS, but resulted in patients who had isolated neuropathic symptoms or asymptomatic signs being classified as “neuropathy free.” Second, all cases of neuropathy were assumed to have developed after exposure to d4T. Most patients were immunodeficient at HIV diagnosis, so some may have had neuropathy before d4T was prescribed, although only two of the 33 patients diagnosed here with neuropathy believed their symptoms may have predated their d4T exposure. However, any misclassification of patients’ d4T neuropathy status was independent of the risk factors considered and thus can be considered random. This may have no impact or could dilute our findings. Hence associations between age, height, and TNF genotype and neuropathy following d4T prescription may be even stronger than described. Our findings also mirror results obtained in Australian whites.

We show that easily measured factors influence risk of neuropathy among patients exposed to d4T. Therefore it is plausible that a simple algorithm could be used to identify those patients at highest risk of neuropathy before d4T prescription, allowing prioritization of these patients for alternative agents. Further study in larger cohorts including patients from additional ethnic groups will confirm the predictive utility of such an algorithm. The simple nature of our proposed model (clinical features able to be tested in any setting plus a single polymorphism) makes this work relevant to resource-limited settings where d4T use remains common and such a tool is most urgently needed.

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Disclosure Statement

No competing financial interests exist.

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