

Urine nevirapine as a predictor of antiretroviral adherence

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Background & objectives: Incomplete adherence is a major contributor to failure of antiretroviral therapy. Although the available methods to monitor adherence to therapy have proved to be predictive of outcomes, the results are variable. We assessed the feasibility of detecting nevirapine (NVP) in spot urine samples to monitor patient adherence to antiretroviral treatment and to study the urinary excretion of NVP in healthy volunteers after oral administration of a single dose of NVP (200 mg).

Methods: Spot urine samples were collected from 50 HIV-infected patients (36 on treatment regimen containing NVP and 14 on drugs other than NVP) and tested for NVP by HPLC in a blinded manner. Sixteen healthy volunteers (9 males and 7 females) were administered a single oral dose of 200 mg NVP and spot urine samples were collected on day '0' before drug administration, and thereafter every 24 h up to 9 days and tested for NVP.

Results: All the urine samples collected from patients undergoing treatment with NVP-containing regimens at different time points after drug administration tested positive for NVP. Thirteen out of 14 samples from patients not on NVP yielded a negative result. The drug was detected in the urine of healthy volunteers up to 9 days. The urinary excretion of NVP was prolonged in females than in males.

Interpretation & conclusion: In view of its long half-life, NVP gets excreted in urine for a long period of time. Hence, testing spot urine samples for NVP may not be a useful measure to monitor patient adherence to treatment.

Key words Antiretroviral adherence - HIV - predictor - urine nevirapine

The use of a potent combination of antiretroviral drugs has led to dramatic reductions in the morbidity and mortality associated with HIV-1 infection¹. However, about 20 per cent of therapy-naive patients fail to achieve adequate virological response during their first year of triple therapy, with approximately 20 per cent more patients

experiencing virological failure during the second year². Variability in response to antiretroviral agents has been attributed, in part, to virological, immunological, pharmacokinetic and adherence differences between patients³. Non adherence to therapy has been shown to be a major contributor to failure of therapy^{4,5}.

Currently available approaches to measure adherence have notable limitations⁶ and individual patient assessments by medical providers do not accurately predict adherence⁷. Urine could serve as an useful biological fluid for detecting drugs/metabolites, particularly to monitor patient adherence to treatment. Though nevirapine (NVP) and its metabolites have been characterized in urine⁸, no attempt has been made to use these as a test for adherence. We, therefore, undertook an investigation to test the usefulness of detecting NVP in spot urine samples to monitor patient adherence to antiretroviral treatment. The reason for choosing NVP was that it is present in the fixed dose triple drug combination of antiretroviral drugs that is commonly used by HIV-infected patients. We also studied the urinary excretion of NVP in healthy volunteers after oral administration of a single dose of NVP.

Material & Methods

Patients: Fifty consecutive HIV-seropositive patients, aged 20 to 54 yr, attending the outpatient clinic at the Tuberculosis Research Centre, Chennai, India, during August - September 2004 took part in this study. Of them, 36 patients were undergoing antiretroviral treatment consisting of NVP (200 mg) and lamivudine (150 mg) with either stavudine (30/40 mg) or zidovudine (300 mg) twice daily. The remaining 14 patients served as controls. On the day of their visit to the clinic for routine examination, the patients were asked to provide a spot urine sample. None of the patients was previously informed about the study. The urine samples were coded and sent to the laboratory for urine NVP test.

Healthy volunteers: The healthy volunteers were 16 (9 males and 7 females) willing staff members working at the Tuberculosis Research Centre. Their age ranged from 28 to 54 yr. A sample of urine was collected (day '0') from all the healthy volunteers followed by administration of NVP (200 mg). Urine samples were collected every 24 h up to day 9. All the urine samples were stored at -20°C until assays were undertaken. Informed written consent was obtained from all the healthy volunteers before they took part in the study.

Determination of urine NVP: Nevirapine was tested in all the urine samples by a high performance liquid chromatography (HPLC) method described previously⁹. In brief, NVP was extracted into ethyl acetate. The organic layer was evaporated to dryness and the dried residue was reconstituted in the mobile phase and injected into the HPLC column. The HPLC system (Shimadzu Corporation, Kyoto, Japan) consisted of two pumps (LC-10ATvp), diode array detector (SPD-M 10AVP) and system controller (SCL-10AVP). A rheodyne manual injector (Rheodyne, Cotati, CA, USA) attached with a 20 µl sample loop was used for loading the samples. Class VP-LC workstation was used for data collection and acquisition. The analytical column was a C₁₈ 250 x 4.6 mm internal diameter, 5µ particle size (Lichrospher 100 RP-19e, Merck, Germany) protected by a compatible guard column.

The mobile phase consisted of 50 mM phosphate buffer, pH 4.6 and acetonitrile (83:17 v/v) containing 0.1 per cent triethylamine (v/v). The UV detector was set at 245 nm. The chromatogram was run for 20 min at a flow rate of 1.0 ml/min at a column temperature of 40°C. Pure NVP solutions prepared in pooled urine were processed along with test samples in a similar manner and run every day. Under these chromatographic conditions, a retention time of 15 min was obtained for NVP. The presence of a visible peak around 15 min was considered to be positive for NVP.

Results & Discussion

Thirty six HIV-positive patients on treatment regimens with NVP were receiving antiretroviral treatment for a period ranging from 2 to 33 months. The time of urine collection after drug administration ranged from 20 min to 14 h. All the urine samples collected from these patients tested positive for NVP, as evidenced by a distinct peak at around 15 min. Standard NVP aided in identifying the peak, in the event of slight day-to-day variations in the retention time of NVP. The method for determination of urine NVP employed in this study was highly sensitive and was able to detect NVP concentrations as low as 0.01µg/ml. Although specificity of the method had been established by us, where interference due to co-administered drugs, namely, lamivudine, stavudine and zidovudine, was ruled out, this aspect

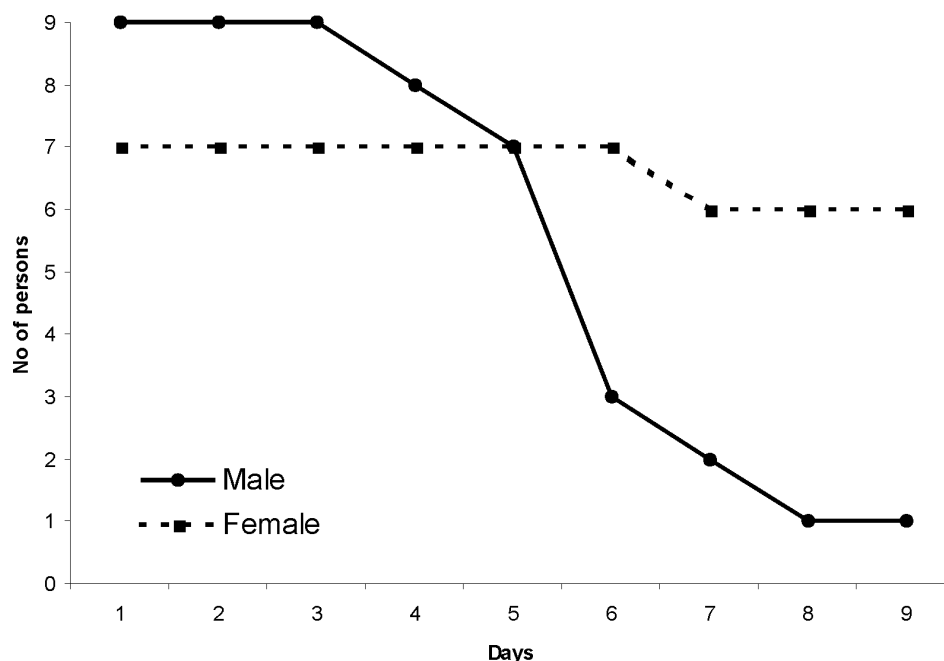


Fig. Urinary excretion of nevirapine in healthy volunteers. Excretion of nevirapine studied in spot urine samples collected from healthy volunteers (9 males and 7 females) up to 9 days following oral administration of a single dose of 200 mg nevirapine.

was further strengthened by the fact that 13 out of 14 urine samples of patients not on NVP treatment, were found to be negative for NVP. The single false positive result could be due to an unknown endogenous compound being eluted with the same retention time as that of NVP. Thus, the accuracy of this method was found to be 98 per cent. The method of Erickson *et al*⁹ to measure NVP and its metabolites formed under *in vitro* conditions, was successfully used to determine NVP qualitatively in urine in this study.

It was observed that all the urine samples collected from 20 min to 14 h, after an oral dose, tested positive for NVP. This could be due to the fact that NVP has a long half - life of 25 to 30 h at steady state. It is therefore expected that NVP would be excreted in urine for a prolonged period. This was confirmed in our study, where we found that 10 out of 16 healthy volunteers excreted NVP up to nine days after a single oral dose of 200 mg NVP. We further observed that majority of the male volunteers stopped excreting the drug by day 6, while all, but one, female volunteers continued to excrete NVP even up to 9 days (Fig.). This probably implies that females retain the drug in their system for a longer time period than males. This is in agreement with an earlier report on

sex-related differences in selected pharmacokinetic parameters, mentioning that females have 20 per cent higher exposure of NVP than males¹⁰.

Several reports have emphasized that non adherence is the main cause for the failure of antiretroviral therapy^{11,12}. Methods used to gauge adherence include patients' self-report and physician assessments, electronic monitoring, pill count, and prescription - refill compliance. These methods have proved to be predictive of outcomes, although the results are variable¹³. Identifying additional accurate predictors of adherence that can routinely be applied in clinical practice may be of clinical value. Some investigators have assessed the antiretroviral drug level in blood as a measure of adherence^{14,15}. Alternatively, urine levels of antiretroviral drugs could serve as predictors to monitor patient adherence to treatment if found to be feasible. This method would be non-invasive and simple to perform. This approach is being used in tuberculosis therapy, where detection of acetyl isoniazid in urine indicates intake of isoniazid within the past 24 h¹⁶.

In this study, we attempted to assess if a simple spot urine test for NVP could help in monitoring patient adherence to antiretroviral treatment.

However, in view of the long half-life of the drug and prolonged excretion in urine, this test may not be useful to check adherence to treatment. One reason why all the 36 patients' urine samples turned out to be positive for NVP could be due to the fact that patients tend to take their drugs without fail on their scheduled visit to the Centre. A few missed doses, a day or two prior to the test day would not be detected by this method. It may therefore, be more appropriate to develop a urine test to check for the presence of lamivudine, since this drug has a shorter half-life and is present in the fixed dose combination pill taken by HIV-infected patients. A simple urine test would go a long way in monitoring antiretroviral adherence in resource-constrained settings.

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