RESEARCH PAPER

# Pharmacokinetics of Nevirapine, Stavudine and Lamivudine in Indian HIV-infected Children Receiving Generic Fixed Dose Combinations

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**Objective:** To determine the trough and two hour plasma levels of nevirapine, stavudine, and lamivudine when administered in fixed dose combinations (FDC).

**Design:** Cross sectional

**Setting:** Tertiary care hospital in Northern India.

**Participants:** 79 HIV-infected children receiving antiretroviral therapy with FDCs for more than month.

**Intervention:** Two-point sampling (0 and 2 hours after the morning dose).

**Outcome measures:** Plasma concentrations of all three drugs were simultaneously assayed by liquid chromatography/mass spectroscopy.

**Results:** Majority (77%) of children were receiving fixed dose combination of stavudine, lamivudine, nevirapine in the ratio of 6:30:50mg. The median (IQR) trough and 2-hour plasma levels (μg/mL) of nevirapine, stavudine and lamivudine were 5.2 (4.0, 6.3) and 7.9 (6.0, 9.7); 0.1 (0.06, 0.16) and 1.1 (0.59, 1.6); 0.1 (0.02, 0.2) and 2.5 (1.4, 3.1), respectively. Very few children had sub-therapeutic plasma drug levels of stavudine (2.5%), lamivudine (7.6%) and nevirapine (10%). Inadequate viral suppression at 6 months follow up was significantly associated with initial high viral load, low CD4 percentage at the time of enrolment in study, and lower doses of lamivudine and stavudine.

**Conclusion:** The currently available generic pediatric fixed dose antiretroviral combinations in India provide adequate drug exposure in majority of children.

**Keywords:** *Antiretroviral, Child, India, Serum levels.*

The availability of pediatric antiretroviral formulations has made possible the scale up of antiretroviral therapy (ART) to HIV-infected children. However, more than 2 million children are still in need of treatment [1]. Though the World Health Organization (WHO) has recently recommended the phasing in of zidovudine based regimen in children, combination of stavudine (d4T) + lamivudine (3TC) + nevirapine (NVP) was widely used because of early availability in pediatric formulations and good short term tolerability, especially in anemic children. A generic fixed dose combination - FDC-6 (d4T:3TC: NVP= 6:30:50 mg) was being provided by the National AIDS Control Organization (NACO) program in India, for a wide weight band ranging from 3 kg to 25 kg as per the simplified, harmonized dosing schedule proposed by the WHO Pediatric Antiretroviral Working Group [2]. There are limited pharmacokinetic data on the use of these formulations in HIV-infected Indian children. The reports on the pharmacokinetics of this particular combination have come from Africa [3,4]. There is no pharmacokinetic study on all three drugs in the fixed dose combination in this ratio for Indian children.

We, therefore, studied the steady state pharmacokinetics of nevirapine, stavudine and lamivudine in HIV-infected children receiving nevirapine-based antiretroviral therapy using fixed dose combination (FDC) of d4T:3TC:NVP in the ratio of 6:30:50 mg. An attempt was also made to look into the predictors of viral suppression in children receiving the above mentioned therapy.

## METHODS

This cross-sectional study was undertaken in All India Institute of Medical Sciences, New Delhi for a period of 12 months in 2009-2010. HIV-infected children who were receiving nevirapine-based antiretroviral therapy in the form of FDCs for at least one month, with an adherence of >95% were included. Children, who had concurrent illnesses like acute bacterial infections,

hepatitis, immune reconstitution inflammatory syndrome (IRIS), tuberculosis, and those not tolerating the drugs were excluded. The study was approved by the Institutional Ethics Committee. Written informed consent was obtained from the p2arents/ guardians of the eligible children.

The children were receiving the drugs as per 2008 WHO and 2010 NACO dosing recommendations [2,5]. The FDCs were generic drugs (Cipla Ltd, India) procured by NACO for the ART clinic of the hospital. The clinical stage and immunological status of each child was recorded [6]. The children were examined for nutritional status by anthropometry and for any concurrent illnesses. The medication history was reviewed and adherence as well as the exact time of administration of medication was determined by interviewing the guardian and also the child, where feasible. In addition, pill count was undertaken at the time of sampling for pharmacokinetic study. The dose and duration of all other medications were recorded.

The sample for trough levels of antiretroviral medications was taken just before the child received the morning dose of FDC so that the interval between last dose in the evening and sampling was 12 hrs. Thereafter, the scheduled dose of FDC was administered and the blood sample taken at 2 hours to determine the peak levels. A light breakfast was given after the dose of medication. Blood (1 mL) was drawn at each time point of estimation in EDTA vials, and plasma was separated within two hours of collection by centrifugation at 4000 rpm. The separated plasma was heated to 56°C on a heating block for 30 min as per the universal precaution to reduce residual infectivity below detectable levels [7] and then stored at -70°C till further analysis.

Nevirapine, lamivudine and stavudine levels were determined by liquid chromatography/mass spectroscopy (LCMS-MS) based on method described earlier [8,9]. Working standards of lamivudine (3TC), stavudine (d4T), nevirapine (NVP) and cytarabine (Internal Standard) were purchased from Sigma- Aldrich Ltd, India, having purity greater than 99%. The concentration range for calibration curve was 19.5- 5000 ng/mL; the sensitivity of the assay was 2.5 ng/mL. The chromatography system consisted of Ultra High Performance Liquid Chromatographic system (UHPLC, Thermo Surveyor system) with a quaternary pump connected to an online degasser and photodiode array detector (PDA). The chromatographic separation was achieved using Purospher C18 (55 mm × 4 mm internal diameter, 3 µm particle size, Merck Millipore International) analytical column. Quantitation was performed using Multiple Reaction Monitoring (MRM) mode to monitor precursor and product ion transitions (m/z) for nevirapine (267.5→226.2), stavudine (225.2→127) and lamivudine (230.1→112). Source-dependent parameters were: gas 1: 30 psi, gas 2: 60 psi, CAD: 9 psi, CUR: 10 psi, ion spray voltage: 5500 V, and temperature: 450ºC.

*CD4 and viral load estimation*: CD4 and viral load were estimated at enrolment, and after six months to determine the immunological status and suppression of viral replication. CD4% and count were enumerated on blood collected in EDTA by whole blood lysis method along with two-color flow cytometry using staining with fluorescent tagged antibodies to CD3 and CD4, on a FACS Calibur flow cytometer (Becton Dickinson, USA). Viral load was estimated by quantifying HIV viral RNA using the CobasTaqman 48 analyzer (Roche Molecular Systems Inc).

*Data management and statistical analysis*: The median blood trough and peak levels of nevirapine, stavudine, and lamivudine were calculated. The proportion of children having sub-therapeutic levels in blood was determined. The determinants of plasma drug levels evaluated were: age, dose, adherence, concurrent medications and nutritional status. The data were managed using Microsoft Access software and analyzed on Stata 9.0 (StataCorp, College Station, TX). Chi-squared test was used to compare the categorical variables. For continuous variables, t test or Wilcoxon ranksum test was used. Any drug level computed by the software below the lower limit of detection of 19.5 ng/mL was taken as 0 ng/mL for statistical analysis. The plasma level, considered therapeutic for nevirapine and stavudine were 3.4 µg/mL and 0.3 µg/mL, repectively. The lower boundary of optimal stavudine trough concentration was taken as 8.09 ng/mL[12]. In case of lamivudine, the cut-off plasma concentration at 3 hour post drug intake is less than 0.21 µg/mL [13].

We also evaluated the determinants of inadequate viral suppression at 6 months after the determination of blood levels of antiretroviral drugs. Children who had a viral load of at least 1000 copies/mL at 6 months of follow-up were considered to be having inadequate viral suppression and were compared with those who had viral load of £1000 copies/mL by bivariate analysis and also a logistic regression model taking initial viral load, initial CD4 count, initial CD4 percentage at the start of study, age, weight-for-age, height-for-age, weight-for-height z-scores, and drug levels as covariates. We performed similar analysis to determine the predictors of complete viral suppression (viral load £47 copies/mL).

## RESULTS

Eighty four children were screened; 79 (58 boys) were enrolled in the study. The baseline demographic and clinical characteristics of these children are given in ***Table* I**. Mean (SD) duration of ART was 32.6 (21.9) months. All children were receiving co2trimoxazole prophylaxis during this period. Two children were on concomitant antiepileptic drugs, two were on inhaled salbutamol and corticosteroids. Some children were also receiving multivitamin (*n*=30) and iron (*n*=16) supplementation.

Sixty one (77%) children were receiving the FDC6 (stavudine 6 mg, lamivudine 30 mg and nevirapine 50 mg), while the rest received the FDC 30 (stavudine 30 mg, lamivudine 150 mg and nevirapine 200 mg). ***Table* II** shows the mean doses of the three antiretroviral drugs received by these children. Nine children were receiving less than the recommended dosing of nevirapine (160 - 200 mg/ $m^2$/dose). Fifty eight children received less than the recommended dose of 1 mg/kg/dose of stavudine [14] while 13 received less than 0.8 mg/kg. Thirteen children were receiving less than 4 mg/kg of lamivudine. ***Table* II** summarizes the median plasma values of the three antiretroviral drugs at trough and two hour time point.

Eight children had less than optimal trough nevirapine concentration. Ninety percent of children achieved the therapeutic plasma level of 3.4 µg/mL for nevirapine [10]. Only two (2.5%) children had a trough plasma level of stavudine below 8 ng/mL and six (7.6%) children had a subtherapeutic plasma level for lamivudine.

***Web Table I*** shows the plasma levels of all three antiretroviral drugs in terms of duration of therapy, age, weight-for-age and height-for-age 'z' scores. The plasma levels of any of these drugs did not vary significantly with age, duration of therapy, undernutrition or stunting. For all three drugs at both time points, the plasma levels were similar in children who received either of the two FDCs.

The CD4% counts after a follow-up of six months improved marginally to mean (SD) 29.35% (8.86%). Mean (SD) CD4 count was almost same as before at 1132.2 (654.33) cells/µL. None of the children had features of clinical failure. At enrolment, 5 children were staged T4 while at 6 months follow up, only 1 child was in T4. Only one child had CD4 count below 100/µL on both occasions six months apart, fulfilling the criteria of immunological failure. The median (IQR) viral load showed a minimal increase to 212 (47- 4157) copies/ mL. Similarly, 11 children had viral load >10000 copies/ mL as compared to five at the time of enrolment. Twenty eight (36.8%) children had undetectable viral copies at the end of study period: seventeen of them were on ART for more than 18 months. Children with a viral load ≥1000 copies/mL at the time of enrolment in the study (the same time when drug levels were studied) were more likely to have a viral load ≥1000 copies/ mL at 6 months (***Table* III**). Also a lower initial CD4% was more significantly associated with poor viral suppression at 6 months of follow-up. A multivariate analysis adjusting for initial viral load, initial CD4 count at the start of study, age, anthropometry and drug levels showed that lower initial CD4 percentage was significantly associated with risk of inadequate viral suppression at the end of study (*P*=0.01).

Complete suppression of HIV virus, with undetectable viral RNA was achieved in 29 (36.7%) children. Children who had incomplete suppression of HIV virus at the end of six months study period were more likely to have inadequate viral suppression (>1000 copies of HIV RNA/ml), *P*=0.001 and a lower CD4 count, *P*=0.01 at the commencement of the study. Children with complete viral suppression also received significantly higher dose of lamivudine and stavudine, though the plasma concentrations of these drugs were not significantly different (***Table* III**). A logistic regression showed that children with complete suppression of HIV virus were more likely to have <1000 copies/mL viral load six months previously (*P*=0.01), and were also more likely to have received a higher dose of lamivudine per kg of body weight (*P*=0.03).

## DISCUSSION

In this study, we observed that the current dosing schedule using fixed dose combinations leads to adequate plasma levels of nevirapine, stavudine and lamivudine. Most of the children had therapeutic plasma levels of these antiretroviral drugs and were found to have viral suppression on subsequent follow up. Children with complete viral suppression received significantly higher dose of lamivudine and stavudine. but the plasma concentrations of these drugs were not significantly different.

The median (IQR) trough plasma levels and 2-hour drugs levels of nevirapine, stavudine and lamivudine in our study were consistent with previously reported values in both adult and pediatric subjects [15-20]. Earlier studies with FDCs showed varied drug exposure. When the adult combination of stavudine 30 mg, lamivudine 150 mg and nevirapine 200 mg were used in

**TABLE I** *General Characteristics of the Study Patients* (N=79)

| *Characteristic* | *Value* |
|---|---|
| Males, No (%) | 58 (73.4) |
| Age (mo), Mean ± SD | 98.6 ± 43.3 |
| Age (mo), at diagnosis, Mean ± SD | 59.4 ± 39.3 |
| Duration of ART (mo), Mean ± SD | 32.6 ± 21.9 |
| WFA z-score, Mean ± SD | -2.32 ± 1.17 |
| z score<-3, *N* (%) | 6 (7.6) |
| HFA z-score, Mean ± SD | -1.96 ± 0.86 |
| z score<-3, *N* (%) | 21 (26.6) |
| WFA z score, mean ± SD | -0.70 ± 0.83 |
| z score<-3, *N* (%) | 1(1.3) |
| BMI (kg/m$^2$), Mean ± SD | 14.93 ± 1.33 |
| Clinical stage (WHO), *N* (%)* | |
| T1 | 45 (56.9) |
| T2 | 2 (2.5) |
| T3 | 27 (34.2) |
| T4 | 5 (6.2) |
| *CD4%, Mean ± SD | 27.5 ± 10.0 |
| CD4%< 15%, *N* (%) | 9 (11.4) |
| *Viral load (copies/mL), Median (IQR) | 47 (47-958) |

*WFA: Weight-for-age; HFA: Height-for-age; WFH: Weight-for-height*
** Values at start of study.*

divided forms, it was found that children may or may not achieve the expected therapeutic serum concentration of nevirapine [15,16,18,21,22]. Recently Fillekes, *et al*. observed that nevirapine levels were sub-therapeutic in 4 out of 15 HIV infected Zambian infants receiving FDC-6. Stavudine and lamivudine plasma levels achieved in these children with body weight <6 kg were adequate [4].

In the Indian population, FDCs have been studied mainly in adults and found to be resulting in adequate plasma concentration [19,23,24]. The pediatric formulation with d4T:3TC: NVP ratio of 10:40:70 was studied in adult male volunteers and found to be bioequivalent as compared to individual liquid formulations [25]. Swaminathan, *et al*. evaluated the factors influencing nevirapine levels in 94 HIV-infected Indian children who were receiving adult and pediatric fixed dose combination as provided by NACO. Sub-therapeutic nevirapine levels were observed in 35% of children, especially the younger ones. Also malnourished state like stunting and the CYP2B6 GG or GT genotype were found to be predictors for low nevirapine concentration [26]. The presently used combination with a ratio of d4T:3TC:NVP 6:30:50 has not been studied concurrently for the pharmacokinetics of stavudine, lamivudine as well as nevirapine in HIV-infected Indian children.

The plasma drug concentrations were not affected by factors like duration of antiretroviral therapy, age or nutritional status of the children. Doses of stavudine and lamivudine appeared to be lower in children older than three years, and if they were stunted. However, this attenuated dosing pattern did not get reflected in the plasma drug concentrations. The children also fared well − clinically as well as immunologically. The clinical relevance of the low dosage in these groups of children is thus doubtful.

A significant number of our children had detectable viremia at 6 month follow up despite receiving the requisite doses of antiretroviral drugs and achieving therapeutic drug concentration in plasma. Those who had ≥1000 copies/mL of HIV RNA and a lower CD4% at the start of study were more likely to have inadequate viral suppression at 6 months follow up. This might indicate a need for longer time for immune recovery in children who are more immunosuppressed, or and have a higher viral load to begin with.

The two time point sampling (0 and 2 hours) for pharmacokinetic analysis was one of the shortcomings of this study. Though limited time point sampling has been earlier validated for pharmacokinetic study of antiretroviral drugs in children [27], a more extensive schedule might have been more informative. Also, for the nucleoside analogs the plasma drug concentration may not reflect actual therapeutic concentration for the drugs as they are phosphorylated into their active metabolites within target cells. Assaying the intracellular phosphorylated component of stavudine and lamivudine

**TABLE II** TROUGH AND 2-HOUR PLASMA LEVELS OF NEVIRAPINE, STAVUDINE AND LAMIVUDINE

| *Antiretroviral drug* | *Dosage, Mean ± SD* | *$C_{0hr}$ (µg/mL), Median (IQR)* | *$C_{2hr}$(µg/mL), Median (IQR)* |
|---|---|---|---|
| Nevirapine | 177.0 ± 15.95 mg/m$^2$ | 5.2 (4.0, 6.3) | 7.9 (6.0, 9.7) |
| Stavudine | 0.92 ± 0.13 mg/kg | 0.1 (0.06, 0.16) | 1.1 (0.59, 1.6) |
| Lamivudine | 4.59 ± 0.65 mg/ kg | 0.12 (0.02, 0.2) | 2.5 (1.4, 3.1) |

*$C_{0hr}$: trough plasma drug concentration, $C_{2hr}$:plasma drug concentration 2 hours after drug administration*

**TABLE III** COMPARISON OF CHILDREN WITH AND WITHOUT VIRAL SUPPRESSION AT 6 MONTHS FOLLOW- UP

| *Characteristic* | *Viral load < 1000 copies/mL (N=48)* | *Viral load ≥1000 copies/mL (N=28)* | *P value* | *Viral load ≤47 copies/mL (N= 29)* | *Viral load >47 copies/mL (N= 50)* | *P value* |
|---|---|---|---|---|---|---|
| Age in months | 96.91 ± 48.32 | 105.04 ± 34.12 | 0.56 | 90.81 ± 43.27 | 103.21 ± 37.26 | 0.22 |
| Boys, n (%) | 36 (75) | 20 (71.4) | 0.79 | 20 (68.97) | 38 (76.6) | 0.49 |
| Medicine received, n (%) | | | 0.41 | | | 0.18 |
| FDC-6 | 35 (72.9) | 23 (82.1) | | 20 (68.97) | 38 (81) | |
| FDC-30 | 13 (27.1) | 5 (17.9) | | 9 (31.03) | 9 (18) | |
| Viral load <1000 copies/mL at enrollment, n (%) | 46 (95.8) | 11 (39.3) | <0.001 | 28 (96.55) | 29 (61.7) | 0.001 |
| CD4% at enrollment | 30.72 ± 9.1 | 22.10 ± 9.01 | <0.001 | 29.37 ± 9.34 | 26.42 ± 10.33 | 0.21 |
| Dose of Nevirapine received (mg/m$^2$), | 177.11 ± 14.41 | 177.07 ± 19.19 | 0.86 | 178.52 ± 14.86 | 176.13 ± 16.23 | 0.52 |
| Dose of Lamivudine received (mg/kg), | 4.64 ± 0.63 | 4.49 ± 0.71 | 0.36 | 4.78 ± 0.63 | 4.48 ± 0.64 | 0.04 |
| Dose of Stavudine received (mg/kg), | 0.92 ± 0.12 | 0.89 ± 0.14 | 0.36 | 0.96 ± 0.13 | 0.89 ± 0.13 | 0.04 |
| Median (IQR) Nevirapine levels (µg/mL) | | | | | | |
| 0 hr | 5.6 ± 2.6 | 5.4 ± 2.9 | 0.86 | 5.4 ± 2.3 | 5.5 ± 2.9 | 0.88 |
| 2 hr | 8.4 ± 3.1 | 8.3 ± 3.1 | 0.91 | 7.4 ± 2.4 | 8.9 ± 3.3 | 0.06 |
| Median (IQR) Lamivudine levels (µg/mL) | | | | | | |
| 0 hr | 0.09 (0.02, 0.1) | 0.14 (0, 0.28) | 0.74 | 0.09 (0.008, 0.18) | 0.14 (0.02, 0.28) | 0.45 |
| 2 hr | 2.2 (1.2, 2.7) | 2.7 (1.7,3.1) | 0.23 | 2.1 (1.2, 2.7) | 2.6 (1.4,3.1) | 0.21 |
| Median (IQR) Stavudine levels (µg/mL) | | | | | | |
| 0 hr | 0.08 (0.05, 0.14) | 0.12 (0.09, 0.19) | 0.08 | 0.07 (0.05, 0.1) | 0.12 (0.07, 0.2) | |
| 2 hr | 1.1 (0.51, 1.6) | 1.03 (0.8, 1.7) | 0.54 | 0.9 (0.51, 1.4) | 1.1 (0.8, 1.7) | 0.15 |

*All values are mean ± SD unless specified.*

**WHAT IS ALREADY KNOWN?**

- Fixed dose combinations of antiretroviral drugs are useful in achieving compliance and adequate drug exposure in children.

**WHAT THIS STUDY ADDS?**

- Generic pediatric fixed dose combination of nevirapine, stavudine, and lamivudine in the ratio of 50: 6:30 provides adequate serum levels of all three drugs in HIV-infected Indian children.

gives a more reliable measure of their therapeutic concentration.

We conclude that the generic pediatric fixed dose combination of nevirapine, stavudine, and lamivudine currently available for Indian HIV infected children through NACO provides adequate drug exposure in majority of children. We need to follow-up for a longer period to assess the duration of suppression of viral replication.

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