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Research Article

Nevirapine-induced Hepatotoxicity in HIV Patients Attending Care and Treatment Clinics in Tanzania

Magessa Mafuru[1], BPharm, Appolinary Kamuhabwa, PhD[1]

[1]Unit of Pharmacology and Therapeutics, School of Pharmacy, Muhimbili University of Health and Allied Sciences, Dar es Salaam, Tanzania

Abstract

Objective

The aim of the study was to determine the prevalence of hepatotoxicity in HIV-infected patients using nevirapine-containing antiretroviral drugs (ARVs) in comparison with those using efavirenz-based ARVs.

Methods

HIV-infected patients who had been using ARVs for at least two weeks were recruited for the study. A total of 373 adult patients (224 taking nevirapine-based regimen and 149 taking efavirenz-based regimens) were screened for elevated liver aminotransferases as markers for liver toxicity.

Results

Mild to moderate hepatotoxicity was observed in 12.1% of patients who were using nevirapine compared to 4% of efavirenz users (P = 0.004). Hepatotoxicity was common (13.2%) in patients who had been using nevirapine for > 12 months than those who were using the same regimens (4.3%) for ≤ 12 months. Patients with CD4 cell count of > 250 cells/mm^3 were 10 times more at risk of developing hepatotoxicity than patients with CD4 cell count of ≤ 250 cells/mm^3. Age, sex, body mass index and duration of use of ARVs were not significantly associated with hepatotoxicity.

Conclusion

Routine monitoring of liver aminotransferances should be conducted in patients taking nevirapine containing ARVs regimen, especially in patients with CD4 cell count of > 250 cells/mm^3.

Keywords: Hepatotoxicity, Nevirapine, ARVs, Liver enzymes.

Introduction

About 39 million people globally are living with the human immunodeficiency virus (HIV) (1). Tanzania with a total population of about 45 million people (2), has 5.1% prevalence of HIV (3). Therefore, about 2.3 million people are living with HIV in Tanzania (3). The high HIV/AIDS related morbidity and mortality in developed countries has been dramatically reduced by the advent of effective combination of antiretroviral therapy (ART) (1). ART slows down the replication of the HIV, thereby causing the suppression of viral particle multiplication and eventually leading to decreased viral load, boosting the immune system and thereby prolonging the patient's life span (1).

The drawbacks to effective use of Highly Active Antiretroviral Therapy (HAART) include the adverse effects associated with the use of these drugs. One of the adverse effects associated with HAART is drug-induced hepatotoxicity and is frequently reported in patients taking nevirapine-containing ARVs (1). Drug-induced hepatic injury is the most common reason for withdrawing of approved drugs from the market, and it also accounts for more than 50% of the cases of acute liver failure in some countries (4). More than 75% of cases of idiosyncratic drug reactions results in liver transplantation or death (4).

Alanine aminotransferases (ALT) and aspartate aminotransferases (AST) are commonly used to measure liver injury in resource-limited settings (1). ALT are enzymes found predominantly in the liver but smaller amounts are also found in the kidneys, heart and muscles. Under normal conditions, levels of ALT in the blood are low but when the liver is damaged, ALT are released into the blood stream and can usually be detected before other symptoms of liver damage such as jaundice. Therefore, levels of ALT in the serum can help in the diagnosis and monitoring of liver disorders (5). Elevations in ALT are also predictive of increased mortality from liver disease and may influence the choice of first-line ARVs to be used in patients (6).

Nevirapine is among the most widely used ARVs in the first-line triple ART, particularly in low- and middle-income countries (7,8). However, hepatotoxicity induced by nevirapine has been of major concern. Two distinct types of nevirapine-associated hepatotoxicity, each with characteristic time courses, have been reported (9). The first type is an immune-mediated hypersensitivity reaction, which develops within 18 weeks of starting nevirapine and it appears to

***Corresponding author:** Prof. AR Kamuhabwa, P.O. Box 65013, School of Pharmacy, Muhimbili University of Health and Allied Sciences, Dar es Salaam, Tanzania, Tel: +255 757 576985; Fax: +255 222150465; E-mail: akamuhabwa@muhas.ac.tz, enali2012@gmail.com

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correlate with baseline CD4 cell count. Most patients with this type of hepatotoxicity will have concomitant flu-like symptoms (fever, myalgia, fatigue, malaise, nausea, and vomiting) with or without skin rash (9). The second type typically occurs after 18 weeks of nevirapine therapy and most likely represents an intrinsic toxic drug effect and does not appear to correlate with baseline CD4 cell count. This delayed hepatotoxicity generally occurs without concomitant constitutional symptoms (9).

Regardless of whether hepatotoxicity occurs early or later, an increase in hepatic aminotransferase levels is most often the first identifiable marker of nevirapine-induced hepatotoxicity. If patients develop clinical hepatitis, they typically display constitutional symptoms and jaundice (9,10). Several studies including case reports on nevirapine-based HAART induced hepatotoxicity and associated factors have been reported in sub-Saharan Africa and other parts of the world (1). However, the reported prevalence of hepatotoxicity have been varying from one study to another, an indication that patient characteristics and related factors play a major role (6,11–13).

Clinical trials and other studies have reported that nevirapine-induced hepatotoxicity is associated with some risk factors such as, sex, BMI, CD4 cell count, co-infection with hepatitis B or C and pregnancy (11,12,14). Based on these observations, in January and March 2005, respectively, the US Food and Drug Administration (FDA) and European Medicines Agency (EMEA) issued warnings in nevirapine package inserts (15). In these warnings, it was recommended that nevirapine should not be initiated in adult women with CD4 cell count of >250 cells/mm^3 and in men with CD4 cell count of >400 cells/mm^3, because of a higher risk of hepatotoxicity (10). In support of these recommendations, a study by Wood (2005) reported that females have a three-fold higher risk of symptomatic nevirapine liver toxicity than males (16). In addition, females with CD4 cell count of >250 cells/mm^3 have a 12-fold higher risk of symptomatic liver toxicity than females with CD4 cell count of <250 (16). Males with CD4 cell count of > 400 cells/mm^3 have a five-fold higher risk of symptomatic liver toxicity than males with CD4 cell count of <400 (16).

However, most of large cohort studies (6,13,17) have not found significant association of these risk factors with the occurrence of hepatotoxicity except co-infection with hepatitis B or C virus. Therefore more studies are required in this area since CD4 cell count is one of the surrogate mark used as the criteria for initiation of ARVs and assessment of patient's treatment responses particularly in low and middle income countries.

In recognition of the hepatotoxicity effects of nevirapine, the U.S department of health and human services guidelines (18) for the use of ARVs in HIV-1 infected adults and adolescents, U.S FDA, and Viramune® manufacturer (Boehringer Ingelheim) recommend that hepatic aminotransferase levels should be monitored very closely in the first 18 weeks of therapy and these tests should continue to be followed in persons who remain on nevirapine therapy. Specifically, hepatic aminotransferase levels (ALT, AST) should be monitored at baseline and at weeks 2, 4, 8, 12, 16 and thereafter every 3 months (18). However, in resource limited country like Tanzania, where there are no adequate diagnostic facilities, poor infrastructure, low standard of health care, and increasing in number of people living with HIV, routine monitoring of liver aminotransferases is rarely done, especially in the public health facilities. To our knowledge there are no published data regarding the prevalence and associated factors of nevirapine-induced hepatotoxicity in Tanzania. Therefore, this study was carried out to determine the prevalence and associated risk factors of nevirapine-induced hepatotoxicity in the rural area with the highest prevalence of HIV in Tanzania.

Methods

Study design

This study was an analytic cross sectional, hospital based, epidemiological study design in which HIV-infected patients who were using nevirapine and efavirenz were assessed for elevation of liver enzymes as markers of hepatotoxicity. This study was conducted for three months from the beginning of January 2014 to the end of March 2014.

Study area

The study was conducted at Iringa Regional Hospital (IRH) in Iringa region, Tanzania. Iringa region is in the Southern Highlands of Tanzania. It has a total population of 941,238 inhabitants (2). Iringa is the second region with the highest prevalence of HIV (9.1%), after Njombe region (14.8%) in Tanzania (3). The regional Hospital (IRH) is one of the 25 regional hospitals in the country. It is a referral hospital to six district hospitals in Iringa region. The hospital has 445 beds with a bed occupancy rate of about 95%. Data of this study were collected at the care and treatment clinics (CTC) of IRH, and blood samples for assay of liver aminotransferases enzymes were taken for analysis at St Egidio DREAM foundation laboratory located in the northern part, about 500m from the Regional Hospital.

Patient recruitment

Systematic sampling technique was used to enroll study participants. The target population was HIV-infected patients who were using nevirapine-based ARVs and efavirenz-based ARVs (as a comparison group). The HIV-infected patients aged 15 years and above attending CTCs in IRH whom had been using ARVs for at least two weeks were enrolled for the study. Critically ill patients, alcoholic patients and patients using anti-TB drugs were excluded from the study. A total of 231 HIV patients taking nevirapine-based ARVs regimen were enrolled to participate in the study. Seven participants were excluded from the study due to alcohol use and/or anti-TB drug use. Therefore a total of 224 patients were used for data analysis.

In addition, a total of 154 HIV infected patients who had been using efavirenz-based ARV regimens were enrolled as a comparison group. Five participants were excluded from the study due to alcohol use and/or anti-TB drug use. Therefore a total of 149 patients who

were using efavirenz were used for data analysis.

Data collection

Blood sample and assay

Cotton wool, methylated spirit, syringes, gloves and vaccuntainer tubes for serum were used for blood sample collection. Blood samples from participants were aseptically collected into 4ml sterile vacuum tubes (Kang Jian®, China) by a phlebotomist. The samples were coded, labeled and transported to St Egidio DREAM laboratory within four hours from time of collection for ALT and AST assay. Blood samples were centrifuged at 3000 rpm for ten minutes to obtain serum. ALT and AST working reagents were prepared according to the Laboratory Guidelines for Screening, Diagnosis and Monitoring of Hepatic Injury (19).

ALT and AST were quantified by absorbance photometry (20), according to the International Federation of Clinical Chemistry Method using commercial automated chemistry analyzer; HumaLyzer 3500. Only unhaemolyzed blood samples were assayed for ALT and AST.

Determination of CD4 cell count

CD4 cell count of study participants were recorded from participant's care and treatment clinic card 1 (CTC_1) or care and treatment clinic card 2 (CTC_2). For those whom their recent CD4 cell count was not available, blood samples were collected for CD4 cell count tests. CD4 cell count was determined by flow cytometry using Becton Dickson FacsCalibur machine, as previously described (21).

Demographic information of patients

Data on socio-demographic and background information were collected by using a questionnaire-checklist. These included age, sex, height, weight and body mass index (BMI) of the patients. Weights of patients were measured by using a digital weighing scale with the patient having no shoes. It was measured up to the nearest 100 grams. Height was measured using a standard height board with the participant having no shoes. The head piece was gradually lowered until it reached the patients head at 90° angle with the measuring scale. The measurement was taken to the nearest 1cm. BMI was calculated as weight in kilograms divided by height in meters squared.

Patient interviews

Patients were interviewed about other co-morbidity, use of any other drugs apart from ARVs, alcohol consumption, use of herbal medicines and pregnancy. Clinical signs and symptoms such as presence of skin rashes, right upper quadrant pain (RUQP) and jaundice were also assessed. The ARVs regimen and duration of use were recorded from ART register, CTC_1 or CTC_2 forms.

Operational definition

Hepatotoxicity was defined as an elevation in transaminases (ALT or/and AST) level above the upper limits of normal (ULN) range. Serum ALT and AST were graded according to the criteria established by the AIDS Clinical Trial Group (6): grade 0 (1.25x ULN), grade 1 (1.25–2.5x ULN), grade 2 (2.6–5.0x ULN), grade 3 (5.1–10x ULN) and grade 4 (10x ULN). For the purpose of this study, the normal range of ALT or AST was set at 5-35 IU/L (8).

Statistical analysis

Data were analyzed using the Statistical Package for Social Sciences (SPSS) computer software version 20. Patient's socio-demographic characteristics and hepatotoxicity specific variables were summarized using frequency tables. The dependent variable of this study was serum levels of ALT and AST as a mark of liver toxicity. Independent variables were age, sex, CD4 cell count, duration on nevirapine use, and BMI. Data were described using mean for continuous variables and proportional for categorical variables. Association between variables was tested by the use of chi-square or Fisher's exact test for proportional and ANOVA for means. Multivariate logistic regression was used to determine independent factors predicting hepatotoxicity. A probability (p) value of less or equal to 0.05 was considered statistically significant.

Ethical consideration

This study was granted ethical clearance from Muhimbili University of Health and Allied Sciences research and publication committee. Permission to conduct the study at Iringa Regional Hospital was sought from the Regional Administrative Secretary. The Helsink declaration of 1964 was observed which requires researchers to protect human subjects. A written informed consent was sought from the patients who participated in the study. Participants were informed about the aim, the benefit, and confidentiality of the study. To ensure confidentiality, patient's code numbers were used instead of patient names.

Results

Patient characteristics

From January 2014 to March 2014, a total of 385 HIV infected patients were enrolled into the study. Of these, 231 patients were using nevirapine-based ARVs regimens and 154 patients were using efavirenz-based ARVs regimens. Seven and five patients on nevirapine and efavirenz arms respectively, were excluded from the study due to alcohol use and or anti-TB drug use. Therefore a total of 373 patients [224 (60%) on nevirapine arm and 149 (40%) on efavirenz arm)] were used for data analysis. Of nevirapine regimen group, 188 (83.9%) patients were females compared to 88 (59.1%) patients in the efavirenz regimen group. The mean age of study participants was 39.51 ± 10.19 years in nevirapine regimen and 40.03 ± 11.54 years in efavirenz regimen group. Generally, participants in this study had comparable socio-demographic characteristics as shown in table 1.

Majority (73.2%) of patients on nevirapine arm had CD4 cell count of ≤ 500 cells/mm^3 compared to 101 (67%) patients on efavirenz arm. The mean CD4 cell count was 412.54 ± 212.15 cells/mm^3 in patients on nevirapine arm compared to 414 ± 226.7 cells/mm^3 on efavirenz arm. Majority of patients, (87.9%) on nevirapine arm and efavirenz arm (91.3%) had been using ARVs for > 12 months. Liver

Table 1. Socio-demographic characteristics of study participants (N = 373)

Characteristics	Category	Nevirapine regimen (n = 224)	Efavirenz regimen (n = 149)
Age (years)	15-35	88 (39.3)	49 (32.9)
	36-55	122 (54.5)	87 (58.4)
	>55	14 (6.2)	13 (8.7)
Sex	Male	36 (16.1)	61 (40.9)
	Female	188 (83.9)	88 (59.1)
BMI (Kg/m^2)	Underweight (<18.5)	18 (8)	13 (8.7)
	Normal (18.5-24)	138 (61.6)	99 (66.4)
	Overweight (25-29)	49 (21.9)	32 (21.5)
	Obese (≥ 30)	19 (8.5)	5 (3.4)

Table 2. Clinical characteristics of study participants (N = 373)

Characteristics	Category	NVP regimen (n = 224)	EFV regimen (n = 149)
CD4 cell count (cells/mm^3)	≤ 250	44 (19.6)	34 (22.8)
	251-500	120 (53.6)	67 (45.0)
	501-1000	55 (24.6)	45 (30.2)
	> 1000	5 (2.2)	3 (2.0)
Duration of ARVs use (months)	≤ 5	4 (1.8)	9 (6.0)
	5 -12	23 (10.3)	4 (2.7)
	> 12	197 (87.9)	136 (91 3)
ALT	Grade 0 (≤ 1.25 x ULN)	197 (87.9)	143 (96.0)
	Grade I (1.25-2.5 x ULN)	26 (11.6)	4 (2.7)
	Grade II (2.6-5 X ULN)	1 (0.4)	2 (1.3)
	Grade III (5.1-10 X ULN)	0 (0.0)	0 (0.0)
	Grade IV (10 X ULN)	0 (0.0)	0 (0.0)
AST	Grade 0 (≤ 1.25 x ULN)	198 (88.4)	144 (96.6)
	Grade I (1.25-2.5 x ULN)	25 (11.2)	3 (2.0)
	Grade II (2.6-5 X ULN)	1 (0.4)	2 (1.3)
	Grade III (5.1-10 X ULN)	0 (0.0)	0 (0.0)
	Grade IV (10 X ULN)	0 (0.0)	0 (0.0)
ARVs Regimen	AZT/3TC/NVP	222 (99.1)	NA
	TDF/FTC/NVP	2 (0.9)	NA
	AZT/3TC/EFV	NA	55 (50.3)
	TDF/3TC/EFV	NA	74 (49.7)

Abbreviations:

ULN, upper limit of normal range; **ALT,** alanine aminotransferases; **AST,** aspartate aminotransferases; **AZT,** zidovudine**; 3TC,** lamivudine; **NVP,** nevirapine; **TDF,** tenofovir; **FTC,** emitricitabine; **EFV,** efavirenz; **NA,** not applicable.

enzymes elevation was of grade 1 to 2 in both arms. Table 2 shows the summary of clinical characteristics of study participants.

Serum aminotransferases (ALT and AST) levels in patients

Mild to moderate elevation of ALT were observed in 27 (12.1%) patients on nevirapine arm compared to 6 (4%) on efavirenz arm. On the other hand, mild to moderate elevation of AST were observed in 26 (11.6%) patients on nevirapine arm compared to 5 (3.3%) patients on efavirenz arm. The mean level of ALT was 34 ± 13.22 IU/L in the nevirapine arm compared to 20.68 ± 14.27) IU/L in efavirenz arm. Twenty six patients had both ALT and AST elevated above the normal range (5-35 IU/L).

Prevalence of hepatotoxicity in patients using nevirapine and efavirenz-based ARVs

The prevalence of hepatotoxicity was 12.1% among nevirapine based regimen users compared to 4% of efavirenz-based regimen users (P = 0.004). Hepatotoxicity was asymptomatic in both groups of patients (i.e there was no signs and symptoms of jaundice, skin rashes or RUQ pain). Figure 1 shows the comparison of hepatotoxicity between nevirapine and efavirenz regimen users.

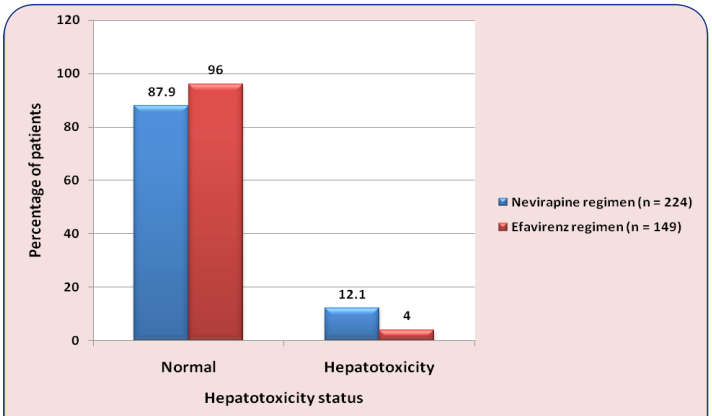

Figure 1. Comparison of hepatotoxicity between nevirapine and efavirenz regimen users

The hepatotoxicity observed was mild to moderate (grade 1 to 2) in both categories of patients. Severe or life threatening hepatotoxicity (grade 3 to 4) was not observed in either group of patients.

Association of hepatotoxicity with patient-related factors

This study enrolled 188 (83.9%) females and 36 (16.1%) males in the nevirapine based group. Hepatotoxicity was approximately equally distributed among females (12.2%) and males (11.1%). Univariate analysis showed no statistical significant differences between occurrence of hepatotoxicity with patient's sex (P = 1.00).

Among the 224 patients enrolled in the study in the nevirapine arm, 138 (61.6%) patients had normal BMI (18.5–24 kg/m^2). However, 18 (8.0%), 49 (21.9%) and 19 (8.5%) patients were underweight (< 18.5 kg/m^2), overweight (25-29 kg/m^2) and obese (≥ 30 kg/m^2), respectively. The mean BMI was 23.67 ± 4.3 kg/m^2. Hepatotoxicity was high (22.2%) in underweight patients compared to normal (12.3%), overweight (8.2%) and obese (10.5%) patients. It was observed that hepatotoxicity decreased steadily as BMI was increasing. However, univariate analysis did not show significant association between occurrence of hepatotoxicity with patient`s BMI (P = 0.474).

Among the enrolled patients in the nevirapine arm, 44 (19.6%), 120 (53.6%), 55 (24.6%) and 5 (2.2%) patients had CD4 cell count of ≤ 250, 251-500, 501-1000 and > 1000 cells/mm^3, respectively. The mean CD4 cell count was 412.54 ± 212.15 cells/mm^3. Hepatotoxicity was significantly higher (18.3%, P = 0.018) in patients with CD4 cell count in the range of 251-500 cells/mm^3 compared to patients with CD4 cell count of ≤ 250 (2.3%) or > 500(7.3%). This was also confirmed in a multiple logistic regression analysis (Table 4) indicating that hepatotoxicity was significantly higher (P = 0.03, Adjusted OR = 10, 95% CI 1.25-80) in patients with CD4 cell count of > 250 cells/mm^3 than in patients with CD4 cell count of ≤ 250 cells/mm^3. Categorization and analysis of CD4 cell count was done using a cut-off point of 350 cells/mm^3 as the criteria for initiation of ART in Tanzania (22). However, the results did not show statistical significant differences (P = 0.97) between patients with CD4 cell count of ≤ 350 cells/mm^3 and those with CD4 cell count of > 350 cells/mm^3 with the occurrence of hepatotoxicity.

Majority of patients (87.9%) were using nevirapine therapy for more than 12 months. The mean duration for the use of nevirapine therapy among patients was 47.93 ± 26.91 months. Hepatotoxity was common (13.2%) in patients who were on nevirapin-based regimen for more than 12 months than those who were using the same regimens (4.3%) for less than 12 months. On the other hand, the univariete analysis indicated no significant differences (P = 0.119) between occurrence of hepatotoxicity with duration of nevirapine use.

Overall, there was no significant association found between age, sex, BMI and duration of nevirapine use with occurrence of hepatotoxicity. However, CD4 cell count levels had an influence in the occurrence of hepatotoxicity. Table 3 shows the summary of the association of these variables.

Among the 188 female patients enrolled into the study in the nevirapine arm, 36 (19.1%) of them had CD4 cell count of ≤ 250 cells/mm^3. Of these, only 1 (2.8%) patient had hepatotoxicity. On the other hand, hepatotoxicity was observed in 22 (14.5%) among 152 (80.9%) patients who had CD4 cell count of > 250 cells/mm^3. There was no significant differences between females with CD4 cell count of ≤ 250 cells/mm^3 and those with CD4 cell count of > 250 cells/mm^3 with the occurrence of hepatotoxicity (P = 0.085). Overall, female sex was not a factor for the occurrence of hepatotoxicity in our study.

Among 36 male patients recruited on nevirapine arm, 18 (50%) patients had CD_4 cell count of ≤ 400 cells/mm^3, in which, hepatotoxicity was observed in 2 (11.1%) patients. Similarly, 18 (50%) patients had CD4 cell count of > 400 cells/mm^3 in which hepatotoxicity was observed in 2 (11.1%) patients. There was no significant differences between males with CD4 cell count of > 400

Table 3. Summary of association of hepatotoxicity with patient-related factors

Variable	Categories	Hepatotoxicity status		Chi-square	P-value
		Normal	Hepatotoxicity		
Age (Years)	15-35	82(93.2)	6(6.8)	7.258	0.029
	36-55	101 (82.8)	21(17.2)		
	> 55	14 (100)	0 (0.0)		
Sex	Male	32 (88.9)	4 (11.1)	0.036	1.00
	Female	165 (87.8)	23 (12.2)		
BMI (Kg/m²)	Underweight (< 18.5)	14 (77.8)	4 (22.2)	2.506	0.474
	Normal (18.5-24)	121 (87.7)	17 (12.3)		
	Overweight (25-29)	45 (91.8)	4(8.2)		
	Obese (≥ 30)	17 (89.5)	2 (10.5)		
CD4 cell count (cells/mm³)	≤ 250	43 (97.7)	1 (2.3)	10.306	0.018
	251-500	98 (81.7)	22 (18.3)		
	501-1000	51 (92.7)	4 (7.3)		
	> 1000	5 (100)	0 (0.0)		
Duration of nevirapine use (months)	≤ 5	4 (100)	0 (0.0)	2.08	0.114
	>5<12	22 (95.7)	1 (4.3)		
	> 12	171 (86.8)	26 (13.2)		

cells/mm³ and those with CD4 cell count of ≤ 400 cells/mm³ with occurrence of hepatotoxicity (P = 1.00).

Multivariate analysis of the factors associated with hepatotoxicity in patients

All patient-related factors on univariate analysis were considered for multivariate analysis so as to control for confounding factors. Table 4 shows the factors associated with hepatotoxicity on multivariate logistic regression analysis. Only CD4 cell count level was the independent factor (P = 0.03, adjusted OR = 10, 95% CI 1.25-80) for occurrence of hepatotoxicity. Patients with CD4 cell count of > 250 cells/mm³ had 10 times higher risk of developing hepatotoxicity than patients with CD4 cell count of ≤ 250 cells/mm³. Female patients had nearly equal probability (adj.OR: 1.37, 95% CI: 0.4-4.7) of developing hepatotoxicity as their male counterparts.

Patients with low BMI (< 18.5kg/m2) were 2.7 times more (adj. OR: 0.3, 95% CI: 0.09-1.43) likely to develop hepatotoxicity than patients with normal BMI or obese patients. Additionally, overweight patients were 0.26 more likely (adj. OR: 0.26, 95% CI: 0.05-1.37) to develop hepatotoxicity compared to underweight patients. However, females with CD4 cell count of > 250 cells/mm³ were 5 times (adj. OR: 5.22, 95% CI: 0.67-40.93) more likely to develop hepatotoxicity compared to females with CD4 cell count of < 250 cells/mm³. On the other hand, male patients with CD4 cell count of > 400 cells/mm³ were 2.57 times more likely (adj. OR: 2.57, 95% CI: 0.19-34.47) to develop hepatotoxicity compared to males with CD4 cell count of < 400 cells/mm³.

Discussion

HIV-1–specific non-nucleoside reverse transcriptase inhibitors (NNRTIs), such as nevirapine and efavirenz, are frequently used in combination with other ARVs for the treatment of HIV infection. However, significant hepatotoxicity has been observed with their use in clinical trials and practice (15). This study has assessed the prevalence of hepatotoxicity (of any grade) in HIV-infected patients who were using nevirapine-containing ARVs in comparison with patients using efavirenz-based ARVs. In this study, the prevalence of hepatotoxicity was three times higher in nevirapine regimen users than in efavirenz regimen users. This is comparable to the prevalence of hepatotoxicity which have been reported in other similar studies (13,17).

The observed hepatotoxicity based on the elevation of liver aminotransferses was mild to moderate (grade 1 to 2), and there was no indication of severe or life threatening hepatotoxicity in the studied patients. However, from the findings of this study, it cannot be conclude that severe hepatotoxicity is uncommon in patients. Because of the study design, patients with severe hepatotoxicity may have not been identified. This study was a cross-sectional study in which the exposure (nevirapine) and measure of outcome (hepatotoxicity) were measured at the same time. Therefore, an explanation could not be provided on whether the elevation of liver enzymes observed may progress to severe levels or return to normal values if the offending drug is not stopped.

Nevirapine has been reported to be more hepatotoxic than efavirenz

Table 4. Factors associated with hepatotoxicity from a multiple logistic regression analysis

Variable	Categories	P-value	Adjusted OR	95% CI of adj. OR
Age (Years)	15-35		1	
	36-55	0.112	2.253	0.83 -6.13
	Above 55	1	0.91	NA
Sex	Male		1	
	Female	0.61	1.37	0.40-4.70
BMI (kg/m²)	Underweight (< 18.5)		1	
	Normal (18.5-24)	0.15	0.37	0.09-1.43
	Overweight (25-29)	0.11	0.26	0.05-1.37
	Obese (≥ 30)	0.24	0.31	0.04-2.19
CD4 cell count (cells/mm³)	≤ 250		1	
	251-500	0.03	10	1.25-80.05
	> 500	0.33	3.1	0.32-29.74
Duration of nevirapine use (month)	≤ 5		1	
	>5≤12	NA	NA	NA
	> 12	NA	NA	NA
CD4 cell count in females	≤ 250		1	
	> 250	0.12	5.22	0.67-40.93
CD4 cell count in males	≤ 400		1	
	> 400	0.48	2.57	0.19-34.47

Abbreviations: **OR**, Odds Ratio; **CI**, Confidence interval; **NA**, Not Applicable; **1**, reference category; **NVP**, Nevirapine.

(17), and two mechanisms have been suggested in nevirapine-associated liver toxicity. The first one is an immune-mediated mechanism, and accounts for cases of transaminase elevations seen in individuals who are also experiencing skin reactions. This occurs a few days to weeks after beginning using nevirapine-containing regimens (23). This hypersensitivity reaction seems to be more common in individuals who have higher CD4 lymphocyte counts. This may explain the unexpectedly high rates and the severity of hepatotoxicity observed in immunocompetent HIV-negative patients who underwent post-exposure prophylaxis with nevirapine (23).

The second mechanism of nevirapine-related liver toxicity, which does not involve other organs, has a delayed onset, often several months, and might represent an intrinsic toxic effect of the drug. If this is the case, it should be predictable and perhaps dose-related. In support of this hypothesis and similar to the findings of the present study, a recent study (17) reported that the incidence of grade 3–4 liver toxicity increased over time in individuals treated with nevirapine.

Although considered to be safe, efavirenz-based regimens have also been associated with hepatotoxicity, lipid disturbances, and psychiatric symptoms (24). Studies of the clinical manifestations of these effects have revealed that some forms of these toxicities resemble disorders induced by mitochondrial dysfunction, but their molecular and cellular mechanisms remain largely unknown (24,25). A study by Apostolova et al (24) reported that efavirenz does induce bioenergetic stress in hepatic cells by inhibiting mitochondrial function through an acute mechanism that is independent of mitochondrial DNA replication. This leads to the accumulation of lipids in the cytoplasm (which damage cellular function) through a mechanism mediated by activation of adenosine monophosphate–activated protein kinase.

In this study, hepatotoxicity was significantly higher in patients with CD4 cell count of > 250 cells/mm³ compared to patients with CD4 cell count of ≤ 250 cell/mm³, irrespective of patient's sex. This observation contradicts with the previous findings which indicated that men and women with CD4 cell count of > 400 cells/mm³ and > 250 cells/mm³, respectively, are at greater risk of developing hepatotoxicity (16). Therefore, based on the findings of this study, patients (whether males or females) with CD4 cell count of > 250 cells/mm³ had 10 times more risk of developing hepatotoxicity than patients with CD4 cell count of ≤ 250 cells/mm³. This explains

the involvement of the immune system in the occurrence of hepatotoxicity. Therefore, close monitoring of patients with CD4 cell count of greater than 250 cell/mm^3 who are using nevirapine based ARVs regimens may be of great benefit.

In this study, hepatotoxicity was higher in underweight patients compared to patients with normal weight, overweight, and obese patients. It was observed that hepatotoxicity decreased steadily as BMI was increasing. This may be due to the fact that the extent of drug metabolism depends on patient's body surface area which is directly proportional to BMI. Therefore, patients with low BMI are more likely to develop drug related adverse effects compared to patients with higher BMI. Similar studies have reported significant association between occurrence of hepatotoxicity and patients with BMI of < 18.5 kg/m^2 (12). However, the results of the present study are in contrast to those reported by Kovari et al (26), who reported that increased BMI (> 25 kg/m^2) was significantly associated with chronic elevated ALT due to increased risk of hepatic steotosis in patients with higher BMI. This difference could be due to differences in the race of the study participants and other patient related factors (27).

This study also showed that, hepatotoxicity was common in patients who were using nevirapine based regimen for more than 12 months than those who were using the same regimen for less than 12 months. The U.S department of health and human services guidelines (18) for the use of ARVs in HIV-1 infected adults and adolescents patients, U.S FDA, and Viramune® manufacturer (Boehringer Ingelheim) have emphasized that the critical period for intensive clinical and laboratory monitoring to detect hepatotoxicity should be in the 12 weeks of nevirapine therapy. However, we noticed that the risk of hepatotoxicity with nevirapine containing regimens increased steadily over time and none of patients had indication of hepatotoxicity in the first 12 weeks of therapy. This observed difference could be due to differences in the cohort of patients who were enrolled in the study. In our study, only 1.8% of patients were on nevirapine therapy for less than 12 weeks. Majority of patients (98.2%) were on nevirapine therapy for more than 12 weeks. Therefore, the small number of patients who were using nevirapine regimen for duration of less than 12 weeks could be the reason for the observed differences in the present study.

In in this study, it was observed that the risk for developing nevirapine related adverse events increased with the duration of nevirapine exposure. This is in line with the alternative mechanism of nevirapine induced hepatotoxicity which is the intrinsic drug toxicity that occurs after long exposure to the drug and does not appear to correlate with baseline CD4 cell count (9). This delayed type of nevirapine toxicity seems to correlate with higher plasma drug concentrations as reported by other researchers. For instance, Sulkowski et al (13) prospectively studied the incidence of severe hepatotoxicity (grade 3 or 4 change in alanine or aspartate transaminase levels) among patients who were receiving NNRTI. In that study, only 32% of patients with severe hepatotoxicity in the nevirapine arm had hepatotoxicity detected during the first 12-weeks of therapy. Therefore, this can easily tell that hepatotocity may occur at any time of nevirapine therapy as it has been reported by other similar studies (17,28).

Conclusion

Based on the findings of this study it can be concluded that hepatotoxicity in HIV-infected patients who are using nevirapine-based ARVs regimens is not uncommon. Liver damage is three times more common in patients receiving nevirapine-based regimens than in those using efavirenz-based regimens. The hapatotoxicity is mild to moderate and may occur at any time of therapy with or without constitution symptoms. It is recommended that routine monitoring of liver aminotransferases should be conducted in patients who are using nevirapine-containing ARVs regimen. This was not a follow-up study, and therefore we could not identify whether the observed mild to moderate elevation of liver aminotransferases could progress to severe hepatotoxicity or return to normal over a period of time if the offending drug is not stopped.

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Disclosure

The authors report no conflicts of interest in this work.

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