Research Article

Patterns and Risk Factors for Alanine Aminotransferase Elevation among HIV Patients on Nevirapine Regimens

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Background: Elevated levels of serum transaminases are often detected in HIV patients. This has often been attributed to hepatic effects of antiretroviral drugs.

Objective: To determine the pattern and risk factors for alanine aminotransferase elevation in HIV patients positive on nevirapine based regimens.

Methodology: We conducted a retrospective cohort study of HIV infected patients on nevirapine containing regimens who attended the Kenyatta National Hospital comprehensive care clinic between May and August 2014. We sampled participants by convenient sampling method. Generalized linear regression was performed to establish patterns and predictors for hepatotoxicity (grade 1-4) which were the primary outcomes of interest. Predictor variables that were included in the analysis include; demographic information, baseline ALT and CD4 levels, ART regimens, co-morbidities and treatment duration.

Results: Risk factors for ALT elevation differed by gender. Predictor variables that were significantly associated with ALT elevation in both sexes included; elevated baseline ALT level [β=10.14 (95%CI 7.34- 12.96); P<0.001], [β=13.52 (95%CI 9.36 –17.68); P < 0.001] and renal disease [β=5.44 (95%CI 2.62 – 8.25); P <0.001], [β=11.52 (95%CI 3.46 – 19.60); P = 0.005] in females and males respectively. Ethnicity had a protective effect in both sexes; [β-6.61(95%CI-9.28, -3.93); P< 0.001] in males and [β-1.20 (95% CI-2.39, -0.01); P= 0.048] in females. Among the different ethnic groups, Nilotes and Cushites had lower ALT levels compared to Bantus.

Other factors that were significant included; smoking (P=0.001), concurrent illnesses (P=0.045), previous adverse drug reactions (P=0.040) in females and a longer duration of anti-retroviral therapy [β 1.81(95%CI 0.89 – 2.73); P < 0.001] in males. Poor adherence had a protective effect [β -1.62(95%CI -3.20, -0.04); P=0.045] among females, whereas initiation on AZT+3TC+NVP had a significant protective effect [β-7.80 (95%CI -13.96, -1.63); P=0.013] in males.

Conclusion: Creatinine and transaminase testing should be done routinely to deal with delayed hepatotoxicity in patients with abnormal ALT baseline levels.

Key words: Alanine aminotransferase, hepatotoxicity, nevirapine.

1. Introduction

HIV associated morbidity and mortality have decreased dramatically since the introduction of life prolonging anti-retroviral therapy. Because of improved life expectancy, non-HIV/AIDs defining diseases and drug related toxicities have emerged key issues in the management and care of people living with HIV/AIDs (Kovari et al, 2010).

Nevirapine is one of the recommended first line anti-retroviral drugs and forms the backbone in HIV management. However, nevirapine can cause potentially life-threatening skin reactions and hepatotoxicity which usually occur within the first 18 weeks of treatment. Six to seven percent of patients on nevirapine based regimen discontinue the use of antiretroviral drugs because of clinically significant hypersensitivity reactions (Kesselring et al, 2009). Hepatotoxicity can be fatal when not recognized early and when treatment is not interrupted in time (Ciccacci et al, 2010).

Screening for hepatotoxicity during ART is primarily based on serum levels of ALT, a liver enzyme that serves as a "proxy" for liver inflammation and damage. However, laboratory tests, while desirable, are not a prerequisite for initiation or for routine follow up of patients on ART in resource constrained settings. Hepatotoxicity from drugs is often difficult to diagnose because the signs and symptoms vary so much from one drug to the next and symptoms often resemble other commonly diagnosed illnesses. Early detection of liver injury may be a challenge in settings where there is no routine ALT monitoring and many patients with hepatotoxicity may be under diagnosed (Baylor & Johann-Liang, 2004).

The bulk of the current knowledge of adverse events associated with anti-retroviral drugs is primary based on data from resource rich countries(Wit et al, 2008). Only few studies have been conducted in Sub-Sahara Africa where the burden of HIV/AIDS is highest. This means available information may not be representative as demographics, genetic factors, comorbidities, nutritional status and concomitant use of other drugs may vary substantially.

It is therefore necessary to determine the frequency and pattern of ALT elevation and identify the risk factors for hepatotoxicity among HIV patients using nevirapine-containing regimen in Sub-Sahara Africa.

2. Methodology

2.1 Study design, site and population

This study was a descriptive right censored retrospective cohort study. The study targeted HIV positive adult patients on nevirapine based regimens seen at Kenyatta National Hospital Comprehensive Care Clinic (CCC) between May and August 2014.

The Hospital is located at the Kenya's capital city of Nairobi and is the largest teaching and referral Hospital in Kenya.

2.2 Inclusion and exclusion criteria

Patients were included into the study if they were HIV positive, on nevirapine-containing regimen for at least 6 months and aged between 18 and 55 years. In addition they were required to provide informed consent at the start of the study. Participants were excluded if they were on nevirapine-containing regimen for less than 6 months, declined to give consent and aged below 18 years or above 55 years.

2.3 Sample size determination and sampling procedure

The sample size was calculated using the formula described by Hulley et al (2013) for estimation of sample size of a dichotomous variable in a cohort study. The calculated minimal sample was 138 based on an expected prevalence of hepatotoxicity of 10% at 95% confidence level.

Participants were sampled by convenient sampling method. Recruitment was done as patients refilled their prescriptions at the CCC pharmacy. All adult patients on nevirapine regimens were invited to participate. Patients were screened beforehand to identify those who met the eligibility criteria. Those who gave informed consent were recruited into the study until the required sample size was achieved.

2.4 Data collection procedure

A pilot study was used to improve the data collection tools. Participants were recruited at the Pharmacy as they waited to refill their prescriptions. Participants were subjected to an interview to obtain information on; self-reported medication related problems, alcohol use, use of herbal and non-prescription preparations, marital status, smoking status and educational level.

A list of patients who agreed to participate was generated and given to the records department for the purpose of retrieving the patient files for data abstraction. The following information was abstracted from the files: demographic characteristics; liver function tests results; history of pre-existing liver disease; CD4 count; history of any skin reaction; renal function tests; any adverse drug event; medication history and documented clinical signs of hepatotoxicity. In addition data on clinical signs of hepatotoxicity was collected.

2.5 Case definitions

Patients were dichotomized as having normal or elevated ALT level if their fold increase was less or greater than1.25 upper limit of the normal (ULN). The severity of liver toxicity was further graded using the AIDs Clinical Trial Group (ACTG) system. In this system, severity is based on the number of times ALT levels is greater than upper limit of the normal (ULN) as follows; grade 0 (normal)< 1.25, grade 1 (mild) 1.25–2.5, Grade 2 (Moderate)2.5–5 , grade 3 (Severe) 5-10, grade 4 (Very severe) > 10. The same cut off 40IU/L was used for both men and women.

Table 1: Demographic and Clinical characteristics of the study cohort

Variables	Median [IQR] or n (%)
Sex	
Male	56 (23.2)
Female	185 (76.8)
Age at diagnosis (years)	39 [35,44]
Weight at diagnosis(kg)	62 [56,70]
Height (cm)	162 [158, 168]
BMI at HAART initiation	
≤18.5	147 (61)
≥18.5	94 (39)
Marital status	
Married	155 (64.3)
Single	57 (23.7)
Divorced	4 (1.7)
Widowed	24 (10.0)
Separated	1 (0.4)
Education	
Primary	48 (19.9)
Secondary	117 (48.6)
Diploma	57 (23.7)
Degree	19 (7.9)
Employment status	
Unemployed	18 (7.5)
Employed	108 (44.8)
Self-employed	115 (47.7)
Alcohol use	
Never	167 (69.3)
Occasionally	72 (29.9)
Regularly	2 (0.8)
Smoking	
No	236 (97.9)
Yes	5 (2.1)
CD4 cell count x10^9/L	206[127-270]
≤250	158 (65.6)
≥251	68 (28.2)
Missing values	15 (6.2)
ALT at initiation of HAART	
Normal	209 (86.7)
Elevated	26 (10.8)
Missing	6 (2.5)
Concurrent illness	
None	182 (75.5)
Hypertension	36 (14.9)
Diabetes	3 (1.2)
PUD	4 (1.7)
Asthma	3 (1.2)
Chronic pain	5 (2.1)
Other conditions	8 (3.6)

ART, antiretroviral therapy; BMI, body mass index, calculated as the weight in kilograms divided by the square of height in meters; IQR, interquartile range ; PUD-peptic ulcer disease; n= proportion per category.

2.6 Data management and analysis

Participant's confidentiality was maintained by not recording their name or clinic number in the data collection forms. Each study participant was allocated a unique identifier that was used throughout the study. Abstracted data was entered in excel® spreadsheet. The raw data generated during the course of the study and the final report was subjected to inspection and quality audit for conformity to set protocols by the investigator.

Data analyses were performed using STATA version 10 (StataCorp, USA). Descriptive data analysis was carried out on all variables. The Shapiro Wilk test was used to determine which continuous variables conformed to normal distribution. Continuous variables that were not normally distributed were summarized as median and interquartile range (IQR).

Counts and percentages were reported for categorical variables and the 95% confidence intervals were reported. Pearson Chi square test and Kruskal Wallis tests were used to compare the distribution of various variables with the main outcome of interest.

Generalized linear regression was used to determine predictors for development of ALT elevation. All variables with a P-value lower than 0.20 at bivariable analysis were entered into a multivariable model (if clinically meaningful) and model building was conducted using a manual forward stepwise selection method. For multi-variable analysis, a P value ≤ 0.05were considered statistically significant.

2.7 Ethical considerations

Ethical approval was obtained from the Kenyatta National Hospital/University of Nairobi Ethics and Research Committee (KNH/UoN, approval reference No: KNH-ERC/A/122).Participants were briefed about aims, procedures, potential risks and benefits of taking part in the study.

Participants were required to understand and voluntary sign consent form before enrolling into the study. To ensure confidentiality of the study participants, all data collection forms were coded and held in safe custody.

Table 2: Regimens at ART initiation

Regimen type	Number of Patients (%)
TDF+3TC+NVP	71(29.5)
AZT+3TC+NVP	78(32.4)
D4T+3TC+NVP	85(35.3)
ABC+3TC+EFV	1(0.4)
AZT+3TC+EFV	3(1.2)
TDF+3TC+EFV	3(1.2)

TDF: Tenofovir; 3TC: Lamivudine; NVP: Nevirapine; AZT: Zidovudine; D4T:Stavudine; ABC: Abacavir; EFV: Efavirenz

3. Results

3.1 Baseline characteristics of the study participants

Overall, 185 (76.8%) of the 241 participants who took part in the study were females. The median age was 39 years [(IQR) 35, 44]. Thirteen (5.4%) had a body mass index of below 18.5kg/m². The median body weight at baseline was 62kg (range 56 to 70kg). One hundred and fifty five (64.3%) were married. Majority of the participants (76.7%) were of the Bantu ethnicity while Nilotes constituted 18%. Seventy two (29.8%) participants reported taking alcohol occasionally (less than twice a month) while two (0.8%) took alcohol regularly. Five (2.1%) participants were smokers. Most patients had attained either primary or secondary education accounting for 68.5% of the study participants.

At the start of therapy 158 (65.6%) patients had CD4 cell count less than 250cells/mm³. The median baseline ALT level was 22 IU/L (range 17 to 32). Most of the participants (91.3%) had a normal ALT level while fourteen (5.8%) had an elevated baseline ALT (above 40UI/L). Most patients were initiated on stavudine based combination (35.3%). The median duration of follow up for the entire cohort was 4.75 years [IQR 3.34 - 6.6]. The prevalence of co-morbidities was less than 10%. The most prevalent conditions were hypertension (14.5%) and chronic pain (2.1%). Baseline characteristics of the study participants are presented in **Table 1**.

3.2 Antiretroviral Regimens

At ART initiation 234 (97%) patients were started on a nevirapine based regimens, the other seven (3%) were initiated on efavirenz-based regimens. Those on efavirenz-based regimens were switched to a nevirapine based regimen at the time of the recruitment into the study. Most patients were initiated on stavudine, lamivudine and nevirapine combination (35.3%). This is presented in **Table 2**.

Ninety two patients switched regimens in the course of their therapy. Most of the patients 70 (29.0%) were switched from D4T+3TC+NVP to TDF+3TC+NVP. The most common reason for regimen switch was development of adverse drug reaction especially peripheral neuropathy (38%) and lipodystrophy (15%) associated with stavudine.

3.3 ALT changes in the study cohort

Patterns of ALT elevation in the course of therapy

At baseline, 209 of the participants (86.7%) had a normal ALT level while 26 (10.8%) had mild elevation. Six patients had no baseline ALT level readings. The median baseline ALT level was 22 IU/L (range 17 to 32).

A median band plot was generated to examine the changes in ALT levels with time in patients with normal baseline values. The pattern looked cyclical with peaks and troughs. The peak levels seemed to increase with time. The trend is presented in **Figure 1**.

In patients who had abnormal ALT levels, lowess plots were generate to establish the trend. A lowess plot is a summary measure of the weighted median of a series of ALT readings. In this group of patients the trend was a gradual decline in ALT levels till about 6 years of therapy, thereafter the ALT levels started rising steadily (**Figure 2**).

Prevalence and severity of ALT elevation

Majority of the participants (67.2%) had normal ALT levels throughout the study. Seventy two (29.9%) had mild elevation and seven (2.9%) developed moderate hepatotoxicity. None of the participants developed severe or very severe hepatotoxicity. This is presented in **Figure 3**.
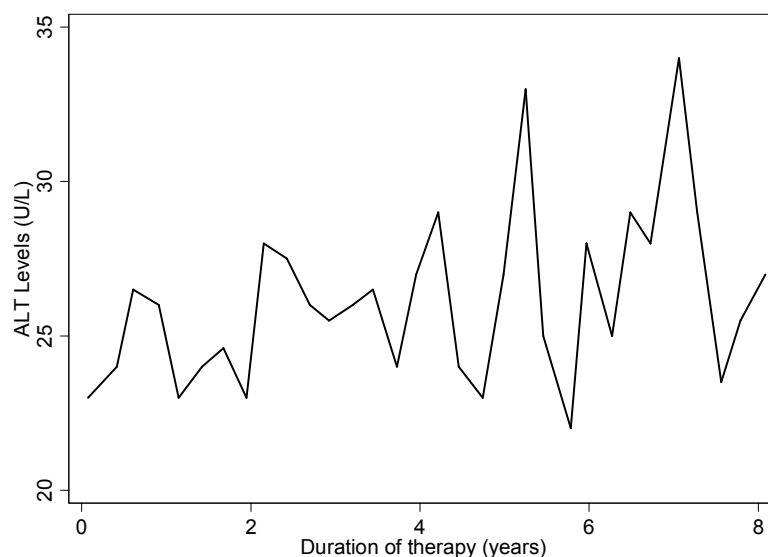

Figure 1: Median band plot of ALT levels over time for patients with normal values at baseline
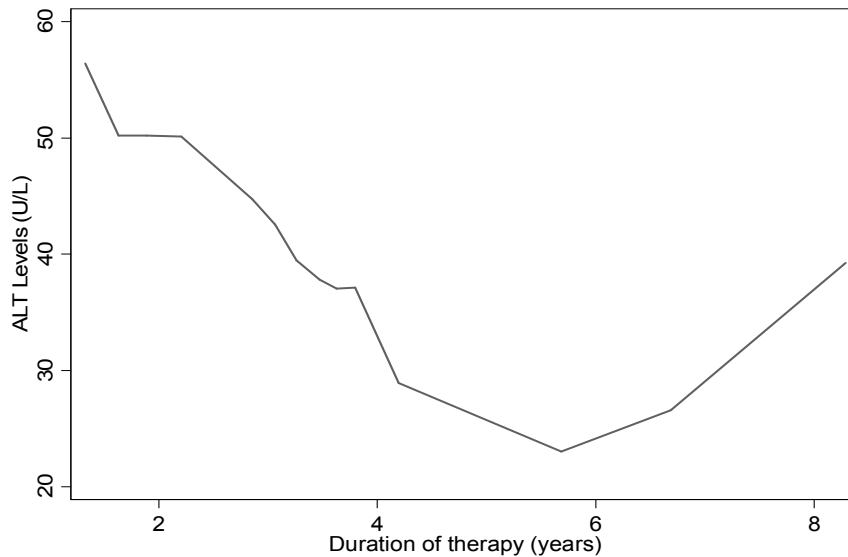
Figure 2: Lowess plot of ALT levels over time for patients with abnormal values at baseline
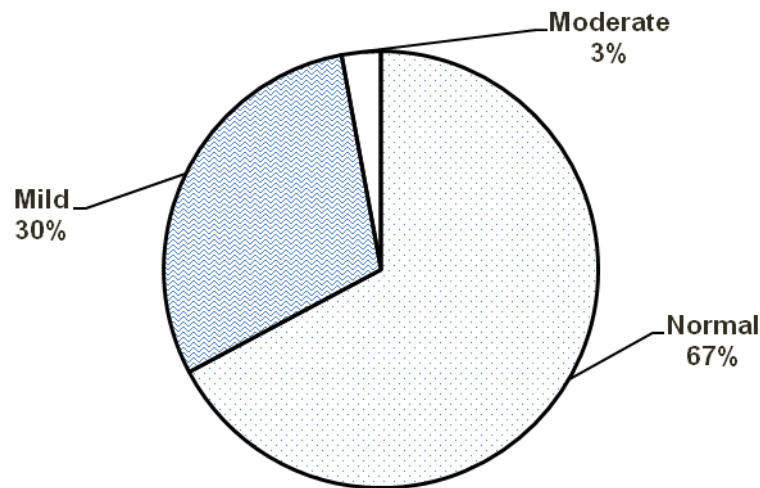

Figure 3: Severity of hepatotoxicity in the study cohort

Comparison of baseline traits of patients with normal and elevated ALT levels during therapy.

The baseline characteristics of the study participants according to ALT elevation are summarized in **Table 3** (Supporting Information). Severity of hepatotoxicity was based on the fold increase in ALT levels. A 1.25 fold increase above the ULN was used as a cut off in this study.

Sex was significantly associated with ALT elevation (P <0.001). Thirty out of a total of 56 males (53.6%) in the cohort had elevated ALT compared to 26.5% females. Age at ART initiation was categorized as those below or above 45 years. Eighteen (41%) of the above 45 years old had an elevation compared to 31.6% among those below 45 years old. A larger proportion of Bantus (35.9%) had an elevated ALT as compared to Nilotes (27.9%). None of the six Cushites had elevated ALT levels.

Thirty eight percent of those with a BMI of <18.5kg/m^2 had elevated ALT as compared to 32.5% of those with BMI of >18.5kg/m^2. Elevation was common among alcohol users (41.7%) as compared to non-users (29.2%). Most smokers had elevated ALT levels (60%) compared to non-smokers (32.2%).

There was an association between abnormal baseline ALT level and subsequent elevation (P <0.001). Ninety two percent of patients with abnormal baseline ALT developed subsequent elevation compared to 25.8% with normal ALT at baseline. CD4 cell count was categorized as low (≤ 250 cells/µL) and high (>250 cells/µL). CD4 count was not a predictor of ALT elevation. Nucleoside/nucleotide backbone used along with lamivudine and nevirapine had a significant impact on the status of liver function (p<0.019). Half of the patients who developed ALT elevation were initiated on d4T+3TC+NVP.

3.4 Identification of risk factors for ALT elevation

Generalized linear regression was done to identify variables predictive of ALT elevation.

In bivariable analysis of the whole cohort; sex, smoking, concurrent illness, elevated baseline ALT level, nucleoside/nucleotide backbone used with nevirapine, duration of therapy and renal disease were significantly associated with ALT elevation (P< 0.05). Ethnic group and poor adherence had a protective effect. The results are presented in **Table 4** (Supporting Information).

During multivariable analysis, there was a statistical interaction between sex and drug regimen. This was interpreted to be a biological interaction since it tended to be additive. Consequently, for subsequent analysis the data was stratified across sex.

In both males and females, elevated baseline ALT level and renal disease were associated with increased risk of ALT elevation. Females who had elevated ALT at baseline had 10 units higher [β 10.14 (95%CI 7.34-12.96); P<0.001] while males had 13.52 units higher [β 13.52 (95% CI 9.36 – 17.68); P < 0.001] than those with normal levels at baseline.

Renal disease which was defined as having an estimated glomerular filtration rate (eGFR) < 50ml/min/1.73² was significantly associated with ALT elevation. Females who developed renal disease had five units higher [(95% CI 2.62 – 8.25); P <0.001] whereas males had 11.52 units higher [(95% CI 3.46 – 19.60); P = 0.005] than those with normal renal function.

Ethnicity had a protective effect in both males and females. Among the different ethnic groups, Nilotes and Cushites had lower ALT levels compared to Bantus. However, ethnicity had a greater effect in males than females. Male Nilotes had 9 units lower [β-9.12 (95% CI -13.17, -5.08); P< 0.001] while Cushites had 13 units lower [β-12.89 (95% CI -25.10, 0.69); P = 0.036] than Bantus. On the other hand, female Nilotes had 4 units

[β-3.56 (95% CI -5.54, -1.59); P< 0.001] whereas Cushites had 8 units [β-7.88 (95% CI -13.04, -2.71); P=0.003] lower than Bantus.

In females, smoking [β11.31 (95% CI 4.46, 18.18); P=0.001], peptic ulcer disease [β16.72 (95% CI 9.48 - 23.97); P<0.001] and previous adverse drug reactions [β 2.02 (95% CI 0.10- 3.95); P=0.040], were associated with increased risk of ALT elevation. Poor adherence had a protective effect [β -1.62 (95%CI -3.20, -0.04); P=0.045].

In males, a longer duration of anti-retroviral therapy was associated with increased risk [β 1.81 (95% CI 0.89 – 2.73); P < 0.001], while initiation on AZT+3TC+NVP had a significant protective effect [β-7.80 (95%CI -13.96, -1.63); P=0.013].

3.5 Prevalence of the clinical signs associated with hepatotoxicity

The clinical signs of hepatotoxicity reported in this cohort were anorexia, abdominal pain and vomiting. None of the patients developed jaundice. Thirteen patients had anorexia, eleven (85%) of whom were females. Eight patients reported vomiting and all of them were females. Abdominal pain was the most widely reported sign with 30 patients.

It was expected that patients who experienced abdominal pain should have showed ALT elevation. Contrary to this expectation, 25 out of 30 patients who experienced abdominal pain had normal ALT levels. There was a negative association between abdominal pain and ALT. Anorexia and vomiting were not statistically significant associated with ALT elevation. The results are presented in **Table 5**.

Table 5: Description of clinical factors associated with ALT elevation

Predictor variable	Normal < 1.25×ULN	ALT elevated > 1.25×ULN	P- Value
Abdominal pain			
No	136 (84.5%)	73 (93.6%)	
Yes	25 (15.5%)	5 (6.4%)	**0.046**
Vomiting			
No	158 (97.5%)	73 (94.8%)	
Yes	4 (2.5%)	4 (5.2%)	0.274
Anorexia			
No	152 (95.0%)	73 (93.6%)	
Yes	8 (5.0%)	5 (6.4%)	0.653

4. Discussion

This study found that the baseline prevalence of ALT elevation (>40 IU/L) was 10.8%; CI (6.84 - 14.73).This implies that 1 in 10 patients had elevated ALT levels before ART initiation. This elevation could be due to HIV illness. Most of the participants in this study (86.7%) had a normal baseline ALT level at ART initiation. Out of

the patients who had normal baseline ALT levels, 30% developed mild elevation while 3% developed moderate elevation throughout the course of therapy. This implies that 3 out of 10 patients on NVP based regimens will develop some form of liver injury. No case of severe or very severe hepatotoxicity was observed in our study. This was in variance with findings of a multicenter study carried in Kenya, Zambia and

Thailand which reported a prevalence of severe hepatotoxicity of 5% (Peters et al, 2010). Other studies have reported a prevalence of severe and/or very severe hepatotoxicity of between 6-15% (Kovari et al, 2010;Stern et al, 2003). This could be explained by the fact that, all our patients were ambulatory and if any patient experienced hepatotoxicity, ARVs had been stopped or were switched to non-nevirapine based regimens.

In patients with normal ALT at baseline, the pattern of ALT change was cyclical with peaks and troughs. The magnitude of the peak levels seemed to increase with time especially from the 5th year of therapy onward. Among patients who had elevated ALT levels at baseline, the trend was a gradual decline in ALT levels until about 6 years of therapy, thereafter the ALT levels started rising progressively. The initial high ALT levels could be attributed to the HIV disease, which is followed by a gradual drop due to protective effect of ART which improved the clinical status and CD4 counts of the patient. HAART therefore has a beneficial effect on patients who have abnormal baseline ALT levels. The delayed rise in ALT levels thereafter could be due to cumulative toxicity and/or decline in liver function with age.

Other studies report that nevirapine-associated hepatotoxicity occurs within the first few weeks to months of starting therapy (Labarga et al, 2010; Stern et al, 2003). In our study, none of the patients developed hepatotoxicity within this period. This may suggest the study cohort was less susceptible to immune-mediated hypersensitivity reaction, which normally develops shortly after starting nevirapine (Kappelhoff et al, 2005). Late occurring hepatotoxicity most likely represents an intrinsic toxic drug effects.

Several unique findings were observed in our study; risk factors for ALT elevation between males and females differed, there were intra-ethnic differences with Bantus being the most susceptible; and initiation on Zidovudine based regimen was protective especially in males.

Intra-ethnic differences in ALT elevation have not been extensively investigated, but a number of studies have reported inter-race variability. Kesselring et al, 2009, found out that Asians were more susceptible to nevirapine induced hepatotoxicity [HR (95% CI) = 2.24 (1.43 – 3.52); P < 0.001] compared to other races. In our cohort, ethnic grouping had a significant effect in ALT levels in both males and females. However, ethnicity had a greater effect in males compared to females. Male Nilotes [β -9.12 (95% CI -13.17, -5.08)] and Cushites [β -12.89 (95% CI -25.10, 0.69)] had significantly lower ALT levels compared to Bantus. The intra-ethnic differences could be due to genetic, environmental or dietary factors. A study has been conducted that compares the distribution of polymorphisms of CYP 450 across the two major ethnic groups (Nilotes and Bantus) of Kenya. The study found a significant variability in the distribution of CYP2D6*4 and CYP2D6*17 between Bantu and Nilotes (Oluka et al, 2014).

Abnormal baseline transaminase levels has been reported to be an independent risk factor for antiretroviral associated hepatotoxicity (Marazzi et al, 2006). Our study has also demonstrated that abnormal baseline ALT levels (> 1.25 times the upper limit of normal)is a risk factor for subsequent ALT elevation in both males and females. This finding are in agreement with a multicenter study conducted in Kenya, Zambia and Thailand which reported that abnormal (≥grade 1) baseline transaminase levels increases the risk of severe hepatotoxicity and rash (Peters et al, 2010).

Although the association between renal disease and hepatotoxicity has not been extensively studied, this study found a correlation between renal disease and ALT elevation. Females who developed renal disease had five units higher [(95% CI 2.62 – 8.25); P <0.001] whereas males had eleven units higher [(95% CI 3.46 – 19.60); P = 0.005] than those with normal renal function. This was a very significant finding although it was not possible to assess whether renal disease precedes liver disease. We speculate that, patients with compromised liver function may accumulate nevirapine which may be toxic to the kidney. Conversion of nevirapine to metabolites may ameliorate it's nephrotoxicity given that there is a paucity of literature of the possible link between nephrotoxicity and hepatotoxicity in patients on nevirapine based regimens.

For the first time we found that poor adherence was associated with a low risk for ALT elevation. We used a very crude measure for poor adherence, patients who reported that they had missed at least one dose the previous week were considered to have poor adherence. Missing doses of ARVs had a protective effect as compared to patients with perfect adherence. This was only significant among females. It is likely that individuals who miss their regular doses of ARVs could be having suboptimal levels of the drugs in plasma.

The type of anti-retroviral drug regimen was found to be one of the predictors of ALT elevation but the findings differed across sex. On bivariable analysis, initiating patients on stavudine based NRTI was associated with increased risk of ALT elevation. However, on stratification across sex, the association was not significant in females. In males, treatment initiation on AZT+3TC+NVP had a significant protective effect [β-7.80 (95%CI -13.96, -1.63)].The finding of our study, contradicts a review of cohort studies investigating the incidence of hepatotoxicity among patients on ARV therapy. This review suggested that the overall rate of ALT elevation is similar among all ARV drugs (Dieterich et al, 2004;Torti et al, 2007).

A longer duration of anti-retroviral therapy was associated with increased risk of ALT elevation among males ([β 1.81(95% CI 0.89 – 2.73); P < 0.001]) but not in females ([β -0.28 (95% CI -0.68, 0.11); P < 0.155]). A study carried out in Spain reported that, patients who tolerate ARV drugs for the first few months of therapy were likely tolerate it in long-term therapy (Kovari et al, 2010)

Smoking and peptic ulcer disease was also associated with increased risk of ALT elevation in females but not in males. However, only two out of a total of 185 females (1.08%) were smokers and about 1.6% had PUD, hence the study was not sufficiently powered to

enable us draw conclusions about the observed association between this two variables and ALT elevation.

From literature, the most important clinical signs and symptoms of drug induced liver injury are; anorexia, vomiting, abdominal pain and jaundice. In this study, anorexia and vomiting were not statistically associated with ALT elevation. One of the likely reasons for this finding is that only a small proportion of the study participants developed and/or reported these signs during the clinical visits. Therefore, the study was not sufficiently powered to find an association between the clinical signs and ALT elevation.

A major strength for this study was that it was carried out in a facility with a capacity to investigate laboratory parameters on regular basis. Also, the sample size of the studied populations was representative of the major ethnic communities in Kenya.

The study had a number of limitations that are inherent to most retrospective observational cohort studies in general. The prevalence of nevirapine-induced hepatotoxicity could not be ascertained precisely. There is a possibility that some patients who developed severe or very severe hepatotoxicity in the course of therapy were either discontinued or switched to other regimens. Secondly, the study relied heavily on pre-recorded information that may have been incomplete, missing or could not be verified, this may have negatively affected the veracity of the study.

5. Conclusion

We conclude that nevirapine based regimens are well tolerated although transaminase elevation might occur in up to one third of HIV infected adult patients on this regimens. Creatinine and transaminase testing should be done routinely to monitor delayed hepatotoxicity in patients with abnormal ALT baseline levels. Impact of renal disease and co-morbidities should be assessed during anti-retroviral therapy. Further studies to establish inter-ethnic variability in clinically relevant polymorphism of drug metabolizing enzymes may be required. This will provide much need pharmacogenetic data specific to African populations.

Conflict of Interest declaration

The authors declare no conflict of interest.

References

Baylor MS, and Johann-Liang (2004). Hepatotoxicity associated with nevirapine use. *J. Acquir. Immune Defic. Syndr.* **35**: 538–539.

Ciccacci C, Borgiani P, Ceffa S, Sirianni E, Marazzi MC, Altan AD, Palombi L (2010). Nevirapine-induced hepatotoxicity and pharmacogenetics: a retrospective study in a population from Mozambique. *Pharmacogenomics*, **11**: 23–31.

Dieterich DT, Robinson PA, Love J& Stern JO (2004). Drug-induced liver injury associated with the use of non-nucleoside reverse-transcriptase inhibitors. *Clin. Infect. Dis.* **38** (Supplement 2): S80–S89.

Kappelhoff BS, Van Leth, Robinson P A, MacGregor TR, Baraldi E, Montella F and Lange JM (2005). Are adverse events of nevirapine and efavirenz related to plasma concentrations. *Antivir. Ther.* **10**: 489–98.

Kesselring AM, Wit FW, Sabin CA, Lundgren JD, Gill MJ, Gatell JM and Reiss P (2009). Risk factors for treatment-limiting toxicities in patients starting nevirapine-containing antiretroviral therapy. *AIDS*, **23**: 1689–1699.

Kovari H, Ledergerber B, Battegay M, Rauch A, Hirschel B, Foguena AK and Weber R (2010). Incidence and risk factors for chronic elevation of alanine aminotransferase levels in HIV-infected persons without hepatitis B or C virus co-infection. *Clin. Infect. Dis.* **50**: 502–511.

Labarga P, Medrano J, Seclen E, Poveda E, Rodriguez-Novoa S, Morello J, and Soriano V. (2010). Safety and efficacy of tenofovir/emtricitabine plus nevirapine in HIV-infected patients. *AIDS.* **24:** 777–779.

Marazzi MC, Germano P, Liotta G, Guidotti G, Loureiro S, da Cruz Gomes and Palombi L. (2006). Safety of nevirapine-containing antiretroviral triple therapy regimens to prevent vertical transmission in an African cohort of HIV-1-infected pregnant women. *HIV Med.* **7**: 338–344.

Oluka MN, Matimba A, Okalebo FA, Osanjo GO, Guantai AN, and Masimirembwa CM (2014). Characterization of inter-ethnic genetic variability of CYP2D6, CYP2C19, CYP2B6, NAT2 and GSTs in the Bantu and Nilotic populations of Kenya and implications for the chemotherapy of infectious diseases. *Afr. J. Pharmacol. Ther.* **3**: 38-46.

Peters PJ, Stringer J, McConnell MS, Kiarie J, Ratanasuwan W, Intalapaporn P and Borkowf CB (2010). Nevirapine-associated hepatotoxicity was not predicted by CD4 count≥ 250 cells/μL among women in Zambia, Thailand and Kenya. *HIV Med.* **11**: 650–660.

Stern JO, Robinson PA, Love J, Lanes S, Imperiale MS & Mayers DL (2003). A Comprehensive Hepatic Safety Analysis of Nevirapine in Different Populations of HIV Infected Patients*. *J. Acquir. Immune Defic. Syndr.* **34**: S21–S33.

Torti C, Costarelli S, De Silvestri A, Quiros-Roldan E, Lapadula G, Cologni G, and Carosi G (2007). Analysis of severe hepatic events associated with nevirapine-containing regimens. *Drug Safety*. **30**: 1161–1169.

Wit FWNM, Kesselring AM, Gras L, Richter C, van der Ende ME, Brinkman K and Reiss P (2008). Discontinuation of nevirapine because of hypersensitivity reactions in patients with prior treatment experience, compared with treatment-naive patients: the ATHENA cohort study. *Clin. Infect. Dis.* **46**: 933–940.