Original Article

Long-Term Effect of Different Classes of Highly Active Antiretroviral Therapy on Transaminases

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ABSTRACT

AIM: The current use of highly active antiretroviral therapy (HAART) for HIV/AIDS patients has increased the recognition of their hepatotoxic effects. The present study is aimed at evaluating the level of aspartate transaminase (AST) and alanine transaminase (ALT) in HIV patients on different classes of HAART, for various durations, which causes its toxicity.

Materials and Methods: The AST and ALT levels were estimated in a total of 340 subjects, of which 290 were HIV positive subjects drawn from patients attending the HIV clinic in two Teaching Hospitals, in Nigeria. 240 of the HIV patients were divided into 3 equal groups of 80 each and placed on non-nucleoside reverse transcriptase inhibitors (NNRTI), nucleoside reverse transcriptase inhibitors (NRTI), and protease inhibitors (PI), respectively, and were monitored for different periods of time (3, 6, 12, and >12 months). 50 of the HIV patients, yet to be placed on anti-retroviral therapy and 50 apparently healthy HIV-negative subjects served as the positive and negative controls, respectively.

Results: A significant increase in enzyme levels was noted at three and six months in the NNRTI group, and at only three months in the NRTI and PI groups, when compared with the controls. However, increased ALT was observed at six months in those on PI. The increased ALT and AST levels noted in the NNRTI group were significant when compared to those on NRTI and PI over a three- and six-month duration.

Conclusion: Liver enzyme activities were generally raised in the first three months of HAART, and further in the NNRTI group, after which they progressively fell to the normal level, with time. The highest degree was observed with NNRTI-based HAART.

Keywords: Duration, effects, HIV-patients, highly active antiretroviral therapy, liver enzymes

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INTRODUCTION

Transaminases, which include alanine transaminase (ALT) and aspartate transaminase (AST) are metabolic enzymes involved in the transfer of an amino group from α-amino acids to α oxoacid, in the presence of a pyridoxal phosphate as a cofactor.[1] Both enzymes are present in high concentrations in liver cells (Hepatocytes). Damage to the liver cell cytoplasmic membrane that may be caused by inflammation or leakage of cytoplasmic contents causes a relatively greater increase in the serum ALT than AST level.[2] On the other hand, if the damage occurs in both the mitochondria and cytoplasmic membrane, there is a proportionally greater increase in both the serum AST and ALT levels. Hence, AST and ALT are referred to as the Markers of 'hepatocellular damage'.[3,4]

Pharmacological agents (antiretroviral drugs) such as Nucleoside reverse transcriptase inhibitors (NRTIs), Non-nucleoside reverse transcriptase inhibitors (NNRTIs), and Protease inhibitors (PI) have been developed to reduce the plasma viral load in HIV patients, as a sequel to understanding the HIV replication cycle.[5]

Low efficacy of single drug therapy has led to the combination of at least three antiretroviral drugs, hence the highly active antiretroviral therapy

(HAART).[6] The introduction of HAART has led to the recognition and characterization of drug-related toxicities.[5] HAART has been shown to lead to both decreased morbidity from HIV infection and increased recognition of both acute and long-term toxicities.[7] Mitochondria damage is thought to be one of the mechanisms of liver injury and the consequent liver enzyme (AST and ALT) elevation is caused by HAART.[3] Studies have shown that HAART-associated liver cell mitochondrial damage and immune reconstitution are heightened in the presence of co-infection with hepatitis B or C.[8]

Liver toxicity is thus a growing problem among HIV patients on HAART; hence, there is a need to monitor the liver enzyme activities in HIV-seropositive patients on different HAART medications, for various periods of time. In the current study we have tested the hypothesis that administration of HAART for different durations of treatment will have an effect on the activities of aspartate transaminase (AST) and alanine transaminase (ALT), which might be dependent on: (1) the class of HAART used, (2) the duration of treatment.

The results of this study will be useful in evaluating and possibly monitoring the patients, particularly in areas of the choice of drug combination and treatment duration, which will lead to reduced hepatic toxicity.

MATERIALS AND METHODS

Subjects

Volunteer HIV-seropositive subjects who attended the HIV clinics at the University of Nigeria Teaching Hospital (UNTH) Ituku-Ozalla, Enugu State, and the Nnamdi Azikiwe University Teaching Hospital (NAUTH) Nnewi, Anambra State, who showed laboratory evidence of HIV infection, with positive confirmatory tests, and who were between 25 and 65 years of age, were candidates for the study. They were not admitted to the study if they showed evidence of abnormal liver function on initiation of therapy. The new cases of HIV-positive subjects that were yet to be placed on anti retroviral therapy (ART) were the candidates for positive control, while the negative control consisted of age-matched apparently healthy HIV-negative volunteers residing in Enugu and Anambra municipalities, South-East Nigeria. Informed consent was given by all the subjects, via filling of well-explained informed consent forms, which protected their identity, in the presence of a witness, before being included in the study. Approval was also given by the institutions ethics committee, to cover the period of one year in which the study was conducted. The approval was to analyze their liver enzyme activities for the said period of HAART intake at the HIV clinics of the hospital.

Study design

A total of 340 subjects were recruited for the study and divided into five groups. The first group, (the positive control group) consisted of 50 new cases of HIV-seropositive subjects, yet to be placed on anti-retroviral therapy, whereas, the negative control (second group) consisted of 50 apparently healthy, HIV-negative subjects.

The third group consisted of 80 HIV-seropositive subjects on NNRTI-based HAART for periods of three months, six months, 12 months, and >12 months, with each period consisting of 20 subjects.

The fourth group consisted of 80 HIV-seropositive subjects on NRTI-based HAART for the same periods as specified earlier, whereas, the fifth group comprised of 80 HIV-seropositive subjects on PI-based HAART, also grouped for the same periods as specified earlier.

Blood samples were collected after three, six, 12, and >12 months for AST and ALT assays. $CD4^+$ counts were also conducted on all the HIV-positive subjects.

All clinical events were evaluated by the clinical team in a blinded fashion and the endpoints were determined. Liver function tests were conducted on the patients, and based on physical examinations, history taking and blood pressure check were performed using the Hawksley random zero sphygmomanometer (Hawksley, Lancing, Sussex), following which the subjects for the study were determined.

All the test study participants were assigned to the treatment during their normal visit to the clinic and they maintained the same groups all through the study period.

Laboratory analysis was carried out at the Chemical Pathology Department, UNTH, Ituku-Ozalla, Enugu, Nigeria. Blood samples were collected after three, six, 12, and >12 months for AST and ALT assays. $CD4^+$ counts were also conducted on all the HIV-positive subjects.

Sample collection and preparation

Blood samples were collected by clean venepuncture from the antecubital fossa into the already labeled plain test tubes, without undue pressure on either the arm or the plunger of the syringe.[9]

The samples were allowed to clot and were centrifuged at 3000 rpm to obtain the sera. The separated clear sera were transferred into sterile bottles and were used for the enzyme assay. When not used immediately, they were stored at −20°C and later used within five days.

Analytical methods

Assay of liver enzymes

Aspartate and Alanine transaminases were assayed using the method of Reitman and Frankel, (1957) and the optical density was read using the Spectronic 20, spectrophotometer. Normal reference values for ALT and AST in normal Nigerian subjects were (3-15 U/L) and (5-18 U/L), respectively.

HIV screening, confirmatory test, and CD4$^+$ T-Cell count

The HIV Tri Line Kits, commercially available (Applied Biosystems Inc, Austria GmbH, Mahlerstrasse 13, 5-7, A-1010, Wien, Austria) were used to detect antibodies to HIV-1 and HIV-2, in the serum samples. The HIV-seropositive samples were confirmed by immunoblot analysis using BIORAD New Lav Blot Kits, commercially available (Biorad Novapath Diagnostic Group, US, Oxnard, CA, USA). The CD4$^+$ count was done by the method of Tuli, *et al*., (2008).

Statistical analysis

All the data analysis was carried out according to a pre-established analysis plan and the mean serum enzyme activities were compared by two tailed t-test and analysis of variance at 95% confidence interval and two degrees of freedom. Results were expressed as mean ± standard deviation (±SD).

RESULTS

The baseline values for aspartate transaminase (AST) and alanine transaminase (ALT) were 11.6 ± 6.3 and 9.0 ± 4.3 U/L, respectively.

The primary endpoint with respect to effect of the different classes of HAART was an initial significant increase in both enzyme activities for the period of three to six months, for all the classes of HAART. This was followed by a progressive fall during the periods of 12 months and >12 months, in comparison with the controls [Tables 1 and 2].

Subjects on PI-based HAART showed significant differences ($P = 0.002, 0.0485$) in ALT when observed in three- and six-month periods, whereas, AST activity was significant only in the three-month period ($P = 0.0034$) compared with the control. Tests of difference between the subjects on NNRTI- and those on NRTI-based HAART, and also between NNRTI - and PI-based HAART, had similar outcomes. Significant differences in ALT and AST activities were observed in the three- and six-month periods, but not in 12 months and above.

Table 3 shows the CD4$^+$ levels of all the HIV-positive patients and the difference was significant ($P = 0.0387$) in

Table 1: Mean ± SD AST and ALT activities (U/L) in patients on NNRTI-, NRTI- and PI-based highly active antiretroviral therapy for different periods of drug intake

Period of treatment (months)	NNRTI-based HAART		NRTI-based HAART		PI-based HAART	
	ALT	AST	ALT	AST	ALT	AST
3	29.4 ± 3.9	25.4 ± 3.5	25.0 ± 4.0	21.2 ± 2.8	26.2 ± 3.8	21.8 ± 3.3
6	21.2 ± 3.7	20.9 ± 2.9	8.4 ± 3.9	9.0 ± 2.8	10.8 ± 2.9	10.2 ± 3.8
12	8.0 ± 2.8	11.6 ± 2.6	6.2 ± 2.9	8.6 ± 2.6	7.0 ± 2.7	9.8 ± 3.7
>12	7.0 ± 2.6	10.8 ± 2.5	6.2 ± 2.8	7.4 ± 2.7	6.4 ± 2.7	8.4 ± 2.5

NNRTI - Non-nucleoside reverse transcriptase inhibitors; NRTI - Nucleoside reverse transcriptase inhibitors; PI - Protease inhibitors; HAART - Highly active antiretroviral therapy; AST - Aspartate transaminase; ALT - Alanine transaminase; SD - Standard deviation

Table 2: Test of difference in mean ± SD of ALT and AST activities (U/L) in control subjects and subjects on NRTI-, NRTI-, and PI-Based highly active antiretroviral therapy for different periods of drug intake

Period of treatment (months)	NNRTI-based (n = 60)		NRTI-based (n = 60)		PI-based (n = 60)		Positive (n = 50)		Negative (n = 50)	
	AST	ALT	AST	ALT	AST	ALT	AST	ALT	AST	ALT
Test (3)	25.4 ± 3.5	29.4 ± 3.9	21.2 ± 2.8	25.0 ± 4.0	21.8 ± 3.3	26.2 ± 3.8	11.3 ± 2.9	8.4 ± 2.9	11.6 ± 6.3	9.0 ± 4.3
Test (6)	20.9 ± 2.9	21.2 ± 3.7	9.0 ± 2.8	8.4 ± 3.9	10.8 ± 2.9	10.8 ± 2.9	na	na	na	na
Test (12)	11.6 ± 2.6	8.0 ± 2.8	8.6 ± 2.6	6.2 ± 2.9	9.8 ± 3.7	7.0 ± 2.7	na	na	na	na
Test (> 12)	10.8 ± 2.6	7.0 ± 2.6	7.4 ± 2.9	6.2 ± 2.8	8.4 ± 2.5	6.4 ± 2.7	na	na	na	na

NNRTI - Non-nucleoside reverse transcriptase inhibitors; NRTI - Nucleoside reverse transcriptase inhibitors; PI - Protease inhibitors; HAART - Highly active antiretroviral therapy; AST - Aspartate transaminase; ALT - Alanine transaminase; SD - Standard deviation; na - not applicable

Table 3: (Mean ± SD) $CD4^+$ counts (cells/l) of all the HIV-positive patients

Period of treatment follow-up (months)	NNRTI-based HAART	NRTI-based HAART	PI-based HAART
3	656.3 ± 57	576.8 ± 40	621.9 ± 55
6	667.6 ± 62	583.3 ± 63	623.2 ± 33
12	671.7 ± 41	588.7 ± 22	630.1 ± 28
>12	732.5 ± 94	654.8 ± 72	745.0 ± 11
Positive control	546.7 ± 10		

NNRTI - Non-Nucleoside reverse transcriptase inhibitors; NRTI - Nucleoside reverse transcriptase inhibitors; PI - Protease inhibitors; HAART - Highly active antiretroviral therapy; SD - Standard deviation

the >12 months group in all classes of HAART compared to the other treatment periods.

DISCUSSION

In the current study we tested the hypothesis that administration of HAART for different durations of treatment will have an effect on the activities of aspartate transaminase (AST) and alanine transaminase (ALT), which might be dependent on the class of HAART used and duration of treatment. The present study revealed a general increase in enzyme activity in all the HIV-positive subjects on HAART for a period of three months. A progressive fall was also recorded in the ALT and AST activities from an earlier elevated level to normal in 12 months of HAART intake, suggesting that HAART exerts hepatotoxic effects on HAART-naïve subjects within the first few months of administration.

Bearing in mind that the normal values for liver enzymes (ALT and AST) in normal Nigerian subjects are (3-15 U/L) and (5-18 U/L), respectively, there was a significant difference ($P = \leq 0.001, 0.0034$) ($P = 0.0038, 0.0054$) in ALT and AST activities during the three- and six-month periods of NNRTI-based HAART intake, but from 12 months, no significant difference was observed when compared with the control subjects.

Subjects on PI-based HAART when compared with the control groups showed a significant difference ($P = 0.002, 0.0034$) in ALT and AST activities in the three-month period and no significant difference in the 12-month and >12 month periods. However, at six months, a significant difference ($P = 0.0485$) was observed only in ALT activity, but not in AST. This probably indicates a decreasing effect on the liver, which is consistent with the activity of ALT tending toward ALT activity in the control groups.

When compared with the controls, the patients on NRTI-based HAART showed significant differences ($P = 0.0031, 0.0038$) in ALT and AST only in three months of intake.

The progressive fall in ALT and AST activities as seen in the results, as also the longer elevated levels of the enzymes, up to six months, associated with NNRTI-based HAART, are in accordance with the finding of, Meldrum (2003), who reported that elevation of liver function tests (especially the transaminases) have been documented for almost all approved antiretroviral drugs and that most of these elevations are mild and may resolve with time, while severe ones can rapidly progress to hepatomegaly, jaundice, and hepatic failure, an effect generally seen with nevirapine®, a non-nucleoside reverse transcriptase inhibitor (NNRTI).

When the mean ALT and AST activities in patients on different HAART were compared, significant differences were observed in the three- and six-month periods, but not in the others, when patients on NNRTI-based HAART were compared with those on NRTI-based HAART. The same observation was also recorded when the comparison was between those on NNRTI and those on PI-based HAART.

On the other hand, no observable significant difference in any of the durations was found when the enzyme levels in patients on NRTI and PI were compared. This may be due to the similarity of NRTI and PI in the lowered hepatotoxic effect, which is supported by the study of Wood *et al.* (2003), which inferred that although most antiretrovirals are hepatotoxic to a certain degree, NRTIs are non-specifically associated mainly with gastrointestinal symptoms, such as, nausea, abdominal pain, and enlargement of the abdomen.

The hepatotoxic effects of HAART, which resolve with time, may be the result of a direct injury to a HAART-naive liver, which declines when the organ gets used to the drug. It could also be related to a previous damage to the liver, which may have been silent until the infiltration of the drug into the liver, leading to leakages of the enzymes into the serum, in the first few months. This also stops with time. However, this study is limited by the number of test subjects available in the clinics, a larger number will no doubt lead to a better outcome.

Further research could be directed at finding the possible effects of the classes of HAART on other organs that are involved in the pharmacokinetics of HAART, with a greater number of test subjects.

CONCLUSION

It is obvious that HAART causes elevation of serum transaminase activities, which resolve in most cases, but in

rare cases, may persist with increasing enzyme activities, which then necessitates discontinuation to begin with.

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