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Research Article

Does chronic alcohol use by HIV-infected patients on d4T/3TC/NVP drug regimen effect the HIV viral load and what is the therapeutic window of the drugs, $CD4^+$ count and WBC count in patients with high viral load during the 9 months period of follow up?

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ABSTRACT

The study investigated the effects of chronic alcohol use on HIV viral load in HIV-infected patients on d4T/3TC/NVP drug regimen during 9 months follow up period. It also determined plasma drug concentrations of d4T, 3TC and NVP; $CD4^+$ and WBC counts for patients with high HIV viral load. A case-control study using repeated measures with serial measurements was used. A total of 41 patients (20 alcohol group and 21 control group) were screened for alcohol use using WHO AUDIT tool and chronic alcohol use biomarkers. Blood sampling was done at 3 month intervals for a period of 9 months. HIV viral load was determined using Roche Amplicor HIV-1 monitor test, version 1.5 (Amplicor). The d4T, 3TC and NVP concentrations were determined by Shimadzu Class-VPTM HPLC Chromatography data system version 6.1. The $CD4^+$ cell count was determined using FACSCalibur flow cytometer. The WBC was determined using automated hematological Coulter CBC-5 Hematology Analyzer system. Results show that % patients with HIV viral load ≥400 copies/ml in control group was highest (23.8%, n=5) at 3 month while in chronic alcohol use group, it was at 0 month (35%, n=7) for both WHO AUDIT tool and chronic alcohol-use biomarkers groups. Generally patients with high viral load ≥400 copies/ml was observed in chronic alcohol use as compared to control group in both WHO AUDIT tool and biomarkers group despite of patients having high steady state d4T, 3TC and NVP plasma drug concentrations in circulation that is available to suppress HIV virus. The high viral load could be associated with the emergence of resistance of the HIV virus and these patients generally had a low $CD4^+$ cell count. Some of these patients had no detectable d4T plasma drug concentrations in circulation and most of them with high viral load had sub-therapeutic NVP plasma drug concentrations in their blood circulation. Chronic ethanol use by HIV-infected patients on d4T/3TC/NVP drug regimen increased HIV viral load and the patients with high viral load had sub-therapeutic NVP plasma drug concentrations and some with undetectable d4T drug concentrations in their blood circulation.

Keywords: Chronic alcohol use, Plasma, HIV viral load, d4T/3TC/NVP regimen

INTRODUCTION

HIV/AIDS is the most devastating disease the world experiences today.[1-3] HIV infect and get integrated into the host genome and the immune system cells especially the cluster of differentiation 4 ($CD4^+$) T cells or the T helper cells and dendritic cells where the virus replicates and acts as a reservoirs.[1-5] The HIV virus destroys the cell mediated and humoral responses of the immune system. However among the many people who consume alcohol chronically are the HIV-infected patients on the d4T/3TC/NVP drug regimen in Uganda.[6-9] In the body, ethanol is broken down to generate a number of potentially harmful byproducts that causes deleterious effects to the body tissues and organs including the immune system.[10,11] The byproducts include the acetaldehyde, acetate, reactive oxygen species (ROS) such as hydroxyl radicals, superoxide anion and hydroxyl radicals and fatty acid ethyl esters (FAEEs).[12-15] The excessive production of the acetaldehyde has been reported to activate the hypothalamic-pituitary-adrenal (HPA) axis similar to that seen in acute stress resulting in production of cortisol in the cascade.[14-18] Acute stress and acetaldehyde causes the release of corticotrophin-releasing factor (CRF) and arginine vasopressin (AVP) by the parvocellular cells of the paraventricular nucleus (PVN). The CRF and AVP act synergistically on the anterior pituitary gland to release the adrenocorticotrophic hormone (ACTH) which then increases the synthesis and release of the glucocorticoids from the adrenal gland.[10] Glucocorticoids suppresses the cell-mediated immunity by inhibiting genes that code for the cytokines IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-8 and IFN-γ, the most important of which is IL-2. These reduce the T cell and B cell proliferation.[10] Acute and chronic alcohol exposure suppresses all branches of the immune responses as well as the protein cytokines that are used by the cells to communicate between cells in the immune system.[10,19] Alcohol consumption is reported to suppress the proliferation of white blood cells and induce an increase in antibody production as well as the $CD4^+$ cell count.[19-21] Ethanol as a diuretic agent may increase the elimination of administered d4T, 3TC and NVP drugs leading to sub-therapeutic plasma drug levels that is required to suppress the HIV virus.[13,22,23] This promotes the emergence of resistance and hence increased replication of the HIV virus leading to increased HIV viral load in blood circulation. Chronic alcohol consumption may also affect the adherence rate to the drugs by the HIV-infected patients thus leading to sub-therapeutic plasma drug concentration.[24-26] The increased production of poor quality drugs (substandard and fake drugs) especially in developing countries like Uganda is also a serious problem in the management of HIV virus in HIV-infected patients.[27,28] The presence of sanctuary sites in the body where the HIV virus can hide from the drugs like the dendritic cells make the HIV virus remain viable and able to replicate thus increasing the HIV viral load in blood circulation of the HIV-infected patients.[29,30] Therefore the excessive chronic alcohol use by the HIV-infected patients may affect the drug regimen and the immune response to the HIV virus and the capacity to suppress the virus in circulation hence making it able to remain viable and replicate rapidly and increase the HIV viral load in blood. The study investigated the effect of chronic alcohol use on HIV viral load in HIV-infected patients on d4T/3TC/NVP drug regimen during the 9 months follow up period. It also determined the plasma drug concentration of d4T, 3TC and NVP; $CD4^+$ and WBC cell counts in HIV-infected patients with high cell count viral load (≥400 copies/ml).

METHODS

Study design, site and population

The study was a case-control study that used repeated measures design with serial measurements model and it was conducted at St. Raphael of St. Francis Hospital, Nsambya, ART Private Clinic on the HIV-infected patients who were exposed to chronic alcohol and at the same time, they were initiated on the d4T/3TC/NVP drug regimen *[triomune 30 (lamivudine (3TC) 150 mg, nevirapine (NVP) 200 mg and stavudine (d4T) 30 mg tablets)]* for the last 6 months. The hospital handles about 1,500 HIV/AIDS patients. The d4T/3TC/NVP drug regimen was selected because during the study period it was one of the first-line drug regimens available in Uganda for the management of HIV-infected patients.

Eligibility and inclusion criteria

All the HIV-infected patients who were included in this study were HIV positive, on d4T/3TC/NVP drug combination regimen for the last 6 months at the time of enrolment. The adherence rates of all the patients recruited were measured using the self-reporting adherence and the pill counts at scheduled visits and all had an adherence rate of above 95%. This was to ensure that the patients were taking their drugs as per the prescription and therefore they are able to maintain the therapeutic steady state plasma drug concentrations in their blood circulation. Also those included were in the age range of 18 to 50 years old. In the test group, they must be exposed to chronic alcohol at the time of recruitment and during the 9 months study period and in the control group, they were not exposed to any type of alcohol at all or for the last 6 to 12 months. All the patients recruited were initiated on antiretroviral drugs for at least the same period of time.

Enrolment of study participants

A total of 41 HIV-infected patients on d4T/3TC/NVP were screened for chronic alcohol use using the WHO Alcohol Use Disorder Identification Test (AUDIT) tool. The 20 patients (13 males and 7 females) were identified to consume alcohol chronically using the tool and were enrolled into the chronic alcohol use group after signing the consent forms. The 21 patients (17 males and 4

females) were identified by the tool as non-alcohol consumers and were enroled in the control group after consenting. The WHO AUDIT is currently an important tool which is non-invasive and it's routinely used worldwide to screen patients on chronic alcohol consumption.[31] The AUDIT tool has a set of 10 questions, each with responses and scores which the individual responds by self-reporting. A total score of 8-15 indicates hazardous alcohol use, 16-19 indicates alcohol use problem and scores above 20 indicates alcohol use dependence.[31] All the patients recruited in the chronic alcohol group had a total score of above 8 according to the WHO AUDIT tool interpretation of the scores. And those enroled in the control group had a total score value of less than 8. However the WHO AUDIT is not sensitive enough to actually detect some of the patients in the control group who were consuming alcohol chronically, therefore the chronic alcohol-use biomarkers (GGT, MCV and AST/ALT ratio) were used to further sort out the patients in the control group who were not detected by the WHO AUDIT tool. The simultaneous elevation of GGT values above 55.0 UI, MCV values above 96 fL and AST/ALT ratio above 2.0 were indicative of chronic alcohol use. About 1mL of whole blood was collected from the cubital vein for all the patients for the analysis of the biomarkers using the automated hematological Coulter CBC-5 Hematology Analyzer system for MCV and the Cobas Intergra 400 Plus analyzer system for GGT, AST and ALT serum enzymes analysis. The elevation of all the biomarkers above the reference ranges indicates chronic alcohol use and this eliminates other confounders that could elevate each of the biomarker. Therefore the 41 HIV-infected patients were again grouped according to the chronic alcohol use biomarkers into 2 arms with the chronic alcohol use arm having 26 patients (22 males and 4 females) and the control group with 15 patients (8 males and 7 females). The patients in both the control and chronic alcohol exposed group were followed-up for 9 months starting from March 2008 to November 2008. All the patients' alcohol use biomarkers were monitored throughout the study period to ensure that there were no reverts and all the patients who participated in the study signed the consent forms. The baseline HIV viral load at time 0 month just before they were initiated on the d4T/3TC/NVP drug regimen for all the patients that participated in the study were collected retrospectively from the patients records.

Whole blood sample collection and HIV viral load determination

About 2mL of whole blood sample from the recruited HIV infected patients on d4T/3TC/NVP regimen were collected at 3 months interval for a period of 9 months into EDTA-containing vacutainer. The plasma was extracted by centrifuging the whole blood at 3000rpm for 10 minutes. The plasma samples in the cryovials were stored at -70°C prior to viral load analysis. The HIV viral load (number of copies/ml) were determined using Roche Amplicor HIV-1 monitor test, version 1.5 (Amplicor), Roche Diagnostics GmbH using the Roche (2006) and Pyne et al. (2010) methods.[32,33] The print-out of each sample was made. All the HIV-infected patients with very high HIV viral load ≥400 copies/ml during the study, their plasma drug concentrations of d4T, 3TC and NVP; $CD4^+$count and WBC counts were analyzed to determine if these have any correlation with the observed high HIV viral load.

Determination of plasma drug concentration of d4T, 3TC and NVP

About 5mL of whole venous blood samples from the patients were collected from cubital vein into a sterile vacutainer with EDTA as anticoagulant every 3 months for a period of 9 (0, 3, 6 and 9) months. The plasma samples were obtained by centrifuging the blood at 3000 rpm for 5 minutes. The plasma was extracted into a clean cryovials and kept into a deep freezer at a temperature of -70°C prior to plasma drug concentration determination

Plasma drug analysis procedures

The individual d4T, 3TC and NVP plasma drug concentrations in the stored plasma were determined using the Shimadzu Class-VPTM HPLC Chromatography data system version 6.1 system with the UV detector using Notari et al. (2006) method.[34]

Chemicals

The pure stavudine, lamivudine and nevirapine that were used as standards were donated by a colleague from the department of Pharmacology and Therapeutics, Gulu University Medical School. Lamivudine was obtained from Iaf Biochem. Int./Glaxo Wellcome, nevirapine from Boehringer Ingelheim and stavudine from Bristol-Myers Squibb. All these drugs were of analytical grade and were used as standards and controls in the study. The acetonitrile and methanol were purchased from BDH chemicals and distributors representative company in Uganda. The KH_2PO_4 was donated by a colleague from the department of Chemistry, Makerere University. All the reagents that were used were of HPLC grade. The distilled water and de-ionized water were produced on-site. The blank plasma, free of any drug was obtained from the Uganda Blood Bank (Nakasero, Kampala-Uganda).

Chromatographic system

The chromatographic system consisted of a Waters 600 pump and a Waters auto sample 717 PLUS equipped with a spectrophotometric UV–vis dual-wavelength system Waters 2487 set at 240 and 260 nm (Milford, MA, USA). The different drug separation was performed at 24.0°C on an analytical C18 Symmetry™ column (250mm×4.6mm I.D.) with a particle size of 5.0 µm (Waters) equipped with a Waters Sentry guard column (20×3.9mmI.D.)

filled with the same packing material (Waters). The 'Millenium'software on the HPLC–UV system was used to process the data.

Mobile phase solutions

The mobile phase was composed of solution A (0.01M KH_2PO_4) and B (acetonitrile). Both solutions were degassed by sparging with helium. The injection volume was 20µL. The mobile phase was delivered at a rate of 1.0 ml/minute and the gradient elution program was used (Table 1).

Table 1: Gradient elution program

Time (minutes)	Flow (mL/min)	KH_2PO_4 solution A (%)	Acetonitrile solution B (%)	pH
0	1	94	6	5.0
10	1	30	70	4.5
15	1	94	6	4.5
20	1	94	6	4.5
25	1	0	100	-

Preparation of the stock, working, and blank plasma solutions

Stock solutions of lamivudine, nevirapine and stavudine (1.0 mg/ml) were prepared by dissolving 5.0 mg of each of the drug in 5.0mL of methanol. Stock solutions were appropriately diluted with methanol for the preparation of working solutions (final concentration ranging between 0.005 and 10µg/ml). The stavudine, lamivudine and nevirapine drug concentration in blank plasma calibration samples ranged between 0.005 and 10µg/ml. The blank plasma was used to mimic the patient plasma samples.

Sample preparation

The plasma samples that were extracted from the HIV-infected patients were cleaned-up by off-line solid phase extraction (SPE) using Oasis HLB Cartridge 1 cc (30 mg) (Waters). The SPE cartridges were conditioned with 1.0mL methanol followed by 1.0mL water Milli-Q. One hundred microliters of methanol were added to 600µL of human plasma, the solution was vortexed for 1.0 min and centrifuged at 5200 rpm for 15.0 minutes, at 24.0°C. The supernatant (ca. 650µL) was diluted by adding water Milli-Q (1.0 mL) and loaded in the cartridge. Then, cartridges were washed with 1.0mL of 5% (v/v) methanol in water Milli-Q. The analytes were eluted by washing cartridges with 550µL 0.01MKH_2PO_4 followed by 2.0mL absolute methanol. The eluate was evaporated in a water bath at 36.0°C under a stream of nitrogen. The extracted sample was reconstituted with 100 µL absolute methanol and transferred to an injection vial. The HPLC-UV detection at 240 and 260 nm and the gradient program was used for stavudine, lamivudine and nevirapine drug separation and concentration determination for each drug.

Calibration curves and recovery

The calibration curves were established over the 0.05, 0.1, 0.5, 1.0, 5.0 and 10µg/ml range for lamivudine and nevirapine and 0.1, 0.5, 1.0, 5.0 and10 µg/ml ranges for the stavudine. The absolute recovery of each of these drugs from plasma was obtained as the peak-area response of the processed samples, expressed as the percentage of the response of the drugs contained in the 20µL injection volume and not subjected to SPE. The average retention time used for d4T, 3TC and NVP drugs are shown in table 2. The areas of the chromatogram peaks for the standards obtained (Figure 1) were used to make a standard calibration curve for each drug that was used to determine the concentration of each drug in the sample. The results of the plasma drug levels were compared with the therapeutic steady state plasma drug concentration reference values obtained from literature (Table 4).

Table 2: Average retention time of d4T, 3TC and NVP drugs used.

ARV drug	Average retention time
Lamivudine	5.02
Stavudine	6.3
Nevirapine	16.5

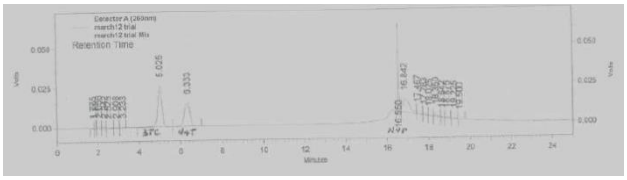

Figure 1: Chromatogram of d4T, 3TC and NVP.

Determination of the CD4$^+$ cell count

The $CD4^+$ counts in the whole blood of the patients were determined using the FACSCalibur flow cytometer (Becton Dickinson-Biosciences, San Jose, CA, USA) and with standard reference method.[35,36]

Determination of the WBC cell count

The same blood sample used for the $CD4^+$ cell count determination was used for white blood cell (WBC) cell count determination using the automated hematological Coulter CBC-5 Hematology Analyzer system.

Data analysis and presentation

Because of the wide variations in the HIV viral load (copies/mL), the values were converted into $\log_{10}$ that were used in plotting the scatter graph to show the distribution of the HIV viral load for the patients recruited into the study during the 9 month follow up period. All the patients with HIV viral load ≥400 copies /ml were correlated with their $CD4^+$ cell count, WBC cell count, the therapeutic steady state (therapeutic window) plasma d4T, 3TC and NVP drug concentrations in the chronic alcohol use and control groups for both WHO AUDIT tool and chronic alcohol use biomarkers methods used for screening the patients for chronic alcohol use.

Ethical consideration

The research work was approved by the Faculty of Medicine Higher degrees, Research and Ethics committee of Makerere University Institution Review Board (IRB) (IRB#-2007-060), IRB of St. Raphael of St Francis hospital, Nsambya (no. IRB 03: 01/03/2008) where the study participants were recruited from and the Uganda National Council for Science and Technology (UNCST)(no. HS 387), a government body that oversee all the research activities done in the country. All the study participants recruited into the study were each duly explained the details of the study and then screened for their eligibility to participate in the study. In this study, a written informed consent was obtained from each patient and that all the procedures used were in accordance with the ethical standards of the responsible conduct on human experimentation (institutional or regional) and with the Helsinki Declaration of 1975, as revised in 1983. They were given study code numbers which were used all through the study period in order to protect their privacy and confidentiality. Their names or any identifier were not used anywhere in the study.

RESULTS

The effect of chronic alcohol use on the HIV viral load in HIV-infected patients on d4T/3TC/NVP drug regimen was determined using the chronic alcohol-use self reporting WHO AUDIT tool and chronic alcohol-use biomarkers. The results show that the % patients with HIV viral load ≥400 copies/ml in the control group was highest (23.8%, n=5) during the 3 month and reduced in the 6 and 9 month follow up period. In the chronic alcohol use group, the HIV viral load ≥400 copies/ml was high at baseline (0 month) (35%, n=7) and remained high at 3 and 6 month (30%, n=6) respectively and reduced during the 9 month (27.8%, n=5) follow up period (Table 3). The distribution of the HIV viral load among the control and the chronic alcohol use varied in both the chronic alcohol-use self reporting WHO AUDIT tool and chronic alcohol-use biomarkers groups; with the majority of the patients with HIV viral load ≥400copies/ml (log 2.8) in the chronic alcohol group throughout the follow up study period for both the chronic alcohol-use self reporting WHO AUDIT tool and chronic alcohol-use biomarkers groups (Figure 2a and 2b). For the patients with high HIV viral load ≥400 copies/ml, they were observed in the chronic alcohol use group as compared to the control group and these patients generally had a low $CD4^+$ count as compared to the control group and the normal reference range values of 415-1550 copies/ml though they had detectable plasma drug levels of d4T, 3TC and NVP in their blood (Table 4). The results in table 4, for the WHO AUDIT tool group, show that patients C113, C32, A017, A32 and A38 had no detectable d4T plasma drug concentrations in their blood circulation while the others had therapeutic steady state plasma drug concentrations above the therapeutic window reference ranges (Table 4). For the 3TC drug, all the patients with high viral load had the therapeutic steady state plasma drug concentrations above the therapeutic window reference ranges (Table 4). For the NVP drug, generally most of the patients with high viral load had sub-therapeutic steady state plasma drug concentrations (Table 4). Also most of the patients with high viral load ≥400 copies/ml had lower $CD4^+$ cell count though their WBC cell count were within the normal reference ranges. The patients C03, C014, C020, C13, C14 in the control group and patients A08, A012, A017, A14, A112, A24, A28, A212, A218 in the chronic alcohol group had a very high HIV viral load and a very low $CD4^+$ cell count (Table 4). Their WBC counts were within the normal reference range of 4.0-11.0 $x10^3$µl (Table 4).

Table 3: Percentage HIV-infected patients with HIV viral load of less than- and above 400 (copies/mL) in blood circulation among the chronic alcohol-use self-reporting WHO AUDIT tool group.

% Patients and viral load (copies/mL)		Time of follow-up (months)			
		0	3	6	9
Control (%)	<400	85.7(n=18)	76.2 (n=16)	94.7 (n=18)	94.7 (n=18)
	>400	14.3 (n=3)	23.8 (n=5)	5.3 (n=1)	5.3 (n=1)
Alcohol (%)	<400	65.0 (n=13)	70.0 (n=14)	70.0 (n=14)	72.2 (n=13)
	>400	35.0 (n=7)	30.0 (n=6)	30.0 (n=6)	27.8 (n=5)

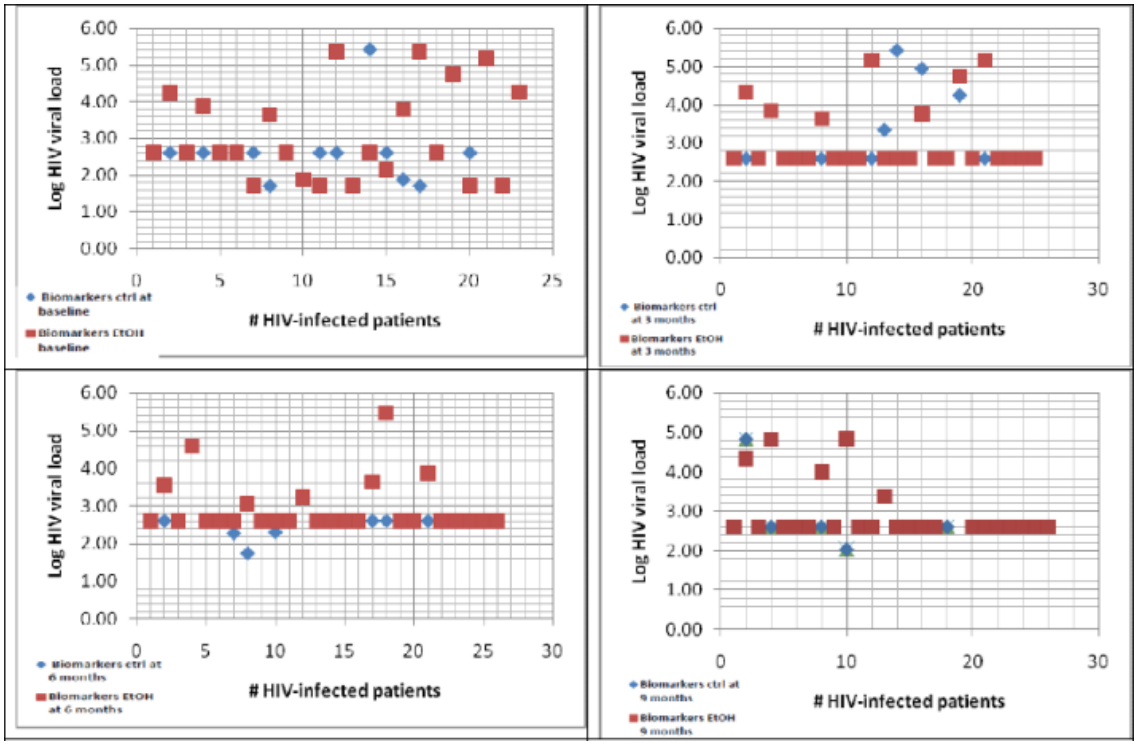

Figure 2a: Distribution of the HIV viral load in the HIV-infected patients in chronic alcohol use group and control group during the 9 month period of follow up in the WHO AUDIT tool group

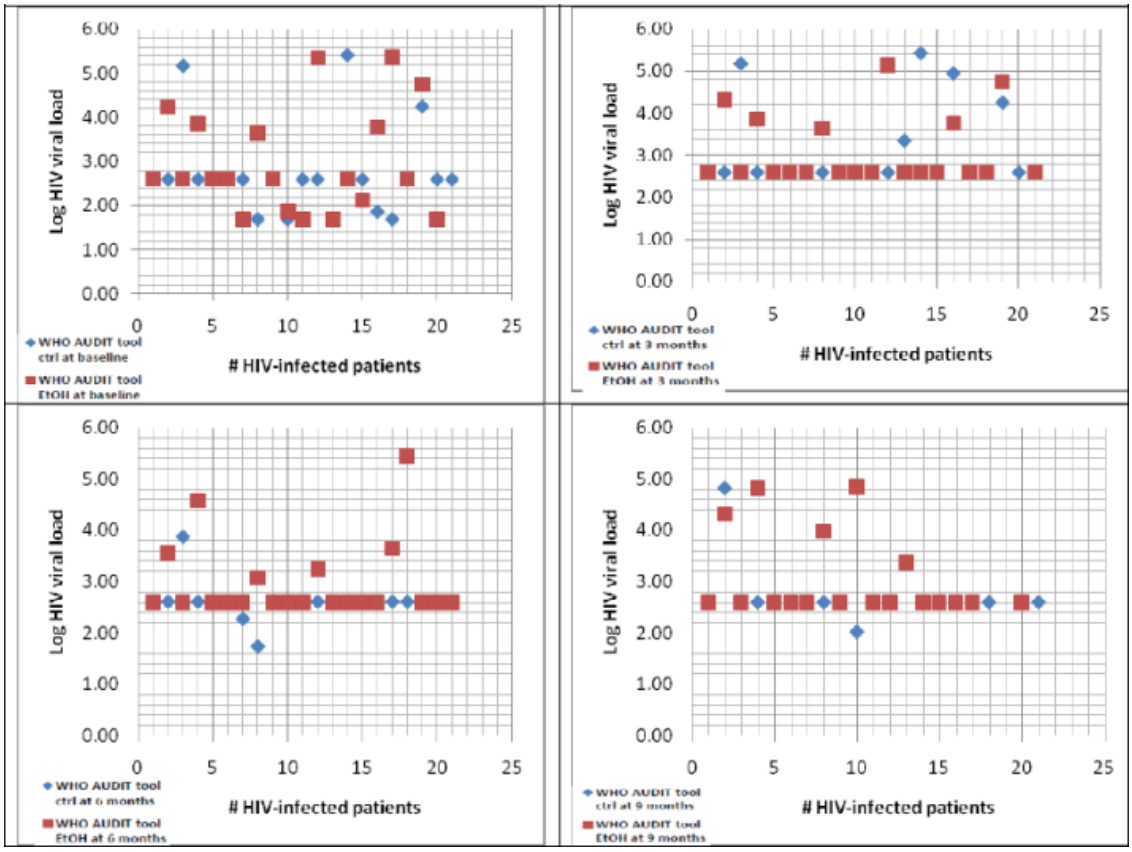

Figure 2b: Distribution of the HIV viral load in the HIV-infected patients in chronic alcohol use group and control group during the 9 month period of follow up in the chronic alcohol use biomarkers group.

Table 4: HIV-infected patients with high HIV viral load above 400 copies/mL and their plasma drug concentrations of d4T, 3TC and NVP; $CD4^+$ count and WBC count during the 9 months period of follow-up in the WHO AUDIT tool reported group.

Time of follow-up (months) Control (C)	Patient code	Plasma drug concentrations (µg/ml)			Therapeutic response		
		d4T	3TC	NVP	$CD4^+$ (cells/µl)	HIV VL (copies/ml)	WBC $x10^3$µl
0	C03	0.50	2.11	6.41	192.00	149000	7.16
	C014	1.12	2.38	4.14	122.00	262000	9.50
	C020	0.38	2.20	5.62	29.00	17900	4.20
3	C13	0.56	2.11	6.41	200.00	112000	6.30
	C113	0.00	2.80	7.60	339.0	2240	5.41
	C114	1.29	2.68	4.54	140.0	152000	3.60
	C116	0.88	4.72	4.26	1271.0	87500	9.50
	C120	0.44	2.80	6.72	114.0	6900	3.40
6	C23	0.61	3.87	5.19	211.0	7400	9.20
9	C32	0.00	2.10	4.49	1133.0	66300	6.60
Alcohol (A)							
0	A02	0.52	2.61	4.05	607.00	16761	6.90
	A04	1.21	5.26	4.12	243.00	7264	5.90
	A08	0.77	3.21	6.54	187.00	4243	7.30
	A012	0.74	4.23	6.81	29.00	223949	4.30
	A016	0.49	3.12	13.74	313.00	6728	2.90
	A017	0.00	2.08	11.61	283.00	229875	4.90
	A019	0.75	3.37	5.58	215.00	46250	6.20
3	A12	0.61	2.84	4.13	529.00	21300	6.50
	A14	1.32	5.60	4.36	144.00	7360	4.30
	A18	0.77	4.02	7.74	247.00	4430	4.60
	A112	0.91	4.36	7.21	58.00	145000	6.20
	A116	0.34	3.32	14.07	326.00	6020	3.50
	A119	0.84	3.58	5.91	235.00	56200	6.20
6	A22	1.86	2.30	4.01	314.00	3600	4.70
	A24	0.70	2.84	4.02	144.00	37400	4.90
	A28	0.80	3.08	7.83	152.00	1180	2.10
	A212	0.81	3.50	4.19	91.00	1770	5.30
	A217	1.01	3.39	4.12	729.00	4470	4.20

	A218	1.03	3.17	4.15	116.00	290000	3.80
9	A32	0.00	3.98	4.91	336.00	21200	4.70
	A34	0.73	4.34	4.81	227.00	66200	4.10
	A38	0.00	2.08	5.74	258.00	9660	4.20
	A310	3.99	14.39	8.41	318.00	70600	4.70
	A313	0.50	2.67	4.72	734.00	2410	5.40
Ref. values		**0.09-0.68**	**0.20-1.42**	**5.3-11.7**	**410-1590**	**Undetectable**	**4.0-11.0**

DISCUSSION

The observed high HIV viral load in the HIV-infected patients in the chronic alcohol use group could be associated with the accumulation of the ethanol metabolites such as acetaldehydes, free fatty acids and the reactive oxygen species that depresses the B-cells and T-cells of the immune system.[10,16,20,37,38] These affect the immune system responses to the HIV infection and hence its capability to suppress the HIV viral load leading to its increased replication and hence the observed increase in the HIV viral load in some patients. Also the acetaldehydes together with the stress the HIV-infected patients experiences are reported to activate the supra-optic and paraventricular nuclei of the hypothalamus. This stimulate the hypothalamo-pituitary-adrenal axis (HPA axis) to release the stress factors especially the glucocorticoids that affects the cytokines and interleukins activity thus affecting the cell mediated and humoral responses of the body to the HIV virus.[10] This reduces the capacity of the immune system to combat the HIV virus leading to its increased replication and hence the observed high viral load in the patients that use ethanol chronically. In the chronic alcohol consumption group, ethanol may affect the CYP450 metabolizing enzyme system that metabolizes these drugs through induction leading to increased metabolism of the drugs especially the NVP. The low NVP plasma drug concentrations observed in some patients leads to sub therapeutic drug concentrations available to suppress the HIV virus.[39-41] Also alcohol has a diuretic effect, this may increase the excretion of the d4T and 3TC which undergo mainly elimination as a free drug by the kidneys and this also reduces the plasma drug concentrations of these drugs (sub therapeutic levels) that are available therapeutically to control the HIV virus replication.[13,22,23] The poor quality of the drugs in cases of undetectable d4T drug concentrations due to lack of the active drug ingredient during the formulation by the pharmaceutical companies could also cause a similar problem of low sub-therapeutic plasma drug concentrations in blood circulation of the patients.[27,28] The other underlying factors leading to the sub-therapeutic plasma drug concentrations in circulation could be due to the use of herbs that could lead to drug-herbal interaction in some of the HIV-infected patients who are using them and not reported.[39,42,43] The herbal medications can induce the CYP450 enzyme system leading to increased elimination (metabolism and excretion) of the drugs from the body hence reducing their half-lives and the available drug concentrations required to suppress the HIV virus in the patients.[39,42,43]

The variation in the HIV viral load in the individual HIV infected patients could also be due to the latent HIV viral reservoir in the infected cells such as the dendritic cells that are established early in infection. The HIV reservoirs have been reported in the maintenance of viral persistence despite highly active anti-retroviral therapy (HAART) and therefore it makes it harder to eradicate the HIV virus completely from the body fluid compartments and tissues as well as in the sanctuary sites.[29,30] Also the observed high HIV viral load in the HIV-infected patients despite of the observed maintenance of the steady state therapeutic plasma drug concentrations of d4T, 3TC and NVP could be due to the rapid emergence of the HIV viral resistance to the administered drugs despite of the patients taking the drugs correctly and consistently as observed in the control group[44-46], and this could also affect the WBC and $CD4^+$ cell counts in the patients as observed. The HIV drug resistance occurs when patients do not respond to prescribed ARVs and as a result, the HIV virus can multiply in the presence of one or more antiretroviral (ARV) drug.[44-46] Also the resistance could be due to the poor treatment adherence to ARVs and this could be due to the ineffectiveness of the methods used in the measurement of adherence rate such as the self-reporting method and the pill count at scheduled visits.[47,48] The shortage of health professionals, limited training, deficient adherence counseling, inconsistent drug supply and weak enforcement of quality standards are among the reported causes of HIV drug resistance.[44-46] Generally results show that chronic ethanol use by the HIV-infected patients on d4T/3TC/NVP drug regimen can affect the patients' response to treatment and hence affects the HIV viral load.

CONCLUSION

The chronic ethanol uses by the HIV-infected patients on d4T/3TC/NVP drug regimen in the chronic alcohol use

group in both the chronic alcohol-use self reporting WHO AUDIT tool and chronic alcohol-use biomarkers groups affected the HIV viral load. This could be due to the effect of ethanol and its metabolites that suppress the bone marrow and the lymphoid system responsible for immunological responses to the viral replication. The diuretic effect of ethanol promotes the excretion of the free drugs especially those which undergo urinary excretion like the stavudine and lamivudine leading to sub therapeutic plasma drug concentrations in blood circulation available to suppress the viral replication. Also the use of herbs by some of these patients could lead to ARVs-herbal interaction and therefore affecting the plasma drug concentrations and hence causing the sub-therapeutic levels. Also poor quality drugs, poor adherence rate and ineffectiveness of the methods of adherence measurements by the patients could influence the plasma drug concentrations leading to the sub-therapeutic drug levels leading to the emergence of resistance thus promoting the HIV viral replication that leads to increased HIV viral load observed in the HIV-infected patients especially the chronic alcohol use group.

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