

Sustained heavy ethanol drinking affects CD4⁺ cell counts in HIV-infected patients on stavudine (d4T), lamivudine (3TC) and nevirapine (NVP) treatment regimen during 9 months follow-up period

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ABSTRACT

Sustained heavy ethanol drinking is a common problem globally and ethanol is one of the most abused drugs among individuals of different socio-economic status including the HIV-infected patients on antiretroviral drugs. Ethanol is reward drug and a CNS depressant especially at high doses. The study determined the effect of sustained heavy ethanol drinking by HIV-infected patients on d4T/3TC/NVP regimen on CD4⁺ cell counts in Uganda using WHO AUDIT tool and chronic alcohol-use biomarkers. A case control study using repeated measures design with serial measurements model was used. The patients on stavudine (d4T) 30 mg, lamivudine (3TC) 150 mg and nevirapine (NVP) 200 mg and chronic alcohol use were recruited. A total of 41 patients (20 in alcohol group and 21 in control group) were screened for chronic alcohol use by WHO AUDIT tool and chronic alcohol use biomarkers. They were followed up for 9 months with blood sampling done at 3 months intervals. CD4⁺ cell count was determined using Facs-calibur Flow Cytometer system. Results were then sorted by alcohol-use biomarkers (GGT, MCV and AST/ALT ratio). Data were analysed using SAS 2003 version 9.1 statistical package with repeated measures fixed model and the means were compared using student t-test. The mean CD4⁺ cell counts in all the groups were lower than

the reference ranges at baseline and gradually increased at 3, 6 and 9 months of follow-up. The mean CD4⁺ cell counts were higher in the control group as compared to the chronic alcohol use group in both WHO AUDIT tool group and chronic alcohol-use biomarkers group though there was no significant difference ($p > 0.05$). Chronic alcohol use slightly lowers CD4⁺ cell count in HIV-infected patients on d4T/3TC/NVP treatment regimen.

KEYWORDS

Sustained Heavy Ethanol Drinking; CD4⁺ Cell Counts; HIV-Infected Patients; d4T/3TC/NVP Drug Regimen

1. INTRODUCTION

Sustained heavy ethanol drinking is a common problem globally including among the HIV-infected patients on ARV treatment regimens. Ethanol is found in various alcoholic beverages including beers, spirits, liquors, wines and traditional home-made brew and is by far the most abused drug for centuries world over [1-3]. The World Health Organization (WHO) estimates that there are about 2 billion people globally that consume alcoholic beverages and it is the leading risk factor to various disease burdens like HIV infection especially in developing countries like Uganda. It is the third risk factor in developed

countries, accounting for 4% of the burden of the diseases [4-6]. In Uganda, alcohol consumption is a serious problem and the country ranked top in the most alcohol consumption among the 189 WHO member countries and in the African region [7-9]. The use of alcohol in Uganda is a widely accepted social activity in both the cultural and ceremonial activities [4,8-10]. In the body, especially in the liver and the gastrointestinal tract (GIT), ethanol is broken down by a number of metabolizing enzyme systems by both the oxidative and non-oxidative pathways to generate a number of potentially harmful by-products which causes deleterious effects to the body tissues and organs [11,12]. The byproducts of ethanol metabolism such as acetaldehyde, acetate, reactive oxygen species (ROS) such as hydroxyl radicals, superoxide anion and hydroxyl radicals and fatty acid ethyl esters (FAEEs) can disorganize the physiological body functions of various tissues, organs such as the liver, haemopoietic, haemostatic systems as well as the immune system resulting in increased or reduced levels of the immune biological markers depending on the metabolic pathway involved [13-17]. The byproducts of the alcohol metabolism can affect the bone marrow and the lymphoid system thus affecting the immune blood cells function [13,18-20]. The ethanol metabolism leads to the generation of free radicals in tissues and also from lipid peroxidation thus depleting the body antioxidants such as the glutathione which is important in the mediation of the immune body responses and thus leading to severe pathological body changes [21-27].

Acetaldehyde as one of the metabolites of ethanol metabolism 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 [11,15,28-30]. Acute stress and acetaldehyde cause 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 [11]. Glucocorticoids suppress both the cell-mediated and humoral immunity by inhibiting genes that code for the cytokines especially interleukins (IL); 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 [11]. Glucocorticoids also act as potent anti-inflammatory agents through the mechanism of lipocortin-1 (annexin-1) synthesis. Lipocortin-1 suppresses phospholipase A2, thereby blocking eicosanoids production and thus inhibits various leukocyte inflammatory events such as epithelial adhesion, emigration, chemotaxis, phagocytosis, respiratory burst and

many others. Glucocorticoids also inhibit the two main products of inflammation, prostaglandins and leukotrienes as well as cyclooxygenase (COX-1 and COX-2) expression which are important in mediating the inflammatory reactions in the body [11]. Acute and chronic alcohol exposure suppresses all branches of the immune responses such as the cells of the immune system as well as the proteins cytokines that are used by the cells to communicate between cells in the immune system [31], thus stimulating or suppressing cell proliferation (replication), production of other cytokines, cytotoxicity and cell migration (chemotaxis) [31]. Alcohol consumption is reported to suppress the proliferation of white blood cells and induce an increase in antibody production as well as the CD4⁺ cells which are a type of T-cell lymphocyte [15, 31-38].

The cluster of differentiation or cluster of designation (CD) is a protocol used for the identification and investigation of cell surface molecules present on the white blood cells. They act by altering the behavior of the cells through cell signaling and cell adhesion. The CD4⁺ cells are important in HIV infection and are a glycoprotein expressed on the surface of T helper cells, regulatory T cells, monocytes, macrophages, and dendritic cells. They are important component of the immune system and are also the cellular receptor for HIV virus. They coordinate the immune system's response to certain micro-organisms including viruses especially HIV [39,40]. The CD4⁺ T lymphocytes occupy the central position in regulating immune functions [40,41]. The HIV attack on the cellular immune system leads to its continued damage eventually making HIV-infected individuals susceptible to various opportunistic infections and cancers. The continued rise in the viral load and a fall in the CD4⁺ cell count show that the virus is replicating at a higher rate [39,40]. The progressive loss of CD4⁺ T lymphocytes eventually results in the loss of the ability of the body to mount desirable immune response to any pathogen and hence the vulnerability to opportunistic infections that are characteristic of HIV-infected individuals. The CD4⁺ T-lymphocyte counts are the most widely used surrogate markers for determining HIV disease progression since they are the targets of HIV and patient staging as well as the therapeutic monitoring of these patients [41,42]. The study therefore determined the effect of chronic alcohol use on the CD4⁺ cell count in the HIV-infected patients on d4T/3TC/NVP drug regimen using the chronic alcohol-use self reporting WHO alcohol-use disorders identification test (AUDIT) tool [43] and the increased levels of chronic alcohol-use biomarkers (liver enzymes such as alanine aminotransferase (ALT), aspartate aminotransferase (AST) and γ -glutamyl transferase (GGT) (AST/ALT \geq 2.0) and mean corpuscular volume (MCV)) [44-46] during the 9 months follow-up period.

2. MATERIALS AND METHODS

2.1. Study Design

The study was a case control study that used repeated measures design model. The study was an effectiveness type where the patients were observed in their natural environment with no confinement and hence it was difficult to quantify the daily alcohol consumption and therefore the WHO AUDIT tool and the chronic alcohol use biomarkers were used to screen the patients for chronic alcohol use during the study. The serial measurements model of CD4⁺ cell count was done on the HIV-infected patients on d4T/3TC/NVP drug regimen at 3 months intervals (0, 3, 6 and 9 months) for a period of 9 months for both the control group and the chronic alcohol exposed group. The d4T/3TC/NVP drug regimen was selected because during the time of the study it was one of the first-line drug regimens available for the treatment of HIV-infected patients in the country.

2.2. Study Site and Population

The study was conducted at St. Raphael of St Francis hospital, Nsambya ART clinic. The hospital handles about 1500 HIV-infected patients. A total of 41 HIV-infected patients who are on Triomune-30 (stavudine (d4T) 30 mg, lamivudine (3TC) 150 mg and nevirapine (NVP) 200 mg drug combination) treatment regimen were screened for chronic alcohol use using the WHO AUDIT tool and chronic alcohol use biomarkers. They were recruited and grouped into two arms with the first arm or the control group consisting of 21 HIV-infected patients who were self-reported for not being exposed to any type of alcohol or to chronic alcohol for the past one year. The second arm had 20 HIV-infected patients who were self-reported to be exposed to chronic alcohol using the chronic alcohol-use self reporting WHO AUDIT tool group and the chronic alcohol-use biomarkers (GGT, MCV, and AST/ALT ratio ≥ 2.0).

2.3. Eligibility Criteria and Enrolment of Study Participants

The study was conducted on the HIV-infected patients who were all initiated on the d4T/3TC/NVP drug regimen for the last 6 months. A total of 41 HIV-infected patients on d4T/3TC/NVP were screened for chronic alcohol use using the WHO AUDIT tool. Twenty patients (13 males and 7 females) were identified to use ethanol chronically and 21 patients (17 males and 4 females) were identified by the tool as non-alcoholic consumers. The WHO AUDIT is a noninvasive method that is routinely used worldwide to screen patients exposed to chronic ethanol [43]. The AUDIT tool has a set of 10 questions, each with responses and scores that the indi-

vidual responded to by self-reporting. A total score of 8 - 15 indicates hazardous alcohol use, 16 - 19 indicates alcohol use problem and scores above 20 indicate alcohol use dependence [43]. 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. The patients enrolled in the control group had a score value of <8 . However, because the WHO AUDIT was not sensitive enough to actually detect some of the patients in the control group who were consuming alcohol chronically, chronic alcohol-use biomarkers (GGT, MCV and AST/ALT ratio) were used to further sort out the patients in the control group. The simultaneous elevation of GGT values above 55.0 UI, MCV values above 96 fL and AST/ALT ratio above 2.0 were indicators of chronic alcohol use [44-46] and these were monitored throughout the 9 months of the study period to ensure that there were no reverts and converts. The baseline serum enzyme concentrations (GGT, ALT and AST) at time 0 month just before they were initiated on the d4T/3TC/NVP drug regimen of all the patients that participated in the study were collected retrospectively from the patients' records. This ensured that these patients in the chronic alcohol-use group were being exposed to alcohol. Then, the 41 HIV-infected patients were again grouped according to the chronic alcohol-use biomarkers into two groups. The chronic ethanol use group had 26 patients (22 males and 4 females) and the control group had 15 patients (8 males and 7 females). The patients in both the control and the chronic alcohol-exposed group were followed up for 9 months starting from March 2008 to November 2008. All the patients who participated in the study signed consent forms.

2.4. Inclusion Criteria

All the patients who were included in this study were HIV positive, on d4T/3TC/NVP drug combination regimen and were reported to have an adherence rate of above 95%. Also those included were in the age range of 18 years to 50 years old. In the test group, they were exposed to chronic alcohol use at that time and in the control group, they must have not been exposed to any form of alcohol at all or for the last 6 to 12 months and all had to consent to participate in the study.

2.5. Whole Blood Sample Collection, Processing and CD4⁺ Cell Count Determination

The whole blood samples from the patients were collected from cubital vein every 3 months for a period of 9 months. About 2 ml of whole blood were collected from each patient's visit into EDTA-containing vacutainer for CD4⁺ cell count determination. The CD4⁺ cell counts were

determined using the FAC-SCalibur flow cytometer (Becton Dickinson-Biosciences, San Jose, CA, USA) and with standard reference method [47,48]. The print-out of each sample was made. The results were entered into the excel spread-sheet from where they were exported to SAS statistical package and analyzed. The results were compared with the standard laboratory reference values.

2.6. Data Analysis

All the data entered in the Microsoft Excel was then further sorted using chronic alcohol-use biomarkers method to produce 2 sets of data which were then compared statistically. It was then imported into the SAS 2003 version 9.1 statistical package (Cary, North Carolina, USA) for statistical data analysis. The data was analyzed at 95% confidence interval. The repeated measures fixed model was used in the statistical data analysis. The student's t-test was used to compare the means of CD4⁺ cell count for HIV-infected patients who were in the chronic alcohol use (chronic alcohol use group) and the control group at different time intervals. The outcome measures were the mean CD4⁺ cell count difference between the chronic alcohol use and non-alcohol use basing on the chronic alcohol-use self-reporting WHO AUDIT tool method and the chronic alcohol-use biomarkers. The p value of less than 0.05 was regarded as statistically significant.

2.7. Quality Assurance

All the study subjects were informed of the study and its purpose and requirement. Then the blood samples were collected into a clean vacutainers by the qualified health care personnel. They were immediately processed as per the standard laboratory procedures of processing of body fluid samples. All the personnel that were involved in the laboratory work were first trained about the methods that were used and they were qualified technical people. The entered data in the Microsoft excel spread sheet were proof read by the principal investigator to

ensure accuracy of the data entered.

2.8. 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. 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 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.

3. RESULTS

The study determined the effect of chronic alcohol consumption on the CD4⁺ cell count in the HIV-infected patients on d4T/3TC/NVP drug regimen using the chronic alcohol-use self reporting WHO AUDIT tool and the chronic alcohol-use biomarkers. The results show that the mean CD4⁺ cell count in the chronic alcohol use group in the 3 and 9 months and in the control group in the 6 and 9 months for the chronic alcohol-use self reporting WHO AUDIT tool group were within the normal reference ranges of 410 - 1590 cells/ μ L (**Table 1**). For the chronic alcohol-use biomarkers group, the mean CD4⁺ cell count in the 3, 6 and 9 months in the control group and in 3 and 9 months in the chronic alcohol use group were also in the normal reference ranges. In the control group for the chronic alcohol-use self reporting

Table 1. Variation of mean CD4⁺ cell counts in the HIV-infected patients on d4T/3TC/NVP drug regimen in the control and chronic alcohol use group with time for chronic alcohol-use self-reporting WHO AUDIT tool group and chronic alcohol-use biomarkers group during the 9 months follow-up period.

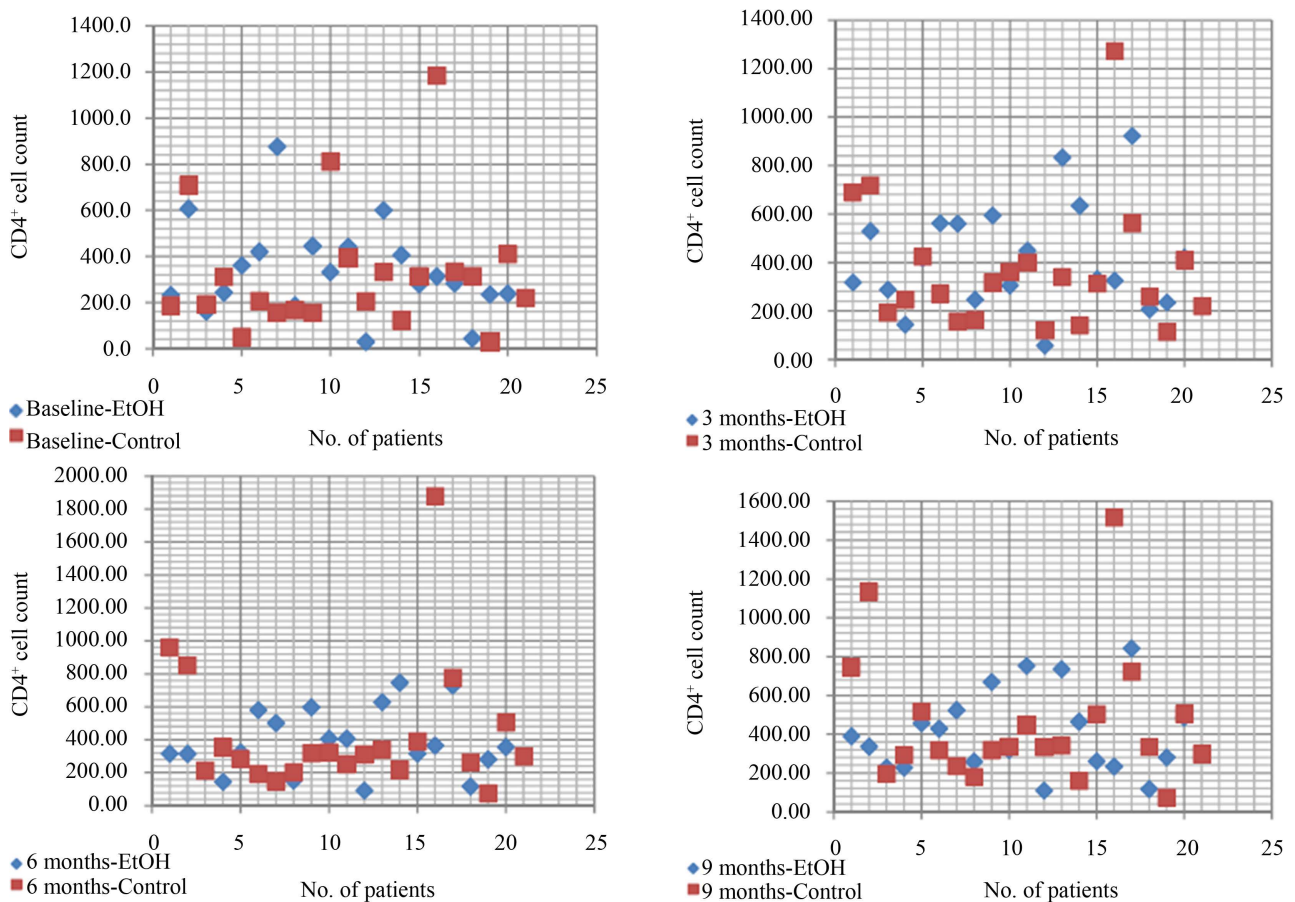
		Chronic alcohol-use self-reporting WHO AUDIT tool group				
Mean CD4 ⁺ cell count (cells/ μ L)		Time of follow-up (months)				Ref
		0	3	6	9	
CD4 ⁺ (cells/ μ L)	Control	334.6 \pm 373.8	390.9 \pm 279.0	481.5 \pm 437.8	501.9 \pm 360.1	410 - 1590
	Alcohol	336.7 \pm 198.5	419.2 \pm 220.7	378.1 \pm 196.7	428.3 \pm 208.7	
p value		0.94	0.72	0.30	0.47	
		Chronic alcohol-use biomarkers group				
CD4 ⁺ (cells/ μ L)	Control	333.1 \pm 261.1	411.2 \pm 310.8	584.2 \pm 496.8	534.5 \pm 420.5	410 - 1590
	Alcohol	337.7 \pm 222.5	400.5 \pm 208.2	350.6 \pm 184.8	430.5 \pm 204.5	
p value		0.84	0.99	0.10	0.23	

WHO AUDIT tool group and the chronic alcohol-use biomarkers group, there was a gradual increase in the mean CD4⁺ cell count while in the chronic alcohol use group, there was an increase in the mean CD4⁺ cell count up to 3 months and then gradually reduced in the 6 months for both groups (Table 1). The variation in the CD4⁺ cell count in the control and chronic alcohol use groups for both the WHO AUDIT tool and chronic alcohol use biomarkers during the 9 months follow up period show that patients with low CD4⁺ cell count below 410 CD4⁺ cells/μL were more in the chronic alcohol group as compared to the control group for both the WHO AUDIT tool and chronic alcohol use biomarkers (Figures 1 and 2). The mean CD4⁺ cell count were generally higher in the control group as compared to the chronic alcohol use group in both the chronic alcohol-use self reporting WHO AUDIT tool group and the chronic alcohol-use biomarkers group but the difference was not statistically significant ($p \geq 0.05$) in both groups (Table 1 and Figure 3). The overall mean CD4⁺ cell count in both the control group and the chronic alcohol use group for both the chronic alcohol-use self reporting WHO

AUDIT tool group and the chronic alcohol-use biomarkers group were within the normal reference ranges but the mean CD4⁺ count in the control group were higher than in the chronic alcohol use group but generally there was improvement in the CD4⁺ cell count during the 9 months follow-up period especially in the control group. Though, however, the difference was not statistically significant ($p \geq 0.05$) in both groups (Table 2).

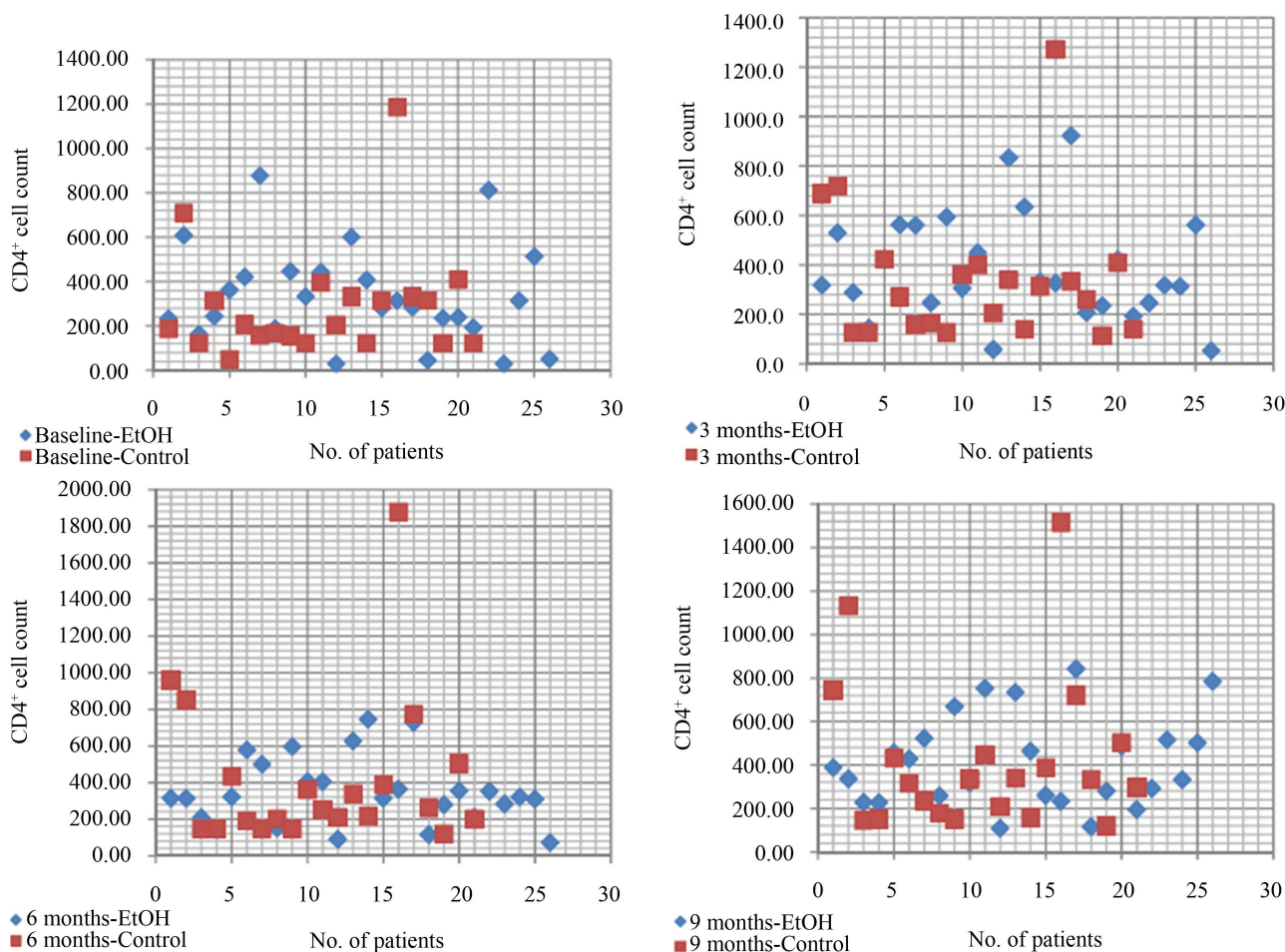
4. DISCUSSION

The study determined the effect of chronic alcohol consumption on the CD4⁺ cell count in the HIV-infected patients on d4T/3TC/NVP drug regimen using the chronic alcohol-use self reporting WHO AUDIT tool and the chronic alcohol-use biomarkers. There was a steady improvement in the mean CD4⁺ cell count from the 0 month to the 9 months of the period of follow-up in the control group and in the chronic alcohol consumption group in both the chronic alcohol-use self reporting WHO AUDIT tool and the chronic alcohol-use biomarkers. The low CD4⁺ cell count in the chronic alcohol use group may



Chronic alcohol Use by WHO AUDIT tool group during 9 months follow-up; EtOH—ethanol; Ref. range 410 - 1590.

Figure 1. Distribution of CD4⁺ cell count in the HIV-infected patients during the 9 months follow-up period in the chronic alcohol-use self reporting WHO AUDIT tool group.



Chronic alcohol Use by alcohol biomarkers group during 9 months follow-up; EtOH—ethanol; Ref. range 410 - 1590.

Figure 2. Distribution of CD4⁺ cell count in the HIV-infected patients during the 9 months follow-up period in the chronic alcohol-use biomarkers group.

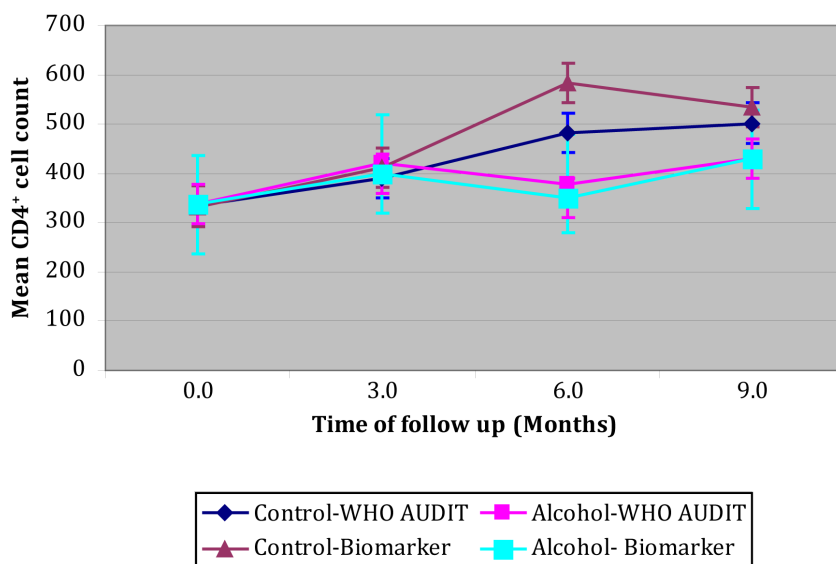


Figure 3. Variation of mean CD4⁺ cell counts in HIV-infected patients on d4T/3TC/NVP with time for chronic alcohol use biomarkers and alcohol self reporting WHO AUDIT tool during the 9 months follow-up period.

Table 2. Effect of chronic alcohol consumption on mean CD4⁺ cell counts in the HIV-infected patients on d4T/3TC/NVP drug regimen for chronic alcohol-use self-reporting WHO AUDIT tool group and chronic alcohol-use biomarkers group during the 9 months follow-up period.

Chronic alcohol-use self-reporting WHO AUDIT tool group			
Mean CD4 ⁺ (cells/ μ L)	Control group	Chronic alcohol consumption	p value
CD4 ⁺ \pm SE (cells/ μ L)	549.7 \pm 75.3	430.7 \pm 70.8	0.258
Chronic alcohol-use biomarkers group			
CD4 ⁺ \pm SE (cells/ μ L)	442.8 \pm 84.7	416.2 \pm 53.6	0.793

have been due to the byproducts of ethanol metabolism such as acetaldehyde, acetate, reactive oxygen species (ROS) such as hydroxyl radicals, superoxide anion and hydroxyl radicals and fatty acid ethyl esters (FAEEs) as well as the increased production of stress factors like the glucocorticoids that may disorganize the physiological body functions of various tissues, organs and especially the liver, haemopoietic, haemostatic systems as well as the immune system [11,13-17]. Also the reduced CD4⁺ cell count in the chronic alcohol use may also be due to the high affinity of the HIV virus to the C-C chemokine receptor type 5 (CCR5) and C-X-C chemokine receptor 4 (CXCR4) receptors found on the CD4⁺ cells to which the virus attach, enter the cell and replicate in them hence destroying the cells leading to a reduction in their numbers. The HIV virus contains the vif factor which is important in supporting viral replication and it is reported that its deficiency in HIV-1 make it unable to replicate in CD4⁺ cells. The HIV virus also contains nef factor which has a number of functions including the induction of the down regulation of CD4⁺ cells and human leukocyte antigen (HLA) class I molecules from the surface of HIV-1-infected cells that provide the escape mechanism for the virus to evade an attack mediated by cytotoxic CD8⁺ T-cells and to avoid recognition by CD4⁺ T-cells. It may also interfere with T-cell activation by binding to various proteins that are involved in intracellular signal transduction pathways as well as being important for the high rate of virus production and the progression of disease [39,49,50]. Also chronic alcohol consumption and its metabolites like the acetaldehyde, acetate, reactive oxygen species (ROS) such as hydroxyl radicals, superoxide anion and hydroxyl radicals and fatty acid ethyl esters (FAEEs) formed in the body can interfere with the hemopoietic process in the bone marrow and the lymphoid tissues leading to reduced production of both the T- and B-lymphocytes such as the CD4⁺ cells [32,35,49]. Alcohol consumption may also affect the level of adherence by the HIV-infected patients on the d4T/3TC/NVP drug regimen which later leads to sub-therapeutic drug levels enabling the HIV virus to attack and replicate in the CD4⁺ cells and destroy them leading to reduced CD4⁺ cell count [7,38,51]. Also alcohol has a diuretic effect in the body and therefore can increase the excre-

tion of the drugs especially the stavudine (d4T) and lamivudine (3TC) which are mainly excreted by the kidneys in urine as a free drug and this reduces the plasma half-life of these drugs hence leading to sub-therapeutic drug concentrations available for the suppression of the HIV viral replication thus leading to a significant increment in the HIV viral load and hence the increased CD4⁺ cell destruction [52,53]. Alcohol can also affect the metabolizing cytochrome P450 enzyme system mainly the CYP2E1 which is inducible by chronic alcohol consumption as well as to a lesser extent the CYP3A4. These enzymes are involved in the metabolism of other drugs like the ARVs drugs especially the nevirapine (NVP) which undergo metabolism and therefore this also leads to sub-therapeutic drug levels available to suppress the HIV viral replication and hence leading to increased HIV viral replication and destruction of the CD4⁺ cells and thus a reduction in the count [51,54-56]. The improved CD4⁺ cell count during the time of follow-up could have been due to the improved close monitoring and adherence of the patients to their treatment and thus attaining the therapeutic window of the drugs that can suppress and reduce the replication of the HIV virus [38,49,50]. Chronic alcohol use by the HIV-infected patients reduces the CD4⁺ cell count in the HIV-infected patients on d4T/3TC/NVP drug regimen during the 9 months follow-up period though the cells were observed to increase with close monitoring and good adherence to the treatment regimen and therefore the CD4⁺ cell counts are important markers of the therapeutic outcome and monitoring the disease progression in the patients.

5. LIMITATIONS OF THE STUDY

1) It was difficult to quantify the daily alcohol consumption for the patients in their natural environment since this was an effectiveness type of study and therefore the WHO AUDIT tool and chronic alcohol use biomarkers are commonly used to screen patients for chronic alcohol use.

2) The use of the WHO AUDIT tool to screen the patients was not sensitive enough to detect some patients on chronic alcohol use and therefore chronic alcohol use biomarkers were used.

3) Since this was a follow-up study, there was some

loss to follow-up of the patients during the 9 months period of follow-up.

6. CONCLUSION

Most of the HIV-infected patients on the d4T/3TC/NVP drug regimen had a CD4⁺ cell count below 200 cells/ μ L during the initial 3 months period of follow-up but after the 9 months period of follow-up most of them had their CD4⁺ cell count improved and some had reached the normal CD4⁺ cell count reference range of 410 - 1590 cells/ μ L in both the control group and in the chronic alcohol use group. The continued closer monitoring of the HIV-infected patients on the d4T/3TC/NVP drug regimen was very vital in the improvement of the CD4⁺ cell count in these patients. The mean CD4⁺ cell counts in the chronic alcohol use group were generally lower than those in the control group during the 9 months period of follow up as observed in the study. Chronic alcohol use and its metabolites affect the bone marrow and the lymphoid system as well as the HPA axis. This then suppresses the normal functioning of the immune system as well as the CD4⁺ cell count production as observed in the study. Chronic ethanol use by the HIV-infected patients reduces the CD4⁺ cell count though the cells may increase with close monitoring and good adherence to the treatment regimen.

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CONFLICT OF INTEREST

The funding of this study was generated by authors only and therefore there are no financial and commercial conflicts of interest to be disclosed for this study.

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