Chronic alcohol use reduces CD4+ counts in HIV/AIDS patients on d4T/3TC/NVP treatment regimen using WHO AUDIT tool and alcohol-use biomarkers

Godfrey S.Bbosa1*, David B.Kyegombe2, William W.Anokbonggo1, Apollo Mugisha3, Jasper Ogwal-Okeng1
1Department of Pharmacology and Therapeutics, Makerere University College of Health Sciences, (UGANDA)
2Department of Pharmacology and Toxicology, Kampala International University School of Health Sciences, Busenyi, (UGANDA)
3Mulago Hospital Complex Clinical Chemistry Laboratory, (UGANDA)
E-mail : smgaddad@gmail.com

ABSTRACT

Alcohol is one of the most abused drugs worldwide by people of different socio-economic status, age groups and including the HIV/AIDS patient on treatment. It is reward drug and a CNS depressant especially at high doses. The study investigated effect of chronic alcohol exposure by HIV/AIDS patients on d4T/3TC/NVP regimen on CD4+ counts in Uganda using WHO AUDIT tool and chronic alcohol-use biomarkers. A longitudinal cohort using repeated measures design with serial measurements model was used. The WHO AUDIT tool was used to screen patients on stavudine (d4T) 30mg, lamivudine (3TC) 150mg and nevirapine (NVP) 200mg for chronic alcohol use. A total of 41 patients (20 alcohol group and 21 control group) were screened for chronic alcohol use. They were followed up for 9 months with blood sampling done at 3 month intervals. CD4+ count was determined using Facsscalibur Flow Cytometer equipment. Results were then sorted by alcohol-use biomarkers (GGT, MCV and AST/ALT ratio). Data was 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+ count in all groups were lower than reference ranges at baseline and gradually increased at 3, 6 and 9 month of follow up. The mean CD4+ count in control group 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/AIDS patients on d4T/3TC/NVP treatment regimen.

KEYWORDS

Chronic alcohol consumption; CD4+ counts; HIV/AIDS patients; d4T/3TC/NVP.
INTRODUCTION

Chronic alcohol use is a common problem globally including among the HIV/AIDS patients on ARV treatment regimens. It is a reward drug and a CNS depressant especially at high doses. Ethanol found in alcohol is consumed in many alcoholic beverages such as beers, spirits, liquors, wines and traditional home-made brew and is by far the most abused drug for centuries world over\cite{1,2,3}. The World Health Organization (WHO) estimates that there are about 2 billion people worldwide consume alcoholic beverages. Alcohol consumption is the leading risk factor for disease burden like HIV/AIDS especially in developing countries like Uganda and the third largest risk factor in developed countries accounting for 4\% of the burden of the diseases\cite{4,6}. In Uganda, alcohol consumption is a serious problem and is ranked a top most country in alcohol consumption among the 189 WHO member countries and in the African region\cite{7,8,9}. The use of alcohol in Uganda is a widely accepted social activity in both the cultural and ceremonial activities\cite{4,5,8-10}. In the body, especially in the liver and the gastrointestinal tract (GIT), ethanol is broken down by a number of metabolizing systems by both the oxidative and non-oxidative pathways to generate a number of potentially harmful byproducts that causes deleterious effects to the body tissues and organs\cite{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 and especially the liver, haemopoietic, haemostatic systems as well as the immune system resulting in increased or reduced levels of the biological markers depending on the metabolic pathway involved\cite{13,14,15}. The byproducts of alcohol metabolism can affect the bone marrow and the lymphoid system thus affecting the immune blood cells function\cite{13,16-18,20}. The production of these byproducts lead to the generation of free radicals in tissues and 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\cite{21-27}.

Acetaldehyde as one of the metabolites of ethanol metabolism has been reported to activate the hypotha

lamic-pituitary-adrenal (HPA) axis similar to that seen in acute stress resulting in production of cortisol in the cascade\cite{11,15,33,35}. 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 adrenocorticotropic hormone (ACTH) which then increases the synthesis and release of the glucocorticoids from the adrenal gland\cite{11}. Glucocorticoids suppress 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\cite{11}. Glucocorticoids also act as potent anti-inflammatory agents through the mechanism of lipocortin-1 (annexin-1) synthesis. Lipocortin-1 both suppresses phospholipase A2, thereby blocking eicosanoid production and inhibits various leukocyte inflammatory events such as epithelial adhesion, emigration, chemotaxis, phagocytosis, respiratory burst and many others. Glucocorticoids also inhibits 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\cite{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\cite{36}, thus stimulating or suppressing cell proliferation (replication), production of other cytokines, cytotoxicity and cell migration (chemoaxis)\cite{36}. 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 lymphocyte\cite{15,29-32,36-38}.

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 cell 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\cite{40,41}. The CD4 T lymphocytes occupy the central position in regulating immune functions\cite{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 rise in the viral load and a fall in the CD4 cell count show that the virus is replicating at a higher rate\cite{40,41}. The progressive loss of CD4 T lymphocytes eventually results in the loss of an ability to mount desirable immune response to any pathogen and hence the vulnerability to opportunistic infections that are characteristic of HIV/AIDS. The CD4+ T-lymphocyte counts are the most widely used surrogate markers for determining HIV/AIDS disease progression since they are the targets of HIV and patient staging as well as for the therapeutic monitoring of these patients\cite{42,43}. The study therefore determined the effect of chronic alcohol use on the CD4+ count in the HIV/AIDS patients on d4T/3TC/NVP drug regimen using the chronic alcohol-use self reporting WHO AUDIT tool and the chronic alcohol-use biomarkers during the 9 month follow up period.

MATERIALS AND METHODS

Study design

The study was a longitudinal cohort study that used repeated measures design model. The serial measurements model was done on the HIV/AIDS on d4T/3TC/NVP drug regimen at 3 month intervals (0, 3, 6 and 9 months) for a period of 9 month for both the control group and the chronic alcohol exposed group.

Study site and population

The study was conducted at St. Raphael of St Francis hospital, Nsambya ART clinic. A total of 41 HIV/AIDS patients who are on Triomune-30 ( stavudine (d4T) 30mg, lamivudine (3TC) 150mg and nevirapine (NVP) 200mg 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/AIDS 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/AIDS 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 \( \geq 2 \)).

Eligibility criteria and enrolment of study participants

A total of 41 HIV/AIDS patients were screened for chronic alcohol use using the chronic alcohol-use self reporting WHO AUDIT tool in which 20 patients were reported as chronic alcohol users group and 21 patients recruited in the control group. These patients were further sorted using the chronic alcohol-use biomarkers in which 26 were recruited in the chronic alcohol use group and 15 patients in the control group. These HIV/AIDS 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 HIV/AIDS patients on d4T/3TC/NVP drug combination regimen, who were recruited in the study, had to have a 95% adherence and this was obtained by both patient self-reporting and pill count. This was to ensure that the patients were taking their drugs as per the prescription. Each HIV/AIDS patient was explained well about what is involved in the study and any questions raised were answered. All those HIV/AIDS patients who accepted to participate in the study were requested to sign a consent form.

Inclusion criteria

All the HIV/AIDS 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 90%. Also those included were in the age range of 18 years to 50 years old. In the test group, they must be exposed to chronic alcohol use at that time and in the control group, they must have not been exposed to any type of alcohol at all or for the last 6 to 12 months. They all signed the consent form.

Whole blood sample collection, processing and CD4+ count determination

The whole blood samples from the recruited HIV/AIDS patients on d4T/3TC/NVP regimen 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+ count determination. The study was carried out at the Joint Clinical Research Clinic (JCRC) using the Facscalibur Flow Cytometer equip-
ment with the standard procedures and the laboratory standard operating procedures (SOPs). The CD4+ counts of the HIV/AIDS patients on d4T/3TC/NVP for both the exposed and non-exposed to alcohol. About 50µL of whole blood from the HIV/AIDS patients were stained with 10µL anti-CD4 PE-labelled mAb (clone EDU2), incubated for 15 minutes at room temperature in the dark, fixed by 800µL fixation buffer and analyzed by a CyFlow SL flow cytometer (Partec, Germany) equipped with a Nd:YAG (532 nm, 50 mW) green laser. Data acquisition, analysis, and real-time display were performed by Windows-based FloMax software. Dual-platform CD4 counts were performed by Sysmex XE2100 hemocytometer and Facs calibur (Becton D.) cytometer. Each CD4+ was counted in the volumetric 1-PF mode using the CyFlow Counter (Partec, Münster, Germany) equipped with photomultipliers for forward (FSC) and sideward scatter (SSC) and for 2 or 3 fluorescence parameters. The device contains a blue solid-state laser that is operated at 488 nm for the excitation of blood preparation. The results were analyzed by using the CyFlow Counter and the FACS-Calibur as a reference system. The print-out for each parameter of each sample was made. The results were entered into the excel spreadsheet from where they were exported to SAS statistical package and analyzed. The results were compared with the standard laboratory reference values for each parameter analyzed.

**Data analysis**

All the data was entered in the microsoft excel and was then sorted using the chronic alcohol-use self-reporting WHO AUDIT tool method for the use of chronic alcohol as well as basing on the 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 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 t-test was used to compare the means for HIV/AIDS 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 difference of the measured parameters 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.

**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 spreadsheet were proof read by the principal investigator to ensure accuracy of the data entered.

**Ethical consideration**

The research work was approved by the Faculty of Health Sciences, Research and Ethics Committee of Makerere University Institutional Review Board (IRB) (IRB#-007-060), 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 human subject and that all the procedures used were in accordance with the ethical standards of the responsible committee 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 study determined the effect of chronic alcohol consumption on the CD4+ count in the HIV/AIDS 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+ count in the chronic alcohol use group in the 3 and 9 month and in the control group in the 6 and 9 month for the chronic alcohol-use self reporting WHO AUDIT tool group were within the normal ref-
Chronic alcohol consumption on CD4+ count in HIV/AIDS patients

For the chronic alcohol-use biomarkers group, the mean CD4+ count in the 3, 6 and 9 month in the control group and in 3 and 9 month in the chronic alcohol use group were also in the normal reference ranges. In the control group for the chronic alcohol-use self reporting WHO AUDIT tool group and the chronic alcohol-use biomarkers group, there was a gradual increase in the mean CD4+ count while in the chronic alcohol use group, there was an increase in the mean CD4+ count up to 3 month and then gradually reduced in the 6 month for both groups (TABLE 1). The mean CD4+ 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≥0.05) in both groups (TABLE 1, Figure 1a, b, c and d, Figure 2 and Figure 3). The overall mean CD4+ 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. Though, however, the difference was not statistically significant (p≥0.05) in both groups (TABLE 2).

DISCUSSION

The study determined the effect of chronic alcohol consumption on the CD4+ count in the HIV/AIDS pa-

**TABLE 1 : Variation of mean CD4+ counts in the HIV/AIDS 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**

<table>
<thead>
<tr>
<th>Mean CD4+ count (cells/µL)</th>
<th>Time of follow-up (months)</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>CD4+ (cells/µL) Control</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>334.6±373.8</td>
<td>390.9±279.0</td>
</tr>
<tr>
<td>Alcohol</td>
<td>336.7±198.5</td>
<td>419.2±220.7</td>
</tr>
<tr>
<td>p value</td>
<td>0.94</td>
<td>0.72</td>
</tr>
<tr>
<td>CD4+ (cells/µL) Control</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>333.1±261.1</td>
<td>411.2±310.8</td>
</tr>
<tr>
<td>Alcohol</td>
<td>337.7±222.5</td>
<td>400.5±208.2</td>
</tr>
<tr>
<td>p value</td>
<td>0.84</td>
<td>0.99</td>
</tr>
</tbody>
</table>

**TABLE 2 : Effect of chronic alcohol consumption on mean CD4+ counts in the HIV/AIDS 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**

<table>
<thead>
<tr>
<th>Mean CD4+ (cells/µL)</th>
<th>Control group</th>
<th>Chronic alcohol consumption</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD4+±SE (cells/µL)</td>
<td>549.7±75.3</td>
<td>430.7±70.8</td>
<td>0.258</td>
</tr>
<tr>
<td>Chronic alcohol-use biomarkers group</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Chronic alcohol-use biomarkers group

CD4+±SE (cells/µL) 442.8±84.7 416.2±53.6 0.793
Figure 1A: Distribution of CD4+ count in the HIV/AIDS patients in the control group at baseline for the chronic alcohol-use self reporting WHO AUDIT tool.

Figure 1B: Distribution of CD4+ count in the HIV/AIDS patients in the control group at 9 month of follow up for the chronic alcohol-use self reporting WHO AUDIT tool.

Figure 1C: Distribution of CD4+ count in the HIV/AIDS patients in the chronic alcohol use group at baseline for the chronic alcohol-use self reporting WHO AUDIT tool.
Chronic alcohol consumption on CD4+ count in HIV/AIDS patients

Figure 1D: Distribution of CD4+ count in the HIV/AIDS patients in the chronic alcohol use group at 9 month of follow up for the chronic alcohol-use self reporting WHO AUDIT tool

Figure 2A: Distribution of CD4+ count in the HIV/AIDS patients in the control group at baseline for the chronic alcohol-use biomarkers group

Figure 2B: Distribution of CD4+ count in the HIV/AIDS patients in the control group at 9 month follow up period for the chronic alcohol-use biomarkers group

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+. The HIV virus also contains nef
factor which has a number of functions including the induction of the down regulation of CD4+ and 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\[40,44,45\]. Also chronic alcohol consumption can 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[29,32,44]. Alcohol consumption may also affect the level of adherence by the HIV/AIDS patients on the d4T/2TC/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[7,39,46]. Also alcohol has a diuretic effect in the body and therefore can increase the excretion 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 levels and hence increased HIV viral replication and hence increased CD4+ cell destruction[47,48]. 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 may also be 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[46,49,51]. The improved CD4+ count during the time of follow up could have been due to the improved 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[40,44,45]. Chronic alcohol use by the HIV/AIDS patients reduces the CD4+ cell count though the cells may increase with good adherence to the treatment regimen and therefore these CD4+ counts are important marker of the therapeutic outcome and they are used in the monitoring of the HIV/AIDS progression of the disease.

CONCLUSION

Most of the HIV/AIDS patients on the d4T/3TC/NVP drug regimen had a CD4+ count below 200 cells/µL in the 3 month period of follow up but after the 9 month period of follow up most of them had their CD4+ count improved and some had reached the normal CD4+ count reference range of 410-1590 cells/µL in both the control group and in the chronic alcohol use group. The continued closer monitoring of the HIV/AIDS patients on the d4T/3TC/NVP drug regimen is very vital in the improvement of the CD4+ count in these patients. Chronic alcohol use and its metabolites affect the bone marrow and the lymphoid system as well as the HPA axis. This then suppress the normal functioning of the immune system as well as the CD4+ count as observed. Chronic alcohol use by the HIV/AIDS patients reduces the CD4+ cell count though the cells may increase with good adherence to the treatment regimen and therefore these CD4+ counts are important marker of the therapeutic outcome and they are used in the monitoring of the HIV/AIDS progression of the disease.

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