Monitoring of CD4$^+$ T-cell counts in HIV infected patients on *Arthrospira platensis* supplement in Kisumu, Kenya

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SUMMARY

Consumption of natural products with high nutritional value can improve nutritional and immune status of HIV patients. *Arthrospira platensis*, is an alga that grows naturally in some tropical lakes. It is rich in nutritional contents and anti-oxidants. This study investigated whether use of *Arthrospira platensis* by HIV positive adults affected their CD4$^+$ T-cell counts. This was a prospective paired study design with two independent groups: the study group and a control group. The findings of individual patients before and after intervention were also paired. Patients with CD4$^+$ T-cell counts above 250 cells/µl were enrolled in Nyanza Provincial Hospital, Kenya. Patients in the study group used *A. platensis* while those in the control group used the standard multi-vitamin supplements. Fifty-eight patients completed the study [28 in *A. platensis* (study) group and 30 in multivitamin (control) group]. The mean CD4+ T-cell counts among patients in the study group increased from 485 ± 163 to 516 ± 181 cells/µl ($p = 0.110$) while in the multivitamin group they declined from 555 ± 221 to 472 ± 174 cells/µl ($p = 0.001$). It was concluded that *A. platensis* increased CD4$^+$ T-cell counts in HIV infected adults and it was well tolerated at a maximum dry dose of 2g/day when used for 1-6 months.

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Introduction

Highly active antiretroviral therapy (HAART) has had remarkable success in the management of HIV infection, though it is difficult to eradicate the infection. The latent form serves as a permanent archive for wild type virus and for drug resistant variants that may arise during treatment [1]. Treatment using HAART aims at reducing the replication of HIV, thus allowing the immune system to recover.

A recent study has shown that most HIV patients on HAART do not infect their sexual partners [2]. These findings led the researchers to recommend early initiation of HIV treatment in order to prevent the spread of the infection.

Before immunosuppression occurs, the aim of management has been to maintain the intact immunity for as long as possible by prevention and treatment of opportunistic infections, together with nutritional counseling and supplementation. Currently in Kenya, an

antibiotic (commonly Co-trimoxazole) and multi-vitamins are used for these purposes respectively [3,4]. Supplementation with multi-vitamins or consumption of products with a high nutritional value has been shown to be beneficial to HIV infected patients [5, 6].

A. platensis contains essential amino acids including the four basic components of the seleno-enzyme glutathione peroxidase, namely selenium, cysteine, glutamine, and tryptophan. It is also rich in phytonutrients, essential fatty acids (EFA's) [7] and C-phycocyanin (a biliprotein) that has anti-oxidant and radical scavenging activities, among other components [8].

Many toxicological studies have proven *Arthrospira platensis's* safety [9] and it is used as a food supplement especially in parts of Mexico, Central Africa, Congo and India [10]. It has been used among the undernourished children, both HIV positive and negative without serious undesirable effects reported [11].

The effect of *Arthrospira platensis* on $CD4^+$ T-cell levels in HIV patients monitored over a period of time was, however, not clear. One study in Yaoundé Cameroon among HIV positive pregnant women was done recently [12], but using a different dose and, probably, form, and the outcome had not been published as this one was ongoing. The current study sought to monitor $CD4^+$ T-cell levels in HIV patients using a specified dose and form of this supplement over a specified period. It was hoped that a clearer effect of this algae in HIV patients from the results of these studies with would emerge.

Materials and Methods

The study was carried-out between October 2009 and June 2010 at patient support centre (PSC) clinic in Nyanza provincial hospital in Kisumu, and the laboratory tests were done at KEMRI/CDC within the Hospital compound. Approval was sought and obtained from KEMRI scientific and ethical committees and the hospital ethics review committee before commencing the study.

The ponds for the growth of the algae were constructed at Kenya medical research Institute, Kisumu. The initial culture of *Arthrospira platensis* algae was obtained from a non-governmental organization running feeding programs that involved adding fresh algae to food (usually porridge), and used by the malnourished children and adults. The culture was then put in a pre-constructed ponds containing water with the required nutrients needed for optimal growth (close to composition of Farouk's medium, used in some bacterial cultures).

After growing for at least 4 weeks, the culture was then sieved, filtered and dried. Dried *Arthrospira platensis* was made to powder using a blender and then put into size "O" capsules, using a manual capsule filler, each holding 500 mg of the powder. These were then packaged in plastic sealable polythene sachets to contain 112 capsules to be taken for 28 days, two capsules taken twice daily with meals (a total of 2g/day). This dose was based on preliminary findings that indicated that this was an effective dose that raised $CD4^+$ T-cell levels without eliciting major adverse events.

Consenting HIV positive adult out-patients with $CD4^+$ T-cell counts above 250 cells/µl (the counts used at

the hospital as cut-off for initiation of ARV therapy) and not on ARV's were enrolled. Patients were assigned into one of the two groups, the study group who used *A. platensis* or a control group who used multivitamins.

Blood samples from each patient were analyzed at enrolment and during clinic visits, often monthly or after three months. The sample obtained before intervention also served as control and paired with sample/s obtained after the intervention. Each subject therefore also served as his/her own control, which allowed change to be detected more easily by controlling for extraneous variation among the observations. Many biological measurements exhibit wide variation among individuals, and the use of paired design was thus especially appropriate.

The formula used to calculate the sample size was $n = 2 \times[(Z_\alpha - Z_\beta)^2/(\Delta)^2$ [13].

Specifications of level of significance (α) for null hypothesis and the desired level of power (β) for an alternative hypothesis permit us to solve for sample size. α, is type I error where a conclusion is drawn that there is a rise in $CD4^+$ T-cell counts after using the supplement while there is not. The desired level of significance used was 0.05, it's z value is 1.96, and the desired level of power of 80% whose lower one tailed z value related to β is -0.84.

Based on preliminary results, the mean $CD4^+$ T-cell counts of 520 ± 173 cells/µl at the beginning of the study was used. An estimated change of 12.5% at the end of the study was assumed. The assumption was that $CD4^+$ counts among patients in the standard multivitamin (control) group would decline by 12.5% to 455 [$520 - 12.5\%$ of 520 ($= 65$)], while those in the study group would rise by a similar margin of 12.5% to 585 ($520+65$).

Δ is the Standardized difference of the means of the two groups, obtained from $(585-455)/ 173$ (s.d) = 0.751

Using the indicated formula, at least 28 participants per group were required, or a total of 56 participants.

All patients followed the routine procedures at the clinic, and enrolment to participate in the study was voluntary but involved meeting specified criteria. Consenting adults with $CD4^+$ T-cell counts above 250 cells/µl were enrolled. The recruited patients were issued with the supplements, either multi-vitamin tablets (control group) or *A. platensis* capsules (study group).

All HIV patients were also put on co-trimoxazole prophylaxis since this was the recommended prophylactic antibiotic for HIV infected patients.

After every 4 weeks (most patients) or 12 weeks (some patients in the control group), patients came to replenish their supplies and also to evaluate their clinical state, asses their treatment adherence and perform scheduled laboratory investigations. The clinical and laboratory monitoring done during these visits were aimed at early detection of opportunistic infections or occurrence of adverse events. Patients found to have any other disease or infection received appropriate prescription to obtain drugs at the hospital pharmacy.

The information obtained was analyzed in SPSS program and T-test performed, which is suitable for paired samples.

Results

Eighty-one patients were enrolled, 58 females (71.2%) and 23 males (28.8%). 58 patients (71.6%) completed

the study, of which, 43 were females and 15 were males. This showed the male: female ratio of about 1:3. The results are as summarized in table 1.

Table 1: Number of patients enrolled and their completion rate

	Completed	Not completed	Total	Completion rate
Males	15	8	23	65.2%
Females	43	15	58	74.1%
Total	58	23	81	71.6%

The mean age for all the patients was 27.4 ± 5.7 years, with that of males being 30.5 ± 7.2 years and that of females 26.4 ± 4.7 years. More than 75% of all the patients enrolled were 30 years old and below.

At the end of the study, the total lymphocyte counts among patients in the study group remained stable, being $1.92 \pm 0.75 \times 10^3$ cells/μl at the beginning of the study and $1.92 \pm 0.47 \times 10^3$ cells/μl (p= 0.994) at the end of the study. The counts among those in the control group declined from $2.33 \pm 0.90 \times 10^3$ cells/μl to $2.06 \pm 0.90 \times 10^3$ cells/μl, which was significant (p= 0.002).

At the beginning of the study, the mean CD4+ T-cell counts among the patients in the study group was 485 ± 163 cells/μl, rising to 516 ± 181 cells/μl at the end of the study (table 2). This change was not statistically significant (p= 0.110). The mean CD4+ T-cell counts among the patients on multi-vitamin (control group) was 555 ± 221 cells/μl at the beginning of the study declining to 472 ± 174 cells/μl at the end of the study. This decline was statistically significant (p= 0.002).

Table 2: Changes in CD4+/CD8+ cell levels among the patients

	Study group			**Control group**		
Parameter	At beginning of study	At end of study	**p-value**	At beginning of study	At end of Study	**p-value**
Total lymphocyte counts, $\times 10^3$ cells /μl (1.50-4.00)	1.92	1.92	**0.994**	2.33	2.06	**0.002**
CD4+ T-cells count (410-1590 cells /μl)	485	516	**0.110**	555	472	**0.002**
CD8+ T-cells count (190-1140 cells /μl)	942	899	**0.533**	1122	982	**0.021**

Discussion

In this study, among the 81 enrolled patients, 11 (13.5%), were lost to follow-up over a period of six months. This is comparable with a previous large study among patients initiated on ARV treatment, which showed that 16% of the patients were lost to follow-up over six months [14].

Among the patients who did not complete the study, 11 (47.8%) failed to return to the clinic on the date of appointment. The other patients, 12 (52.2%), were

excluded because of other different reasons. A higher proportion of the females (74.1%) completed the study as compared to the males (65.2%). The reasons for these difference was not clear.

A male: female ratio of about 1:3 was a general reflection of the HIV clinic attendance.

The average age for all patients in the current study was 27.4 years. An earlier study on HIV patients before initiation of anti-retroviral treatment showed that the average age for the patients was 38 years [15]. That study was done about five years earlier than the current one. The possible explanations to this apparent 10-year difference in the average ages could be because HIV infection is now occurring in younger population.

Another observation was that the females tended to be younger than the males, where the average age of the females in this study was 26.4 years while that of the males was 30.5 years.

HIV infection, in most patients, ultimately leads to depletion of $CD4^+$ T-cells. It is not uncommon for $CD4^+$ T cell counts to drop as low as 10 cells/μl or even 0, yet the patients may survive for months or even for more than 1 year [16]. The situation has become increasingly common as patients are treated more aggressively including treatment of opportunistic diseases or are given prophylactic treatment against them. Ultimately, without proper treatment, patients who progress to this severest form of immunosuppression usually succumb to opportunistic infections or neoplasms.

There have been reports that an abnormally high percentage of $CD8^+CD38^+$ T-cells predict decline of $CD4^+$ T-cells and the rate of disease progression in HIV-1 infected adults [17, 18] and pediatric patients [19]. The biological basis for the negative prognostic value of $CD8^+CD38^+$ T-cells is unknown. In the current study, patients with high $CD8^+$ T-cells predicted a decline of $CD4^+$T-cells. It could not, however, be established whether these were $CD8^+CD38^+$ T-cells as this was a later finding during analysis of the results and tests to estimate the counts of $CD8^+CD38^+$ T-cells had not been included in the protocol.

The dosage of *A. platensis* used in this study was based on the available information. In Kisumu town, the area of the current study, *A. platensis* was being used as food supplement for malnutrition by some adults and children, where 10g of fresh *A. platensis,* obtained after draining away the growth medium by sieving and filtering, was used. This amount was added to a cup of sugarless porridge for adults and 5g for children, and then drank once in the morning. Alternatively, 5g of dried and powdered *A. platensis* was given to adults to be used at home daily for the same purpose [IMSAM, Unpublished report].

A study on healthy human subjects also indicated that using 4.2 g/day of *Arthrospira platensis* was safe [10].
To test a hypothesis that there is no difference in $CD4^+$ T-cell counts before and after using the supplements, T-test was used. In the *A. platensis* group, the statistical analysis of the $CD4^+$ T-cell counts before and after using this supplement was that, from a statistical table, with an area of 0.05 in two tails and with the degrees of freedom (df) 27, the critical value for the *t* distribution is 2.05 (or -2.05), obtained from a t-table. An α value of 0.110 was obtained in the study group, which is >0.05. Using SPSS to find the t-value in the

study group, a *t* value of -1.652 was obtained, which lies in the range between -2.05 and $+2.05$ and therefore we accept the null hypothesis that there is no difference between $CD4^+$ T-cell counts before and after using *A. platensis* and reject the alternative hypothesis that there is a difference.

In the multivitamin group, the statistical analysis of the $CD4^+$ T-cell counts before and after using this supplement was that α value of 0.002 was obtained, which is much less than 0.05, and a df of 29. The obtained *t* value of $+3.437$ lies outside the interval -2.05 and $+2.05$ and therefore we reject the null hypothesis that there is no difference between CD4 counts before and after using the supplement and accept the alternative hypothesis that there is a difference.

Conclusions

In conclusion, these results showed that there was a non-significant increase in CD4 T-cell counts among HIV infected adults taking *Arthrospira platensis* (study group) supplement after using for a period of one to six months. There was also a significant decrease in CD4 T-cell counts among HIV infected adults taking multi-vitamins (control group). *Arthrospira platensis* was also well tolerated by HIV infected adults at the dose of 2 g/day for up-to six months.

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