Nutritional Supplements Can Delay the Progression of AIDS in HIV-Infected Patients: Results from a Double-Blinded, Clinical Trial at Mengo Hospital, Kampala, Uganda

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Abstract

Objective: To examine the immunologic, biochemical and clinical effects of two combinations of nutritional supplements on the progression to AIDS of HIV-infected patients not receiving anti-retroviral treatment.

Design: A prospective randomized, double-blinded clinical trial.

Methods: 249 female and 61 male HIV-infected outpatients of Mengo Hospital, Kampala, Uganda, with baseline CD4 cell counts above 200, who were not receiving anti-retroviral drugs, were randomized into Groups A and B. Group A patients received capsules containing 37 nutrients, while those in Group B were given capsules of 30 nutrients. Both nutritional combinations were designed to increase glutathione peroxidase levels. All patients were instructed to take two capsules, three times daily with meals for 52 weeks. 310 patients began the year long study and 263 completed it. The loss to follow-up, therefore, was some 15.2 percent. Serum glutathione peroxidase levels and CD4 cell counts were measured at baseline, after 28 weeks and at the trial's end which was 52 weeks after patients began taking nutrients. Patient weights were recorded at six weekly intervals. Karnofsky scores were used to establish changes in the quality of life and were measured at the beginning of the trial and at 30 and 52 weeks. The study examined the effects of these two combinations of nutrients on biochemical and immunologic parameters, that is, serum glutathione peroxidase levels and CD4 cell counts as the study's primary endpoint. Secondary end points were weight changes and patient assessed quality of life.

Results: The mean/median serum glutathione peroxidase levels in Group A (37 nutrients) increased from 3825/3628 U/L (International Units) at baseline to 8894/8575 U/L at the trial's end ($p<0.000$). Similarly, patients in Group B (30 nutrients) had an increase in mean/median serum glutathione peroxidase levels from 3862/3602 to 9839/9203 U/L over the length of the trial ($p<0.000$). The mean/median CD4 cell counts rose from 400/347 mm^3 to 446/388 in Group A and from 400/335 to 446/394 mm^3 in Group B ($p<0.000$). Mean weight increases over 52 weeks were 1.0 kg in Group A and 1.4 kg in Group B, while Karnofsky scores in Group A rose from 81 to 85 and in Group B from 82 to 86. Wilcoxan Signed Ranks Test and the Sign Test both indicated that all these measured increases within both groups were statistically significant ($p<0.01$). Indeed, for both glutathione peroxidase levels and CD4 cell counts $p<0.000$ in all cases. There were, however, no statistically significant differences between the measured parameters, (serum glutathione peroxidase, CD4 cell counts, weight and Karnofsky scores) in Group A compared with those of Group B ($p>0.05$), or between

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males and females ($p>0.05$).

Conclusions: Specific nutritional supplements, designed to raise patients' glutathione peroxidase levels, appeared to be able to significantly increase CD4 cell count recovery in HIV-infected patients receiving no other medications. This increase in CD4 cell count was associated with an improvement in quality of life and an increase in body weight. The supplements tested were generally well tolerated and appear to hold promise for the prophylaxis of HIV/AIDS. Further nutritional clinical studies appear warranted.

Key Words: HIV, nutritional supplements, selenium, glutathione peroxidase, CD4 cell count, Karnofsky scores

Introduction

There is clearly a relationship between the global diffusion of HIV/AIDS and the nature of local diets.[1] In HIV infection, for example, deficiencies of specific nutrients have been shown to be associated with more frequent opportunistic infections, faster progression of disease and higher AIDS mortality.[2-4] Furthermore, Fawzi and co-workers[5] have reported an increase in CD4 cell count in HIV-infected patients receiving micronutrients.

Selenium appears to play a key role in this relationship. Ogunro and colleagues,[6] for example, have shown that as HIV/AIDS progresses, both plasma selenium levels and mean erythrocyte glutathione peroxidase activity declines. Consequently they suggest that selenium supplementation would be of immense benefit to HIV-1/AIDS infected patients. Kaiser and co-workers[7] also have recently demonstrated that micronutrient supplements, including selenium, N-acetyl cysteine and L-glutamine significantly increased CD4 cell count in HIV-infected patients receiving highly active anti-retroviral therapy (HAART). Indeed, Foster[8] has argued that the major symptoms seen in AIDS are due to extreme deficiencies of selenium and the three amino acids, cysteine, glutamine and tryptophan, caused by production of a homologue of glutathione peroxidase by HIV.

The major objectives of this clinical trial were to establish whether nutritional supplementation could slow the decline of HIV-positive patients to AIDS and improve their CD4 cell counts and quality of life, as shown by Karnofsky scores. If so, the study sought to determine whether such improvements were associated with increases in serum glutathione peroxidase levels.

Method

Study Design

This was a prospective, randomized, double blinded, clinical trial designed to determine the affects of two nutritional supplement mixtures on HIV-1 disease progression, in HIV-infected patients who were not receiving any anti-retroviral treatment. These mixtures were designed to increase the body's production of glutathione peroxidase to determine whether elevated levels of this selenoenzyme changed the natural history of HIV infection.

After the trial had received ethics approval from the Mulago Ethical Review Committee (ERC) and the Uganda National Council of Science and Technology, it was given the International Standardised Randomised Controlled Trial Number 42274642. Before their enrollment, informed consent was obtained from study participants, all of whom were outpatients of the Mengo Hospital in Kampala, Uganda. The trial lasted one year.

Study Subjects and Study Site

Enrollment began in June 2005 and concluded in October of the same year. This individual recruitment process took 2 weeks and so, although patients were involved with the trial for 54 weeks, they actually received the assigned supplements for 52 weeks. By the end of enrollment in October, 310 HIV-positive patients had been recruited. This study size was ad-

equate to bring about a power of 80 percent at a 95 percent confidence interval. 249 of the enrolled patients were female and 61 male. In women, the median age at the trial's start was 36.0 years (25 percentile 30.0 years, 75 percentile 40.0 years). In men, the median age was 39.0 years (25 percentile 33.7 years, 75 percentile 45.5 years). Using a random block design to achieve patient randomization, participants were given one of two nutrient supplement combinations identified as A or B, to be taken three times daily with meals, for 52 weeks (**Table 1,** p.132). Patients who were pregnant, had a baseline CD4T lymphocyte count of 200 or less, or who were receiving anti-retroviral treatment were excluded from trial participation. However, patients who suffered from other additional illnesses, such as tuberculosis, were accepted for enrollment. All clinical staff and student assistants were unaware of the patient group treatment assignments. The labels were attached to all bottles of nutrients by an external co-ordinator in Canada who also kept the code necessary to identify members of the two groups. All trial participants agreed to return, at six weekly intervals, to Mengo Hospital to receive nutritional supplements and for measurement of CD4 cell counts, serum glutathione peroxidase, weight and quality of life assessments as required.

Study Medications

Two nutritional combinations were designed and encapsulated specifically for use in this trial. The medications given to patients in Group B consisted of 30 nutrients with a filler of organic sugar (Table 1). This combination of nutrients had been found, in small open trials, to stimulate appetites in HIV-positive patients[9]. The logic behind this approach was to establish whether greater appetite was sufficient to encourage HIV-positive individuals to increase their consumption of local foods to a point at which enough selenium and amino acids were digested to normalize glutathione peroxidase levels. Serious loss of appetite is a common symptom of HIV/AIDS.[10]

The capsules taken by Group A patients contained the same thirty appetite stimulating supplements, but also included the additional seven nutrients shown in Table 1. The latter nutrients were designed to directly promote the body's production of glutathione peroxidase and, in addition to amino-acid rich desiccated beef liver, included L-selenomethionine, N-acetyl cysteine, L-glutamine, hydroxytryptophan, alpha lipoic acid and ascorbic acid. Patients receiving this mixture of supplements did not have to rely on their own diet to provide the selenium, cysteine, tryptophan and glutamine thought necessary to boost body glutathione peroxidase levels.[11]

Capsule sizes and dosages were identical and their appearances were extremely similar. Both nutrient supplements were taken three times daily (six capsules in total) with food.

Clinical and Laboratory Evaluations

Potential study patients underwent an initial screening at Mengo Hospital. This involved collection of demographic data, medication history and a laboratory testing of CD4T cell levels. Eligible participants then returned to this hospital for baseline and follow-up visits on a six weekly basis for 52 weeks. Glutathione peroxidase levels were measured at baseline on entering the trial, after 30 weeks and at the end of the trial, as were CD4T cell counts. Patients were also weighed during each of their hospital visits. Karnofsky quality of life scores were taken at baseline and at 30 an 52 weeks. Laboratory measurements of serum glutathione peroxidase levels took place at the Mengo Hospital Laboratory, while CD4T cell counts were conducted by the Hematology Laboratory at Mulago Hospital.

Table 1. Nutritional combinations used in Mengo Hospital HIV-Positive Outpatient Trial (Groups A and B).

Amount per Serving (Three servings needed per day)	Group A	Group B
Calcium	23 mg	23 mg
Magnesium	23 mg	23 mg
Boron	0.2 mg	0.2 mg
Zinc	1.1 mg	1.1 mg
Vanadium	2 mcg	2 mcg
Copper	100 mcg	100 mcg
Chromium	8 mcg	8 mcg
Manganese	620 mcg	620 mcg
Silica	770 mcg	770 mcg
AEP Iron (2-amino ethanol phosphate)	600 mcg	600 mcg
Iodine	2.9 mcg	2.9 mcg
Strontium	25.7 mcg	25.7 mcg
Molybdenum	0.3 mcg	0.3 mcg
Vitamin A	390 IU	390 IU
Provitamin A	390 IU	390 IU
Vitamin D_3	31 IU	31 IU
Vitamin B_1	1.9 mg	1.9 mg
Vitamin B_2	1.9 mg	1.9 mg
Vitamin B_3	7.8 mg	7.8 mg
D-Calcium Pantothenate	7.8 mg	7.8 mg
Vitamin B_6	1.9 mg	1.9 mg
Vitamin B_{12}	8 mcg	8 mcg
Vitamin C (calcium ascorbate)	23 mg	23 mg
Vitamin E (D Alpha Tocopheryl Succinate)	5 IU	5 IU
Vitamin K (Phytonadione)	23 mcg	23 mcg
Biotin	8 mcg	8 mcg
Folic Acid	31 mcg	31 mcg
Choline	4 mg	4 mg
Inositol	4 mg	4 mg
P.A.B.A. (Para Amino Benzoic Acid)	2 mg	2 mg
Dessicated Beef Liver (undefatted)	400 mg	Nil
L-Glutamine	180 mg	Nil
Hydroxytryptophan L-S (5-HTP)	180 mg	Nil
N-Acetyl Cysteine	180 mg	Nil
Alpha Lipoic Acid	30 mg	Nil
Ascorbic Acid	40 mg	Nil
L-Selenomethionine	200 mcg	Nil
Organic sugar as a filler	Nil	Yes

Data and Collection and Statistical Analyses

At Mengo Hospital, patient data was stored in an Access database and data collection forms were created using Access database software. After collection, the data were moved from Access to the statistical package SPSS for analyses.

All statistical analyses were conducted by the Statistical Consulting Centre of the University of Victoria, British Columbia, Canada which recommended the use of Wilcoxan Signed Ranks Tests and the Sign Test to establish the statistical significance of data from baseline to the study's 52 week conclusion.[12]

The study's primary end points were to examine the effects of the two nutrient mixtures on serum glutathione peroxidase levels and on CD4 cell count. Secondary end points were weight changes and general health status as measured by Karnofsky scores. Of particular interest was any delay in the progression of AIDS and the need to start associated anti-retroviral treatment.

Results

310 patients began the trial, 160 of whom were randomly assigned to Group A and 150 to Group B. During its 52 week duration, 47 patients exited the programme, 29 from Group A and 18 from Group B. This represented a loss to follow-up of some 15.2 percent. In Group A, 3 left because of pregnancy, 14 were lost to follow-up, 4 withdrew consent, 2 died from tuberculosis and one from esophageal cancer. Two further patients died from unknown causes and another from severe anemia. In Group B, over the course of the trial, one patient left because of pregnancy, 6 were lost to follow-up, one withdrew consent, four died of tuberculosis, two from cancer and four of undiagnosed illnesses.

As a consequence, 81.9 percent of patients in Group A and 88.0 percent of those from Group B completed the year long trial. The statistical analyses which follow refer to these patients. Beyond simple measures of central tendency, Sign Test and Wilcoxan Signed Rank Tests were used to establish whether the obtained results were statistically significant, at least at the 0.01 level.

Immunological, Biochemical Parameters

From baseline over the one year period of follow up, 92 patients in Group A experienced an increased in the CD4 counts, while the remaining 39 showed a decrease (**Table 2**, p.134). Nevertheless, the mean/median CD4 count for Group A as a whole rose from 400/347 to 446/388 cells per mm^3. The Wilcoxan Signed Ranks Test and the Sign Test both indicated that these Group A mean/median increases were statistically significant (p=0.000).

Similarly, from base measurement to week 52, 89 patients in Group B showed an increase in their CD4 counts, while the remaining 41 displayed a decrease. The mean/ median CD4 count for Group B, rose from 400/335 to 446/394 over the year. Again the Wilcoxan Signed Ranks Test and Sign Test both established these gains to be of statistical significance (p=0.000).

At the beginning and end of the 52 week trial, glutathione peroxidase levels were measured in 92 patients from Group A. 77 of these patients had experienced an increase in the serum levels of this selenoenzyme, while the remaining 15 showed decreases. As a whole, 92 patients in Group A, for whom serum glutathione peroxidase levels were measured, showed mean/median increases from 3825/3628 U/L at the beginning of the 52 week long trial to 8894/8573 U/L at its end (p=0.000). Similarly, serum glutathione peroxidase levels were measured in 92 members of Group B at the start and endpoint of the trial. 81 of these had experienced an increase and 11 a decrease in serum levels of this selenoenzyme. This group, as a whole, showed rises in mean/median serum glutathione

Table 2. Changes (increases/decreases/no change) in CD4 cell counts, serum glutathione peroxidase, weight and Karnofsky scores from baseline measurement to 52 weeks.

Group A		CD4 cell count	Serum glutathione peroxidase	Weight	Karnofsky scores
	Increase	92	77	69	68
	No Change	0	0	17	27
	Decrease	39	15	41	34
Group B					
	Increase	89	81	76	71
	No Change	0	0	8	27
	Decrease	41	11	42	31

peroxidase levels from 3862/3602 U/L to 9839/9203 U/L over the 52 week trial. Both the Sign Test and Wilcoxan Signed Rank Test established that these increases were of statistically significance (p=0.000)

Clinical Parameters

Over the 52 weeks, 67.7 percent of the patients in Group A either increased, or remained the same in weight. Mean weight increased from 60.1 kg to 61.1 kg. Both the Wilcoxan Signed Rank Test (p=0.001) and the Sign Test (p=0.01) showed this gain to be statistically significant. Similarly, 66.7 percent of the patients in Group B either increased or remained the same in weight over the year long trial. Mean weight for Group B rose from 62.0 kg to 63.4 kg, during the 52 weeks. Again both Wilcoxan Signed Ranks Test (p=0.000) and the Sign Test (p=0.002) demonstrated that weight gain was of statistical significance.

Quality of life, as measured by the Karnofsky scores[13] also had risen during the duration of the trial in both groups. In Group A, for example, these scores rose or remained the same in 73.6 percent of patients, while the mean of this measure increased from 81 to 85 (p=0.000 and p=0.001). In Group B scores rose or remained unchanged in 76.0 percent of patients, while the mean rose from 82 to 86 (p=0.000 in both statistical measures).

Comparison of Groups A and B and of Males and Females

Four Analysis of Variance (ANOVA) models were used to compare changes in CD4 cell counts, serum glutathione peroxidases, weight and Karnofsky scores, over 52 weeks, using two factors; nutritional supplements and gender. Neither of these two factors were significant at the 0.05 level. That is, during this trial, there were no statistically significant gender differences in the results obtained, nor were there any statistically significant differences in changes in CD4 cell count,

serum glutathione peroxidase, weight or Karnofsky scores between Group A and B patients. In summary, both genders showed similar immunologic, biochemical and clinical improvements, regardless of which of the two nutritional combinations they were taking.

Discussion

The major objective of this clinical trial was to determine whether either, or both, of the nutritional supplement mixtures, described in Table 1, could slow or reverse the progression to AIDS of HIV-infected patients who were not taking anti-retroviral drugs. When the trial ended, the majority of patients from both Groups A and B felt that this goal had been achieved. Many patients, for example, described significant appetite increases, together with the return of their ability to walk long distances. Most also reported being happier. The quantitative data supported these claims. It has been long established that selenium and associated glutathione peroxidases are essential components of the human immune system.[14] In the trial described here, two related but distinct, nutritional treatments, were both able to increase serum glutathione peroxidase levels in HIV-infected outpatients by roughly a factor of 2.5, over a 52 week period. Such increases would be very unexpected in HIV-positive patients who are not receiving anti-retroviral drugs since serum selenium and glutathione peroxidase levels both normally decline as HIV/AIDS progresses.[15-16]

Simultaneously, the CD4 cell counts, indicative of an improving immune system, rose in both treatment groups. Such improvements are also atypical of HIV-positive patients not taking anti-retroviral drugs. To illustrate, a healthy CD4 cell count is somewhere between 500 and 1500 cells per cubic millimeter of blood. Normally, as observed at Mengo Hospital and confirmed elsewhere,[17] in HIV-positive patients not receiving anti-retroviral, the count decreases on average about 50 to 100 cells each year. If this is the case, one might have expected CD4 cell counts (mean/median) to have fallen to some 325/272 in patients in Group A and 325/260 in Group B after the completion of the 52 week trial. In fact, these measures of central tendency were, as described, 446/338 and 446/394 at trial's end. That is, both nutrient groups had mean and median CD4 cell counts that were roughly 120 cells per cubic millimeter of blood higher than expected in HIV-positive patients not receiving anti-retroviral treatment. Increases in weight and Karnofsky Score were also indicative of a general clinical improvement in health of both treatment groups. While these relationships do not prove that increasing serum glutathione peroxidase levels cause improvements in CD4 cell counts, weight gains and higher personal evaluation of health they are certainly consistent with this hypothesis. These analyses strongly support providing nutritional supplements for all HIV-1/AIDS patients, although it is clear that further clinical trials are required to establish optimum dosages and nutrient combinations.

Conclusions

This trial shows that both nutrient combinations (Table 1), taken for 52 weeks by HIV-positive patients who were receiving no anti-retroviral drugs, can significantly slow their decline into AIDS. The associated improvement is associated with increases in serum glutathione peroxidase levels, CD4 cell counts, body weights and improvements in quality of life scores. These results are consistent with those of smaller open trials using nutritional supplement which have been conducted elsewhere in Sub-Saharan Africa.[18] It seems clear that inadequate nutrition plays an extremely important role in the progression into AIDS of HIV-infected patients. These results also are consistent with Foster's model[19] of the development

of AIDS which suggests that deficiencies of glutathione peroxidase play a key role in the process.

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