Effect of a liquid nutritional supplement on viral load and haematological parameters in HIV-positive/AIDS patients

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ABSTRACT

The effect of a nutritional supplement on the immune status and haematological parameters of HIV-positive/AIDS patients is tested using standard procedures. This clinical trial of 35 patients consists of a baseline visit and three months of supplementation from April to September 2003. Results showed that viral load decreased significantly ($P<0.002$) with time following supplementation. Mean cell volume (MCV) and mean cell haemoglobin concentration (MCHC) increased significantly ($P<0.002$ and $P<0.0002$, respectively), reflecting the positive effect of the supplement on these haematological parameters. Supplementation had no effect on $CD4^+$ T-cell count, which decreased significantly with disease progression. Owing to certain limitations of the study (small sample size, short duration and the late stage of HIV infection), further studies are needed to confirm the effect attributed to the supplement.

KEY WORDS: Acquired immunodeficiency syndrome. Dietary supplements. HIV infections. Immunity.

Introduction

Evidence from different studies[1–3] indicates that carefully chosen supplementation can enhance quality of life. Micronutrient supplementation has been advocated for human immunodeficiency virus (HIV)-infected persons, especially in low-income countries such as South Africa.[4–6] Micronutrient supplementation has been shown to be associated with a significant slowing of HIV disease progression, better preservation of $CD4^+$ T-cell count and lower viral loads.[7,8] There is evidence that nutritional intervention assists in maintaining and optimising nutritional status and immune function, prevents the development of nutritional deficiency, loss of weight and lean body mass, promotes response to medical treatment, and increases longevity in HIV-infected persons.[9–16]

It has been demonstrated[17] that micronutrient supplementation may be an important prophylactic and therapeutic measure for HIV-1 infected persons and is possibly one of the few potential therapies that could improve a patient's quality of life by maintaining strength, comfort, level of functioning and self image. For example, daily β-carotene supplementation (180 mg, one month) in HIV-positive persons resulted in a small but statistically significant increase in total white blood cell count and a percentage change in $CD4^+$ T-cell count in one trial.[18] In another study,[11] supplementation with multivitamins resulted in a significant increase in $CD4^+$ and $CD8^+$ T-cell counts. In the same study, haemoglobin levels were significantly increased. In a clinical trial conducted in Canada,[19] it was observed that daily mega doses of vitamins C and E over three months resulted in a clinically important reduction in viral load.

However, studies on nutritional supplementation in HIV-positive persons are scarce in South Africa,[20] and it is against this background that the effect of a liquid nutritional supplement on the immune status and haematological parameters of HIV-positive/acquired immune deficiency syndrome (AIDS) patients is examined in the work reported here.

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Materials and methods

This study involved an open-labelled, multiple-dose clinical trial consisting of 50 HIV-positive /AIDS volunteers. Of the 50 volunteers who met the inclusion criteria, three dropped out, 10 died and two relocated during the three-month period of the study. Of the 35 patients that completed the food frequency questionnaire and had their anthropometric parameters measured at baseline, only 29 completed the study.

Approval from the Ethics Committee (ETOVS 32/03) of the Faculty of Health Sciences, University of the Free State, was obtained and all patients signed informed consent forms for inclusion in the study. Study patients were between 18 and 65 years of age, HIV-positive, not on antiretroviral therapy, willing to undergo a pre-and post-study physical and medical examination, have a $CD4^+$ T-cell count of 100–350 cells/mm^3. For ethical reasons, a placebo group was not included in this study but results from screening visits served as internal controls.

The study involved a baseline visit and monthly visits to Medi Inn clinic and Tsepo House (home-based care) from April to September 2003. The duration of the study was similar to that reported by Allard *et al*.[19]

Sample collection and processing

Blood (10 mL) was collected from each patient into EDTA sampling tubes for the determination of full blood count, $CD4^+$ T-cell count and viral load. Blood samples were kept at room temperature and processed within four hours. Haematological parameters were performed on all patients using a Beckman Coulter analyser. T-cell subset numbers were determined by flow cytometry (Beckman Coulter).

A quantitative HIV-1 RNA polymerase chain reaction (PCR) was performed on batched samples using an HIV-1 monitor assay (Roche Molecular Systems) according to the manufacturer's instructions for the detection and quantitation of viral load. In each case, 50 µL each prepared RNA sample was used for PCR. Following amplification and detection of the PCR product, the starting HIV-1 RNA load in each sample was calculated in accordance with the internal quantitation standard, with results expressed as HIV-1 RNA copies/mL plasma.[21]

Supplementation

The supplement comprised extract of hypoxis (500 mg), grape fruit seed extract (4 mg), sitosterol and sitosterolin (28 mg), β-carotene (1 mg), vitamin E (12.5 mg), vitamin B_6 (7.5 mg), vitamin B_1 (3.75 mg), vitamin B_2 (10 mg), vitamin B_{12} (3 µg), nicotinamide (5 mg), vitamin C (50 mg), olive green leaf extract (35 mg), folic acid (325 µg) and natural antioxidant (biocydin, 52 mg).

Following determination of baseline haematological and immunological parameters and viral load, patients were given 7.5 mL test supplement twice daily (07.00–09:00 and 16:00–19:00). Daily intake of supplement was monitored by members of the South African Red Cross Society, Bloemfontein, and the medical staff at Tsepo House.

Follow-up visits

Patients visited the clinic on a monthly basis. Blood samples were taken and full blood count and $CD4^+/CD8^+$ T-cell counts were determined. Viral load estimation was repeated at the end of the study. Haematological and immunological parameters were also repeated at the end of the study to determine the possible influence of the supplement on these values.

Compliance with the regime was ensured by counting the supplement units on a daily basis and at each visit, and by reminding the patients of the need to follow the protocol. Compliance with supplement intake by patients was monitored by assistants of the South African Red Cross Society, Bloemfontein.

Statistical analysis

The results for the study were analysed by an independent biostatistician at the University of the Free State, Bloemfontein, South Africa, using SAS.[22] The results are presented in mean, median and standard deviation. Significance was set at $P<0.05$.

Results

Table 1 shows the mean, standard deviation, median and *P* value of haematological parameters for the study group at baseline and on the final visit. Table 2 demonstrates the differences by gender.

Table 1. Haematological parameters in HIV-positive/AIDS patients at baseline and final visit.

	Baseline ($n=35$)			Final visit ($n=29$)			*P* value
	Mean	SD	Median	Mean	SD	Median	
RBC (10^9/L)	4.0	0.6	4.1	4.4	1.7	4.0	NS
Haemoglobin (g/dL)	12.1	1.9	12.0	11.9	1.7	11.9	NS
Hct	0.37	0.1	0.36	0.38	0.1	0.36	NS
MCHC (g/dL)	32.8	0.9	33	33.6	1.0	34	<0.0002
ESR (mm/h)	83.7	32.8	86	86.7	24.1	83	NS
MCV (fL)	91.8	5	92	87.7	4.9	90	<0.002
MCH (pg)	30.2	5	31	30.1	2.4	30	NS
RDW	15.1	1.5	14.	15.2	2.0	14.5	NS
WCC (10^9/L)	5.7	3.9	4.9	4.8	1.6	4.8	NS
Neutrophils (10^9/L)	2.7	2.6	2.2	2.2	1.0	2.2	NS
Lymphocytes (10^9/L)	2.1	0.8	1.9	1.9	0.9	1.9	NS
Monocytes (10^9/L)	0.6	0.4	0.5	0.4	0.3	0.4	<0.001
Eosinophils (10^9/L)	0.3	0.4	0.2	0.3	0.3	0.2	NS
Basophils (10^9/L)	0.1	0.5	0.02	0.03	0.02	0.02	NS
Platelets (10^9/L)	254.3	82.3	252	239.6	74.4	234	NS

$P<0.05$ significant.

NS: not significant; SD: standard deviation.

RBC: red blood cells; Hct: Haematocrit; MCV: mean cell volume; MCH: mean cell haemoglobin; MCHC: mean cell haemoglobin concentration; ESR: erythrocyte sedimentation rate; WCC: white cell count; RDW: red cell distribution width.

Table 2. Haematological parameters of HIV-positive/AIDS patients at baseline and final visit by gender.

	Baseline $n=35$			Final visit $n=29$		
	Mean	SD	*P* value	Mean	SD	*P* value
RBC (10^9/L)						
Male	4.4	0.8	0>0.05	4.4	2.0	0>0.05
Female	3.9	0.6		4.4	2.0	
Haemoglobin (g/dL)						
Male	13.7	2.5	<0.03	13.4	2.6	0.1
Female	11.6	1.5		11.4	3.1	
Hct						
Male	0.41	0.1	<0.02	0.39	0.1	0>0.05
Female	0.36	0.04		0.38	0.1	
MCHC (g/dL)						
Male	33.5	0.9	<0.05	33.8	1.3	0>0.05
Female	32.1	0.9		33.5	0.9	
ESR (mm/h)						
Male	95.9	24.9	0>0.05	87.4	18.8	0>0.05
Female	80.1	34.4		86.4	26.2	
MCV (fL)						
Male	90.8	4.3	0>.05	92.8	4.6	<0.05*
Female	89.2	3.9		91.6	4.9	
MCH (pg)						
Male	33.5	2.2	<0.01	32.5	1.8	<0.05*
Female	30.0	1.9		29.1	2.1	
RDW						
Male	15.6	1.7	>0.05	13.1	2.0	0>0.05
Female	16.0	2.3		15.7	2.1	
WCC (10^9/L)						
Male	5.9	3.8	0>0.05	4.8	1.6	0>0.05
Female	4.8	2.7		4.4	1.3	
Neutrophils (10^9/L)						
Male	2.9	2.6	0>0.05	3.0	3.2	0>0.05
Female	2.4	1.9		2.3	2.6	
Lymphocytes (10^9/L)						
Male	2.3	0.8	0>.05	2.4	0.9	0>0.05
Female	1.8	0.7		2.1	0.9	
Monocytes (10^9/L)						
Male	0.4	0.6	0>0.05	0.5	0.1	0>0.05
Female	0.5	0.7		0.6	0.3	
Eosinophils (10^9/L)						
Male	0.3	0.2	0>0.05	0.3	0.3	0>0.05
Female	0.4	0.3		0.2	0.2	
Basophils (10^9/L)						
Male	0.1	0.5	0>0.05	0.02	0.01	0>0.05
Female	0.1	0.5		0.03	0.02	
Platelets (109/L)						
Male	258.5	83.1	0>0.05	241.2	76.1	0>0.05
Female	250.1	79.7		238	74.7	

P<0.05 significant.

Table 3. Immunological parameters of HIV-positive/AIDS patients at baseline and final visit.

	Baseline $n=35$			Final visit $n=28$			*P* value
	Mean	SD	Median	Mean	SD	Median	
Total T-cell count/mm^3	1615	736.3	1543	1495.7	758.9	1400	0.07
CD4+ T-cell count/mm^3	203.9	83.5	176	188.2	96.9	164	<0.03
CD8+ T-cell count/mm^3	1407.2	672.2	1254	1267.1	686.9	1228	0.09
CD4/CD8 ratio	0.2	0.1	0.2	0.2	0.1	0.2	0.7
Viral load/mL	374302	300299	292000	279367	244877.6	193000	<0.002

SD: standard deviation

$P<0.05$ significant

Table 3 shows the mean, standard deviation, median and P value of immunological parameters at baseline and on the final visit. $CD4^+$ T-cell count ($P<0.03$) and viral load ($P<0.002$) showed significant differences between the baseline and final visit. The supplement showed no positive effect on $CD4^+$ T-cell count, which decreased significantly with disease progression ($P<0.03$); however, the supplement demonstrated a significantly positive effect on viral load ($P<0.002$). Table 4 demonstrates the differences by gender. The supplement appeared to have a positive effect on $CD8^+$ T-cell count by gender but this was not significant ($P<0.05$).

Discussion

The current study would appear to be the first clinical trial of its type in the Free State Province of South Africa and the results demonstrated that daily supplementation of micronutrients (antioxidants/minerals) and a sterol/sterolin mixture significantly decreased the viral load in an HIV-positive population. Researchers recommend the benefit of nutritional intervention programmes, including supplementation with antioxidant nutrients, as part of the comprehensive care for HIV-positive/AIDS people.[10,23,24] In this study, levels of these antioxidants were not determined because it was not the primary focus. However, a previous study performed in the Free State by Van Staden *et al*.[4] reported reduced levels of these antioxidants in the blood of HIV-positive persons. It was therefore appropriate to test the effect of a liquid product containing sterol/sterolin and antioxidants on the immune status and haematological parameters of HIV-positive/AIDS patients.

In vitro evidence implicates oxidative stress in the stimulation of HIV replication through activation of the necrosis factor in a human T-cell line,[25] and the addition of antioxidant vitamins blocks the activation of the necrosis factor and inhibits HIV replication.[25–27] Similar effects may occur *in vivo*, as demonstrated by the significant reduction in HIV viral load observed following supplementation in the present study.

This finding is similar to that reported by Allard *et al*.,[19] who observed that daily vitamin C and E supplementation over three months resulted in reduction in viral load. In another study, Bouic *et al*.[3] indicated that HIV-positive subjects on a sterol/sterolin mixture experienced a decrease in average viral load.

The supplement in the present study contained sterol/sterolin mixture and antioxidants/minerals. Whether the significant reduction in viral load was due to the sterol/sterolin mixture or to its combination with the other supplement components (antioxidants/minerals) needs to be clarified in future studies.

Kanter *et al*.[2] reported delayed onset of AIDS and death following supplementation with multivitamins. The current study did not measure progression of HIV infection or survival rate; however, evidence[6,13,28] shows that one of the factors determining disease progression in HIV infection is the rate of virus replication. In decreasing the viral load significantly, supplementation shows its positive effect on the quality of life and the rate of disease progression in those with HIV/AIDS.

A central feature in the pathogenesis of HIV infection is the depletion of $CD4^+$ T-lymphocytes.[29,30] $CD4^+$ quantitation measures this depletion and provides important information about the immune status of HIV-1-infected persons. Here, the $CD4^+$ T-cell count decreased significantly during the course of study. Decrease in $CD4^+$ T count reflects the reduction in immune status.

With a decrease in viral load, it was expected that $CD4^+$ T count would increase, but this did not occur. The quantity and function of $CD4^+$ T cells depends on the production of new $CD4^+$ T cells. If production is impaired, damaged $CD4^+$ T cells cannot be replaced and therefore a decrease in viral load may not necessarily result in a corresponding increase in the $CD4^+$ T-cell count.

Compared with viral load, measurement of $CD4^+$ T cells might be misleading in predicting HIV progression;[30–34] therefore, viral load seems a better predictor of HIV replication and the rate of decline in immune status in HIV-infected persons.

Although AIDS-defining complications are common once the $CD4^+$T-cell count is <200 cells/mm^3, the association between the $CD4^+$ T-cell count and the risk of death in persons with AIDS is not well defined. In the present study, 10 of the patients whose $CD4^+$T-cell count fell below 200 cells/mm^3 died during the course of the study. However, a few of the patients who had a $CD4^+$T-cell count <200 cells/mm^3 survived to the end of the study.

$CD8^+$ T lymphocytes are believed to mediate antiviral activity against HIV.[35] This was first reported in 1986 when investigators noted that depletion of $CD8^+$ T cells from peripheral blood of HIV-infected persons resulted in a marked increase in viral replication. Replacement of the $CD8^+$ T cells caused a dose-dependent suppression of viral replication, and an increase in the $CD8^+$T-cell count correlated with a decline in viraemia and inversely

Table 4. Immunological parameters of HIV-positive/AIDS patients by gender.

	Baseline $n=35$			Final visit $n=29$		
	Mean	SD	*P* value	Mean	SD	*P* value
Total T-cell count/mm^3						
Male	1617	737.1	0>0.05	1543	697	0>0.05
Female	1498	746.3		1344	701.9	
CD4+ T-cell count/mm^3						
Male	204.2	83.8	0>0.05	188.6	86.2	0>0.05
Female	202.4	81.2		182.2	90.1	
CD8+ T-cell count/mm^3						
Male	1411	671.3	<0.02	1255	675.3	0>0.05
Female	1390	686.5		1247	668.4	
CD4/CD8 ratio						
Male	0.16	0.1	0>0.05	0.18	0.1	0>0.05
Female	0.18	0.1		0.17	0.1	
Viral load/mL						
Male	374308	300301	0>0.05	279371	244876.2	0>0.05
Female	374304	300304		279373	244878.1	

$P<0.05$ significant

correlated with disease progression.[35] In the present study, CD8+ T cells showed no correlation with decrease in viral load.

HIV infection is known to reverse the $CD4^+/CD8^+$ T-cell ratio (normal range: 0.72–3.14). A $CD4^+/CD8^+$ T-cell ratio of 0.15 is said to correlate with a $CD4^+$T-cell count of 200 cells/mm^3. A high baseline $CD8^+$T-cell count and subsequent fall seems to occur as a result of HIV-mediated immune hyperactivation.[34] The median $CD4^+/CD8^+$ T-cell count ratio of 0.2 reported in the present study is similar to the figure quoted above and tends to correlate with the level of the $CD4^+$T-cell count. The present study also indicated that the $CD4^+/CD8^+$ T-cell count ratio was reversed. The reversed ratio is believed to be associated with a significant decline in $CD4^+$ T-cell count.

In the present study, anaemia was defined as haemoglobin below 12.0 g/dL (Table 1). The haemoglobin level in all the patients was slightly above 12.0 g/dL at baseline and proved to be more or less stable through the course of the study. It is possible that haemoconcentration contributed to the haemoglobin level reported in this population. Anaemia is a common manifestation of HIV infection and occurs at all stages of HIV-associated disease. It remains a significant problem despite recent advances in antiretroviral therapy and the introduction of highly antiretroviral therapy (HAART) to routine clinical practice.[36]

Among HIV-infected patients, anaemia has been linked to decreasing levels of $CD4^+$T-cell counts (more severe with $CD4^+$ T-cell count <200 cells/mm^3), increased plasma HIV RNA levels, and a history of clinical AIDS-defining conditions. Anaemia in HIV-infected persons has also been linked to abnormally low levels of erythropoietin, decreased erythropoietin production, increased erythrocyte destruction, ineffective erythrocyte production and the use of multiple medications.

Haemoglobin level in HIV-infected patients is expected to decrease as the disease progresses. In the present study, the level was maintained and it is possible that this was due to the use of the supplement, which contained folic acid, vitamin C and vitamin B_{12}, all of which could have helped to maintain the haemoglobin level.

Folic acid is essential in the formation and maturation of red blood cells.[37] Vitamin C is a reducing agent and prevents the oxidation of iron, thus making more iron available. The effect of vitamin B_{12} in red blood cell synthesis is well known.

There was no significant difference between the median white cell count and median differential count in the patients before and after supplementation. Baseline total T-lymphocyte count and total T-lymphocyte count following supplementation did not show significant differences (Table 1). Lymphopenia and neutropenia are common in HIV-positive/AIDS, especially in Africa;[34] however, these phenomena were not observed in the present study.

A high incidence of thrombocytopenia has been reported in some HIV-positive patients. Prevalence of 5–12% has been reported in AIDS-related complex (ARC) patients compared with a prevalence of 30% in patients with AIDS.[38] The mechanism of thrombocytopenia in HIV infection appears to be due to the presence of an autoantibody acting against a platelet membrane protein. This protein appears to be approximately 25 kDa in size and is present on normal platelets. It may resemble an HIV precursor protein, but the exact mechanisms for platelet destruction remain unclear. Patients in the present study did not show thrombocytopenia, as the platelet counts were within the reference range (Table 1). This could be due to the small sample size.

The present study showed that supplementation significantly reduced viral load and had an effect on some haematological parameters, suggesting clinical benefit worthy of larger clinical trials. Use of antiretroviral therapies is generally limited in developing countries, particularly those in sub-Saharan Africa, and thus supplements are an important adjunct to treatment and might show appreciable

benefit, perhaps similar to the effect of vitamin A supplementation on childhood mortality in developing countries. □

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