1. Introduction

Nutritional deficiencies have long been recognized as an important problem among HIV-1-seropositive individuals. They have a great demand for nutrients because of the stress placed on their immune systems. Moreover, poor nutrition may also affect drug effectiveness or increased toxicity [34-37]. It has been shown that micronutrient deficiencies are associated with more rapid HIV-disease progression and higher HIV-1 related mortality [38-40]. Supplementation of micronutrient has delayed time to AIDS and improved survival, suggesting that supplementation could offer a simple and relatively inexpensive strategy to slow HIV-1 progression [41, 42].

HIV infected patients present changes in components of the antioxidant defense system, which may be the result of excessive production of oxygen-derived species during the development of the disease (Pace and Leaf, 1995) and that cells infected with HIV can enhance production of $O_2^{-}$ [43]. This phenomenon combined with a deficiency in key antioxidant enzymes superoxide dismutase and catalase, and a decreased concentrations of the antioxidant vitamins [44] may lead to severe oxidative stress in HIV-infected patients. Humans infected with human immunodeficiency virus (HIV) have been shown to be under chronic oxidative stress [44-46], which is the result of imbalance between free radical (or pro-oxidant) production and antioxidant action. In HIV infection, oxidative stress may be caused by both overproduction of reactive oxygen species (ROS) and a simultaneous deficiency of antioxidant defenses [47, 48]. Oxidative stress induced by ROS play a critical role in the stimulation of HIV replication and the development of immunodeficiency [49, 50].
Many studies have focused on the role of nutritional supplements to attenuate signs and symptoms of HIV. Of these, some have reported favorable results, while many others have reported no benefit of the selected nutrient. Despite these mixed findings, recommendations for the use of nutritional supplements for the purposes of attenuating HIV are rampant. Based on this background, we have assessed the antioxidant status among HIV-infected patients on oxidative stress after antioxidant supplementation.
2. Subjects and methods

2.1. Subjects

Open clinical trial study was implemented to access antioxidant status among HIV-infected male volunteers in Latvia. Twenty six HIV-positive males (age 35.3 ± 2.5) whose serostatus are known were studied. They were recruited among two non-governmental HIV infected patients’ support organizations by “snow-ball” methodology using gatekeepers as contact persons. All participants in the research study were volunteers and their agreement to participate was get through their gatekeepers. The HIV-infected subjects represented a broad range of disease progression. None of the screened subjects had (CD4) T cell counts less than 200x10^9/L.

Exclusion criteria for the study groups were as follows: they were over 18 years old, have not used antioxidants as food supplement two months before the study, had no active opportunistic infections or malignancies, had readily mobile, and were no drug users. Any information of partner identifications was not used in the written information.

For the control group 10 uninfected males were selected among uninfected friends and relatives of HIV-infected individuals. Control subjects had no acute or chronic illness and were not taking any medications or nutritional supplements.

HIV-infected individuals used food supplements—antioxidant cocktail for 6 month, including 250 mg L-carnitine (Bio-Carnitine™), 800 μg vitamin A, 15 mg vitamin E, 90 mg vitamin C, 2 mg vitamin B₆, 15 mg Zn, 100 mg CoQ₁₀ and 75 μg selenium (organic) (Bio-Selenium™+Zn) a day. All subjects underwent an initial screening and after 6 months that included an anthropometric (weight and height) and biochemical (complete blood count, bilirubin, albumin, from liver panel-alanine aminotransferase (ALT) and alkaline aminotransferase (AST), from lipid profile total holesterol and triglicerides. All patients were evaluated with regard to the blood antioxidant system, specifically superoxide dismutase (SOD), catalase (CAT) selenium-dependent glutathione peroxidase (GSH-Px, trace element selenium, and α-tocopherol).

Participants will be involved in the study only after obtaining informed consent. The study protocol was approved by the ethics committee of the Latvian Institute of Cardiology for Clinical and Physiological Research, Drug and Pharmaceutics Product Clinical Investigation.

2.2. Laboratory analysis

After overnight fasting, venous blood samples were collected from all study subjects. Biochemical determinations were done at the hospital laboratory. CD4+ and CD8+ cell count was estimated by FACScan flowcytometry (BD Becton Dickimon). Alanine aminotranferase (ALT) and alkaline aminotranferase were estimated by kinetic reaction (Hitachi 917, Roche Diagnostics), bilirubin, albumin and total protein by two point colour reaction (Hitachi 917, Roche Diagnostics). From lipid profile total cholesterol and triglycerides were estimated by using fermentative colour reaction (Hitachi 917, Roche Diagnostics). Blood antioxidant system was evaluated at Riga Stradinš University laboratory. Selenium and α-Tocophrol concentrations
in plasma were measured using fluorometric method. Other measurements were also included such as catalase and GSHPX.

2.3. Statistical analyses

Using the SPSS 14.0 for Windows software standard version, statistical analyses were performed. All group data are expressed as means ± SEMs. The HIV-positive group was compared with the seronegative control subjects by using one way ANOVA. The minimal level of significance was identified at \( P < 0.05 \). All patients were evaluated with regard to the blood antioxidant system, specifically superoxide dismutase (SOD) and glutathione peroxidase.

3. Results

Subject characteristics, including CD4+ cell count, CD8+ cell count, and CD4/CD8 ratio, weight, body mass index (BMI, in kg/m\(^2\)), are shown in Table 1. At initial screening HIV-infected subjects were significantly lower weight and BMI compared to healthy subjects. There were insignificant changes between initial screening and after 6 months. The range of CD4+ cell counts was broad, from 282 to 1830 x 10\(^6\) cells/L at the initial screening and from 323 to 1687 after 6 months CD4/CD6 ratio was from 0.1 to 1.7 at the initial screening and from 0.1 to 3.4 after 6 months of using food supplements, CD8+ cell counts was from 272 to 4525 at the initial screening and from 222 to 4851 after 6 months.

|                  | Baseline          | After 6 months    |
|------------------|-------------------|-------------------|
|                  | n  | Mean±SE  | n  | Mean±SE  |
| Weight           |     |          |     |          |
| HIV-infected     | 26 | 73.1±2.1 | 13 | 74.7±2.8 |
| Healthy uninfected | 10 | 82.4±2.6 | 9  | 83.3±2.7 |
| p<0.05           |     |          |     |          |
| BMI              |     |          |     |          |
| HIV-infected     | 26 | 22.5±0.6 | 13 | 23.0±0.8 |
| Healthy          | 10 | 24.7±0.7 | 9  | 25.1±0.7 |
| p<0.05           |     |          |     |          |
| CD4+             |     |          |     |          |
| HIV-infected     | 25 | 698.4±74.3 | 21 | 743.6±87.4 |
| CD8+             |     |          |     |          |
| HIV-infected     | 25 | 1641.9±188.1 | 21 | 1341.0±216.1 |
| CD4+/CD8         |     |          |     |          |
| HIV-infected     | 25 | 0.6±0.1  | 21 | 0.9±0.2  |

**Table 1.** Effect of antioxidant supplementation on body weight, body mass index and immune function in HIV patients.

Antioxidant status is shown in Table 2. At the baseline HIV-positive patients had significantly lower GSHPX and CAT concentrations compare to healthy subjects (p<0.05), but did not differ after 6 months using food supplements. There were insignificant differences in SOD and MDA
measurements and α-Tocopherol concentration at the initial screening, but differed significantly after 6 months compared to HIV-infected and healthy individuals. Changes of biochemical measurements (alanine aminotransferase, alkaline aminotransferase, bilirubin, albumin and total protein, total cholesterol and triglycerides) were not significant between groups of HIV-infected and healthy individuals as well as at the initial screening and after 6 months after use of food supplements. Additionally, two HIV-infected individuals from 26 involved in the study reported that after the use of antioxidant cocktail, gingival bleeding was stopped. One reported this symptom for two years before the study and one reports half a year this problem before study.

| Measurements | groups | Baseline | After 6 months |
|--------------|--------|----------|----------------|
|              |        | n | Mean±SE | n | Mean±SE | p  |
| GSHPX        | infected | 25 | 46.5±1.1 | 19 | 45.6±1.3 | NS |
|              | healthy | 10 | 50.9±1.6 | 9 | 49.5±0.7 | NS |
|              | p       |     | 0.036 |     |        | NS  |
| CAT          | infected | 26 | 1023.5±164.1 | 19 | 350.3±22.3 | 0.01 |
|              | healthy | 10 | 317.4±34.6 | 9 | 377.7±13.1 | NS |
|              | p       |     | 0.013 |     |        | NS  |
| SOD          | infected | 25 | 1074±68.3 | 19 | 1419.3±56.6 | 0.001 |
|              | healthy | 10 | 1293.7±36.6 | 9 | 1172.8±30.4 | 0.023 |
|              | p       |     |        |     |        | 0.008 |
| TAS          | infected | 24 | 1.0±0.0 | 17 | 1.5±0.0 | 0.001 |
|              | healthy | 10 | 1.4±0.0 | 9 | 1.4±0.0 | NS  |
|              | p       |     |        |     |        | NS  |
| VITE         | infected | 26 | 10.8±0.8 | 19 | 13.2±1.2 | NS |
|              | healthy | 10 | 9.2±1.0 | 9 | 7.4±0.1 | NS  |
|              | p       |     |        |     |        | 0.003 |
| SE           | infected | 25 | 47.4 | 21 | 64.0±3.7 | 0.000 |
|              | healthy | 10 | 51±3.0 | 9 | 53.9±3.1 | NS  |
|              | p       |     |        |     |        | NS  |
| MDA          | infected | 24 | 3.5±0.1 | 17 | 5.1±0.3 | 0.000 |
|              | healthy | 10 | 3.4±0.2 | 9 | 3.4±0.2 | NS  |
|              | p       |     |        |     |        | 0.000 |

Table 2. Effect of antioxidants supplementation on antioxidant enzymes, selenium, vitamin E and lipid peroxidation in HIV patients.
4. Discussion

There has been increasing interest in the application of antioxidants to many diseases as information is constantly gathered linking the development of HIV to oxidative stress [51]. Antioxidants are believed to function interactively and synergistically to neutralize reactive oxygen species (ROS). Reactive oxygen species include molecules like hydrogen peroxide; ions like the hypochlorite ion; radicals like the hydroxyl radical; and the superoxide anion, which are an ion and a radical.

All subjects from the current study are at low serum selenium level (which is about 80 – 120 μg /L in Europe), and stayed as that even after use of antioxidants supplements, including selenium. Similar trend was also notice in the selenoenzyme glutathione peroxidase (GSHPx) level among HIV patients. Low serum selenium was defined as a serum level ≤ 85 μg/l [52]. Selenium deficiency, more than any other nutrient, has been documented to correlate with progression and mortality of HIV [53]. Selenium is needed for the proper functioning of the immune system, and appears to be a key nutrient in counteracting the development of virulence and inhibiting HIV progression to AIDS [54]. When taken as a supplement, selenium modulates the cellular response to oxidative stress, inducing a faster restoration of the endogenous antioxidative defence system against the production of reactive oxygen species [55]. Glutathion peroxidase controls the interacellular level of hydrogen peroxide, reducing the formation of reactive oxygen species that can induce lipid peroxidations with consequent damage to the cellular membranes [56].

![Figure 3. Selenium level of HIV infected individuals and healthy subjects (μg Hb)](image-url)
The loss of CD4 T lymphocytes is a central factor in the progression of HIV infection to AIDS. The key role of these cells in regulating and amplifying the immune response means that any decline in their number results in deficits in both humoral and cell-mediated immunity [57]. Both the CD4 percentage and the CD4:CD8 ratio are also affected by changes in the number of CD8 cells, which tends to rise through the course of HIV infection [58]. This study demonstrates that after supplementation, there is an increase in CD4 T cells in HIV patients. Several research studies have indicated that the apoptosis of CD4 cells contributing to HIV progression does not result solely from HIV infection, but largely from antioxidant imbalances in the host [43, 59, 60]. Activation of latent HIV state can be stimulated in the presence of reactive oxygen species (ROS) through the stimulation of oxygen-responsive transcription factors, specifically NF-kB, which induces HIV replication in the infected T-lymphocyte.

The mean serum malondialdehyde (MDA) concentrations in this study were significantly elevated in HIV infected patients. Serum concentration of total serum antioxidant status (TAS) was increased after supplementation. Our data indicate that severe oxidative stress occurs in the serum of HIV patients in comparison with controls ones and the inclusion of antioxidants in the therapeutic approach in managing HIV patients may prevent the additional free radicals damage. It has been shown that cells infected with the HIV undergo a significant amplification of $O_2^-$ generation. This phenomenon combined with a deficiency in key antioxidant enzymes (SOD and CAT) and a decreased concentration of the antioxidant vitamins, may lead to severe oxidative stress in HIV-infected patients. Consequently these conditions may in turn be responsible for the DNA base modifications observed in this study [43]. Serum MDA adducts also tended to correlate inversely with expression of CD127 on T cells was shown [61]. Study in placenta, umbilical cord blood and infant blood in HIV/ART-exposed infants compared with uninfected controls [62] showed that placental mitochondria malondialdehyde (MDA) concentrations and mtDNA content in placenta and cord blood were similar between groups. Supplementation with antioxidant vitamins prevents oxidative modification of DNA in lymphocytes of HIV-infected patients [44]. Furthermore, as an antioxidant, vitamin E [63] and atherosclerotic lesions contain oxidized lipids [64], therefore, supplementation with vitamin E would decrease heart disease risk. It has been reported that α-tocopherol concentrations in serum are regulated by the α-tocopherol transfer protein and are dependent on serum lipid concentrations because α-tocopherol is non-specifically transported by lipoproteins [63]. Serum micronutrient concentrations along with surrogate markers of atherosclerosis in a cohort of HIV infected adults were studied [65](Falcone et al. 2010), the highest tertile of serum vitamin E concentration was associated with higher common and internal carotid intima-media thickness (c-IMT) and coronary artery calcium (CAC) scores [65]. The authors concluded that elevated serum vitamin E values may increase the risk of cardiovascular complications in HIV-infected adults [65].

There appears to be a possible role for antioxidants supplements. However, these supplements cannot effectively eliminate HIV signs and symptoms and the optimal dosage of these nutrients is (whether used alone or in combination) has to defined
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