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Influence of HIV infection and the use of antiretroviral therapy on selenium and selenomethionine concentrations and antioxidant protection

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ABSTRACT

Objective: The aim of the present study was to evaluate whether HIV infection and antiretroviral therapy (ART) use are associated with oxidative stress, concentrations of selenium and selenomethionine, and antioxidant protection.
Methods: Individuals were classified as HIV negatives: control group (CG; n = 40); HIV positives: group 1 (G1; taking ART for >5 y, n = 40) and group 2 (G2; taking ART for <5 y, n = 40). Plasma and erythrocyte selenium, selenomethionine, glutathione (GSH), glutathione peroxidase activity (GPX), and malondialdehyde (MDA) were evaluated.
Results: Selenium deficiency (plasma selenium 45 μg/L) was not observed in any of the participants, and plasma selenium in CG (69.4 μg/L) was lower than in G1 and G2 (88.4 and 72.5 μg/L, respectively). G1 and G2 showed higher concentrations of MDA and GPX and lower concentration of GSH than CG. Multiple linear regression analysis indicated an association of MDA, GPX, and GSH with HIV status. CG participants showed higher concentrations of selenomethionine than G1 and G2 individuals and we observed a significant negative correlation between the concentration of selenomethionine and the use of ART.
Conclusions: Prolonged ART use seems to increase the selenium in plasma, but influences the reduction of selenomethionine. HIV infection was associated with increased oxidative stress and appears to affect in protective activity of GPX. Finally, more studies are required to further address the importance of selenium and selenometabolites in the pathogenesis of infection and metabolism of HIV-positive individuals in prolonged use of ART.

Introduction

Oxidative stress has been widely documented in patients infected by HIV; once viral infections promote prolonged activation of the immune system contributed to the increase of the production of reactive oxygen species [1–3]. Several studies linked the use of antiretroviral therapy (ART) as a potential contributor to increase the reactive oxygen species (ROS) [4]. Furthermore, the use of ART has been associated with adverse effects that are related to important metabolic changes [5,6].

The level of oxidative damage in HIV-infected individuals can be influenced by both the extent of oxidative stress and the

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activity of antioxidant defenses [7]. Increase in oxidative stress may accelerate disease progression, increasing HIV replication through signaling of nuclear factor (NF)-κB as well as the mutation rate of the viral RNA genome, leading to greater damage to the host [2,3]. On the other hand, balance in the activity of antioxidant defenses, originated or not from diet and antioxidant enzymes, protect against the oxidative stress and may delay progression of the HIV disease [2,7].

Selenium is an essential micronutrient that has been widely associated with an important role in HIV infection, mainly due to its involvement in regulating oxidative stress, intimately linked with the redox state of the cell and the redox regulation of genes, which are important for multiple immune functions [1,8]. Moreover, by incorporating selenoproteins, particularly glutathione peroxidase (GPX) and thioredoxin reductase, selenium has been shown to be a potent regulator in both the NF-κB activity and transcription of HIV [1,8].

The beneficial effects of selenium to human health are strongly dependent on their chemical form and concentration [9]. Therefore, it highlights the importance of chemical speciation in identifying and quantifying a number of metabolites that contains selenium [10].

The characterization of this metabolism and verification of the individual's health status can help in establishing new therapeutic strategies. However, currently studies that evaluate the selenium in HIV patients who have been using ART for a long time are limited. Additionally, no data is available related to the selenium metabolites and HIV infection. Thus, the present study aimed to evaluate whether HIV infection and use of ART are associated with oxidative damage, concentrations of selenium and selenomethionine, and antioxidant protection of glutathione (GSH) and GPX.

Methods

Study population

This was a prospective, observational, cross-sectional study conducted at the Clinics Hospital of Ribeirão Preto Medical School, University of São Paulo (HC/FMRP-USP). The study was approved by the Research Ethics Committee of the institution (process 551.355) and all participants signed the informed consent.

Recruitment of the study group patients was in the Special Treatment Unit in Infectious Diseases in HC/FMRP-USP. The control group was made up of staff and students in the different areas of the HC/FMRP-USP.

We evaluated 120 adults of both sexes. Of this total, 80 HIV-positive individuals using ART made up the study group and were divided into group 1 (G1), composed of 40 participants taking ART for >5 y and group 2 (G2), composed of 40 participants taking ART for <5 y. The arrangement of HIV-positive individuals in G1 and G2 according to the ART usage time was based on a prior analysis of the profile of patients attending in UETDI, in which the average of ART usage time was about 5 y. The control group (CG) was composed of 40 healthy HIV-negative individuals.

The exclusion criteria for participation in the project were drug users, the use of a supplement containing selenium in its composition, adjacent morbidities, thyroid disease, malabsorption syndrome, diabetes mellitus, renal failure, and chronic inflammatory diseases such as rheumatic and autoimmune diseases.

Anthropometric measurements

Body weight (kg) was measured on an electronic scale accurate to 0.1 kg and height in stadiometer accurate to 0.1 cm. Body mass index (BMI; in kg/m^2) was calculated for each participant.

Evaluation of selenium dietary intake

Selenium dietary intake was assessed by food record consecutively over 3 d. The information was processed in nutritional analysis program Dietpro®, 5 i version. For recommendation of selenium consumption, we considered Dietary Reference Intakes (DRIs) [11].

Quantification of viral load and CD4 + lymphocytes count

The quantification of viral load was determined by the Abbott Real Time method and was considered undetectable when the values were below 50 copies/mL [12].

The count of CD4+ lymphocytes was determined by the flow cytometry method using the kit Multitest® and Calibur® cytometer FACS (Becton Dickinson, San Jose, CA, USA) and the classification criteria for HIV/AIDS from the Centers for Disease Control and Prevention [13] were used.

Biochemical evaluation

The collection of blood samples was performed in the Clinical Research Unit (UPC) of HC/FMRP-USP after 8 h of fasting and the separation of whole blood to obtain serum, plasma, and erythrocytes occurred immediately after collection. The samples were stored at –80° until the time of analysis.

Evaluation of oxidative damage: Malondialdehyde

According to a previous methodology, to obtain the dosage of malondialdehyde (MDA), 100 μL plasma sample was used [14]. To this was added 300 μL of 10 mM solution of 1-methyl-phenylindole in acetonitrile and methanol (2:1, v/v) and 75 μL of HCl pure (37%). The Eppendorf tubes were then vortexed and incubated in a water bath at 45°C for 40 min. After the bath, the samples were cooled in ice and then the Eppendorf tubes were centrifuged at 4000*g* for 10 min. The supernatant was read for absorbance at a wavelength of 586 nm. The MDA concentration was calculated by comparing it to a curve 1,1,3,3-tetramethoxypropane (TMP) hydrolyzate.

Reduced glutathione

GSH activity was determined using a previously adapted method that proposed that 25 μL of plasma be used. One mL of Tris-EDTA, and 25 μL of DTNB concentration of sulfhydryl groups were calculated using a standard curve of GSH [15].

Glutathione peroxidase activity

GPX activity was measured using a previously adapted method in erythrocytes [16]. The method was based on the reaction in which GPX catalyzes the oxidation of GSH by a hydroperoxide. In the presence of GPX nicotinamide adenine dinucleotide phosphate (NADPH), oxidized GSH is converted to the reduced form with a concomitant oxidation of NADPH+. The decrease in absorbance at 340 nm was then measured.

Selenium

The determination of the total concentration of selenium in plasma and erythrocytes was performed according to a previously described method, by an inductively coupled plasma mass spectrometry (ICP-MS), fitted with a dynamic reaction cell (Perkin Elmer Sciex, Norwalk, CT, USA) [17]. Samples were diluted in the ratio 1:50 with a solution containing Triton X-100 0.01% (v/v), HNO_3 0.05% (v/v) and 10 mg/L-1 rhodium (Rh) as an internal standard. The concentration of the analytical calibration standards ranged from 0 to 50 μg/L.

Selenium speciation: Selenomethionine

The chemical speciation of selenium for identification of metabolite selenomethionine was performed in plasma of 15 individuals, 5 in each group, according to the previously adapted method [18]. The standards of seleno amino acids: seleno-L-methionine (S-3132), Se-(methyl)-selenocysteine (M-6680), and Protease XIV (P5147) were obtained from Sigma-Aldrich (St. Louis, MO, USA). For the preparation of plasma samples, 20 mg of protease XIV was added to a 900 μL sample and then vortexed. This mixture was incubated at room temperature for 24 h. After incubation, 100 μL of HNO_3 (1 N) was added to the mixture, vortexed, then centrifuged at 3500*g* for 10 min. The supernatant fraction was then filtered using a 0.25-μm filter. The sample was diluted five times and after dilution, 100 μL was injected into the high-performance liquid chromatography ICP-MS for selenomethionine speciation using metilselenocysteine as an internal standard.

For the chemical speciation of selenium, we used a pump LC Perkin Elmer Model G-200, a six-port injector (Rheodyne 9725) and a reverse-phase column (C8, 3 μM, 4.6 mm × 33 mm, Column Brownlee, PerkinElmer, Boston, MA, USA) constitute of the liquid chromatography system. The output from the chromatographic column was coupled to ICP-MS nebulizer. The instrumental conditions were Radio frequency power: 1400 W; plasma flow rate: 15 L/min; nebulizer flow rate: 1.25 L/min; standard resolution; scan mode: time resolved acquisition; residence time: 500 ms; and monitored isotope: mass 82. The mobile phase was composed of water-methanol (97: 3, v/v) and the flow rate was 1 mL/min.

Statistical analysis

Categorical variables

For categorical variables we applied the χ^2 exact test and the results were presented as frequencies and percent.

Comparison between groups

Comparison of plasma and erythrocyte selenium, GSH concentration, GPX, and MDA among CG, G1, and G2 groups was conducted by analysis of variance

(ANOVA) with Tukey as post hoc test. For selenomethionine, comparison between the same groups was performed using the Kruskal–Wallis method with Dunn as post hoc test. The statistical software was SPSS version 22.0. Results were expressed as a mean and SD. For all analyses $P \leq 0.05$ was considered significant.

Regression analysis

Previously, we calculated the correlation coefficients between the variables of interest to verify the existence of linear relationships. For this analysis, we used the Pearson correlation coefficient, except for the selenomethionine variable in which we used the Spearman correlation coefficient.

The variables were divided into six groups: personal characteristics (age, sex and BMI), HIV status (positive or negative), selenium intake, antioxidants (plasma selenium, GSH, and GPX), oxidative stress (MDA), and selenium speciation (selenomethionine). The erythrocyte selenium was only used as a variable in the analysis of GPX.

Regression analysis aimed to identify predictors for antioxidant variables and oxidative stress. First, we used HIV status (HIV-positive [G1 and G2] and HIV-negative [CG]; n = 120), personal characteristics, and selenium intake as predictors for the antioxidant variables in a multiple-linear regression analysis. These same variables, including antioxidants, were used as predictors for oxidative stress. Second, we performed multiple-linear regression keeping the same predictors but using only HIV-positive individuals (G1 and G2; n = 80), to determine the influence of antiretroviral therapy (ART) in all the response variables.

Results

The baseline characteristics of the participants are displayed in Table 1. The average age of study participants was 40.7 ± 11.3 y. The sample consisted of 59 men (49.2%) and 61 women (50.8%). Regarding the anthropometric measurements performed using BMI, participants were classified as eutrophic (18.5–24.9 kg/m^2) and overweight (25–29.9 kg/m^2). All groups presented adequate selenium intake in accordance with the recommendation of 45 μg/d from the DRIs [12]. Selenium-containing foods most frequently consumed among the groups were Brazil nuts and whole grains (consumption observed only in CG), chicken egg, bread, red meat, chicken, white rice, pinto beans, cow's milk, and pasta.

The mean exposure time to HIV in G1 and G2 was 161 and 87.8 mo, respectively. All HIV-positive patients were on stable ART for an average time of 147.2 mo in G1 and 33 mo in G2. The CD4 T-cell count in HIV patients was >200 cells/mm^3 and HIV-1 RNA level <1000 copies/mL. These parameters indicate that HIV-positive individuals in the present study were immunologically stable and were not considered patients in advanced stages of the disease. Additionally, patients had no significant adjacent morbidities.

Table 1
Age, sex, anthropometric and clinical characteristics of the participants according to the study group

Variable	HIV-negative (CG)	HIV taking ART >5 y (G1)	HIV taking ART <5 y (G2)
n	40	40	40
Age, y	30.8^a ± 7.4	47.2^b ± 7.1	44.1^b ± 11.2
Male, n (%)	10^a (25)	23^b (57.5)	26^b (65)
Female, n (%)	30^a (75)	17^b (42.5)	14^b (35)
BMI, kg/m^2	24.4 ± 4.4	26.1 ± 4.8	24.8 ± 4.1
Selenium intake, μg	78.3^a ± 82.9	45.6^b ± 23.4	47.7^b ± 35.5
Exposure time to HIV, mo	–	161^a ± 46.5	87.8^b ± 89.9
Duration of ART use, mo	–	147.2^a ± 39.2	33^b ± 19.3
Count CD4 cells, cell/mm^3	–	693.9^a ± 297.5	416^b ± 272.1
Viral load <50 copies/mm^3, n (%)	–	38 (95)	36 (90)

ART, antiretroviral therapy; BMI, body mass index; CG, control group; G1, group 1; G2, group 2
Analysis of variance. Values are expressed as mean ± SD. For the variables of sex, male and female, and viral load, values are expressed in percentage and χ^2 test was used. Different superscript letters (a, b) indicate significant differences with $P < 0.05$

Table 2
Selenium concentration in plasma and erythrocyte, GPX, GSH, and MDA of the participants according to the study group

Variable	HIV-negative (GC)	HIV-positive ART >5 y (G1)	HIV-positive ART <5 y (G2)
n	40	40	40
Plasma selenium, μg/L	69.4^a ± 26.1	88.4^b ± 37.4	72.5^a ± 24.3
Erythrocyte selenium, μg/L	105.1 ± 40.1	102.1 ± 34.5	91.5 ± 27.4
GSH, nM/mL	2.5^a ± 0.2	2.0^b ± 0.3	2.1^b ± 0.6
MDA, nmol/mL	4.2^a ± 1.4	7.6^b ± 2.6	8.5^b ± 2.2
GPX, U/mg Hg	87.0^a ± 19.9	115.1^b ± 29.5	111.5^b ± 41.8

ART, antiretroviral therapy; CG, control group; G1, group 1; G2, group 2; GPX, glutathione peroxidase; GSH, glutathione; MDA, malondialdehyde.
Analysis of variance. Values are expressed as mean ± SD. Different superscript letters (a, b) indicate significant differences between the means with $P < 0.05$

The comparison of plasma and erythrocyte selenium, GSH, MDA, and GPX between study groups is displayed in Table 2. The concentrations of plasma and erythrocyte selenium were adequate in all groups. Plasma selenium was higher in HIV-positive individuals who used ART for a longer time (G1), whereas erythrocyte selenium did not change between groups. In HIV-positive groups (G1 and G2), higher oxidative stress was indicated by lower concentrations of GSH and higher concentrations of MDA when compared with HIV-negative individuals. Interestingly, GPX was significantly higher in HIV-positive individuals (G1 and G2) than in HIV negatives (CG).

Multiple-linear regression analysis was performed with all study participants (CG, G1, G2) and indicated that the variables HIV status, personal characteristics and selenium intake were not associated with plasma selenium (data not shown). For GSH, just the variable HIV status was identified as significant predictor, indicating an association between HIV-positive individuals and lower concentrations of GSH. For GPX, the variables HIV status and erythrocyte selenium were identified as significant predictors, indicating that GPX concentration is higher in HIV-positive individuals and directly proportional to erythrocyte selenium (Table 3). HIV status also was identified as a significant predictor for MDA, indicating an association between HIV-positive individuals and higher concentrations of MDA (Table 3).

Multiple-linear regression analysis with HIV-positive patients (G1 and G2), including the use of ART as a predictor variable, did not indicate it as a predictor of any antioxidant or oxidative variables.

Table 3
Multiple linear regression analysis for antioxidant (GPX and GSH) and oxidative stress (MDA) with all study participants (N = 120)*

	Coefficient	SE	*P*-value
GPX			
HIV status	29.543	8.213	<0.001
Erythrocyte selenium	0.291	0.090	0.002
GSH			
HIV status	−0.432	0.130	0.001
MDA			
HIV status	4.131	0.568	<0.001

GPX, glutathione peroxidase; GSH, glutathione; MDA, malondialdehyde
HIV status, personal characteristics, and selenium intake were used as predictor variables in the analysis for antioxidant variables (GPX and GSH). These same variables, including antioxidant ones, were used as predictors for oxidative stress (MDA)
* Only predictors with significant coefficients are presented for each response variable.

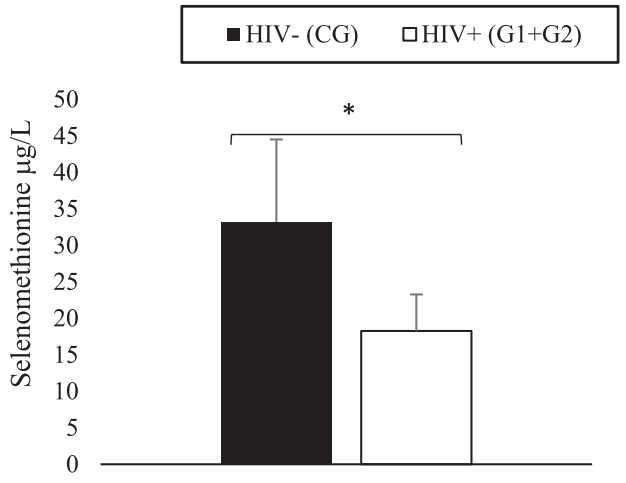

Fig. 1. Identification of selenomethionine via speciation of selenium. Kruskal–Wallis test. Values are expressed as mean + SD. The concentration of selenomethionine was significantly higher in individuals in HIV-negative (CG) when compared with HIV-positive individuals (G1 and G2). *$P = 0.01$. CG, control group; G1, group 1; G2, group 2.

The concentration of selenomethionine (Fig. 1) was significantly higher in HIV-negative individuals (CG = 33.2 µg/L) compared with HIV-positive groups (G1 = 20.6 µg/L and G2 = 16.0 µg/L; $P = 0.015$). A negative correlation also was observed between the concentration of selenomethionine and the use of ART ($R = -0.52$; $P = 0.048$).

Discussion

In the present study, individuals did not present plasma selenium deficiency, and patients in the HIV-positive group who used ART for a longer time showed higher plasma selenium concentrations compared with other groups. Moreover, a positive correlation between the use of ART and plasma selenium concentration was observed ($R = 0.41$; $P < 0.001$; data not shown). Recent studies corroborate the results obtained in the present study, demonstrating the absence of selenium deficiency in HIV-positive individuals, not only related to the use of ART but also the prolonged exposure has been associated with adequate concentrations of selenium in plasma [7,19,20].

Plasma selenium is not a single entity. It has several components that currently are defined as two selenoproteins (selenoprotein P and the extracellular GPX3) that specifically contain selenocysteine; selenium incorporated nonspecifically as selenomethionine in lieu of methionine in albumin and other proteins; and a small amount of non-protein bound Se [21]. In a non–selenium-deficient cohort, as in the present study, it is to be expected that the two selenoproteins in the plasma are maximally expressed, leaving differences in total plasma selenium concentrations to changes in protein-bound selenomethionine as albumin and, perhaps, other plasma proteins [20]. In accordance with a previous study, we hypothesized that the use of antiretroviral resulted in increased turnover of proteins in the cells, which increased the availability of selenomethionine from tissue protein for incorporation into plasma proteins during their synthesis [20].

GSH and MDA were associated with HIV status. In HIV-positive individuals, higher oxidative stress was indicated by lower concentrations of GSH and higher concentrations of MDA in comparison with HIV-negative individuals. These results are consistent with studies in the literature showing that in HIV-positive individuals GSH deficiency is common and could be associated with a decrease in levels of GSH-synthetic enzymes [22,23] and the concentrations of MDA could be a reliable marker for oxidative stress in HIV infection [7,24,25].

Interestingly, GPX activity was associated with HIV status in the present study. GPX activity was significantly higher in HIV-positive individuals than in HIV negatives as reported in previous studies [7,26,27]. A possible explanation for this result may be that GPX1 has been considered one of the major antioxidant enzymes, and together with catalases and peroxiredoxins, it has been implicated in protection of cells from oxidative damage by degrading toxic H_2O_2. Despite this, GPX1 appears to play a protective role under conditions of oxidative stress, but it is dispensable under unstressed conditions, consistent with its low position in the hierarchy of selenoprotein expression [28]. Additionally, the HIV infection promotes the increase in erythropoiesis during oxidative stress, which in turn increases levels of expression of the GPX1, the predominant form of the enzyme in erythrocytes [24]. Moreover, studies have shown that both GPX and thioredoxin reductase are potent regulators of NF-κB, which is the first cell factor involved in transcription regulation by HIV, but in different functions. It is reasonable, therefore, that HIV could have evolved to participate directly in regulatory processes, by encoding their own selenoproteins [1].

To our knowledge, this is the first study with data for speciation of selenomethionine in HIV-positive individuals. Although numerous studies have tested the total serum selenium levels, there are no published data measuring the serum concentrations of selenomethionine. Quantification and speciation of selenium can be of great value to identify how much and what forms of selenium act as chemoprotectors in HIV infection and other diseases.

In the present study, the selenomethionine concentrations were higher in HIV-negative individuals compared with HIV-positive patients. A possible explanation for this result is based on the chemical form of ingested selenium. HIV-negative individuals had a larger and more frequent consumption of Brazil nuts and whole grains, rich in selenomethionine, in which the bioavailability is higher, compared with HIV-positive individuals. Interestingly, selenomethionine concentrations were negatively associated with length of use of ART, indicating that the prolonged use of ART by HIV-positive individuals in the present study could justify the lower selenomethionine concentrations compared with HIV-negative individuals. It is worth emphasizing that the data for analysis of selenomethionine in this study are based on a small sample.

Conclusion

Prolonged use of ART seems to increase selenium concentration in plasma, but it influences the reduction of selenomethionine. Interestingly, the concentration of selenomethionine can be related to the chemical form of selenium intake. HIV infection was associated with increased oxidative stress and appears to affect GPX activity, suggesting a protective antioxidant action in this condition. This elevation also suggests that underlying selenium status was sufficient to allow for this apparent increase. Finally, more studies are required to further address the role of selenium and selenometabolites as important players in the infection pathogenesis and metabolism of HIV-positive individuals in prolonged use of ART.

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