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Serum selenium, plasma glutathione (GSH) and erythrocyte glutathione peroxidase (GSH-Px)-levels in asymptomatic versus symptomatic human immunodeficiency virus-1 (HIV-1)-infection

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Objectives: Antioxidant defense status was investigated in HIV-infected patients by measuring serum selenium, erythrocyte glutathione peroxidase (GSH-Px) activity, plasma thiol (-SH) and glutathione (GSH) concentrations along with the assessment of the clinical stage and surrogate markers of HIV-disease.
Design, setting and subjects: Serum selenium levels were determined cross-sectionally in 104 sequentially selected HIV-infected patients (83 outpatients and 21 patients with ongoing AIDS defining events). The patients were classified into three stages of the disease, I, II and III according to the 1993 Centers For Disease Control (CDC) classification system for HIV-infection. GSH-Px activities, plasma SH and plasma GSH concentrations were determined in a subset of 24 patients at stage I and 12 patients at stage III with an active AIDS-defining disease.
Results: Mean serum selenium levels were lower in CDC stage II (68.7 ± 20.9 µg/l; $P < 0.01$; $n = 34$) and stage III (51.4 ± 14.7 µg/l; $P < 0.01$; $n = 37$) HIV-infected patients than in healthy subjects (89.2 ± 20.9 µg/l; $n = 72$) and stage I patients (82.3 ± 20.5; µg/l; $n = 33$). Serum selenium levels were positively correlated with CD4-count ($r = 0.42$; $P < 0.001$; $n = 104$) and inversely with levels of soluble tumor necrosis factor receptors type II ($r = -0.58$; $P < 0.01$; $n = 35$), neopterin ($r = -0.5$; $P < 0.001$; $n = 80$) and β2-microglobulin ($r = -0.4$; $P < 0.001$; $n = 94$). Hepatitis C virus (HCV) and HIV-coinfected patients at CDC stages I and II showed markedly lower selenium concentrations compared to HIV-infected patients without concomitant HCV-infection. Serum selenium and GSH-Px activity in hospitalized AIDS patients was significantly lower as compared to asymptomatic patients and healthy subjects, whereas plasma SH and GSH concentrations were lower in both, asymptomatic -and AIDS-patients, than in the controls.
Conclusion: The results show that stages I–III of HIV-disease are characterized by significant impairments of antioxidative defenses provided by selenium, GSH-Px, SH-groups and GSH.
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Descriptors: serum selenium; glutathione peroxidase; plasma thiols; glutathione; HIV-infection; AIDS.

Introduction

Evidence presented so far suggests that an imbalance between diminished host antioxidant defenses and increased formation of oxygen radicals and proinflammatory cytokines create a state of 'oxidative stress' in HIV disease (Halliwell and Cross, 1991; Matsuyama *et al*, 1991; Müller, 1992; Revillard, 1992; Greenspan, 1994; Matsumoto *et al*, 1993; Favier *et al*, 1994; Pace and Leaf, 1995). At the same time malnutrition frequently leads to a multilevel antioxidant deficiency which further heightens oxygen radical formation.

The essential trace element, selenium, acts as an integral constituent of the antioxidative enzyme glutathione peroxidase (GSH-Px, EC 1.11.1.9), which detoxifies hydrogen peroxide (H_2O_2) and organic lipid peroxides at the expense of reduced glutathione (GSH). The oxidized form, glutathionedisulfide (GSSG), in turn regenerated by the glutathione reductase which uses NADPH as redox equivalent provided by the pentose phosphate shuttle. Oxygen radicals, H_2O_2) and proinflammatory cytokines, such as tumor necrosis factor alpha (TNF-α), play a crucial role in HIV disease by directing intracellular signalling processes towards increased proviral transcription via activation of the cytoplasmic nuclear transcription factor kappa B (NFκB); furthermore TNF-α itself is a strong activator of NFκB (Schreck *et al*, 1992). Consequently, the accumulation of oxygen radicals and proinflammatory cytokines in HIV-infected cells might lead to a steady increase of viral load. On the other hand, antioxidant thiol compounds such as the endogenous tripeptide GSH (Ho and Douglas, 1992; Staal *et al*, 1993) and the cysteine prodrug N-acetylcysteine (NAC) have been shown to suppress the NFκB-mediated proviral transcription in vitro. Notably, plasma GSH and SH-groups were found to be decreased in the early stage of HIV-infection (Buhl *et al*, 1989; Eck *et al*, 1989). In addition to GSH, adequate concentrations of selenium are essential for optimal functioning of the GSH-Px-system in order to maintain a reductive cytoplasmic milieu in HIV-

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infected cells. This might aid the suppression of viral replication. Indeed, studies have supported a link between low selenium concentrations, low GSH-Px-activity and increased NFκB activation and viral transcription (Christensen and Pursey, 1994; Sandstrom *et al*, 1994; Sappey *et al*, 1994). In addition to its function as an antioxidant, immunemodulating properties, have been attributed to selenium (Petrie HT *et al*, 1989; Perez *et al*, 1991; Stabel and Spears, 1993; Kiremdjian-Schumacher *et al*, 1994; Roy *et al*, 1994). The HIV-gene product, Tat (trans-acting transcriptional activator) in addition to being essential for virus replication, down regulates cellular manganese superoxide dismutase (MnSOD), consequently resulting in an increase in superoxide radical concentration (Flores, 1993; Westendorp, 1995; Ehret *et al*, 1996).

To date only limited data exists on plasma concentrations of antioxidants with respect to surrogate markers of HIV-disease and proinflammatory cytokines (Coodley *et al*, 1993; Delmas-Beauvieus *et al*, 1996). In this study we determined selenium and SH-dependent antioxidants in a broad spectrum of HIV-infected patients and in healthy controls by measuring serum selenium, plasma thiol (-SH) and GSH concentrations and erythrocyte glutathione peroxidase (GSH-Px) activity. At the same time surrogate markers of HIV-disease, such as the $CD4^+$ count, neopterin, β2-microglobulin (Fahey *et al*, 1990) and the soluble tumor necrosis factor receptor 75 (sTNFR 75) (Godfried *et al*, 1993, 1994) were assessed to evaluate disease status.

Patients and methods

Patients

One hundred and four unselected HIV-infected patients, sequentially presenting to the clinic's immunological outpatient department constituted the study group. Serum selenium concentrations were determined in all patients. Twenty-one patients with acute opportunistic infections (OI) and/or HIV-associated tumors required hospitalization. All patients were classified according to the Centers for Disease Control (CDC)′93 classification system for HIV-infection into groups I (asymptomatic), II (symptomatic, non AIDS), and III (AIDS), (Centers for Disease Control, 1993). Characteristics of the patients are given in Table 1. None of the subjects had received selenium supplementation for at least one year. HIV-infected patients received standard antiviral therapy and prophylaxes appropriate to their clinical and CD4 status. Plasma-SH groups, GSH levels and GSH-Px activity in erythrocyte hemolystate were determined in a subset of the patients (26 CDC I outpatients, 12 hospitalized CDC III patients with ongoing OI) if fresh samples were available. Adequate number of fresh samples from CDC II patients were not available and were therefore not investigated.

Table 1 Patients characteristics according to the CDC′93 classification

Risk factors	*Patient group (n)/sex/age*		
	CDC I (33) (23 m; 10 f) 34 (20–69)	*CDC II (34) (26 m; 8 f) 35 (26–55)*	*CDC III (37) (31 m; 6 f) 33 (25–56)*
Drug addicts	4	7	4
Homosexual/bisexual	8	11	23
Hemophiliac	4	7	8
Heterosexual, others	17	9	2

m = male; f = female.

Healthy controls

Seventy-two randomly selected age-matched blood donors from the Bonn area served as a control group. These inidividuals showed no serological evidence for HIV and/or HCV-infection and no abnormal laboratory findings (blood count, serum transaminases and creatinine were within the normal range). Serum selenium was determined in all control subjects; plasma SH, GSH and GSH-Px-activity in erythrocyte hemolysate were determined in a randomly selected subset of 25 healthy controls.

Informed consent was obtained from each patient before blood was drawn. The study was approved by the local ethics committee and conformed the guidelines of the 1975 Declaration of Helsinki.

Determination of serum selenium

Selenium was determined by atomic absorption spectrometry (AAS) using a 1100 Perkin Elmer atomic absorption spectrometer with a mercury hydride system (Welz *et al*, 1983).

Preparation of plasma and washed erythrocyte hemolysate

Blood from healthy subjects and HIV-infected patients was collected in 10 ml monovettes containing 1.6 mg/ml of EDTA (Sarstaedt, Germany). Plasma was obtained by centrifugation of the monovettes at 5000 g for 10 min at 4°C. Aliquots of 1.0 ml were pipetted into Eppendorf tubes, tightly closed with the lid, dropped into liquid nitrogen and stored at −80°C till ready for use. The erythrocyte pellet was washed 3 times with isotonic NaCl and hemolyzed by the addition of 9 ml of Milli Q-water; after thoroughly mixing by tilting the contents several times the tubes were stored at −80°C till further use.

Determination of GSH-Px in erythrocyte hemolysate

The method was essentially that described by Wendel in 1981, however, we introduced slight modifications. Haemoglobin concentration in the erythrocyte hemolysate was determined by mixing 100 µl of the hemolysate and 1.0 ml of Drabkin's reagent (KCN, 1.54 mM, was dissolved in 1 mM potassium dihydrogen phosphate and $K_3[Fe(CN)_6]$, 1.2 mM, was dissolved in 0.25 mM potassium dihydrogen phosphate). The two solutions were mixed 1:1 ;v/v just before use, since premixing the two components leads to an unstable mixture. Absorption was read at 546 nm in a Perkin-Elmer 550S spectrophotometer. The blank consisted of water instead of the hemolysate. The concentration of haemoglobin was calculated by the formula $A_{546} \times 12.47 = mg/(Hb \times ml)$ and adjusted to a concentration of 3 mg Hb/ml with water. The reaction mixture for GSH-Px assay consisted of potassium phosphate, 250 mM, glutathione disulfide (GSSG)-reductase 12 units, NADPH, 10 mM, and 100 mM GSH solution in water. Enzyme assay was carried out by pipetting 75 µl of the reaction mixture and 100 µl of the hemolysates or water (serving as blank) in 96 wells microtest plates (Greiner, Nuertingen). After 5 min at 23°C, the enzymatic reaction was started by adding 50 µl of a solution of tert-buryl hydroperoxide (stock solution of 12.5 mM diluted 1:10) and the extinction of the sample was registered at 340 nm every minute for a period of 10 min in an Rainbow SLT microplate reader (SLT Instruments, Crailsheim). The decrease in extinction was linear from 1–7 min. The GSH-Px activity was read from a standard curve using different activities of GSH-Px, obtained from Sigma, Munich, from 10–150 U/l. Enzyme activity determinations were carried out in triplicate.

Table 2 Surrogate markers of HIV disease in CDC′93 I–III and correlation with the corresponding serum selenium concentrations

Parameter	*n*	*Healthy controls*	*n*	*CDC I*	*n*	*CDC II*	*n*	*CDC III*	$\sum n$	*r vs selenium*
CD4-cells (/μl)	(20)	902 ± 334	(34)	533 ± 205	(33)	231 ± 148	(37)	50 ± 94	(104)	0.42^{***}
β-Microglobulin (μg/ml)	(20)	1.9 ± 0.8	(31)	2.8 ± 1.1	(32)	3.2 ± 1	(33)	4.4^{*} ± 1.9	(96)	-0.4^{***}
Neopterin (μg/l)	(20)	1.5 ± 0.4	(26)	3 ± 1.7	(32)	3.9 ± 2.2	(26)	7.8^{*} ± 3.4	(84)	-0.51^{***}
sTNFR II (μg/ml)	(15)	4 ± 0.7	(15)	6.8 ± 2.1	(10)	6.8 ± 3.1	(10)	11.3^{*} ± 6.6	(35)	-0.58^{**}

P value for t-test: $^{*} < 0.01$ *vs* CDC I.
P value for linear regression analysis: $^{**} < 0.01$; $^{***} < 0.001$.
Linear regression analyses did not comprise the group of healthy controls which is a randomly selected subset of the 72 selenium controls.

Determination of thiols in plasma
The plasma samples were thawed at room temperature (22°C); 50 μl of plasma was rapidly added to 850 μl of Tris-HCl buffer (400 mM), pH 8.2 followed by the addition of 100 μl of 2.5 mM DTNB [5.5 dithio-bis(2-nitrobenzoic acid)] obtained from Sigma, Munic. DTNB was dissolved in 400 mM Tris-HCl buffer, pH 8.2, containing 0.5 mM EDTA. The Tris buffer was gassed with N_2 for 15 min before use. After 10 min the extinction of the mixture was read in the Rainbow SLT microplate reader using the 412 nm filter. The extinction of the two blanks, plasma alone and DTNB alone, were subtracted from the extinctions of the test probes. The concentration of thiols in the samples were read from a standard curve using different concentrations of GSH (50–1000 μM). The extinction of plasma samples and blanks were determined in triplicate.

Determination of GSH in plasma (Tietze, 1969)
One hundred μl of freshly thawed plasma was pipetted into Eppendorf tubes containing 200 μl of a 10% solution of TCA (tricholoroacetic acid), vortexed and centrifuged at 4000 × g for 10 min at 10°C. To 200 μl of the supernatant 700 μl of 400 mM Tris-HCl buffer, pH 8.9, was added followed by the addition of 100 μl of 2.5 mM DTNB dissolved in 40 mM Tris-HCl buffer pH 8.9. After 10 min at room temperature the extinction of the samples was measured at 412 nm in a Perkin-Elmer 550S spectrophotometer. Blank consisted of DTNB instead of plasma; its extinction was subtracted from the test sample extinction before matching it with the standard curve described above.

Plasma proteins were determined according to the method of Lowry *et al*, 1951 with bovine serum albumin as reference protein.

T-cell subsets were determined on a Becton/Dickinson-FACScan-flowcytometer using Simultest IMK-lymphocyte antibodies [CD4, CD8, CD3] from Becton/Dickinson, Heidelberg, Germany. The absolute numbers of $CD4^{+}$ cells were calculated on the basis of automated white blood-cell counts using an autodiffer (H1, Technicon, USA).

Soluble TNFR 75 levels in serum were determined using a commercially available enzyme amplified sensitivity immunoassay (EASIA)-kit (Medgenix Diagnostics, Fleurus, Belgium) and a Multiscan Titertec MCC 340 ELISA-reader.

Neopterin determinations were performed by radioimmunoassay, with a commercially available kit (Henning Inc., Berlin, Germany).

β2-Microglobulin determinations were performed on a Multiscan Titertec MCC 340 ELISA-reader, using a commercially available ELISA kit (synELISA-kit by ELIAS, Freiburg, Germany).

Statistical analysis
The data were analyzed by one-way analysis of variance (ANOVA), multiple range test (Duncan) with post-hoc (Bonferoni) adjustment, simple and multiple regression analysis, using an SPSS-PC^{+}-software package (SPSS, Inc.). Simple and multiple linear regression analyses were performed between serum selenium levels and surrogate markers for HIV-disease progression (CD4-count: $n = 104$, sTNFR 75: $n = 35$, neopterin: $n = 94$ and β2 microglobulin: $n = 80$). The sample size for linear regression varied due to the availability of suitable aliquots of the patient's sera. The data are given as mean ± standard deviation except the age of the patients and the controls, which is given as median ± range.

Results

Serum selenium in CDC I–III and controls
Serum selenium concentrations markedly decreased with the disease stages of HIV-infection (Figure 1). The mean serum selenium levels of stage III (51.4 ± 14.7 μg/l) and stage II patients (66.7 ± 20.9 μg/l) were significantly lower as compared to healthy individuals (89.2 ± 20.9 μg/l) and CDC I patients (82.3 ± 20.5 μg/l), respectively ($P < 0.01$). The mean of CDC II patients was also significantly lower as compared to group III ($P < 0.01$). Box plots of serum selenium concentrations in controls, and CDC groups I-III are shown in Figure 1.

Correlation of selenium levels with surrogate markers of HIV-infection
A positive correlation was found between $CD4^{+}$-count ($r = 0.42$; $P < 0.001$; $n = 104$) and selenium levels. A negative correlation was found between serum selenium concentrations and sTNFR 75 ($r = -0.58$; $P < 0.001$; $n = 35$), serum neopterin ($r = -0.5$; $P < 0.001$; $n = 80$) and β2-microglobulin ($r = 0.001$, $n = 94$; see Table 2). Multiple regression analysis revealed that neopterin and β2 microglobulin levels were independently correlated with serum selenium, whereas CD4-count was not an independent factor with selenium. ($P < 0.01$; multiple *r*: 0.541, $n = 78$). This suggests that serum selenium is more related to OIs than to the CD4 count.

Subgroup-analysis of serum selenium levels
Twenty eight patients (stage I, $n = 8$; II, $n = 9$ and III, $n = 11$) were coinfected with the hepatitis-C virus (HCV)

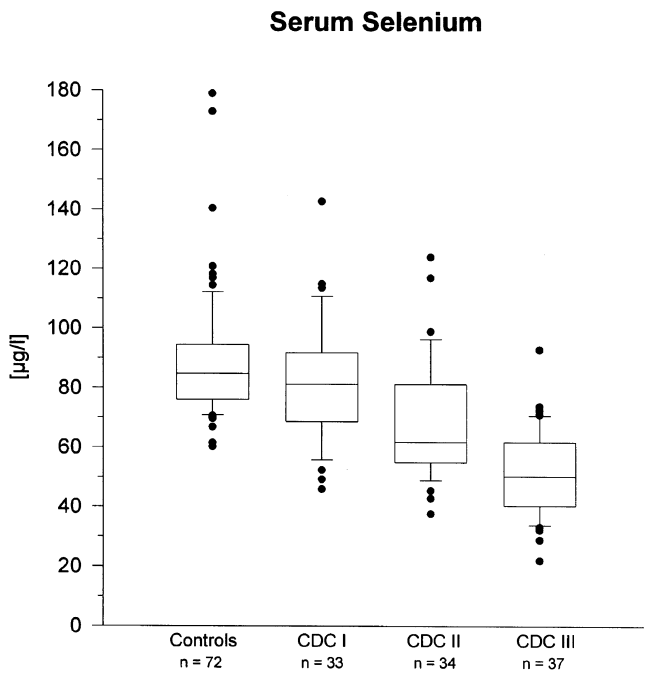

Figure 1 Box-plots of serum selenium in healthy subjects and CDC′93 I–III (the lower boundary of the box indicates the 25th percentile, the line within the box marks the median, and the upper boundary of the box indicates the 75th percentile. Error bars above and below the box indicate the 90th and 10th percentiles. In addition, outlying points are graphed). All groups were different from each other (P value <0.01) except CDC I *vs* controls.

as confirmed by ELISA-testing for HCV antigens and/or by the polymerase chain reaction. The mean serum selenium level in these patients taken as one group, was markedly lower (57.7 ± 16.3 *vs* 70.8 ± 23.5 µg/l; $P < 0.001$; $n = 28$ *vs* 76) as compared to that of the remaining HIV-infected individuals. All differences in serum selenium concentrations between CDC I–III groups and controls in the remaining study group were still significant ($P < 0.001$) after exclusion of the HCV coinfected patients (data not shown). When mean selenium concentrations between HCV coinfected and non-HCV-infected HIV patients were compared with the respective CDC-groups, significant differences were apparent for CDC I and II-patients (59.3 ± 15.6 *vs* 73.3 ± 20.6 µg/l; $P = 0.0012$; $n = 8$ *vs* 25 and 63.5 ± 14.3 *vs* 88.3 ± 18.6 µg/l; $P = 0.021$; $n = 9$ *vs* 25, respectively), whereas CDC III-patients (51.6 ± 14.6 *vs* 51 ± 15.3 µg/l; $P = 0.91$; $n = 11$ *vs* 26) did not differ in their selenium levels. Twenty one patients of group III with either acute OI and/or AIDS-defining tumors (kaposi sarcoma or lymphoma) had to be admitted to hospital for treatment. In this group, the mean serum selenium level was markedly lower (43.6 ± 11.6 *vs* 61.6 ± 12 µg/l; $P < 0.001$) as compared to that of the remaining 16 stage-III patients, who were outpatients with stable clinical conditions at the time of selenium determination.

Comparison of GSH-Px-activities, plasma SH-group- and GSH concentrations in patients at stages CDC III, CDC I and controls

Erythrocyte GSH-Px activities, plasma SH-group- and GSH levels of 12 hospitalized AIDS-patients at stage III were compared with those of 26 stage-I patients and 25 healthy subjects. The mean of GSH-Px activities in AIDS-patients was significantly lower as compared to stage I-patients and healthy subjects. GSH-Px activity tended to be higher ($P = 0.1$) in asymptomatic HIV-positive individuals than in healthy subjects. Thus, GSH-Px-activities of AIDS-patients and asymptomatic patients (CDC I) were positively correlated with the corresponding serum selenium levels ($r = 0.42$; $P < 0.05$; $n = 38$), which underlines the dependency of GSH-Px activities on selenium levels. Plasma SH-group- and GSH-levels were significantly lower in both, asymptomatic HIV-infected individuals at CDC stage I and AIDS-patients as compared to healthy subjects. Total protein content in serum (data not shown) and in plasma of asymptomatic patients was significantly higher as compared to that of healthy subjects. The mean plasma GSH concentration in hospitalized AIDS patients and in healthy controls did not differ ($P = 0.17$) on the basis of gram (g) protein. (Table 3; box plots of GSH-Px activities, plasma SH-group-, GSH- and total protein concentrations are shown on Figures 2a–f).

Discussion

Our study demonstrates that serum selenium concentration declines with disease progression in HIV-infected individuals; these results agree with the observations of previous investigators (Dworkin *et al*, 1988; Olmsted *et al*, 1989; Beck *et al*, 1990; Cirelli *et al*, 1991; Dworkin, 1994).

Serum selenium concentrations according to CDC′93 subgroups

The extent of decrease of serum selenium is augmented in the presence of an underlying OI and/or HIV-associated neoplasms. One explanation is that a variety of factors, such as fever, extreme tiredness, esophageal and oropharyngeal candidiasis, abdominal pain and diarrhea, lead to

Table 3 GSH-Px activities, plasma SH and GSH in critically ill patients with AIDS, asymptomatic HIV-infected individuals and healthy controls

	AIDS (n = 12)	*Asymptomatic HIV (n = 26)*	*Healthy controls (n = 25)*
Selenium (µg/l)	46.2 ± 15.5^{d}	73.53 ± 16.2^{c}	90.5 ± 23.1
GSH-Px (U/gHb)	17.7 ± 5.4	25.4 ± 7.8^{b}	$22.2 \pm 5.9^{a,e}$
Plasma-SH (µmol/l)	300 ± 104.1^{d}	439 ± 68^{c}	474.6 ± 64.3
Plasma-SH (nmol/g protein)	3926.7 ± 1015.2^{d}	4393.2 ± 655.4^{d}	5384.5 ± 819.4
GSH (µmol/l)	12.4 ± 6.8^{c}	14.6 ± 4.8^{d}	18.2 ± 5.7
GSH (nmol/g protein)	179.4 ± 65.6	147.2 ± 54.5^{d}	212.1 ± 81.2
Plasma protein (g/l)	76.4 ± 13.2	100.5 ± 12.4^{d}	88.4 ± 8.9

[a] <0.05; [b] <0.01 *vs* Active AIDS; [c] <0.05; [d] <0.01 *vs* Healthy controls; [e] $=0.1$ *vs* Asymptomatic HIV.
AIDS = acute AIDS-defining opportunistic infection and/or disease according to the CDC′93 classification system, which required hospitalisation.
The group of healthy controls is a randomly selected subset of the 72 selenium controls.

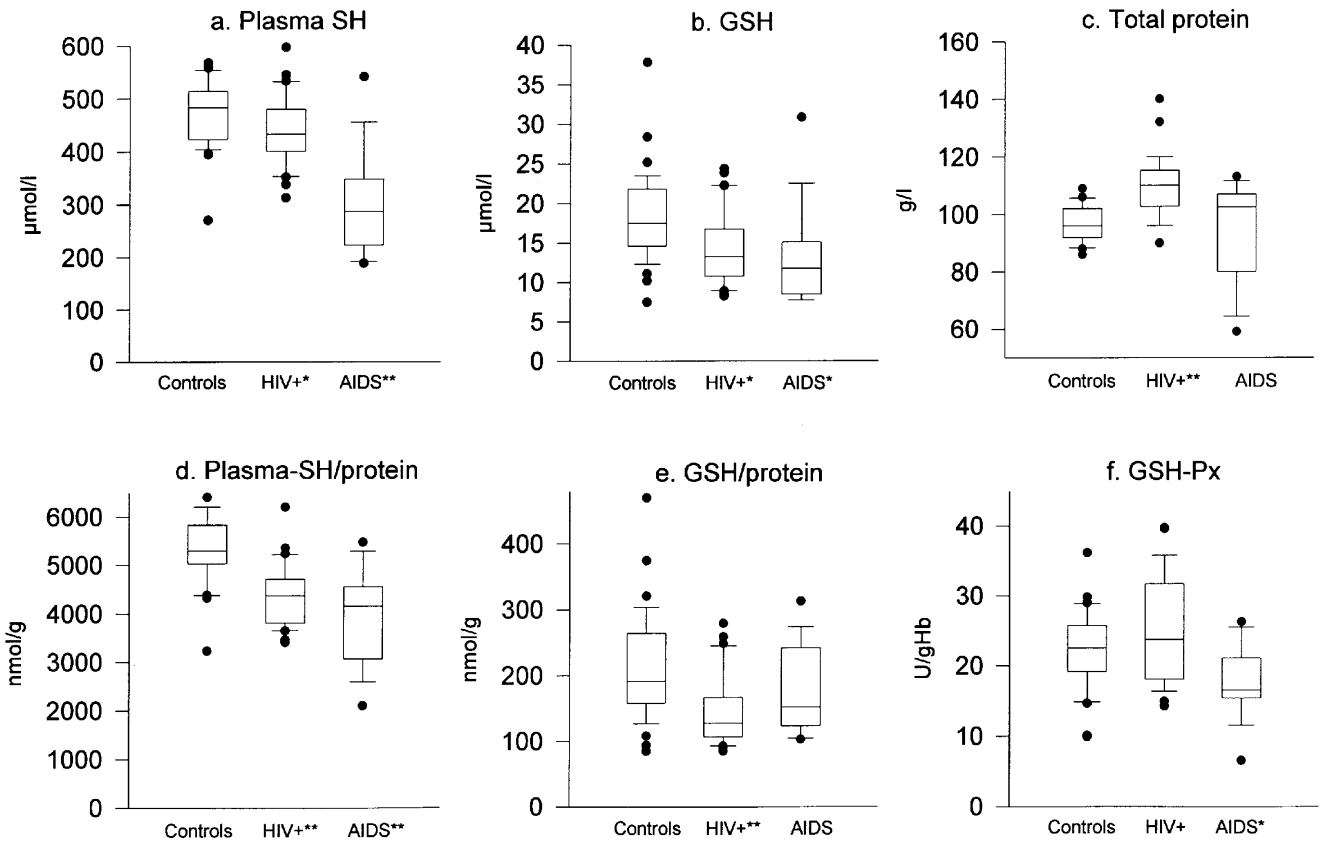

Figure 2a–f Box-plots of plasma-SH groups, -GSH, total plasma protein content and erythrocyte GSH-Px activity in control subjects ($n = 25$), CDC I ($n = 26$) and CDC III ($n = 12$); (the lower boundary of the box indicates the 25th percentile, the line within the box marks the median, and the upper boundary of the box indicates the 75th percentile. Error bars above and below the box indicate the 90th and 10th percentiles. In addition, outlying points are graphed). $^{*}P < 0.05$; $^{**}P < 0.01$ *vs* controls.

malnutrition resulting in the markedly low serum concentrations in patients with OI. Notably, also stage-II patients with symptomatic HIV-infection, but without AIDS-defining events and no obvious nutritional deficiencies, had significantly lower selenium levels than asymptomatic HIV-infected individuals at state I and healthy individuals. Patients at stage I and II coinfected with HIV and HCV displayed significantly lower selenium concentrations than patients exclusively infected with HIV at the respective stage. Liver dysfunction, as a consequence of HCV-infection, might decrease biosynthesis of selenium-binding proteins such as the selenoprotein P and plasma GSH-Px exacerbating selenium deficiency.

Correlation of serum selenium with surrogate markers of HIV-disease progression

Selenium was independently correlated with β2-microglobulin and neopterin but not with the CD4 count. Thus, it seems that low selenium levels in our patients are more closely related to the presence of OI and/or to the fact of dual infection with HIV and HCV than to the CDC stage alone. In a recent study a multivariate analysis revealed that serum selenium concentrations and CD4 count were predictable for death and for the prevalence of OI whereas p24 antigen and β2 microglobulin levels were not (Constans *et al*, 1995).

Plasma GSH concentrations in CDC I, III and healthy controls

Plasma SH- and GSH-levels were low in asymptomatic HIV-positive individuals at CDC state I and in patients with AIDS (CDC III). Increased consumption of GSH by the GSH-Px reaction and/or decreased release into the circulation from the liver, the site of GSH production, might account for the low plasma concentrations. Considering the role of GSH in immune function the loss of thiol compounds, especially of GSH, represents a critical feature of HIV-disease. In fact, low concentrations of GSH in $CD4^{+}$ and $CD8^{+}$ lymphocyte subsets (Roederer *et al*, 1992, 1993; Drge, 1993), in peripheral blood mononuclear cells (PBMC) (de Quay *et al*, 1992), or in plasma and bronchoalveolar lavage (Buhl *et al*, 1989; Borum *et al*, 1992) of individuals infected with HIV have been reported. The present knowledge about the subcellular distribution of GSH in human cells suggests that both, a sufficient level of GSH and a certain balance between GSSG and GSH is crucial to maintain normal cellular immune function. However, in contrast in a more recent study on the intracellular redox state of GSH in $CD4^{+}$ lymphocytes of HIV-infected patients, total cellular GSH appeared to be elevated as compared to that of non-infected individuals, while at the same time a decrease in the intracellular ration of GSH to GSSG was observed (Aukrust *et al*, 1995). The authors expressed the concentration of GSH per 10 million cells; other studies have shown that a GSH-deficiency in lysates of PBMC from HIV-infected patients was apparent only, when expressed as nmol/mg of protein, but no difference was found when expressed as nmol per 10 million cells (Barditch-Crovo *et al*, 1995; Munir *et al*, 1996). These results may be explained due to increased intracellular protein content and/or due to the presence of a greater proportion of larger PBMCs in the blood of HIV-infected

individuals. In our study the total plasma protein concentrations of asymptomatic HIV-infected individuals at state I were higher as compared to that of healthy subjects. This difference is caused mainly by the increased production of gamma globulins, a typical feature of HIV disease. Albumin did not differ among the study groups with the exception of lower albumin concentrations in CDC-III patients versus CDC-I patients and healthy controls (data not shown). Nevertheless, in our study, plasma GSH and -SH were low when expressed either as μmol/l or nmol/g protein. Another aspect of this context that may be considered is the action of the HIV-gene product Tat on plasma GSH. This protein depletes GSH or T-cells presumably by suppressing the expression of MnSOD. This leads to increased concentration of the superoxide anion which renders the cells more sensitive to TNF-toxicity (Flores *et al*, 1993; Westendorp *et al*, 1995; Ehret *et al*, 1996).

Thus, the question of the sites of loss of GSH, factors affecting de-novo synthesis and homeostasis (Helbling *et al*, 1996), the intra and extracellular redox sensitivity of GSH in different types of lymphocyte subsets and macrophages and its enhanced oxidation need to be investigated to evaluate the role of GSH in HIV-infection.

Erythrocyte GSH-Px activities in CDC I, III and healthy controls

In the present study erythrocyte GSH-Px activity was lower in patients with ongoing AIDS-indicative diseases than in healthy controls and CDC I patients. This is in accordance with a previous report from the group of Dworkin in 1989, albeit in our study population the correlation between selenium and the GSH-Px activities is somewhat weaker. This is not surprising, because about 50% of selenium in human serum is bound to the selenoprotein P, originating from the liver, whereas GSH-Px bound selenium accounts for only 25–30% of total serum selenium. There was no compromise of GSH-Px activity and concordantly no selenium deficiency in asymptomatic HIV-infected individuals. Thus, it might be speculated that in end-stage AIDS, a breakdown of GSH-Px activity occurs, when serum selenium levels fall below a certain threshold value of about 55 μg/l.

Conclusions

Selenium deficiency is common in advanced HIV-disease and should be corrected, irrespectively, whether caused by malnutrition and/or by the presence of OI. Controlled studies in addition to antiretroviral treatment should be directed at keeping a maximum replete micronutrient and vitamin status enabling suppression of 'oxidative stress' and possibly viral propagation.

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