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Zinc Serum Level in Human Immunodeficiency Virus-Infected Patients in Relation to Immunological Status

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ABSTRACT

In human immunodeficiency virus (HIV) infection, serum level of zinc, an important micronutrient for immune function, is frequently diminished. The aim of this study was to determine the zinc status in relation to immunological parameters and disease stage in 79 HIV-1 seropositive patients. The median serum level of zinc was within normal limits (12.5 µmol/L) but in 23% of patients, zinc deficiency was seen. Decreased serum zinc was associated with a low CD4 cell count, high viral load, and increased neopterin and IgA levels. According to current treatment recommendations, the majority of patients received antiretroviral triple therapy. Zinc levels in treated and untreated patients were comparable. Referring to disease stage (CDC classification, 1993), the mean zinc level was highest in stage C and lowest in stage A. In conclusion, even under antiretroviral triple therapy, zinc deficiency is still of great importance in HIV infection, and zinc substitution in zinc deficient individuals should be taken into account to optimize therapeutical success.

Index Entries: Zinc; human immunodeficiency virus; immunology; antiretroviral therapy.

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INTRODUCTION

Zinc is an essential trace element for the immune system with particular importance for cellular immune function. A balanced zinc status is necessary for both T-cell maturation in the thymus and proper functioning of peripheral lymphocytes. In zinc-deficiency states, a diminished T-cell count and decreased function of T-helper- and T-killer-cells as well as impaired function of macrophages are seen. By pharmacological zinc substitution, observed disturbances are mostly reversible *(1–3)*.

In human immunodeficiency virus (HIV)-infected patients, the serum level of zinc is frequently diminished, and the prevalence of zinc deficiency ranges from 25% to 36% *(4–10)*. Zinc deficiency in HIV infection is associated with a higher risk of systemic bacterial infections *(6)* and increased HIV-1-related mortality *(11)*. Reasons for the observed zinc deficiency may be malnutrition, intestinal malabsorption, and increased urinary loss of zinc. Furthermore, redistribution of zinc into the liver, as seen within the acute-phase reaction, may also play a role.

Apart from direct inhibition of viral proliferation, antiretroviral triple therapy has a profound influence on the immune status of the patient *(12)* and, thus, might also play a role in the regulation of zinc homoeostasis. However, data about zinc status in patients undergoing antiretroviral triple therapy are still missing. In this study, we determined the serum concentration of zinc in HIV-1-infected patients antiretrovirally treated according to current treatment recommendations *(13)*. A relationship between zinc status and immunological parameters, like viral load, CD4 cell count, neopterin, and immunoglobulin A, nutritional status and actual antiretroviral therapy were assessed.

PATIENTS AND METHODS

Subjects

The study population consisted of 79 patients, 20 female and 59 male, with a mean age of 39 yr (range: 24–69 yr), documented to be HIV seropositive by Western blot and polymerase chain reaction. All patients who attended our outpatient clinic between January and June 1998 were included in the study. Patients who suffered from clinically and microbiologically documented acute infection other than HIV infection (n = 5) were, however, excluded because lowered zinc serum levels resulting from redistribution of serum zinc into the liver can be assumed. All patients denied taking any zinc supplementation in the past. Modes of transmission were as follows: heterosexual contacts in 29% (23/79), homosexual contacts in 28% (22/79), patient from HIV highly endemic region in 10% (8/79), intravenous drug abuse in 8% (6/79), transfusion of blood products in 4% (3/79), and unknown in 21% (17/79). Patients

were classified into stages A (n = 42; 53%), B (n = 17; 22%), and C (n = 20; 25%) according to the CDC classification *(14)*. Sixty-six percent of patients (56/79) received antiretroviral therapy. Of these patients, 52 (93%) were taking triple therapy and 4 (7%) were taking only one drug or a combinations of two drugs. When considering the respective stage, 52% (22/42) of patients classified into stage A, 88% (15/17) into stage B, and 95% (19/20) into stage C received drug treatment.

Determination of Immunological and Nutritional Parameters

Venous blood samples were collected in pyrogen-free sterile tubes. Serum samples were stored at –70°C until quantification of zinc. All other parameters were determined in the fresh specimen. Serum levels of zinc were determined by atomic absorption spectrometry with a Perkin-Elmer (Norwalk, CT) 1100 B atomic absorption spectrometer. The normal range for serum zinc was 11.5–18 μ*M*; values between 10.5 and 11.4 μ*M* were determined as borderline levels. To rule out misinterpretation of zinc concentrations caused by a different status of hydration in the patients, zinc levels were normalized by the respective serum osmolarity. Osmolarity was determined by a cryoscopic osmometer, using a Gonotec osmomat 030 (Gonotec, Berlin). A serum osmolarity of 290 mosm/L was considered as the physiological reference value.

The HIV viral load in plasma was determined by quantitative polymerase chain reaction (Amplicor, Version 2.1, Hoffmann LaRoche, Basel). Minimal detectable concentration was 50 copies/mL. CD4 cells were enumerated by flow cytometry (FACS Calibur, Becton Dickinson, Heidelberg) in EDTA blood using three-color direct immunfluorescence with monoclonal antibodies (Becton Dickinson). Neopterin in serum was analyzed by commercially available enzyme-linked immunosorbent assay (ELItest, Brahms Diagnostika, Berlin) with a reference value up to 10 nmol/L. Serum IgA and C-reactive protein (CRP) were determined by nephelometry (Dade Behring, Liederbach, Germany). The reference range was 0.7–4.0 g/L for IgA. The minimal detectable limit for CRP was 3.44 mg/L and the upper limit of the physiological reference range was 5 mg/L. The body mass index (BMI) was calculated by the formula weight/(height)^2. The normal values for BMI were 19–24 kg/m^2 for female and 20–25 kg/m^2 for male patients.

Statistical Analyses

Data are generally expressed in medians to consider a small sample size. Significances of differences were analyzed by the Mann-Whitney rank sum U-test. In Figs. 1 and 2, box plots display the median and the 10th, 25th, 75th, and 90th percentiles of the respective serum level. Values outside the 10th and 90th percentiles are plotted as points. Statistical

correlations between zinc and immunological parameters were assessed by the Spearman Rank Correlation Test. ρ corrected for ties and tied *p*-values were determined (presence of a maximum of eight ties within each individual data set).

RESULTS

Zinc and Immunological Status of the Study Population

The median zinc level of the study population, normalized by the respective serum osmolarity, was 12.5 μ*M* (mean: 12.6 μ*M*, S.D. ± 2.8) and thus within normal limits (Table 1). However, zinc levels < 10.5 μ*M* were observed in 23% of patients (Fig. 1). Characteristics of immunological markers, such as CD4 cell count, viral load, neopterin, IgA, and CRP, as well as body mass index, are presented in Table 1.

Relationship Among Zinc, Immunological Parameters, and Nutritional Status

A possible relationship between zinc and immunological parameters that have a predictive value in HIV infection was assessed. Zinc serum level was positively related to CD4 cell count: Patients with a CD4 cell count below 200 cells/μL had the lowest median zinc level (11.9 μ*M*), whereas patients with a CD4 cell count exceeding 500 cells/μL showed higher zinc serum levels (12.9 μ*M*, Fig. 2A). An inverse association was found for serum zinc and plasma viral load. Patients with a viral load below the detection limit of the applied assay (i.e., below 50 copies/mL) had the highest zinc levels with a median of 14.3 μ*M*, whereas zinc levels in patients with moderate (50–10,000 copies/mL) and high viral loads (> 10,000 copies/mL) were much lower (Fig. 2B). Zinc levels were comparable in patients with normal neopterin (< 10 nmol/L) and in patients with moderately elevated (10–20 nmol/L) neopterin concentrations (median 12.9 versus 12.4 μ*M*). However, in patients with a highly elevated serum neopterin (> 20 nmol/L), the median zinc level was diminished to 11.6 μ*M*, near the lower limit of the reference range (Fig. 2C). A similar distribution was found for serum IgA: The median serum zinc levels were much lower in patients with elevated IgA (> 4 g/L) than in patients with normal IgA levels (11.5 versus 12.6 μ*M*, Fig. 2D).

Apart from a stratification of the immunological parameters in different clinically related subgroups, a possible correlation between zinc and investigated parameters in the whole plot of original values was evaluated. However, there were no significant statistical correlations found (zinc versus [1] CD4 cell count: $r = 0.14$, $p = 0.23$; [2] plasma viral load: $r = -0.05$, $p = 0.67$; [3] neopterin: $r = -0.16$, $p = 0.16$, and [4] IgA: $r = -0.11$, $p = 0.33$).

Table 1
Immunological and Nutritional Characteristics of the Study Population

median; range (mean ±SD)	Stage A (n = 42)	Stage B (n = 17)	Stage C (n = 20)	total (n = 79)
Zinc (µmol/l)	**12.4**; 7.1 - 18.3 (12.3 ±2.8)	**12.3**; 6.4-16.0 (12.4 ±2.8)	**13.5**; 9.5-19.6 (13.4 ±2.5)	**12.5**; 6.4-19.5 (12.6 ±2.8)
CD4 cell count (cells/µl)	**388**; 44-1367 (385 ±226)	**217**; 26-766 (253 ±182)	**195**; 4-542 (230 ±154)	**280**; 4-1367 (317 ±211)
Plasma viral load (10^3 copies/ml)	**5.3**; 0.05-419.3 (36.8 ±75.9)	**1.3**; 0.05-96.3 (15.5 ±26.9)	**2.1**; 0.05-89.0 (16.7 ±27.2)	**1.6**; 0.05-419.3 (27.1 ±58.8)
Neopterin (nmol/l)	**9.9**; 4.6-22.7 (11.3 ±4.7)	**11.4**; 7.6-58.0 (14.4 ±11.6)	**10.3**; 5.9-48.3 (13.8 ±10.2)	**10.4**; 4.6-58.0 (12.6 ±8.2)
IgA (g/l)	**2.2**; 0.3-5.6 (2.3 ±1.3)	**3.3**; 1.9-10.2 (4.1 ±2.2)	**2.7**; 0.3-7.1 (3.0 ±1.8)	**2.5**; 0.3-10.2 (2.9 ±1.8)
CRP (mg/l)	**3.4**; 3.4-29.0 (4.7 ±4.3)	**3.4**; 3.4-208.0 (20.3 ±51.8)	**3.4**; 3.4-27.5 (6.0 ±5.5)	**3.4**; 3.4-208.0 (8.4 ±24.6)
BMI (kg/m^2)	**23.4**; 17.6-46.9 (24.6 ±5.1)	**23.5**; 19.6-35.7 (24.6 ±4.6)	**24.4**; 17.4-33 (24.1 ±3.3)	**23.9**; 17.4-46.9 (24.5 ±4.5)

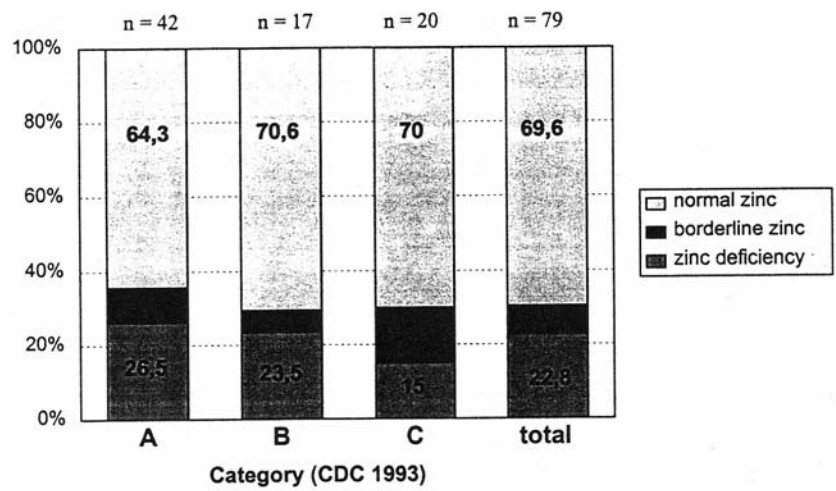

Fig. 1. Zinc status in HIV positive patients (n = 79). Percentage of patients with normal, borderline, and zinc deficiency in the whole study population and categorized into stages A, B, and C according to CDC classification 1993 are shown.

In order to determine activation of the acute-phase response, the serum concentration of CRP was measured. The majority of patients had CRP levels below the detection limit of the applied assay (59/79; 75%). Because of the unequal distribution of CRP in the study population (see Table 1), we refrained from further analyses regarding association with

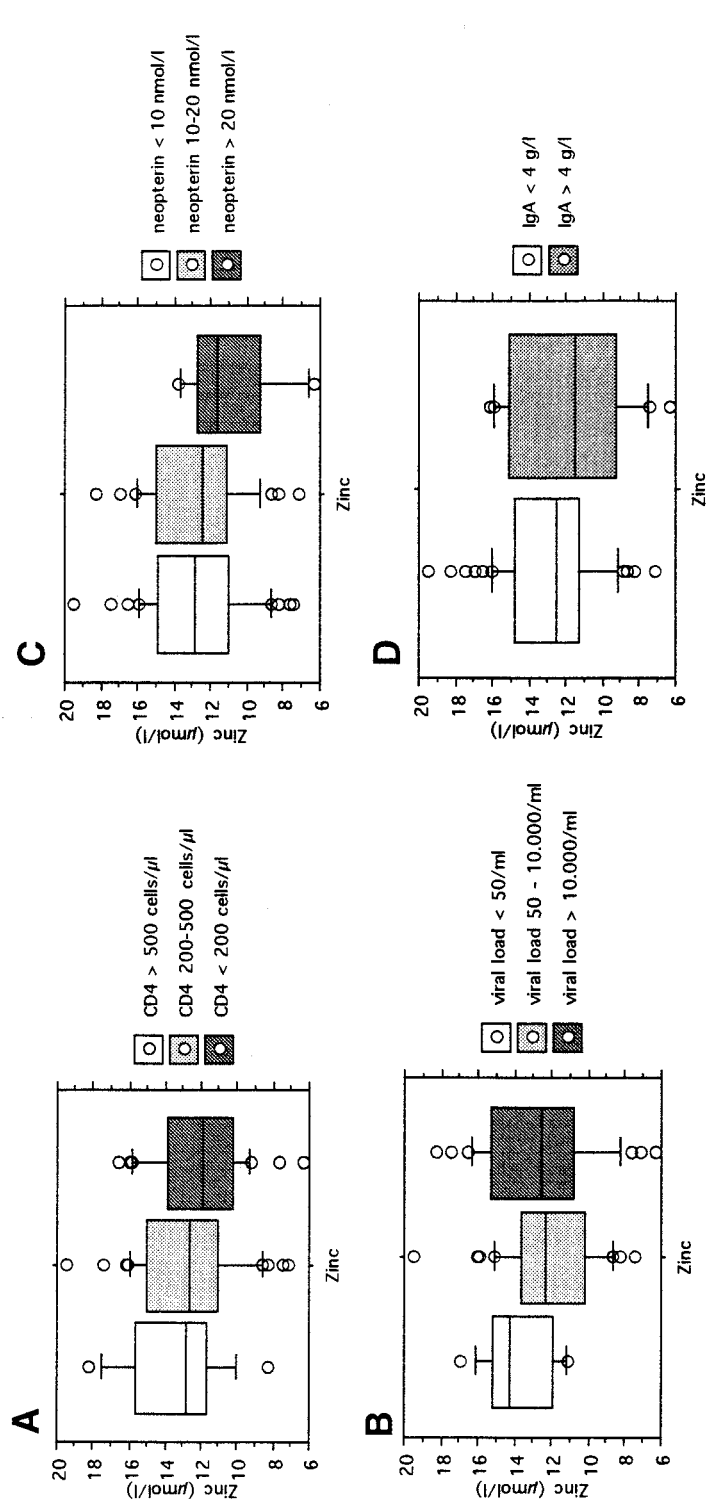

Fig. 2. Serum levels of zinc (µmol/L) in dependence on immunological parameters in HIV-positive patients (n = 79). CD4 cell count (cells/µL, **A**), plasma viral load (copies/mL, **B**), neopterin (nmol/L, **C**), and IgA (g/L, **D**), categorized into different subgroups, are presented.

the zinc level. The range of CRP was noticeable large in stage B patients, although there were no patients with obvious clinical infection included. Classification into stage B was mostly the result of oropharyngeal candidiasis or oral hairy leukoplakia.

Because the nutritional status might have an influence on the zinc level, we determined the body mass index (BMI) of the patients. The median BMI of the whole population was 23.9 kg/m^2 (mean 24.5 kg/m^2 ± 4.5) and, thus, within the normal range. When patients were classified into those exhibiting zinc deficiency and those with normal zinc serum level, no difference in BMI was observed (24.2 kg/m^2 [mean 24.6 kg/m^2 ± 3.7] versus 23.3 kg/m^2 [mean 24.4 kg/m^2 ± 4.7]).

Influence of Antiretroviral Therapy on Zinc Level

Because an influence of therapy on zinc level is reasonable to assume, we assessed the relationship between antiretroviral drug treatment and zinc status. In patients who did not receive antiretroviral therapy, the median zinc serum levels were comparable to those measured in patients undergoing triple therapy (12.4 µ*M* [mean 12.5 µ*M* ± 2.8] versus 12.5 µ*M* [mean 12.7 µ*M* ± 2.7]). Zinc deficiency was seen in 22% of patients without therapy (5/23) and 25% of patients treated with triple therapy (13/52). Compared to untreated subjects, patients receiving triple therapy had a significantly lower CD4 cell count and viral load ($p < 0.01$ and $p < 0.05$, respectively; data not shown). Because of the small size of the study population, we refrained from stratification into further groups regarding the intake of different antiretroviral drugs and duration of therapy.

Stratification of the Study Population According to Clinical Stages of the CDC Classification (1993)

To investigate the association between zinc status and disease progression, zinc levels in the different disease stages (CDC A/B/C) were assessed separately. Interestingly, the highest median serum zinc was found in stage C patients (13.6 µ*M*) and the lowest in stage A (12.5 µ*M*, Table 1). Whereas in stage C, only 15% of patients had serum zinc levels below 10.5 µ*M*, zinc deficiency was seen in 26% of stage A and 24% of stage B patients (Fig. 1). Distribution of the immunological parameters in the three groups is presented in Table 1. Apart from a negative association between CD4 cell count and disease progression, no obvious relationship between disease stage and immune status have been detected. In the three patient categories, the BMI was comparable (Table 1).

DISCUSSION

Micronutrient deficiencies have already been reported early in the HIV pandemic *(7–8,15,16)*. As an essential trace element for the immune system *(1–3)*, zinc plays an important role for immune function in HIV infection *(4–9)*. In the present study, the zinc status in HIV-infected patients, the majority treated with antiretroviral triple therapy, was evaluated.

In accordance with earlier studies, the mean zinc level of the study population was within normal limits and comparable to healthy controls, as investigated previously with the same method *(17)*. To avoid unintentional variations caused by the differences in hydration, zinc concentrations were normalized by the serum osmolarity. Surprisingly, we revealed zinc deficiency in 23% of patients. This percentage is comparable to that described earlier for untreated HIV patients *(6–8)* and clearly exceeds that of healthy controls *(17)*. It shows that zinc deficiency in HIV-infected patients has not grossly changed after the introduction of antiretroviral triple therapy in 1996.

In this study, the relationship between zinc level and immunological parameters *(18,19)* was evaluated. High serum zinc was associated with a high CD4 cell count (> 500 cells/μL), low viral load (< 50 copies/mL), and normal neopterin and IgA levels. The lowest zinc levels were found in patients with viral loads exceeding 10.000 copies/mL, CD4 cells below 200 cells/μL, and enhanced levels of neopterin and IgA. Thus, by investigation of new surrogate markers, we confirmed earlier results that revealed a negative correlation between serum concentrations of zinc and β_2-microglobulin *(9)*. Although statistical correlations between zinc and investigated parameters, based on the whole plot of original values, were not found, observed associations seen after stratification of the data in different immunological and clinical related subgroups (Fig. 2) strongly suggest a clinical significance of the obtained data. Reasons for the observed zinc deficiency may not only be possible differences in nutrition and zinc redistribution but also increased urinary loss of zinc, as a correlation between serum neopterin concentration and urinary output of zinc in HIV-infected patients has been reported *(20)*. Furthermore, increased incorporation of zinc into viral nucleocapsid zinc-finger proteins during enhanced viral proliferation *(21)* leading to diminished serum zinc level may also be speculated.

To evaluate a possible relationship between zinc status and disease progression, patients were stratified into clinical stage A/B/C according to CDC classification. Although in an earlier study an association between zinc level and disease stage following the Walter Reed classification has not been found *(15)*, a significant decline of serum zinc with disease progression has been reported later *(5)*. Surprisingly, the median

zinc level in the present study was highest in stage C patients and in this group, only 15% of patients exhibited zinc deficiency. These data seem to contradict the above-mentioned relationship between zinc and immune status because low CD4 cell count and high viral load (which were associated with low serum zinc) can be assumed in stage C patients. However, apart from CD4 cell count, immunological parameters were not different in the three disease stages. For instance, the mean viral load was *not* higher in stage C than in stage A patients. Because nowadays triple therapy can lead to reconstitution of immune status even in progressed patients, classification into clinical stage A/B/C, which has already been established before the introduction of specific antiretroviral therapy, does not necessarily reflect immunological status of the patient. Therefore, A/B/C classification does not seem to be appropriate for evaluation of immunological parameters in HIV patients.

As a possible explanation for higher zinc levels in stage C patients, an influence of antiretroviral triple therapy may be reasonable to assume because the percentage of treated patients was highest in this group. However, treated and untreated patients had similar serum zinc levels and exhibited zinc deficiency in comparable percentages. The observed differences in zinc serum level might also be caused by redistribution of zinc into the liver, as seen in inflammatory and acute-phase reactions *(22,23)*. Increased levels of cytokines and their respective receptors have been determined in HIV patients *(18,19)* and may contribute to redistribution of zinc via enhanced induction of liver metallothionein. However, CRP was comparable in the different stages in our study. Obvious differences in nutritional status have been excluded by measurement of the BMI, but, nevertheless, different compositions of food might play a role. Finally, also limited interpretation of the data resulting from small sample size and inhomogenous composition of the study population has to be considered. For example, differences in the distribution of zinc deficiency among HIV-infected homosexual men and drug users have been described recently *(10)*.

In conclusion, even under antiretroviral triple therapy, micronutrient deficiencies are still of great importance. Data about pharmacological zinc application in HIV-infected patients are, however, still scarce. Apart from positive effects after administration of oral zinc alone or as an adjunct to zidovudine therapy *(16,24)*, poorer survival has been reported also *(25)*. Whether this observation is related to impairment of immune function, as described for the application of zinc in high dosages [i.e., 300 mg zinc/d *(26)*], remains to be clarified. Thus, further studies are necessary to determine the mechanisms and clinical significance of altered zinc status and pharmacological application of zinc in order to optimize therapeutical success in HIV infection.

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