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Relationship of Trace Element, Immunological Markers, and HIV1 Infection Progression

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ABSTRACT

Trace elements (selenium, zinc, copper), β_2 microglobulin levels, CD_4, and CD_8 cell counts have been determined in 80 HIV_1 seropositive patients. The study group consisted of 19 females and 61 males with age mean of 35 ± 10 yr, at stage IV of infection (CDC—Atlanta classification) and treated by AZT. No severe renal or liver diseases or hypoalbuminemia were observed in this group.

Se values were significantly lower than in normal adults, 48.3 ± 17 µg/L vs 71 ± 12 µg/L; Zn was moderately diminished, 1 ± 0.2 mg/L vs 1.2 ± 0.2 mg/L, whereas copper values were in the normal range, 1.2 ± 0.3 mg/L vs 1.1 ± 0.5 mg/L. Se or Zn deficiency was found in 60 and 30 subjects, respectively. Blood Se and Zn decreases were associated in 23 patients. Moreover, all patients showed higher β_2 microglobulin values than the upper normal limit of 2.4 mg/L. Negative correlations were found between Zn and β_2 microglobulin ($p < 0.005$) and between Se and β_2 microglobulin ($p < 0.05$). Moreover, there was a positive correlation between Se and Zn values ($p < 0.05$).

Nineteen subjects died 1 yr later (group I), and 61 remained alive (group II). With respect to the clinical evolution, a significant difference between both groups was found in Se and β_2 microglobulin levels as well as in CD_4 cell counts. The correlations previously observed persisted in group II, whereas no correlation was noted in group I. In addition, the patients of group I had significantly lower Se values, which were below 30 µg/L in 10 cases.

These results confirm the prevalence of abnormalities in Se and Zn levels and their relationships with nonspecific markers of immune system activity in more advanced HIV disease. Impairment of trace

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element status and mainly Se status appeared, at least partially, to reflect the disease activity/progression and subsequently the immune dysregulation.

Index Entries: AIDS; HIV_1 seropositive; serum selenium level; plasma zinc level; serum copper level; trace elements and HIV_1 disease activity/progression.

INTRODUCTION

Patients with AIDS display multiple abnormalities of blood trace elements, such as Cu, Zn, and Se, which are believed to be important in maintaining immune functions *(1,2)*. The decrease of Zn and Se may involve malnutrition, gastrointestinal malabsorption, lowering of their carrier protein synthesis, tissue redistribution, or increase of losses *(3,4)*. Some investigators have reported that the modifications of serum trace element levels could be correlated to the disease progression *(5,6)*. These results were not confirmed by other authors *(7)*.

The aim of this study was to determine (1) the relationship between the blood Zn, Cu, and Se concentrations and β_2 microglobulin values, which is widely considered as a marker of the disease progression *(8)*, and (2) the clinical interest of such trace elements in predicting the disease progression.

SUBJECTS AND METHODS

Subjects

In the first stage of the study (month 0), trace elements and β_2 microglobulin concentrations, and CD_4 and CD_8 leukocyte counts were determined in a group of 80 patients. This group, was comprised of 61 men and 19 women, with an age mean of 35 ± 10 yr, at stage IV of the disease (CDC—Atlanta Classification). Epidemiologic risk factors for AIDS included 36 homosexual, 18 heterosexual, 3 patients with hemophilia, 13 drug adducts, and were unknown for 10 subjects. All these patients, who have not been hospitalized over the 6 mo prior to the study, were undergoing treatment by AZT (200 or 250 mg 3 × t/d).

These patients had normal renal and liver functions as evaluated by serum creatinine levels below 15 mg/L, and normal aminotransferase activity. They had no hypoalbuminemia.

Methods

Selenium was measured by graphite furnace atomic absorption spectrometry. The analytical characteristics of the method included a detec-

tion limit of 5 μg/L, interday coefficient of variation of 5.1%, and range of normal values 60–83 μg/L. Values under 50 μg/L were considered as an index of deficiency.

Zn was determined by flame atomic absorption spectrometry: detection limit was 0.07 mg/L, coefficient of variation was 3.2%, and normal range was 1–1.4 mg/L. One considered that a moderate deficiency was present with values under 0.6 mg/L *(9)*.

Cu was evaluated by flame atomic absorption spectrometry. Detection limit was 0.06 mg/L, coefficient variation was 2.9%, and normal values extended from 0.8 to 1.6 mg/L.

β_2 microglobulin was determined by a radioimmunoassay method with threshold detection of 0.1 μg/L, coefficient of variation of 8%, and reference values under 2.4 mg/L. CD_4 and CD_8 were counted by standard flux cytometry. In the second stage of the study, the results obtained at month 0 were re-examined according to the clinical evolution in the ensuing year.

STATISTICAL ANALYSIS

The data were expressed as mean ± SEM. Results in patient group were compared to reference values by Student's *t*-test. Regression line and correlation coefficient were calculated with Stat-View II program.

RESULTS

Results Obtained at Month 0 in 80 Patients

The data are summarized in Table 1. In the study group, decreased Se and Zn levels were observed in 75 and 37.5% of cases, respectively. Among these subjects, the decrease under normal range in both trace element concentrations was found in 23 patients. Serum Cu concentrations were in the normal range in 76 subjects (95%), but β_2 microglobulin was increased in all the patients.

Significant negative correlations were found between Zn and β_2 microglobulin levels ($y = 0.037\ x + 1.218$, $r = -0.340$, $p < 0.005$) and between Se and β_2 microglobulin values ($y = 2.379\ x + 59.862$, $r = -0.248$, $p < 0.05$). Furthermore, a significant positive correlation between Zn and Se concentrations was observed ($y = 0.003\ x + 0.91$, $r = 0.255$, $p < 0.05$).

However, Zn was moderately diminished since no value was lower than 0.6 mg/L, whereas Se diminution was more noticeable. Indeed, 10 patients exhibited an important Se deficiency characterized by level below 30 μg/L.

Table 1
Se, Zn, and Cu, Immunological Parameters in Serum of HIV1 Seropositive Patients (Stage IV) (Influence of the Disease Evolution: Group I Patients Died, and Group II Patients Alive 12 Mo After the Beginning of the Study)

	All patients $n = 80$	Group I $n = 19$	Group II $n = 61$
Selenium µg/L	48 ± 17^{++}	41 ± 11.6	51 ± 16**
Zinc mg/L	1 ± 0.18^{+}	1.4 ± 0.2	1 ± 0.18
Copper mg/L	1.2 ± 0.3	1.3 ± 0.4	1.2 ± 0.2
β_2 microglobulin mg/L	4.5 ± 1.7^{+++}	5.5 ± 1.8	4.2 ± 1.6**
	0–1500^{+++}	0–188	14–1500***
CD_4/CD_8 ratio	0.02–2.2^{+++}	0–2.2	0.02–1.2*

Significant difference between group I and group II; *$p < 0.05$, **$p < 0.01$, *** $p < 0.001$.
Significant difference between values obtained in all patients and normal values; $^{+}p < 0.05$, $^{++}p < 0.01$, $^{+++}p < 0.001$.

Re-examination of Data 12 Mo Later According to the Disease Evolution

Nineteen subjects (group I) died 1 yr later (5 women and 14 men), whereas 61 subjects (group II) were alive (14 women and 47 men). The data corresponding to both groups, which were obtained at month 0, were then reanalyzed. Compared to group II, patients with short survival delay (group I) exhibited significantly lower Se values and CD_4 cell counts as well as higher β_2 microglobulin concentrations (Table 1). Furthermore, the aforementioned correlations between the different parameters were found in group II, but not in group I. Indeed the significant negative correlations between Zn and β_2 microglobulin ($p < 0.005$), between Se and β_2 microglobulin ($p < 0.05$), and the significant positive correlation between Zn and Se ($p < 0.05$) were only observed in subjects with a survival time above 12 mo.

DISCUSSION

A wide variety of specific nutrient deficiencies have been reported in HIV seropositive patients *(5–10)*. By compromising, immune functions and host defense mechanisms, such abnormalities, have been considered as contributing factors in the disease progression.

Our findings confirm and extend previous data. In our study group, serum Cu concentration was not modified. Moreover, no difference was observed according to the 12-mo clinical evolution. Some authors have reported significantly higher Cu values in progressor HIV1 seropositive

patients than in control group or in nonprogressor HIV seropositive subjects *(5)*. It may be hypothesized that opportunistic infective processes, which occur during HIV infection, may play a role in the increase in serum Cu levels *(5,6)*.

Plasma Zn values were moderately diminished, and our data are in agreement with those of Beck et al. *(10)*. Such a decrease may reflect changes in Zn distribution *(11)*. On the other hand, an important fact is that all patients receive AZT treatment, which is known to induce Zn diminution *(12)*.

A lot of reports *(13)* described Se deficiency in HIV seropositive subjects. We have found that concentrations of Se were lower than normal in 75% of cases and below 30 μg/L in 10 subjects (12.5%). This change may be, at least in part, of nutritional origin. However, Beach et al. *(5)* described adequate and quite frequently high levels of intake for many essential nutrients. Furthermore, the role of intestinal malabsorption seems to be insignificant for two reasons. First, in response to Se oral supplementation, blood Se levels quickly increased *(14)*. Second, impairment in Zn and Cu status subsequent to nutritional or absorption mechanisms should be associated with Se deficiency. In addition, no interaction between Zn intake and plasma Zn levels was found by Graham et al. *(6)*

Another possibility is that Se deficiency reflects the activity of antioxidant defense systems, especially the generation of free radicals *(15)*. An increase of these free radicals might stimulate the TNFα synthesis *(16)*, which in turn could partially explain the loss of body mass and cachexy observed at the end stage of the disease. In the some way, TNFα itself is known to increase the production of free radicals *(17)*. We cannot exclude the hypothesis that low Se values are related to free radical scavenger deficiency, such as glutathione *(18)* or vitamin E *(19)*.

We found that the Zn and Se, which are positively correlated together, are also negatively correlated to β_2 microglobulin values, markers of immune system activation. These relations suggest that blood Zn and Se are markers of disease activity progression rather than being casually related. The plausibility of this hypothesis is further supported by recent data showing that some trace element modifications observed in HIV seropositive subjects could be potentially attributable to a direct effect of HIV$_1$ activity *(5)*.

The fact that these relationships disappeared in the group of patients with a survival time below 12 mo is interesting. The lack of correlation between these parameters suggests a total immune derangement and argues for an increased risk factor of unfavorable evolution.

In conclusion, the measurement of trace elements, especially Se, may be a useful marker to predict the HIV infection progression. Better understanding of the mechanisms of trace element abnormalities and the strategies to maintain optimal trace element balance could, thereby, improve the immune function and life quality of these patients.

REFERENCES

1. N. Fabris, E. Mocchegiani, M. Galli, L. Izato, A. Lazzarini, and M. Moroni, *JAMA* **259,** 839 (1988).
2. B. M. Dworkin, W. S. Rosenthal, G. P. Wormser, and L. Weiss, *J. Parenteral. Enteral. Nutr.* **10,** 405 (1986).
3. R. S. Beach, E. Mantero, A. Atienza, F. Van Riel, and M. Fordyce-Baum, *Arch. AIDS Res.* **3,** 225 (1989).
4. G. T. Kensh and M. J. G. Farthing, *Annu. Rev. Nutr.* **10,** 475 (1990).
5. R. S. Beach, E. Mantero-Atienza, G. Shor-Posner, J. J. Javier, J. Szapocznik, R. Morgan, H. E. Sanberlich, P. E. Cornwell, C. Eisdorfer, and M. K. Baum, *AIDS* **6,** 701 (1992).
6. N. M. H. Graham, D. Sorensen, N. Odaka, R. Brookmeyer, D. Chan, W. C. Willett, J. S. Morris, and A. J. Saah, *J. Acquired Immune Defic. Syndrome* **4,** 976 (1992).
7. A. Cirreli, M. Ciardi, C. De Simone, F. Sorice, R. Giordano, L. Ciaralli, and S. Constantini, *Clin. Biochem.* **24,** 211 (1991).
8. B. Hofmann, Y. Wang, W. G. Cumberland, R. Detels, M. Bozorgmehri, and J. L. Fahey, *AIDS* **4,** 207 (1990).
9. J. Neve and A. Peretz, *J. Pharm. Belg.* **43,** 466 (1988).
10. K. W. Beck, P. Schramel, A. Hedl, H. Jaeger, and W. Kaboth, *Biol. Trace Element Res.* **25,** 89 (1990).
11. R. M. Walter, M. H. Oster, T. J. Lee, N. Flynn, and C. L. Keen, *Life Sci.* **46,** 1597 (1990).
12. G. Shor-Posner, E. Mantero-Akenza, R. Beach, J. J. Javier, A. Basit, C. Cabrejos, H. E. Samberlich, D. Feaster, and M. K. Baum, *CR VII International Conference on AIDS—Florence* June (1991).
13. B. M. Dworkin, W. S. Rosenthal, G. P. Wormser, L. Weiss, M. Nunez, C. Joline, and A. Herp, *Biol. Trace Element Res.* **15,** 167 (1988).
14. L. Olmsted, G. N. Schrauzer, M. Flores-Arce, and J. Dowd, *Biol. Trace Element Res.* **20,** 59 (1989).
15. D. J. M. Malvy, *4th International Congress on Trace Elements in Medicine and Biology. Trace Element and Free Radicals in Oxidative Diseases,* Chamonix (France), April 5–9 (1993).
16. O. Yoshie, T. Majima, and H. Saito, *J. Leukocyte Biol.* **45,** 10 (1989).
17. N. Yamauchi, H. Kuriyama, N. Watanabe, H. Neda, M. Maeda, and Y. Niitsu, *Cancer Res.* **49,** 1671 (1989).
18. F. J. T. Staal, S. W. Ela, M. Roederer, M. T. Anderson, L. A. Herzenberg, and L. A. Herzenberg, *Lancet* **339,** 909 (1992).
19. A. Contelle, D. Bonnefont-Rousselot, J. Delattre, O. Lopez, M. C. Jaudon, S. Herson, and J. Emerit, *Presse Médicale* **21,** 1809 (1992).
20. W. R. Beisel, *Med. Clin. North Am.* **60,** 831 (1976).