Correlation of CD4+ T cell count with serum Zinc, Copper and Selenium in HIV positive individuals

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Abstract

The basic role of micronutrients in the pathogenesis of HIV infection still remains a major lacuna in scientific knowledge. Hence, the present study was carried out with an objective to document-
Mean level of serum Zinc, Copper and Selenium in patients with HIV infection vis-à-vis healthy controls, and also to find, Correlation of CD4+T cell count with serum Zinc, Copper and Selenium in HIV infected individuals
This case-control study was carried out with 50 subjects in either arm matched for age and sex. Cases were patients tested positive for HIV by ELISA using two different antigens whereas the controls were healthy subjects. The mean serum level of Zinc shows a positive correlation with CD4 cell count. The serum Se levels declined progressively with falling CD4 cell counts (p<.001). Zn and Se supplementation can be used as a boost in standardized HAART therapy and nutritional programs for HIV positive patients.

INTRODUCTION

Major advances have been made in understanding the biology of HIV infection and significant progress in therapy has occurred in last few decades, the basic role of host nutrition in the pathogenesis of HIV infection still remains a major lacuna in scientific knowledge. Immune function is highly dependent on nutritional status because of large mass and high rate of cellular turn over of immune system make it a major use of nutrients. With the Opportunistic infection, as an added burden in HIV/AIDS patients, the nutrient requirement may increase many fold. Though HAART has considerably facilitated the management of HIV/AIDS where it is available, this costly treatment remains unobtainable in most part of the world. In the developing countries where nutritional problems are already common and expensive drugs are generally unavailable, the identification and correction of micronutrients deficiency may be blessing in disguise. Research study to understand this aspect is of paramount importance. Hence, the present study was carried out with an objective to document:

- Mean level of serum Zinc, Copper and Selenium in patients with HIV infection vis-à-vis healthy controls, and

- Correlation of CD4 cell count with serum Zinc, Copper and Selenium in HIV infected individuals.

MATERIAL AND METHODS

The present study was carried out in Institute of Medical sciences and it's associated SSH Hospital in collaboration with Research Laboratory for Advanced Studies, Department of chemical Engineering, Institute of Technology, Banaras Hindu University.

Selection of Cases: The Subjects were drawn from the patients attending hospital medical outdoor and /or admitted to indoor ward.

Inclusion Criteria:

- All patients who were tested positive for HIV in serum (positivity was determined by ELISA using two different Antigens/Two different test)

- Patients having AIDS defining illness (WHO guidelines)

Exclusion Criteria:

- HIV negative Subjects who tested negative for HIV-1 by ELISA technique

- Patients who did not voluntarily give consent for inclusion in the study.

- Those who didn't fulfill the selection criteria.

- Patients already on Multivitamins/ zinc supplementation in previous 3 months before enrolment in this study

METHODOLOGY

After successful screening a total number of 50 cases were thus included. Another set of 50 individuals' age and sex matched who were non suspects and also not related to the patient were also evaluated. Their sera samples were tested and if found negative for HIV-1 were regarded as controls. All the cases and controls were counseled and were included only when they willingly give consent to enrollment. A detailed history and examination were done and all routine bio-chemical investigations were carried out. Few specific investigations were done on demand of specific situation of a particular patient. Estimation of Zinc, Copper and Selenium in serum was done in all patients at base line and also in healthy controls. All measurements of trace elements in serum was done by atomic absorption spectrometry (Atomic absorption Spectrophotometer ELICO, India, Model No. SL173). All patients had CD4 counts estimation done at base line. Immunophenotyping of lymphocytes was carried out by FACS count (Becton Dickinson, Singapore (BD). Lymphocytes were stained according to the protocol suggested by the manufacturer. In brief, 1 ml of heparinized blood was mixed with 10 µL of monoclonal anti bodies [AntiCD3-FITC, anti CD-PE]. RBCs were lysed using lysing solution and after incubation for 30 minutes, the cells were washed and fixed with PBS. Height, weight were measured and BMI was calculated in each patient. Patients so enrolled wee called at 3 months and 6 months. At every follow up visits CD4 counts was done and trace elements were measured. All patients were offered ART and specific therapy for Opportunistic infections. Data thus obtained were presented in the form of mean and standard deviation. Necessary statistical tests like student's T-test and regression analysis were applied wherever needed.

RESULTS

Figure 1

Table 1: Baseline comparison of cases and control included in the study

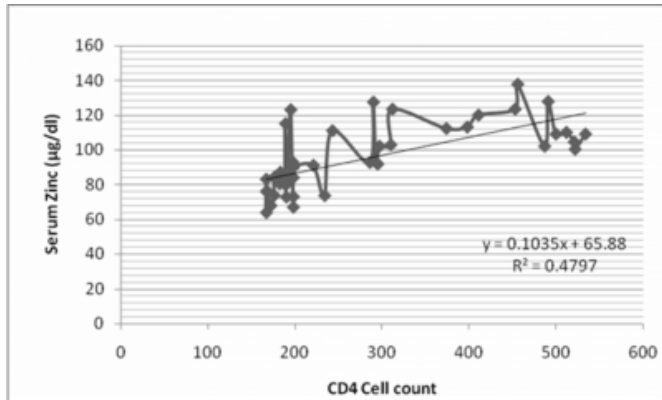|		Controls (N=50)	Cases (N=50)	p-value*
Sex	Males	43	43	
	Females	07	07	
Age in years (Mean ± SD)	Males	31.60±7.05	31.70±7.00	
	Females	31.28±8.69	31.10±8.88	
BMI (Kg/mt²)		18.23±3.20	16.98±4.23	>0.05
Serum Zinc (Zn) (µg/dl)		136.55±9.30	92.60±18.55	<0.001
Serum Copper (Cu) (µg/dl)		104.74±5.06	119.95±7.94	<0.001
Serum Selenium(Se) (µg/dl)		113.34±4.27	72.87±22.20	<0.001
* Mean difference is significant at $\alpha = 0.05$				

Out of 50 cases 43 cases were male and 7 cases wee female. Mean age for cases was 31.70±7.00 years and 31.10±8.88 years in males and females respectively, whereas for control it was 31.60±7.05 years and 31.28±8.69 years in males and females respectively. There was no statistical significant difference between the BMI of Control and Cases (p>0.05). As per 1993 CDC guidelines, majority 56% of patients had advance HIV disease i.e. stage C (Table-1). A total of 18 (36%) patients were asymptomatic HIV sero-positive cases. The stages e.g. A, B and C are commensurate with low (<200 cells/µl), moderately low (200-500cells/µl) and high (>500 cells/µl) CD4 cell count respectively. Irrespective of sex more than 56% of our cases had a mean CD4 cell count below 200cell/µl (Table-2).

Patients with HIV/AIDS were found to have a mean serum zinc level of 92.60±18.55 µg/dl as compared to 136.55±9.30 µg/dl in controls. The difference with the healthy control subjects was highly significant (p<0.001) (table-1). Hypozincemia became very sever with progression of disease (Table-2). Serum Zn level was 83.12±12.29 µg/dl in patients with CD4 cells count < 200/dl as compared to 108.05±16.95 µg/dl and 106.68±4.11 µg/dl respectively with CD4 cell count 200-500cell/dl and >500 cell/dl (p<0.001).

Figure 2

Figure 1: Scatter-plot showing correlation between serum Zinc and CD4 cell counts in HIV infected cases

There was a moderate degree of correlation between serum zinc and CD4 cell count (r=0.6926, r^2 =0.4797) (Figure-1). Similarly marked Zn deficiency has been observed in HIV infection at various stages of illness. Serum zinc level was 111.58±12.68 µg/dl in patients of stage A as compared to 83.12±12.29 µg/dl in patients with stage C (p<0.001).

Figure 3

Table 2: Serum level of micronutrients in accordance to CD4 cell count and stage of the disease in cases

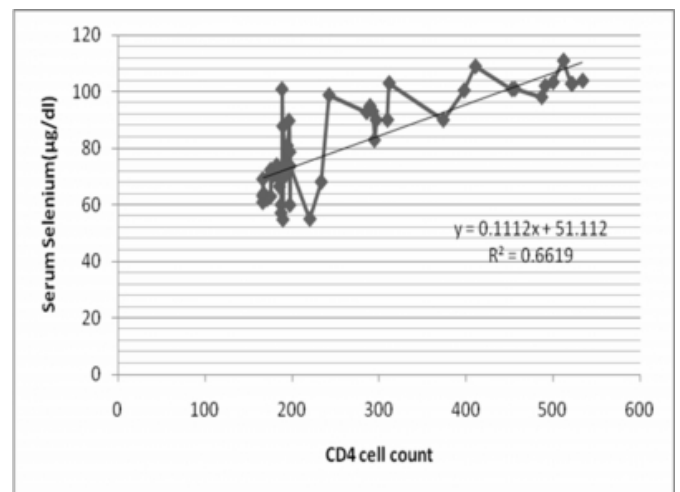| Micronutrients	CD4 cell counts (Cells/µl)			
	<200 (N=28)	200-500 (N=17)	>500 (N=05)	p-value*
Serum Zinc(Mean ± SD)	83.12(±12.29)	108.05(±16.95)‡‡	106.68(±4.11)‡‡	<0.001
Serum Copper (Mean ± SD)	124.30(±8.24)	116.63(±10.69)	120.68(±6.14)	>0.05
Serum Selenium(Mean ± SD)	70.62(±10.21)	92.26(±13.40)‡‡	104.90(±4.01)‡‡§	<0.001
Copper: Zinc ratio (Cu/Zn) Mean ±(SD)	1.52(±0.23)	1.10(±0.21)‡‡	1.18(±0.06)‡‡	<0.001
	Stage of the disease			
	Stage A (N=18)	Stage B (N=04)	Stage C(N=28)	
Zinc (Zn) (Mean ± SD)	111.58(±12.68)	92.05(±15.23)††	83.12(±12.29)††	<0.001
Copper (Cu) (Mean ± SD)	120.41(±10.94)	118.03(±5.14)	124.30(±8.24)¶	>0.05
Selenium(Se) (Mean ± SD)	98.46(±7.40)	78.55(±20.63)††	70.62(±10.21)††	<0.001

(Note: All statistical calculation were done at a significance level of 5%)
* p- value obtained from One Way ANOVA test, ‡‡ p- value <0.001, when compared with CD4 cells count <200(cells/ µl), § p- value <0.05 when compared with CD4 cells count <200-500 (cells/ µl), †† p- value<0.001, when compared with stage A, ¶ p-value <0.05, when compared with stage B

The mean level of serum Cu in control (Healthy subjects) was 104.74±5.06µg/dl. The mean serum copper in HIV infected cases was 119.95±7.94µg/dl and was significantly higher than healthy controls (p<0.001) (Table-2). Overall 84.62% patient had high mean serum Cu as compared to healthy controls. All patients in stage A and B and 84% in Stage C had high serum Cu. The rise in serum Cu level was inversely related to severity cef stage of disease i.e. higher serum level in early stage of the disease with CD4 cell count >200 cell/µl (p<0.001), but the relation ship was not

sustained in the late stage of disease (Table-2). In our study Cu/Zn in cases with severe Zn deficiency (<75µg %) was 1.75±0.24 as compared to1.28±0.24 when serum zinc level is >75 mg/dl (p=0.025). Cu:Zn ratio in patients with CD4 cell counts <200 was significantly lower as compared to cases with CD4 count >200 indicating higher serum copper levels in early phase of the disease (Table-2). The serum Selenium (Se) value in healthy controls was 113.34±4.27µg/L as compared to HIV cases in whom the mean serum Se values recorded a fall to the tune of 30-40 µg/L with a mean of 72.87±22.0 µg/L (p<0.001).

Figure 4

Figure 2: Scatter-plot showing correlation between serum selenium and CD4 cell counts in HIV Infected cases

Severe Se deficiency was found only in Stage B and C of HIV. 25% of stage C patients had severe serum Se deficiency as defined by serum level <60µg/L. The present study overall 9 cases (18%) cases had severe Se deficiency (<60µg/L). Severe Se deficiency was present only in the late stage of the disease and with low CD4 cell counts. Lower the CD4 cell count lower is the serum selenium concentration in HIV +ve cases.

DISCUSSION

The present analysis confirmed that in Indian patients with HIV there is male dominance and male/ female ratio varying from 2.4:1 to 3:1 [1,2]. It may be due to the fact that in our society females prefer to stay indoor and don't come forward to seek medical aid until very late. In our study we have noticed that the more severe the diseases the less will be the CD4 cells count. This data are in tune with the data reported from India as well as from India [1]. The obvious implication of these observation is, that clinical diagnosis is usually made late in our setup. It is neither mandatory nor

economically viable to propose the use of CD4 cell count for these purposes of diagnosing stage of HIV infection/AIDS in our set up. In deed for countries like India and others in south-east Asia there is a need to revise these definition criteria immediately to add sensitivity and specificity reliance on CD4 count be promoted. Micronutrients play a critical role in the proper functioning of the immune system. Thus in HIV where there is profound immune-suppression, there occur deficiency of many micronutrients. Our results also indicate towards this truth. In HIV cases there is zinc deficiency, an essential element for the functioning of CD4 cell counts. In our study we have seen that serum Zn level were directly proportional to the CD4 cell count, lower the CD4 cell count, lower is the Zn level. Similarly marked Zn deficiency has been observed in HIV infection at various stages of illness [3] . There fore Zn deficiency may be a co-facto for progression of disease. Many studies have reported that Zn deficiency is not a common contributory factor for HIV/AIDS or clinical expression and that HIV infection doesn't induce Zn deficiency [4] . Serum Copper (Cu) value have been reported to be significantly higher in infection and inflammatory states [5] . This rise has been attributed to an increase hepatic synthesis and release of Ceruloplasmin. The mean level of serum Cu in control (Healthy subjects) was 104.74±5.06μg/dl compared to 119.95±7.94μg/dl in cases. The value matches well with the values reported from else where in the county and abroad [5,6,7,8] . The values were not affected by age, sex or community. Serum Cu is high in both symptomatic as well as in asymptomatic cases but prevalence is more in early stage of disease Though many studies have corroborated to the finding obtained in our study, only few correlated it to the stage of the disease and/or CD4 cell counts. A study on HIV patients with opportunistic infection had resulted that in these patients there was a significantly higher serum Cu concentration [9,10] . In contrast Heise et al, 1989 didn't demonstrate any difference in serum Cu level in HIV infected individual as compared to healthy individuals [11] . To some extent our study has shown that the rise of serum Cu correlated inversely with diminution in serum Zn level. However, this relationship was not sustained when the disease was advanced.

Many investigators have utilized plasma Cu/Zn ratio for clinical assessment of Zn deficiency in several disease [12] . The mechanism of alteration in plasma Cu/Zn ratio is still elusive [13,14] . In our study Cu:Zn ratio in patients with CD4 cell counts <200 was significantly lower as compared to cases with CD4 count >200 indicating higher serum copper

levels in early phase of the disease. A study involving 121 HIV positive homosexual men concluded that Cu: Zn ratio>1 was associated with increased mortality [15] . This plasma Cu: Zn ratio may be a useful predictor of survival and disease progression in HIV patients.

The values of serum selenium obtained in our study were independent of age and sex and matched to those observed else where in the world [16]. Selenium deficiency is responsible for early progression of disease and mortality in HIV [17] . Se deficiency has been documented in both HIV and AIDS patients in both plasma and red blood cells. Similar to our study many researchers have reported a low level of serum Se in HIV infected cases [18,19,20,21] . In our study severe Se deficiency was present only in late stage of disease and with low CD4 cell counts. This was in contrast to the findings that frank deficiency of Se in 14.8% patients and marginally low levels in 57.4% of asymptomatic HIV positive males, but some of the researchers documented that Se deficiency was severe only in Cases with AIDS and not in asymptomatic HIV cases [19,23] . Association of lower Se levels with progression of disease has been documented in several studies. Similarly in our study we have got a strong correlation between serum Selenium and CD4 cell count (r=0.8078). Previously several researches have documented that selenium deficiency in HIV patients was independent of malabsorption [20,24] and there is a correlation between both CD4 cell and serum Se with mortality and OI in HIV positive individuals [23] . Some of the research had shown that HIV positive patients with low serum Se level had significant 20 fold risk of OI than hose with adequate serum Se [26] .

CONCLUSION

Existence of micronutrient deficiency state has been established, and our study does add to this knowledge. Zinc has potential to boost immune system in more tan one way. Replacement therapy with Zn known to reverse the process to some extent, the therapy itself is very economical too. Depletion of Se is another hall mark in this disease and poses high risk for OI. As more HIV infected individuals around the world are initiated on effective anti-HIV therapy, the need to maximize durability of viral suppression will become increasingly important. Data are needed on the role that micronutrient status may play on low-level viral replication among subjects on therapy. For HIV-infected individuals with adequate viral suppression, but inadequate CD4+ cell counts, micronutrients could play a role in boosting the immune response. Thus Zinc and Selenium

supplementation alone or in combination with other micronutrients can be used to give a boost to HAART therapy and an also be a part of nutritional program in HIV positive patients.

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