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Nutritional Status and Serum Zinc Levels in HIV Infected Individuals Compared to Control Subjects Undergoing Anti Retro Viral Therapy (ART)

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Abstract:

Human immunodeficiency virus (HIV) infected individuals are prone to malnutrition due to increased energy requirements, enteropathy and catabolism. Trace element such as zinc have major role in maintaining a healthy immune system. HIV-infected subjects receiving ART and pre-ART subjects were taken and Nutritional assessment was done after an interview and physical examination based on clinical and anthropometric parameters. Body mass index (normal range 18.5–27 kg/m2 based on age) of less than 16, 16–16.9 and 17–18.4 kg/m^2 were considered as severe, moderate and mild malnutrition respectively. Fully automated analyzer measured serum level of zinc. Mild, moderate and severe malnutrition were detected in 14.28%, 7.14%, 7.14% of the HIV-infected subjects receiving ART and 64.28 % of mild malnutrition were found in pre-ART subjects. The study concluded that malnutrition and serum zinc deficiency are common in HIV-infected patients and early evaluation of nutritional status of these subjects and providing appropriate nutritional support and mineral supplementation along with the specific anti-retroviral treatment are recommended.

***Key words**: ART, HIV, Malnutrition, Zinc.*

Introduction:

Human immunodeficiency viruses (HIVs) are lentiviruses (a member of the retrovirus family) evolved to establish chronic persistent infection with gradual onset of clinical symptoms. Humans and chimpanzees are the only hosts for these viruses. CD4 T cells are also known as helper cells and play a vital role in maintaining the integrity of the human immune system. A primary target of HIV is CD4 T cells which are preferentially depleted during the course of the disease. CD4 T cell counts is very crucial for monitoring the rate of progression to AIDS, both for initiating prophylaxis for opportunistic infections as well as monitoring the impact of antiretroviral therapy (ART) [1]. Despite dramatic advances in basic virology and clinical management, HIV infection has developed into a worldwide pandemic, with tens of millions of individuals infected by the virus and many millions more affected by it.

Human Immunodeficiency Virus (HIV) infection is a major health problem in the world and HIV infected individuals are vulnerable to malnutrition due to several factors including inadequate nutrient intake (anorexia, gastrointestinal complications such as nausea and vomiting, oral and esophageal sores), nutrient loss (mal absorption and / or diarrhea), metabolic alteration (increased protein turn over and changes in fatty acid metabolism) and drug - nutrient interactions [2]. Although malnutrition is more frequent at the end of HIV infection course, it can also occur at the onset of the disease, before severe immune suppression [3].

Figure 1 provides an overview of the interrelationship between nutritional status, HIV infection, and the impact on immune function. In HIV infection, zinc plays specific roles as an anti-oxidant, immune - modulator and a possible direct anti-viral agent. Zinc, in vitro, has been found to inhibit cell death mediated by tumor necrosis factor (TNF), a cytokine linked to cellular apoptosis and wasting syndrome in HIV. The HIV virus binds to zinc ions in T-cells in order to produce pro-viral peptides which form the basis of new infectious viral particles. The HIV-1 protease

enzyme then cuts the viral chains to form new infectious viral particles, which are released into the circulation and infect new immune cells. Depressions in blood zinc levels in HIV/AIDS may reflect the presence of acute infections; serum zinc levels decrease as hepatic zinc uptake increases, reflecting zinc's role as an acute-phase reactant. Opportunistic infections have been shown to lower serum zinc levels, with depressed serum a level lasting long after the infection is resolved [4].

The present study aimed to compare the mean serum zinc level in HIV Sero positive patients receiving Antiretroviral therapy (on ART) and the patients who were not initiated the ART (Pre ART) with control subjects and to correlate the serum Zinc levels with malnutrition and to discuss the impact of nutrition interventions on HIV progression and mortality.

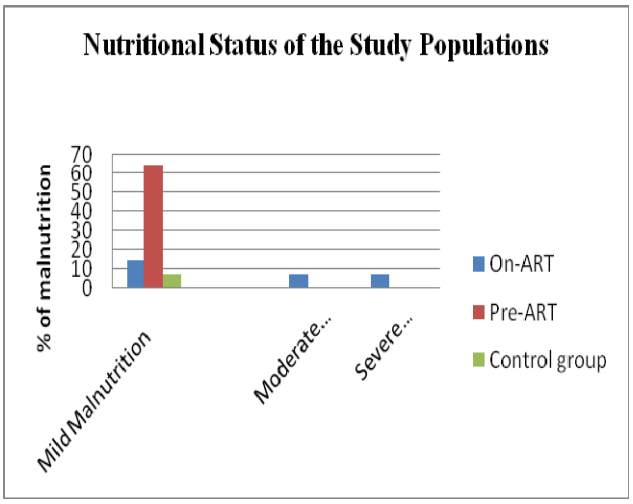

Figure 1: Nutritional status of the study populations

Materials and Methods:

Materials

HIV Spot Kit supplied from National AIDS control organization (NACO), India. BD Tritest CD3 / CD4 / CD5 reagent was a gift sample from Clontech Discovery Labware Immunocytometry Systems, Pharmingen, India. Commercial Zinc Kit obtained from Crest Biosystems, Goa, India.

Sampling Strategy

Totally 42 subjects were enrolled in this study, 28 HIV infected subjects of which 14 patients were receiving anti retro viral treatment (on-ART) (7F, 7M; mean of ages =40.29 ±10) and 14 subjects were not initiated anti retro viral treatment (Pre-ART) (10F, 4M; Mean of ages = 32.6 ± 6.4). 14 control individuals (4F, 10M) with the mean age of 43.14 ± 20.4 years were included in this study.

The control subjects were healthy males and females related to HIV infected individuals (WHO accompanied HIV infected patients) without any medical problem at the time of study or history of any chronic disease and with negative anti HIV antibody test.

Data collection

In primary study, baseline information was collected from subjects on a range of sub economic and demographic characteristics including geographic area where they were raised, education level attained, household ownership of assets and marital status.

Nutritional Assessment

Nutritional status of each patient was assessed using anthropometric parameters. Body weight was determined to the nearest 0.1 kilogram using adult balance and standing weight was determined to the nearest one centimeter (cm) using Standiometer provided by National AIDS control Organization (NACO). Body Mass Index (BMI) was calculated using the following formula [5].

$$\text{BMI (kg/m}^2\text{)} = \frac{\text{Body weight (kg)}}{\text{Height (m}^2\text{)}}$$

BMI (normal range 18.5-27 kg / m^2 based on age) of less than 16, 16-16.9 and 17-18.4 kg/m^2 were considered as severe, moderate and mild malnutrition respectively [6]. Weight changes during past six months up to 10% body weight loss was considered as severe weight loss [7]. According to definition of Centre of Disease Control and prevention (CDC), wasting defined as an involuntary weight loss of greater

than 10% of baseline body weight during the past six months [8].

Clinical assessments including medical history and physical examination were conducted to identify any sign or cause of malnutrition, physical appearance, opportunistic infections and diarrhea symptoms of gastrointestinal distress such as nausea and vomiting, medications, use of herbal supplements and functional status were considered in clinical assessment.

Estimation of Cd4 Count in Serum

Immunophenotyping was done by using BDFACS caliber Flow Cytometer. Immunophenotyping characterizes cells at different stages of development through use of fluorescent labeled monoclonal antibodies against surface markers [9].

Specimen collection

1ml of blood was collected aseptically by vein puncture into a sterile EDTA (Ethylene Diamine Tetra Aceticacid). BD Vacutainer ™ blood collection tube. Pipette out 20 µl of BD Test CD3 / CD4 / CD45 reagent into bottom of the tube. Cap the tube and vortex gently to mix, incubated for 15 minutes in dark at room temperature (20-25^{0}C) then added 450µl 1xBD FACS lysing solution (containing Diethylene glycol and Formaldehyde) [10] to the tube and again vortex gently to mix and incubated for 15 minutes at room temperature.

The sample is placed in flow cytometer. The flow cytometer equipped with 488nm laser capable of detecting light scatter and 3color fluorescence with emission detectable in 3 ranges 515-545nm, 562-607nm and >650nm. BD Multiset version 2.0 software was used to generate the CD4 count and absolute lymphocyte count results. Results were reported as the percentage of positive cells per lymphocyte population. Optical density (OD) was given as the number of positive cells per µl of blood (absolute count). Estimation of serum zinc done by colorimetric method using Rx Daytona fully automated analyzer.

Estimation of serum zinc by calorimetric method

Five ml of fasting blood samples were collected from all HIV infected and healthy subjects. Blood samples were centrifuged at 3000g for 5 minutes. The collected serum was stored at -70^{0}C until analysis. All glass wares and bottles used for separation of serum and further analysis were previously soaked in 10% nitric acid and rinsed thoroughly with deionized water. Commercial zinc kit contains buffer reagent (L1), colored reagent (L2) and zinc standard (S) (200mg/dl) [11]. Working reagent was prepared by mixing the L1 and L2 reagents.

Four dry clean test tubes were taken and labeled as Blank (B), Standard (S), Sample blank (SB) and Test (T). 1ml of zinc buffer reagent (L1) was pipetted out into B, S and T test tubes. 0.05 ml of distilled water was added into blank test tube and 0.05 ml of zinc standard was added into standard test tube and finally 0.05ml of sample was added into test. All the test tubes were mixed well and incubated at room temperature for 5 minutes. Absorbance of blank (Abs B), Standard (Abs S), sample blank (Abs SB) and test samples (Abs T) was taken against distilled water. The results were read out with the help of fully automated analyzer Rx Daytona. Yellow filter with wavelength of 570nm and light path of 1cm was used and readings are recorded in µg/dl [12].

$$\text{Zinc in mg /dl} = \frac{\text{Abs. T} \times 200}{\text{Abs. S}}$$

For this study, Zn deficiency was defined as a serum level < 67 µg/ dl. Using the cut-off referenced by Bender and Bender [13] for normal plasma Zn (67–183 µg/ dl).

Statistical Analysis

Data was analyzed using SPSS software version 17.0. Normal distribution of data were assessed using kolmogorov-Smirnov test and independent sample t-test was used to compare numeric

variables such as age, weight, height, serum zinc between HIV infected patients on Anti retro viral therapy and patients not on treatment compared with healthy subjects.

Results:

Twenty eight HIV-infected adult subjects with the mean age of 36.46 ± 8.269 years and 14 control with the mean age of 43.14 ± 20.4 years were included in this study. HIV infected subjects were sub-classified into two groups based upon the anti retro viral treatment. 14 patients were receiving anti retro viral treatment (on ART) with the mean age of 40.29 ±10 years old and 14 subjects were not initiated anti retro viral treatment (Pre-ART) with the mean age of 32.6 ± 6.4 years included in this study.

Table 1: HIV infected and Control group Baseline Characteristics

Parameters	HIV Infected Group		Control Group	p-value
	Receiving Treatment (on- ART) (mean ± SD) n=14	Untreated (pre-ART) (mean ± SD) n=14	(mean ± SD) n=14	
Number of subjects Age (year)	40.29 ± 10.110	32.64 ± 6.428	43.14 ± 20.444	<0.0001
Height (Cm)	158.07 ± 9.161	154.36 ± 6.640	159.36 ± 6.172	
Weight (Kg)	53.57 ± 8.864	46.86 ± 7.5 84	63 ± 8.209	
BMI (kg/m2)	21.457 ± 3.124	19.65 ± 2.697	24.407 ± 2.516	
CD4 (Cell/mm3)	449.21 ± 236.50	454.89 ± 168.426	974.43 ± 105.16	
ALC (cells/ (µl)	2360.14 ± 953.08	2541.64 ± 618.41	7421.7 ± 1819.4	

Table 2: Nutritional Status of the Study Populations

Category	HIV Infected Group (%)		Control group (%)	p-Value
	On-ART	Pre-ART		
Mild Malnutrition	14.28	64.28	7.14	<0.001
Moderate Malnutrition	7.14	0	0	
Severe Malnutrition	7.14	0	0	
Normal	71.42	35.71	92.8	

Table 3: Serological profile of study subjects

Zinc in µg/dl	HIV Subjects		Control
	Receiving Anti Retroviral therapy(on-ART)	Untreated (Pre-ART)	
0-10	0	0	0
11-20	0	0	0
21-30	2	1	0
31-40	1	7	0
41-50	6	5	0
51-60	5	1	0
61-70	0	0	4
71-80	0	0	7
81-90	0	0	3

Table 4. Mean ± SD of serum Zn concentrations and prevalence of deficiency in HIV infected and Control group

Parameter	HIV Infected Group		Control Group
	Receiving treatment (on-ART)	Untreated (pre-ART)	
Zinc concentration (µg/ ml)	46.68 ± 8.813	39.27 ± 8.564	76.955 ± 6.999
Number of individuals with zinc deficiency	14	14	1
p- value	0.898	0.998	0.843

Nutritional status of study population was determined using body mass index as shown in Figure1. Subjects were classified according to degree of malnutrition. Graph 1 shows that mild malnutrition was more in pre-ART group compared to control group. Persons receiving ART were less prone to mildmalnutrition and thus very less percentage of these subjects were effected with severe and moderate malnutrition.

Serum zinc levels were evaluated for all study groups. Normal zinc level (60-120µg/dl). Maximum number of HIV subjects receiving ART had zinc levels between 51-60µg/dl as shown in figure 2, whereas untreated subjects were having zinc levels between 31-40 µg/dl.

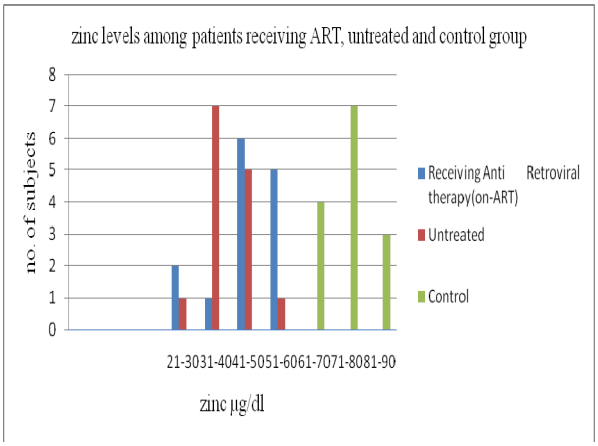

Figure 2 : Zinc levels among patients receiving ART, untreated and control group.

Figure 3 shows that the mean zinc levels in ART subjects were higher than Pre- ART subjects but zinc levels in both ART and Pre-ART subjects were less than normal value. Maximum number of control subjects had normal zinc level (71-80 µg/dl).

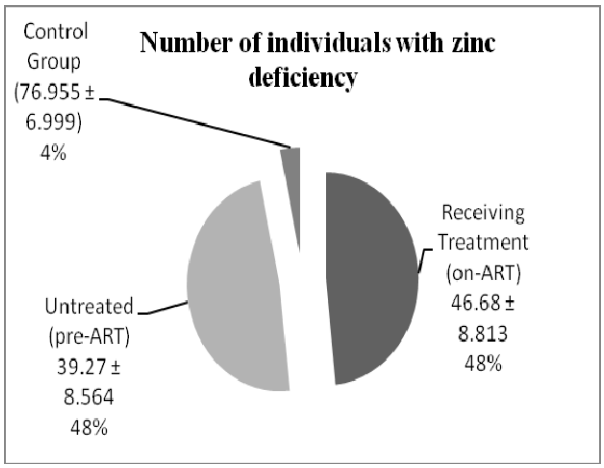

Figure 3: Number of individuals with zinc deficiency

Normal zinc level is 60-120µg/dl. The study subjects having zinc level less than 60 µg/dl considered as zinc defecieny. Figure 2 shows that zinc defeciency occurred in both ART and Pre- ART group as compared to control group. The prevalance of zinc defeciency found to be more in HIV infected group compared to control.

Discussion:

In this study 28 HIV-infected subjects were evaluated for nutritional status and serum zinc levels and the results thus obtained were compared with 14 HIV sero-negative control subjects. Comparisons of baseline characteristics including age, weight, height, BMI, CD4 lymphocytes count and absolute

lymphocytes count of HIV-infected and healthy subjects were shown in Table1. None of the HIV infected individuals express any significant past medical problem and history of vitamin or mineral supplementary consumption. Most of the HIV infected individuals were pale, 60% of them were anemic (serum hemoglobin level of less than 12 g/dl) and all of them had some degree of temporal atrophy.

Gastrointestinal symptoms including nausea, vomiting, diarrhea and decreased appetite were observed in 9% of patients [14]. Significant and severe recent weight losses were detected in 3% of HIV patients receiving ART (on-ART) and 7% of ART untreated patients (Pre-ART) respectively. The most prevalent route of HIV infection in these patients was sexual contact. Most of the subjects in present study were illiterate, unemployed and married with an insufficient monthly income [15]. Due to educational, socioeconomic and behavioral characteristics, they were more vulnerable to malnutrition [16]. Malnutrition is a significant clinical problem in HIV infected individuals and wasting has been associated with disease progression and increased mortality. Although malnutrition is usually encountered at the advanced phase or end of the HIV-infection course, however, as seen in this study it may also occur in the first stages of the HIV-infection as well. As anthropometric measurements provide inexpensive and non invasive method to evaluate nutritional status [17] these parameters were used in this study to evaluate nutritional status of HIV-infected individuals. The nutritional status of the study populations were shown in Table 2. The results of this study showed mild, moderate to severe malnutrition in 14.28%, 7.14%, 7.14% of the HIV-infected subjects receiving ART and 64.28 %of mild malnutrition were found in untreated (pre-ART) subjects. None of the pre-ART subjects express any moderate or severe malnutrition. 71.42% of on-ART, 35.71% of pre-ART and 92.8% of control subjects were found to be normal. 7.14% of control subjects had mild malnutrition. Based on CDC definition, 12% of the patients had wasting syndrome. Mild malnutrition was the most prevalent type of malnutrition in HIV-infected individuals observed in 39.28% of patients followed by both moderate and severe malnutrition observed in 7.14%, 7.14% of the patients respectively. The Mean serum Zinc concentrations and prevalence of deficiency in HIV infected and healthy individuals were shown in Table 4. The mean concentrations of zinc in HIV-individuals receiving ART and untreated patients (46.68 ± 8.813, 39.27 ± 8.564) were significantly lower than control group (76.955± 6.3339). Based on World Health Organization nutritional recommendations for HIV infected persons, adequate nutrition is critical for health and survival for all subjects regardless of HIV infection condition [18]. Following presentation of the study results, nutritional assessment is a part of clinical assessment of HIV infected individuals. Because adequate intake of zinc is essential for maintaining immune system function, HIV-infected individuals are particularly susceptible to the effects of zinc deficiency. Decreased serum zinc concentrations have been associated with advanced disease and increased mortality in HIV patients. This study results revealed that the zinc levels were lowered in HIV patients irrespective of antiretroviral treatment compared to control. Study results also revealed that malnutrition and serum zinc deficiency are common in HIV-infected patient.

Conclusion:

Specific role of these micronutrients remain largely undefined. Depressions in blood zinc levels in HIV/AIDS may reflect the presence of acute infection. Declining plasma and serum levels in HIV patients made us to undertake the estimation of serum levels in the study population from ART centre. The present study shows that malnutrition and serum zinc deficiency are common in HIV-infected patients irrespective of antiretroviral therapy and early evaluation of nutritional status of these subjects and providing appropriate nutritional support and mineral

supplementation along with the specific anti-retroviral treatment are recommended. Various studies shown that zinc supplementation in AIDS patients decreased the incidence of opportunistic infections. However, the HIV virus also requires zinc, and excessive zinc intake may stimulate the progression of HIV infection. These contradictory results indicate that further research is necessary to determine optimal zinc intakes for HIV-infected individuals. Long term studies about serum zinc level in HIV/AIDS patients are necessary. Further longitudinal independent risk factor for increased morbidity and mortality.

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