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RESEARCH ARTICLE

TO DETERMINE THE ZINC LEVEL AND ITS CORRELATION WITH CD4 COUNT BEFORE AND AFTER ZINC SUPPLEMENTATION IN HIV INFECTED PATIENTS

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Abstract

Introduction: Human immunodeficiency virus (HIV) infection is a state of profound immunodeficiency. Zinc is a known to maintain a healthy immune system. Low levels of zinc in plasma predict a threefold increase in mortality related to HIV, while adequate levels of the zinc in the blood have been associated with a slow disease progression, decrease in viral load and HIV RNA, increase in CD4 count, a decrease in the risk for diarrhea, bacterial and opportunistic infections.Zinc deficiency which is common among the HIV infected persons, is overlooked So, we conducted this study to analyze the level of serum zinc level in HIV infected patients and to find out correlation between serum zinc level and CD4 count before and after 6 months of zinc supplementation.

Methods: This study enrolled 54 HIV infected patients above 18 years of age who visited the Medicine outpatient department or the ART Centeror admitted to the medicine ward, Regional institute of Medical Sciences (RIMS), Imphalfor a period of 2 years(October 2019 to October 2021). Serum zinc level, CD4 count, HIV viral load before and after 6 months of zinc supplementation and other routine blood investigations were done. Zinc deficiency was defined by serum Zinc level (< 70mcg/dl).Zinc supplementation (12 mg/day for female, 15 mg/day for male) had been done for the duration of 6 months in the form of oral zinc sulfate.

Result: A total of 54 HIV infected patients under ART were enrolled in our study. As per WHO clinical staging, majority of the patients (79.6%) belonged to stage1, the mean duration ± SD of HIV was 10.9815 ± 8.1229 years. Most of them (46.3%) have CD4 count in the range of 200-500 cells/mm3 while maximum patients (70.4%) had viral load <50 (Target not detected). Majority of the patients (50%) had serum zinc level in the 60-70 mcg/dl while 18.5% of them had zinc level < 50 mcg/dl. There was statistically significant improvement in level of serum zinc and CD4 count, but no improvement in viral load after 6 months of supplementation of zinc.The association with CD4 count was not statistically significant.

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Conclusion: The present study showed that the majority of patients (92.6%) had zinc deficiency at baseline. There was increase in the mean serum zinc level, CD4 count after 6months of zinc supplementation but no improvement in viral load. Zinc deficiency was reduced to only (31.5%) patients. Hence, this study conclude that zinc deficiency is common among HIV patients and zinc supplementation as an adjunct therapy can significantly improve zinc deficiency and CD4 count in HIV infected patients on ART without any adverse side-effects of zinc supplementation thereby decreasing the risk for bacterial and opportunistic infections.

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Introduction:-

HIV directly weakens the immune system of the person resulting in malnutrition; thereby worsening the effect of HIV and contributing to more rapid progression to AIDS. Although adequate nutrition including minerals and vitamins cannot cure HIV infection, but it increases resistance to superinfection and disease progression. Maintaining a good nutrition status is important to support the overall health and immune function of people living with HIV while suboptimal nutrition results in immunological deficiencies(1,2).Nutrition is a significant component of comprehensive care for individuals livingwith HIV particularly in settings where malnutrition and food insecurity are prevalent. People with HIV infection and AIDS requires nutrients that contain macronutrients (carbohydrates, proteins, fats) and micronutrients (vitamins and minerals) in sufficient amount in order to build strong immune system in prevention of the emergence of opportunistic infections. (3). Nutritional supplements taken concurrently with ART can promote weight gain, improve immune response and improve physical activity in HIV-positive patients that present at ART initiation with weight loss (4). .

Of all the minerals, Zinc is recognized as an important mineral in maintaining a healthy immune system factor. Low levels of zinc in people living with HIV can increases mortality, while adequate levels of the zinc in the blood retards the disease progression and a decrease emergence of opportunistic infections (5).Zinc deficiency is prevalent among the I.V drug HIV abusers. Zinc nutritional supplementation increases in HLA Dr + cells and stimulates lymphocyte transformation (5,6).A number of immunological impairments owing to zinc deficiency such as decreased CD4 lymphocytes. Zinc deficiency is associated with increased risk of diarrhea, pneumonia, malaria (7). Moreover, zinc has been reported as an antioxidant in cell cultures andanimal models and zinc supplements delay the progression of HIV patient on ART (8). The importance of proper zinc nutrition for immunity and its demonstrated benefits invarious infectious disease had motivated investigations of zinc nutrition in PLHIV. Previousstudies conducted by Asikin et al (3) and Contreras-Martinez et al(9) concluded that zincsupplementation in HIV patients resulted in increased CD4 count.Dearth of similar study of Zinc deficiency in PLHIV in Manipur state instigated us to conduct,analyze the level of serum zinc level in HIV infected patients and to find out correlation between serum zinc level and CD4 count before and after zinc supplementation.

Material And Methods:-

This study was conducted at the Department of Medicine and ART Centre in collaboration with Department of Biochemistry of Regional institute of Medical Sciences (RIMS), Imphal, India, for a period of 2 years (October 2019 to October 2021). Serum zinc levels were measured in 54 HIV positive patients.

Inclusion Criteria

HIV positive patients aged >18 years who visited the Medicine outpatient department or the ART Center, RIMS, or were admitted to the medicine ward were included in the study after obtaining informed consent.

Exclusion criteria

Included terminally ill patients, those with co-morbid conditions (liver failure, renal failure, sepsis, malignancy), pregnancy females, HIV positive patients already on zinc supplementation and those not giving consent.

Study variables
The study included demographic data such as age, sex, past history, high risk behavior/modes for transmission, co-morbidities, co infection with hepatitis B, C, socio-economic status, BMI and ART drug history.

All the routine examination was done as per NACO recommendation and after informed consent. A total confidentiality was maintained by coding of patient's data throughout the study. Serum zinc level before and after zinc supplementation, CD4 count before and after zinc supplementation, HIV viral load and other routine blood investigations (complete hemogram, kidney function test, liver function test, random blood sugar and fasting lipid profile) were done.

Study tools
Included Viral load PCR, Randoximolaautoanalyser (manufactured 2007, UK),Quantichrom Zinc Assay kit (DIZN-250) and Beckman spectrophotometer.

Conversions: 1 µM zinc equals 6.5 µg/dl or 0.065 ppm (65 ppb)(10)

Operational definition
Zinc deficiency was defined by serum Zinc level (< 70mcg/dl)(11)

Intervention
Zinc supplementation (12 mg/day for female, 15 mg/day for male) had been given for the duration of 6 months in the form of oral zinc sulfate with monitoring by nutritionist in ART centre as patient was come every month to collect ART drug.

Statistical Analysis:
Statistical analysis was performed using SPSS (version 27.0; SPSS Inc., Chicago, IL, USA) and Graph Pad Prism version. Mean, standard, two-sample t-tests, paired t-tests and chi-squared test ($\chi 2$ test) were used for statistical hypothesis. All p-values < 0.05 were considered significant.

Approval of Research Ethics Board and Informed consent:
The study was approved by Research Ethics Board Regional Institute of Medical Sciences, Imphal.(Reference No-A/206/REB-Comm (SP)/RIMS/2015/559/37/2019).

Results:-

A total of 54 HIV infected patients under ART were enrolled in our study after informed consent for a period of 2 years. The baseline characteristics and their mean of the study subjects were given in table I and II respectively.The mean age ± SD of study subjects was 41.5 ± 12.0748 years with majority of them (31.5%) in age group 41-50 years. Majority of them were males (55.6%) while females were 44.4%. The mean BMI ± SD of the patients was 21.47 ± 2.0466 kg/m2). In present study majority of them (75.9%) were not associated with co-morbid illness, (16.7%) patients were hypertensive and (7.45%) were diabetic. The most common route of HIV transmission in our study was heterosexual route (55.6%) followed by IVDU (22.2%), unknown (7.4%), mother to child (5.6%), sex worker (5.6%) and blood transfusion (3.7%). Majority of the study subjects (85.2%) did not have Co- infection while co-infection with hepatitis B was present in 5.6%, hepatitis C in 5.6% and pulmonary TB was seen in 3.7%. As per WHO clinical staging, majority of the patients (79.6%) belonged to stage1 followed by stage 2 (16.7%) and stage 3(3.7%). The mean duration ± SD of HIV was 10.9815 ± 8.1229 years. Most of them (46.3%) have CD4 count in the range of 200-500 cells/mm3 while maximum patients (70.4%) had viral load <50 (Target not detected/TND).Patients were mainly on first line ART (77.8%) while 22.2% patientswere on second line ART. Most of them were on (TENOFOVIR+LAMIVUDINE+DOLUTEGRAVIR)/TLD as First line ART regimen while 6(11.1%) patients were on(ABACAVIR+LAMIVUDINE+DOLUTEGRAVIR)and 6(11.1%) patients were on (ZIDOVUDINE+LAMIVUDINE+DOLUTEGRAVIR) as Second line ART regimen. Majority of the patients (50%) had serum zinc level in the 60-70 mcg/dl while 18.5% of them had zinc level < 50 mcg/dl. There was statistically improvement in level of serum zinc and CD4 count after 6 months of supplementation of zinc.Therefore, after 6 months of zinc supplementation, the mean ± SD serum zinc level increased from baseline60.6667 ± 7.8162 to 70.2278 ± 3.0610 and the mean ± SDCD4 count increased from baseline 460.1296 ± 213.8162 cells/mm3 to 504.0000 ± 223.2176 cells/mm3, given in table III and IV respectively. .

Table I:- Baseline characteristics of study subjects.(N=54).

Characteristics	Study patients (N = 54), n (%)	P -value
Age (in years)		
≤30	12(22.2%)	
31-40	12(22.2%)	0.38978
41-50	17(31.5%)	
51-60	13(24.1%)	
Gender		
Male	30(55.6%)	0.25014
female	24(44.4%)	
Education		
College	20(30.7%)	
Secondary school	17(31.5%)	0.54186
Primary school	16(29.6%)	
Illiterate	1(1.9%)	
Co- morbidity		
HTN	9(16.7%)	
T2DM	4(7.4%)	<0.00001
Nil	41(76%)	
Occupation		
Govt employee	11(20.4%)	
House worker	7(13%)	<0.00064
Nil	28(51.9%)	
Self employed	8(14.8%)	
Mode of HIV transmission		
Blood transfusion	2(3.7%)	
Heterosexual	30(55.6%)	
IVDU	12(22.2%)	0.00038
Mother to child	3(5.6%)	
Sex worker	3(5.6%)	
Unknown	4(7.4%)	
Distribution of coinfection		
Hep B	3(5.6%)	
Hep C	3(5.6%)	<0.00001
Nil	46(85.2%)	
Pulmonary TB	2(3.7%)	
CD4 count (cells/mm3) at baseline		
<100	1(1.9%)	
100-200	6(11.1%)	0.56192
200-500	25(46.3%)	
>500	22(40.7%)	
CD4 count (cells/mm3) after 6 months		
<100	1(1.9%)	
100-200	5(9.3%)	< .00001
200-500	24(44.4%)	
>500	24(44.4%)	
Viral load(copies/ml)		
Target not detected (<50)	38(70.4%)	<0.00001
400-1000	10(18.5%)	
>1000	6(11.1%)	
Viral load(copies/ml) after 6		

months		
Target not detected (<50)	38(70.4%)	<0.00001
400-1000	11(20.4%)	
>1000	5(9.3%)	
Serum zinc (mcg/dl) baseline		
<50	10(18.5%)	
50-<60	13(24.1%)	
60-<70	27(50%)	0.00528
≥70	4(7.4%)	
Serum zinc(mcg/dl) after 6months		
<70	17(31.5%)	0.00012
≥70	37(68.5%)	
Line of ART regime		
First line	42(77.8%)	<0.00001
Second line	12(22.2%)	
ART regime		
TLD	42(77.8%)	
ALD	6(11.1%)	<0.00001
ZLD	6(11.1%)	
Cholesterol level(mg/dl)		
<200	47(87%)	<0.00001
>200	7(13%)	
Triglyceridelevel(mg/dl)		
<150	49(90.7%)	<0.00001
>150	5(9.3%)	

Abbrevations -T- tenofovir, L- lamivudine, D- dolutegravir ,A- abacavir,Z-zidovudine

Table II:- Mean of baseline characteristics(N=54).

Parameters	Mean
Age (years)	41.5000 ± 12.0748
Duration of HIV(years)	10.9815 ± 8.1229
Mean BMI (kg/m^2)	21.4778 ± 2.0466
Haemoglobin (g/dl)	12.5185 ± 1.7024
TLC (per mcl)	7462.2222 ± 1499.2585
Platelet count (per microliter in lakh)	3.0967 ± .7729
RBS (mg/dl)	115.5370 ± 36.5290
Bilirubin (mg/dl)	0.6963 ± 0.3090
Albumin (g/dl)	3.8185 ± 0.5147
Urea (mg/dl)	25.8519 ± 8.9472
Creatinine (mg/dl)	0.7000 ± 0.3629

Table III:- Distribution of mean CD4 COUNT cells/mm^3 at different time interval(N=54).

	Number	Mean	SD	Minimum	Maximum	Median
CD4 Counts cells/mm3 at baseline	54	460.1296	213.8162	99.0000	1124.0000	461.0000
CD4 count cell/mm^3 after 6 months	54	504.0000	223.2176	98.0000	1056.0000	500.0000

Table IV:- Distribution of mean Serum zinc mcg/dl at different time interval(N=54).

	Number	Mean	SD	Minimum	Maximum	Median
Serum zinc mcg/dl at baseline	54	60.6667	7.8162	46.0000	72.3000	62.7000
Serum zinc mcg/dl after 6months	54	70.2278	3.0610	60.7000	75.0000	70.5000

Discussion:-

Despite adequate ART with complete virological suppression,15-18 % of the patients do not achieve successful immune recovery. This phenomenon is called "immunologic failure or immunovirological discordance (IVD)"(12).Poor immune recovery can be due to level of poor baseline immunosuppression, age and nutrition. Intake of specifically Zinc which has immunomodulatory role are successfully used to prevent immune failure and reducing risk of IVD by 4 %.(12).Zinc is the structural component of a wide variety of proteins, neuropeptides, hormone receptors and polynucleotides. Zinc is necessary for the function of immune system. Zinc-dependent hormones/ enzymes such as Cu, Zn superoxide dismutase are important for human body antioxidant defensemechanism, and a necessary ingredient for the formation of thymulin hormone. Thymulin hormone helps in T-lymphocytes formation.Zinc deficiency results in shrinkage of the thymus, impaired cell-mediated cutaneous sensitivity, decrease thymocytes and lymphopenia. This process causes reduction of T-helper 1 (Th1) cells maturation, inhibits IL-2 and IFN-γ production. Primary and secondary antibody responses are reduced in zinc deficiency, especially those needing T-cell help example red blood cells. Also, antibody response and the generation of splenic cytotoxic T cells after immunization are reduced (13).

Additionally, Zinc inhibits tumor necrosis factor (TNF) which plays an important role in development of wasting in AIDS(14,15). Low plasma zinc levels dysregulated cell apoptosis and reduces immunity thereby increasing the risk for bacterial and opportunistic infections.Its Antiviral role has been determined in in-vitro studies such as free virus inactivation, inhibition of denudation, viral genome transcription and interference in polyprotein processing (12) .

The present study showed that the majority of HIV patients (92.6%) had zinc deficiency at baseline which was consistent with the studies by Madueke NM et al(16),Emokpae MA et al(17),Ademasu A et al(18) andFufa H et al (19) .The mean serum zinc level (mean ± SD) of patients was (60.6667 ± 7.8162) mcg/dl which is comparable to mean serum zinc level of (57.27 $\pm$ 8.32) mcg/dl in a study conducted by Wiranguna A et al(20).After 6 months of zinc supplementation there was statistically significant improvement in serum zinc level and CD4 count.After 6 months of zinc supplementation, there was increase in the mean serum zinc level (mean ± SD) of patients (60.6667 ± 7.8162) mcg/dl to (70.2278 ± 3.0610) mcg/dl and zinc deficiency was finally noted in only (31.5%), which was comparable to the study by Jeykumar W et el(21) and Isa L et al(22).At baseline the mean CD4 count was (460 $\pm$ 213.81) cells/mm^3, majority of the patients (87%) had CD4 count >200 cells/mm^3 and (13%) patients had CD4 count <200 cells/mm^3. After 6 months of zinc supplementation the mean CD4 count showed statistically significant ($p<0.05$) increase (504 $\pm$ 223.21) cells/mm^3, (88.8%) patients had CD4 count >200 cells/mm^3 and (11.2%) patients has CD4 count <200 cells/mm^3 which was at par with studies conducted by Asikin A et al(3) and Contreras-Martinez H et al(9).Similar findings in regards of the CD4 countsi.e significant improvement in CD4 count was not observed in a study byHadadi A et al(23) and Silvaetal(12) despite zinc supplementation.At baseline, majority of the (70.4%) patients had viral load < 50 copies/ml (TND) but there was no significant change in viral load seen after zinc supplementation which is consistent with a study conducted by Baum MK et al(24).

Decreased serum zinc was associated with a low CD4 cell count, high viral load (25). HIV RNA is inversely correlated with both CD4+ and zinc level values thereby decreasing the risk of opportunistic infections in HIV with zinc supplementation (26). This applies to adults and children with HIV infection (27).Koch J et al(28) reported in his study that patients with zinc deficiency had a significantly higher incidence of bacterial infection than did patients with normal zinc. However, a low zinc level was not associated with the length of HIV seropositivity, CD4 count, or degree of malnutrition.

Zinc supplementation at nutritional levels delayed immunological failure and decreased diarrhea over time(24). This evidence supports the use of zinc supplementation as an adjunct therapy for HIV-infected adult cohorts with poor viral control.

Conclusion:-

Zinc deficiency was a common prevalence among the PLHIV in our state. Low levels of zinc in plasma predict a threefold increase in mortality related to HIV, while adequate levels of the zinc in the blood have been associated with a slow disease progression, decrease in viral load and HIV RNA, increase in CD4 count, a decrease in the risk for diarrhea, bacterial and opportunistic infections.There was statistically significant positive correlation of HIV with co-morbidites, occupation, modes of transmission of HIV, co-infection with hep B, C, viral load while association with CD4 count was not statistically significant.Therefore, recommends early evaluation of serum zinc

deficiency and zinc supplementation along with anti-retroviral treatment and forunder nourished adult people living with HIV/AIDS. .

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Conflict of interest:
None declared.

Ethical Approval:
The study was approved by the Institutional Ethics Committee.

References:-

1. Onyango AC et al. Assessing nutrient and nutrient status of HIV seropositive patients attending clinic at Chulaimbo Sub-district Hospital, Kenya. J NutrMetab.m2012;2012(3):1-6.
2. Gedle D et al. Food insecurity ans its associated factors among people living with HIV/AIDS receiving antiretroviral therapy at Butajira Southern Ethiopia. J Nutr Food Sci. 2015;5(2):1-6.
3. Asikin A et al. Zinc sulfate increases lymphocyte CD4 count in HIV/AIDS patients at ICUID Dr. Soetomo hospital Surabaya. Fol Med Indones. 2012;48:17-9.
4. Evans Det al. Impact of nutritional supplementation on immune response, body mass index and bioelectrical impedance in HIV-positive patients starting antiretroviral therapy. Nutr J. 2013 Aug 6;12(7):111-9.
5. Baum MK et al. Zinc status in human immunodeficiency virus type 1 infection and illicit drug use. Clin Infect Dis. 2003;37(2):117-23.
6. Zazzo JF et al. Effect of zinc on the immune status of zinc-depleted AIDS related complex patients. Clin Nutr. 1989;8:259–61.
7. Kupka R, Fawzi W. Zinc nutrition and HIV infection. Nutr Rev. 2002;60(3):69-79.
8. Prasad AS. Zinc: mechanism of host defence. J Nutr. 2007;137(5):1345-9.
9. Contreras-Martinez H et al. Effect of zinc on immune recovery in HIV patients. CES Med. 2017 Jun;31(1):3-13.
10. https://bioassaysys.com/wp-content/uploads/DIZN.pdf. QuantiChromTM Zinc Assay Kit (DIZN-250) Quantitative Colorimetric Zinc Determination at 425nm
11. Suter PM et al editors. Harrison's principle of internal medicine.20th ed. New York; McGrawhill; 2018. p.2309-18.
12. Silva M et al (2021) Role and effects of zinc supplementation in HIV-infected patients with immunovirological discordance: A randomized, double blind, case control study. PLoS ONE 16(1): e0244823. https://doi.org/10.1371/journal. pone.0244823
13. Baum MK et al. Zinc status in human immunodeficiency virus infection. J Nutr. 2000; 130:1421-3.
14. Flieger D et al. Zn^{2+} inhibits both tumor necrosis factor-mediated DNA framentation and cytolysis. Int J Cancer. 1989; 44:315–19.
15. Beutler B, Cerami A. Cachectin more than a tumor necrosis factor. N Engl J Med. 1987;316:479–85.
16. Madueke NM et al. Comparative study of plasma zinc and selenium levels amongst human immunodeficiency virus (HIV) positive and negative subjects. Afr J Food Sci Technol. 2015;6(8):253-8.
17. Emokpae MA. Correlation of copper/zinc ratio with superoxide dismutase activity and neutrophil-to-lymphocyte ratio in human immunodeficiency virus type 1(HIV-1) infected subjects. HIV AIDS Rev. 2018;17(1):18-23.
18. Ademasu A et al. Serum zinc deficiency and associated factors among pre-ART and on-ART adults at FelegeHiwot Referral Hospital, Bahir Dar, Northwest Ethiopia. Int J Nutr Food Sci. 2014;3(4):311-7.
19. Fufa H et al. Nutritional and immunological status and their associations among HIV-infected adults in Addis Ababa, Ethiopia. Food Nutr Bull. 2009;30(3):227-32.
20. Wiranguna A et al. Comparison of plasma zinc levels among HIV-positive and HIV-negative subjects infected with condyloma acuminate. Asian Pac J Cancer Prev. 2019;2(3):943-9.
21. Jeykumar W et al. Estimation of zinc in HIV/AIDS patients. Indian J Transm Dis. 2006;27(2):89-91.
22. Isa L et al. Blood zinc status and zinc treatment in human immunodeficiency virus-infected patients. Int J Clin Lab Res. 1992; 22:45-7.
23. Hadadi Aet al. The effect of selenium and zinc on CD4 (+) count and opportunistic infections in HIV/AIDS patients. Acta ClinicaBelgica. 2020 May 3;75(3):170-6.

24. Baum MK et al. Randomized controlled clinical trial of zinc supplementation to prevent immunological failure in HIV-infected adults. Clin Infect Dis. 2010;50(12):1653–60.
25. Wellinghausen N et al. Zinc serum level in human immunodeficiency virus-infected patients in relation to immunological Status. Biol Trace Elem Res. 2000;73(2):139-49.
26. Mocchegiani E, Muzzioli M. Therapeutic application of zinc in human immunodeficiency virus against opportunistic infections. J Nutr. 2000;130:1424-31.
27. Zeng L, Zhang L. Efficacy and safety of zinc supplementation for adults, children and pregnant women with HIV infection. Trop Med Int Health. 2011;16(12):1474-82.
28. Koch Jet al. Zinc levels and infections in hospitalized patients with AIDS. Nutr. 1996;12(7-8):515-8.