

# Effect of Vitamin-D, Zinc and Copper Supplementation in HIV-Positive Pregnant Women and Co-Relation with CD4 Counts- A Double Blinded Placebo Controlled Study

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**Abstract:** Title: Effect of vitamin-D, zinc and copper supplementation in HIV-positive pregnant women and co-relation with CD4 counts- a randomized double blinded placebo controlled study. Background: The main aim of this study was to evaluate the effect of vitamin D, zinc and copper supplementation in HIV-positive pregnant women and its co-relation with CD4 counts. Methods: A total of 195 HIV-positive pregnant women were selected from the antenatal clinic of Silchar medical college, Silchar. At their 1<sup>st</sup> visit (booking visit), the baseline levels of vitamin D, zinc and copper were estimated along with routine antenatal tests the patients. They were randomized into four groups, one of which received vitamin-D, 2<sup>nd</sup> one zinc and 3<sup>rd</sup> one copper supplementation. The 4<sup>th</sup> control group received placebo. The CD4 counts were determined at 36 weeks of gestation and 6 weeks after delivery. Statistical analysis was done using Chi-square and Mann-whitney tests and the relationships between supplementation of vitamin-D, copper and zinc on CD4 levels were analyzed and conclusions were drawn. Results: vitamin D, zinc and copper supplementation produced significant improvement in CD4 counts in all three groups as evidenced by higher CD4 counts in all three groups compared to the control. The greatest effect was obtained with vitamin-D supplementation. Zinc and copper appear to be less important than vitamin-D. Conclusion: In this study, supplementation of vitamin D, copper and zinc were significantly associated with improvement in mean CD4 counts. CD4 counts denote the level of immunity of the patient and their levels are used to determine the severity and stage of the disease.

**Keywords:** HIV, vitamin-D, zinc, copper

## 1. Introduction

HIV is a immune-depleting disease characterized by gradual depletion of CD4 cells. The CD4 cells with CD-56 antigen are the primary targets for action of HIV<sup>1</sup>. Over years, disease progression in HIV-positive patients has been correlated with CD4 counts<sup>2</sup>. The sole aim of the anti-retroviral therapy has been to keep the viral load below detectable levels (<50 copies) and improve the CD4 counts (>500/dl)<sup>2</sup>. Off late HIV has evolved into a very unpredictable disease, with rapid or slow progression despite the initiation of adequate anti-retroviral therapy.

Most studies have tried to evaluate the underlying phenomena which, governs the progression of HIV to AIDS. One such possible co-factor is serum vitamin-D level (25-OH-D). vitamin D has been known to be an important immune-regulator. Vitamin-D dependant receptors have been discovered on the mono nuclear cells of the immune system<sup>3</sup>. Vitamin D also plays a role in infection prevention, although the mechanism of such action is not completely known<sup>4</sup>. Deficiency of vitamin-D has been known to increase the severity of a number of infections including tuberculosis<sup>5,6</sup>.

Zinc is crucial for the cell-mediated immune system by modulating the functions of the T-lymphocytes by TH1 cytokine production and B-lymphocyte activation with antibody production. It also plays an important role in DNA, RNA replication and prevention of apoptosis<sup>7,8</sup>.

Copper improves interleukin-2 production and T-cell proliferation. It also potentiates the oxidative killing of pathogens by generating superoxide ions<sup>9</sup>. Both copper and zinc dismutases and cytochrome-c oxidases are integral in human immune system<sup>9,10</sup>.

The present study evaluates this co-relation in a critical manner to see if there is a relationship between vitamin-D, zinc and copper supplementation and the CD4 counts and if so, then, vitamin-D, zinc and copper must be made an integral part of disease management in HIV-positive mothers to improve CD4 counts and slow down the disease progression.

## 2. Materials and Methods

The study was conducted during a three year period from August-2011 to July-2014 in the department of obstetrics and gynaecology, silchar medical college, Silchar. Ethical statement: the study method was cleared by the ethical committee of silchar medical college, Silchar. Verbal consent was obtained from the participants after explaining the procedure, due to high illiteracy rates.

Method: A total of 195 patients participated in the study who, were selected from 448 HIV-positive antenatal cases attending the antenatal clinic of department of O&G, silchar medical college, silchar. All the patients were HIV-positive, with similar baseline CD4 counts and serum vitamin-D, zinc and copper levels. At the 1<sup>st</sup> visit (booking visit), their baseline CD4 counts, serum vitamin-D, zinc and copper levels were estimated. A total of 448 patients, who attended

the clinic over three years were seen and those with gross vitamin -D, copper or zinc deficiency were excluded. Patients with severe HIV disease (WHO category four and AIDS) cases were also excluded from the study. The suitable cases (n=195) were divided into three groups by sealed envelope technique:

Group A- (n=49): those who received 400 I.U/day of vitamin-D supplementation.

Group B-(n=49): those who received 15mg/day of zinc supplementation.

Group C-(n=48): those who received tablet copper sub-acetate 22 mg/day.

Group D-(n=49): controls who received placebo.

Pre-designed interviews were done during the 1<sup>st</sup> antenatal visit to collect information on age, education, socio-economic status, associated symptoms, morbidities, hospitalization etc. adequate baseline medical examination and tests of blood, urine, stool and vaginal swabs were taken. The disease was staged according to WHO guidelines.

The patients were instructed to take their supplements regularly as advised. They were started on anti-retroviral therapy as per schedule of National AIDS control organization (NACO), India guidelines.

The CD4 counts were re-evaluated at 36 weeks of gestation and at six weeks post partum, irrespective of the outcome of pregnancy. All women received Iron-Folic acid supplementation and tetanus toxoid injection according to national guidelines. Blood samples from the patients were evaluated for serum vitamin-D, copper and zinc levels using fully automated chemi-luminescence assay system (ADVANTAGE). Absolute CD4 counts were evaluated by fluorescent -cytometry technique (FACS-COUNT SYSTEM). The levels were evaluated at the 1<sup>st</sup> visit (booking), at 37 weeks of gestation and six weeks after delivery. Statistical analysis was done using Chi-square and Mann-whitney tests, and the relationship between supplementation of vitamin-D, copper and zinc on CD4 counts were analyzed at 37 weeks of gestation and at six weeks post-partum compared to the baseline levels at the 1<sup>st</sup> visit. A p-value of <0.05 was taken as statistically significant. The criteria for normal serum vitamin-D level was taken as > 30ng/ml; zinc > 70mcg/dl and copper >90mcg/dl<sup>11</sup>.

### 3. Results

A total of 195 patients were selected for participating in the trial from 448 patients seen in the HIV clinic of the antenatal out patient department, over a period of three years 2011-2014.

#### Baseline Characteristics:

Pre-designed interviews were carried out and the cases were interviewed regarding their age, education, socio-economic status, associated symptoms, morbidities, hospitalization etc. The following table shows the age distribution among the four groups and it is clear that they were similar in terms of their age distribution ( **table 1** )

**Table 1:** Age distribution of patients

Age group	Group A (n=49)	Group B (n=49)	Group C (n=48)	Group D (n=49)
<20 years	04	03	04	04
20-25 years	35	34	34	32
25-29 years	10	12	10	13
>30 years	-	-	-	-

According to the WHO guidelines, staging of the cases was done and those with stage IV disease and overt AIDS were excluded from the study (**Table 2**).

**Table 2:** WHO staging at 1<sup>st</sup> visit

WHO staging	Group A (n=49)	Group B (n=49)	Group C (n=48)	Group D (n=49)
I	45	42	44	46
II	03	07	03	03
III	01	-	01	-

CD4 counts at the 1<sup>st</sup> visit were determined, which, formed a baseline against which the subsequent changes in CD4 counts were compared to. The following table shows the baseline CD4 counts at the 1<sup>st</sup> visit (**Table 3**).

**Table 3:** CD4 count at 1<sup>st</sup> visit

CD4 count	Group A (n=49)	Group B (n=49)	Group C (n=48)	Group D (n=49)
>500	47	44	40	46
350-500	02	05	08	03
<350	-	-	-	-

The levels of serum vitamin D was determined and it was found that the four groups were similar in terms of distribution of cases with baseline vitamin D levels (**Table4**).

**Table 4:** vitamin D levels at 1<sup>st</sup> visit

Serum Vitamin D level (ng/ml)	Group A (n=49)	Group B (n=49)	Group C (n=48)	Group D (n=49)
<30	02	04	02	02
≥30	47	45	46	47

The following tables (**tables 5 and 6**) show that the four groups had similar distribution of cases with baseline copper and zinc values.

**Table 5:** zinc levels at 1<sup>st</sup> visit

Serum zinc level(mcg/dl)	Group A (n=49)	Group B (n=49)	Group C (n=48)	Group D (n=49)
<70	03	02	04	01
≥70	46	47	44	47

**Table 6:** Copper levels at 1<sup>st</sup> visit

Serum copper levels(mcg/dl)	Group A (n=49)	Group B (n=49)	Group C (n=48)	Group D (n=49)
<90	04	03	03	04
≥	45	46	46	45

Thus, the co- factors of variation in baseline characteristics among the four groups are found to be similar. The patients were followed up with routine antenatal care and treatment for HIV infection was given according to standard guidelines issued by the National AIDS control Organization, India in compliance with the WHO guidelines. The optimum treatment and regularity of dosage was strictly

adhered to, in order to maintain the uniformity. Their CD4 counts were again evaluated at 37 weeks of gestation (Table 7).

**Table 7: Mean CD4 counts at 37 weeks of gestation**

Groups	Mean	Standard deviation(SD)	Statistical implication
A(n=49)	778.3	78.8	Vit.D v/s control (p=0.0001, significant)
B(n=48)	763.4	69.7	Zinc v/s control (p=0.0074, significant)
C(n=49)	722.6	70.9	Copper v/s control (p=0.0404, significant)

Mode of delivery was governed by the obstetric indications and anti-retroviral therapy was continued during labour as well as continued after delivery. The baby was also started on anti-retrovirals after birth as per guidelines the CD4 counts were again evaluated at 6 weeks post partum (Table 8).

**Table 8: Mean CD4 counts at 6 weeks post partum**

Groups	Mean	Standard deviation	Statistical significance
A(n=49)	1040.6	74.4	Vit.D v/s control (p=0.0001, significant)
B(n=48)	863.4	70.7	Zinc v/s control (p=0.0041, significant)
C(n=49)	914.3	76.3	Copper v/s control (p=0.0357, significant)

From the above tables, it is clear that the mean cd4 counts at 37 weeks in the three groups were significantly higher than control (p=0.0001,0.0074,0.0404). Again at six weeks post partum, the mean cd4 counts were significantly higher (p=0.0001,0.0041,0.0357).

#### 4. Discussion

HIV is an important cause of mortality across the globe. Considerable impetus on the control of progression of the disease and prevention of its transmission has been laid<sup>1,2</sup>. In this study, supplementation of vitamin D, copper and zinc were significantly associated with improvement in mean CD4 counts. CD4 counts denote the level of immunity of the patient and their levels are used to determine the severity and stage of the disease<sup>1</sup>.

Vitamin D decreases the surface expression of cd4 antigens in pro-myelocytic leukemia cell lines and monocytes. There have been reports of vitamin D's role in innate immunity by toll like receptors. Also, pre-treatment with vitamin-D in in-vitro studies showed decreased rates of HIV infection<sup>3-6</sup>.

The present study found a significant relationship with improvement in CD4 counts with vitamin D supplementation. Zinc and copper, on the hand modulate the oxidant killing system of the macrophages and monocytes. Zinc and copper are also essential for cell renewal and epithelial integrity. The oxidant killing mechanism is of utmost importance in prevention of opportunistic infections. They also improve the immunoglobulin production. The present study found a significant improvement in CD4 counts with zinc and copper supplementation as compared to control group<sup>7-10</sup>.

Improvement of CD4 counts denotes slowing down the progression of the disease and better prevention of opportunistic infections and other HIV-associated morbidities<sup>12</sup>. Larger randomized trials are needed to evaluate whether routine supplementation of vitamin D, zinc and copper should be recommended in HIV positive cases and specially, pregnant women.

#### 5. Conclusion

Vitamin D, zinc and copper supplementation produced significant improvement in CD4 counts in all three groups as evidenced by higher CD4 counts in all three groups compared to the control. The greatest effect was obtained with vitamin-D supplementation. Zinc and copper appear to be less important than vitamin-D.

#### 6. Conflicts of Interest: none

#### 7. Acknowledgements: To the Patients

#### 8. Contribution to authorship

**9. Details of ethics approval:** the study was cleared by the ethics committee of Silchar Medical College and Hospital, Silchar

#### 10. Funding: Self

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### Abbreviations

- 1) AIDS- acquired immunodeficiency syndrome
- 2) CD- cluster differentiation
- 3) dl- decilitre
- 4) HIV- human immune deficiency virus
- 5) IL- interleukin
- 6) mcg- microgram
- 7) ng- nanogram
- 8) SD- standard deviation
- 9) th1- T helper cell 1
- 10) vit.- vitamin
- 11) v/s- versus
- 12) WHO- world health organization.

