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# Serum levels of vitamin D, magnesium, calcium, iron, and total iron binding capacity in HIV-infected patients

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## Abstract

**Introduction:** Alterations of vitamins and minerals have been seen in patients with the human immune virus (HIV) in numerous studies. This study aimed to evaluate serum levels of vitamin D, magnesium, calcium, iron, and total iron binding capacity (TIBC) in HIV-infected patients compared with controls in western Iran.

**Material and methods:** Ninety-eight HIV-infected prisoners and 98 healthy controls were selected for the study in 2016. Cluster of differentiation 4 (CD4) was checked in all HIV-infected patients. Age, sex, vitamin D, calcium, magnesium, iron, and TIBC were checked for each person in both groups.

**Results:** The mean age (range) of the HIV-infected and the healthy control groups was 40.11 years (21-68 years) and 45.59 (18-85 years), respectively. Vitamin D, calcium, iron and TIBC levels were significantly different in the two groups. There was a significant correlation between calcium and iron levels and the CD4 count.

**Conclusions:** There were significant differences in vitamin D, calcium, iron, and TIBC levels in the HIV-infected patients compared to the healthy controls. Therefore, the results show the correlation between these levels and the incidence of HIV. In addition, the significant difference between calcium and iron levels with the CD4 counts can enhance the correlation.

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**Key words:** HIV, vitamin D, magnesium, calcium, iron.

## Introduction

Cluster of differentiation 4 (CD4) T lymphocytes, monocytes and macrophages are infected by the human immune virus (HIV). HIV reduces the number and function of CD4 cells, alterations that impact both cell-mediated and humoral immunity [1]. The prevalence of HIV is rising worldwide because people on antiretroviral treatment are living longer. New infections decreased from 2002 to 2012 and also global acquired immune deficiency syndrome (AIDS)-related mortality decreased from 2005 to 2012 because almost 9.7 million people in low-income and middle-income countries had started antiretroviral treatment in 2012 [2]. A meta-analysis in Iran from January 1996 to March 2012 showed that HIV prevalence varied from 0% in the general population to 17.25% in people who inject drugs [3]. The number of people

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living with HIV worldwide reached nearly 35.3 million in 2012 [4]. Vitamin D deficiency is a very common disorder, regarding both Western and developing countries [5]. Vitamin D insufficiency and HIV infection are both risk factors for chronic disorders and so it is essential to study vitamin D status in patients with HIV infection [6]. Vitamin D deficiency has been correlated with enhanced risk of several disorders or diseases such as cardiovascular disease, falls and fractures, diabetes and obesity, some cancers, and tuberculosis [5, 7, 8]. Changes in calcium and bone metabolism have been mentioned in a lot of studies in HIV-infected patients [9]. Iron deficiency is the chief cause of anemia in the developing world [10]. In HIV-infected patients, anemia is a general clinical finding and iron deficiency may increase the development of low hemoglobin levels [11]. Calcium and magnesium are two important ions in bone metabolism [12]. In a study with a large number of HIV-infected participants 53.7% and 26.8% of patients had osteopenia and osteoporosis, respectively [13]. This study aimed to evaluate the serum levels of vitamin D, magnesium, calcium, iron, and total iron binding capacity (TIBC) in HIV-infected patients compared with healthy controls in western Iran.

## Material and methods

In this case-control study that was approved by the Ethics Committee of Kermanshah University of Medical Sciences, Kermanshah, Iran, 98 HIV-infected prisoners or patients and 98 healthy controls were selected for the study in 2016. Age, sex, vitamin D, calcium, magnesium, iron, and total iron binding capacity (TIBC) were checked for each person in both groups. Also, CD4 was checked in all HIV-infected patients. For determination of vitamin D and elements in serum, 5 ml of blood was taken from each participant in sterile conditions and then serum samples were isolated by centrifugation.

### Measurement of vitamin D

We used the enzyme-linked immunosorbent assay (ELISA) method for measurement of 25(OH) vitamin D in serum. The AccuBind ELISA Microwells kit (Monobind Inc. Lake Forest, CA 92630, USA) with product code 7725-300 was used by an ELISA reader (DYNEX Technologies, USA). The sensitivity was 0.67 ng/ml according to the brochure of the kit. The vitamin D level was defined to three different degrees [sufficiency (> 30 ng/ml or 75 nmol/l), insufficiency or mild deficiency (20-30 ng/ml or 50-75 nmol/l) and deficiency (< 20 ng/ml or < 50 nmol/l)] [14].

### Measurement of calcium, magnesium, iron, and total iron binding capacity

We used a photometric assay with the ARSENAZO III method from Pars Azmoon diagnostic kits (Pars Azmoon Co., Iran). Measurement of elements was done with an Erba Mannheim XL-600 autoanalyzer. The normal ranges for calcium, magnesium, iron and TIBC were 8.5-10.1 mg/dl, 1.8-3.0 mg/dl, 60-170 mg/dl, and 240-450 µg/dl, respectively.

### Statistical analysis

The analysis of data was done by IBM SPSS version 21 software (SPSS Inc., Chicago, USA). Independent-samples *t*-test was used for the comparison of significant differences between the means of two groups and one-way ANOVA for more than two independent (unrelated) groups (CD4 status and vitamin D degrees). *P*-value (2-tailed) < 0.05 was considered statistically significant.

## Results

The mean age ± SD (range) of the HIV-infected and the healthy control groups was 40.11 ± 7.73 (21-68) years and 45.59 ± 18.61 (18-85) years, respectively. In the HIV-infected group, 85 patients (86.7%) and in the control group, 86 controls (87.8%) were male. The mean age ± SD (range) of CD4 in the HIV-infected group was 400.09 ± 287.72 cells/mm$^3$ (37-1472 cells/mm$^3$). Table 1 shows the correlation of variables between two groups. Vitamin D, calcium, iron, and TIBC levels were significantly different between groups ($p < 0.05$). Deficiency of vitamin D in the HIV-infected group was more than in the control group. Also, calcium,

**Table 1.** The relationship of variables between two groups

| Variables | HIV-infected group, *n* = 98 | Control group, *n* = 98 | *p*-value |
|---|---|---|---|
| Vitamin D, ng/ml | | | |
| Mean ± SD | 29.56 ± 27.27 | 30.63 ± 18.66 | 0.818 |
| Range | 4.2-142.2 | 3-99 | |
| Vitamin D status, *n* (%) | | | |
| Sufficiency | 31 (31.6) | 49 (50.0) | 0.026 |
| Insufficiency | 23 (23.5) | 14 (14.3) | |
| Deficiency | 44 (44.9) | 35 (35.7) | |
| Magnesium, mg/dl | | | |
| Mean ± SD | 2.16 ± 0.11 | 2.19 ± 0.32 | 0.349 |
| Range | 1.9-2.5 | 1.7-3.2 | |
| Calcium, mg/dl | | | |
| Mean ± SD | 9.73 ± 0.62 | 9.37 ± 0.63 | < 0.001 |
| Range | 7.4-10.7 | 6.2-10.6 | |
| Iron, mg/dl | | | |
| Mean ± SD | 100.34 ± 56.79 | 81.94 ± 19.21 | 0.003 |
| Range | 11-378 | 31-131 | |
| TIBC, µg/dl | | | |
| Mean ± SD | 336.70 ± 48.12 | 305.97 ± 58.95 | < 0.001 |
| Range | 225-461 | 119-439 | |

*SD – standard deviation, TIBC – total iron binding capacity*

Table 2. The relationship of variables with CD4 status

| Variables | CD4 count, cells/mm$^3$ | | | *p*-value |
|---|---|---|---|---|
| | < 200, *n* =25 | 200-500, *n* = 47 | > 500, *n* = 26 | |
| Age, years | | | | |
| Mean ± SD | 40.76 ± 7.75 | 40.10 ± 7.85 | 39.50 ± 7.74 | 0.847 |
| Range | 21-61 | 28-68 | 25-60 | |
| Vitamin D, ng/dl | | | | |
| Mean ± SD | 26.17 ± 19.38 | 32.51 ± 29.00 | 28.74 ± 30.72 | 0.628 |
| Range | 6.6-79.8 | 4.2-138.7 | 4.2-142.2 | |
| Magnesium, mg/dl | | | | |
| Mean ± SD | 2.16 ± 0.11 | 2.17 ± 0.12 | 2.15 ± 0.90 | 0.822 |
| Range | 1.9-2.4 | 2.0-2.5 | 2.0-2.3 | |
| Calcium, mg/dl | | | | |
| Mean ± SD | 9.45 ± 0.64 | 9.75 ± 0.66 | 9.96 ± 0.41 | 0.012 |
| Range | 8.4-10.6 | 7.4-10.7 | 9.0-10.7 | |
| Iron, mg/dl | | | | |
| Mean ± SD | 73.72 ± 48.99 | 100.25 ± 46.57 | 126.11 ± 69.43 | 0.004 |
| Range | 11-221 | 15-214 | 34-378 | |
| TIBC, mg/dl | | | | |
| Mean ± SD | 319.60 ± 54.19 | 342.74 ± 48.88 | 342.23 ± 37.10 | 0.120 |
| Range | 225-439 | 227-461 | 253-421 | |

*CD4 – human immune virus, SD – standard deviation, TIBC – total iron binding capacity*

iron, and TIBC levels were higher in the HIV-infected group compared with the control group.

The correlation of variables with CD4 count is shown in Table 2. There was a significant correlation of calcium and iron levels with CD4 count ($p < 0.05$). Therefore, the patients with CD4 < 200 cells/mm$^3$ had lower calcium and iron levels compared with CD4 = 200-500 cells/mm$^3$ and CD4 = 200-500 cells/mm$^3$ compared with CD4 > 500 cells/mm$^3$. Calcium levels have statistically a significant difference, but the difference may not be clinically significant. Meanwhile, iron and TIBC must be interpreted according to the type of anemia.

## Discussion

This study showed that the levels of calcium, iron, and TIBC in the HIV-infected patients were significantly higher than the controls and also vitamin D insufficiency and deficiency were more frequent in HIV-infected patients. The patients with CD4 < 200 cells/mm$^3$ had lower calcium and iron levels compared with 200-500 and > 500 cells/mm$^3$. The overall estimated prevalence in people living with HIV and vitamin D deficiency is high (range, 70.3 to 83.7%) [15]. Eckard *et al.* [16] reported that most HIV-infected patients (median age, 11 years) had vitamin D deficiency or insufficiency compared with age- and sex-matched controls. Dao *et al.* [8] among HIV-infected adults and Conesa-Botella *et al.* [17] among HIV-infected individuals confirmed this result. One study [6] on 113 HIV-infected children with age ≤ 24 years and 54 age-and phototype-matched healthy controls concluded that the mean serum vitamin D levels in the HIV-infected group were significantly higher than the control group. Our study showed that vitamin D deficiency and insufficiency are more prevalent in HIV-infected patients. Therefore, a lot of studies have shown that vitamin D deficiency can be a risk factor in HIV-infected patients. One study [18] on 828 HIV-infected patients and 549 controls reported that mean serum calcium levels in the HIV-infected group were significantly lower than in the control group ($p < 0.0001$), but in the study of Shadrack *et al.* [19], the HIV-infected patients have higher serum calcium than in the controls.

Sixty-two males with HIV (HIV-1) or AIDS and 120 healthy males of the same age group (31-45 years) were selected in one study [20], the results of which indicated significantly lower levels of serum calcium and magnesium of patients in comparison to the healthy controls ($p < 0.01$). A meta-analysis reported in 2009 showed bone absorptiometry results indicating osteoporosis and osteopenia in 15% and 52% of HIV patients, respectively, and suggested that a calcium disorder may be a common risk factor for osteoporotic fractures in HIV-infected patients [21]. Banjoko *et al.* [22] selected 80 HIV-1 patients and 50 age- and sex-matched seronegative controls. Serum iron and TIBC were significantly higher in the patients compared with the control. Salhi *et al.* [23] found that high serum ferritin levels were

correlated with more rapid progression of HIV disease and suggested that iron excess may have had an adverse impact. A meta-analysis reported that vitamin D and calcium combination is better than using a single drug in fracture prevention [24]. Conesa-Botella *et al.* [17] reported that vitamin D deficiency was common before highly active antiretroviral therapy (HAART) and after 12 months on HAART, vitamin D level had a significant decrease. A study showed that there was a significant association of the level of magnesium in the plasma in both genders with HIV/AIDS infection [25]. Another study [26] reported that serum magnesium in HIV/AIDS patients was low, and the present study confirmed it.

Two small cross-sectional studies [27, 28] reported that vitamin D deficient HIV-infected patients had significantly lower CD4 counts. Our study showed mean vitamin D was lower in the group of CD4 < 200 cells/mm$^3$, but the correlation was not significant. In HIV patients in the HAART era, 9.2% of patients of CD4 < 200/mm$^3$ had low serum calcium compared with 0.5% of CD4 > 200/mm$^3$ ($p < 0.002$) [29]. In HIV-seropositive women (age 18-25 years), serum iron concentration was higher at low CD4 levels [1]. The present study confirmed the results about calcium levels, but not about iron levels.

In conclusion, there were significant differences in vitamin D, calcium, iron, and TIBC levels in the HIV-infected patients compared to the healthy controls. Therefore, the results show a correlation between these levels and the incidence of HIV. In addition, the significant difference between calcium and iron levels with CD4 counts can enhance the correlation. In future studies, researchers should pay attention to changes in levels of these serum factors in HIV-infected patients with control of other factors affecting HIV infection (age, sex, associated diseases, drug use, and nutrition habits) and even ethnicity/race.

## Conflict of interest

The authors declare no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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