Serum Levels of Vitamin D, Magnesium, Calcium, Iron, and TIBC in HIV-Infected Patients

Alireza Janbakhsh 1, Feizollah Mansouri 1, Siavash Vaziri 1, Babak Sayad 1, Mandana Afsharian 1, * and Ronak Miladi 1
1Department of Infectious Disease, School of Medicine, Kermanshah University of Medical Sciences, Kermanshah, Iran
*Corresponding author: Imam Reza Hospital, Kermanshah University of Medical Sciences, Kermanshah, Iran. Tel: +98-8334276315, Email: mandanaafsharian@yahoo.com

Abstract

Background: Vitamin D insufficiency and HIV infection are both risk factors for chronic disorders. Several studies on small groups of male HIV-infected patients have reported alterations of calcium and bone metabolisms. This study aimed to evaluate serum levels of vitamin D, magnesium, calcium, iron, and TIBC in HIV-infected patients compared with controls in the west of Iran.

Methods: In a case-control study approved by the Ethics Committee of Kermanshah University of Medical Sciences, 98 prisoners with HIV and 98 controls were selected in 2016. Age, sex, vitamin D, calcium, magnesium, iron and total iron binding capacity (TIBC) were checked for all participants in both groups. Also, CD4 was checked in all HIV-infected patients.

Results: The mean age ± SD (range) of HIV-infected and control groups was 40.11 ± 7.73 (21.68) and 45.59 ± 18.61 (18.85) years, respectively. Vitamin D, calcium, iron, and TIBC levels were significantly different in these two groups (P < 0.05). Vitamin D deficiency was higher in the HIV-infected group than that in the control group. Furthermore, calcium, iron and TIBC levels were higher in the HIV-infected group compared with those in the control group.

Conclusions: This study showed that calcium, iron and TIBC levels were significantly higher in HIV-infected patients compared with those in the control group. Moreover, vitamin D insufficiency and deficiency were higher in HIV-infected patients. The patients with CD4 < 200 cells/mm³ had less calcium and iron levels compared with 200 - 500 and > 500 cells/mm³.

Keywords: Vitamin D, Magnesium, Calcium, Iron, TIBC, HIV

1. Background

HIV infects cluster of differentiation 4 (CD4) T-lymphocytes, monocytes, and macrophages. As a result of this infection, the number and function of CD4 cells are reduced and both cell-mediated and humoral immunity are affected (1). HIV prevalence is increasing worldwide because people on antiretroviral therapy live longer. Nevertheless, new infections have decreased from 3.3 million in 2002 to 2.3 million in 2012. Global AIDS-related deaths peaked at 2.3 million in 2005 has decreased to 1.6 million by 2012. An estimated 9.7 million people in low-income and middle-income countries had started antiretroviral therapy by 2012 (2). According to a systematic review and meta-analysis in Iran from January 1996 to March 2012, HIV prevalence varied from 0.00% in the general population to 17.25% in injecting drug users (3). The number of persons living with HIV worldwide reached approximately 35.3 million in 2012 (4).

Vitamin D deficiency is a very common disorder, affecting both Western and developing countries (5). Vitamin D insufficiency and HIV infection are both risk factors for chronic disorders; so, it is important to consider vitamin D status in HIV-infected patients (6). Vitamin D deficiency has been associated with an increased risk of falls and fractures, diabetes and obesity, cardiovascular disease, some malignancies, and tuberculosis (5, 7, 8). Alterations of calcium and bone metabolisms have been reported in several studies on small groups of male HIV-infected patients (9). Iron deficiency is the leading cause of anemia in the developing world (10). Anemia is a common clinical finding in HIV-infected patients. In this regard, iron deficiency or redistribution may contribute to the development of low hemoglobin levels (11).

The aim of this study is to evaluate serum levels of vitamin D, magnesium, calcium, iron, and TIBC in HIV-infected patients compared with healthy people (i.e., controls) in the West of Iran.

2. Methods

In a case-control study approved by the Ethics Committee of Kermanshah University of Medical Sciences, 98...
prison patients with HIV and 98 controls were recruited 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. In order to determine vitamin D and elements in serum, 5 mL blood was taken from each participant in sterilized conditions and then serum samples were isolated by centrifugation.

2.1. Measurement of Vitamin D
We used the enzyme-linked immunosorbent assay (ELISA) method for measuring 25(OH) Vitamin D in serum. Accu bind ELISA Microwells kit (Monobind Inc. Lake Forest, CA 92630, USA) with product code: 7725-300 was used by ELISA reader (DYNEX Technologies, USA). The sensitivity was 0.67 ng/mL according to the manufacturer’s instruction. The vitamin D level was defined to three different degrees: sufficiency (≥ 30 ng/mL or 75 nmol/L), insufficiency (20 - 30 ng/mL or 50 - 75 nmol/L), and deficiency (< 20 ng/mL or < 50 nmol/L) (12).

2.2. Measurement of Calcium, Magnesium, Iron, and TIBC
We used Photometric assay with ARSENAZO III method from Pars Azmoon diagnostic kits (Pars Azmoon Co., Iran). The elements were measured using Erba® Mannheim XL-600 autoanalyzer. The normal ranges for calcium, magnesium, iron, and TIBC were 8.5 - 10.1 mg/dL, 0.97 - 16.2 mg/dL, 20 - 30 mg/dL, 50 - 75 nmol/L, and 60 - 170 mg/dL, and 240 - 450 μg/dL, respectively.

2.3. Statistical Analysis
The data were analyzed with IBM SPSS version 21 software (SPSS Inc., Chicago, USA). Independent Samples T-test was used for comparing the significance of differences between the means of two groups while one-way ANOVA was employed to compare such differences for more than two independent (unrelated) groups (CD4 status and vitamin D degrees). P value (2-tailed) < 0.05 was considered as statistically significant.

3. Results
The mean age ± SD (range) of HIV-infected and control groups was 40.11 ± 7.73 (21-68) 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 males. The mean age ± SD (range) of CD4 in HIV-infected group was 200 - 500 cells/mm³ (37 - 1472 cells/mm³). Table 1 shows the correlation of variables between the two groups. Vitamin D degree, calcium, iron, and TIBC levels were significantly different in the two groups (P < 0.05). Deficiency of vitamin D was more in the HIV-infected group than in the control group. Also, calcium, 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 between calcium and iron levels with CD4 count (P < 0.05). Therefore, the patients with CD4 < 200 cells/mm³ had less calcium and iron levels compared with CD4 = 200 - 500 cells/mm³ and CD4 = 200 - 500 cells/mm³ compared with CD4 > 500 cells/mm³.

4. Discussion
This study showed that calcium, iron, and TIBC levels were significantly higher in HIV-infected patients compared with controls and also vitamin D deficiency and the deficiency was more in HIV-infected patients. The patients with CD4 < 200 cells/mm³ had less calcium and iron levels compared with 200 - 500 and > 500 cells/mm³.

The overall estimated prevalence in people living with HIV and vitamin D deficiency is high, ranging from 70.3 to 83.7% (13). Eckard et al. (14) reported that most HIV-infected patients (median age: 11 years) had vitamin D deficiency or insufficiency compared with age- and sex-matched controls. This result was confirmed by Dao et al. (8) on HIV-infected adults and Conesa-Botella et al. (15) on HIV-infected individuals. One study (6) on 113 HIV-infected children (age ≤ 24 years) and 54 healthy age-matched and phototype controls revealed that mean serum vitamin D concentrations were significantly higher in an HIV-infected group than the control group; in contrary, our study showed no significant difference in mean vitamin D between two groups. Therefore, many studies show that vitamin D deficiency can be a risk factor in HIV-infected patients. One study (16) on 828 HIV-infected patients and 549 controls reported that mean serum calcium levels were significantly lower in an HIV-infected group than the control group (P < 0.0001). In comparison, in the study of Shadrack et al. (17), HIV-infected patients showed a higher serum calcium than in controls.

A total of 62 males with HIV-1 and 120 healthy males of the same age group (31-45 years) were investigated in (18), where significantly lower levels of serum calcium and magnesium of patients were observed compared to the healthy controls (P < 0.01) (18). A meta-analysis of studies published between 1966 and 2005 showed osteoporosis in 15% of HIV patients and osteopenia in 52% and suggested disorder in calcium may be a conventional risk factor for osteoporotic fractures in HIV-infected patients (19). Banjoko et al. (20) selected 80 HIV-1 patients and 50 seronegative age- and sex-matched controls and reported that serum iron and TIBC are significantly higher in the patients compared with the control. Salhi et al. (21) showed high serum ferritin concentrations associated with more rapid progression of HIV disease and suggested that iron excess may have an adverse influence in this regard. Our study confirmed this result.
Meta-analyses have shown that vitamin D plus calcium association is superior to the use of a single drug in fracture preventions (22). Conesa-Botella et al. (15) showed 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. After a 12 months follow-up, replacement of low dose once daily oral vitamin D with calcium in treatment-experienced HIV patients with vitamin D deficiency can elevate the vitamin D level (23).

Two small cross-sectional studies (24, 25) reported that vitamin D deficient HIV-infected patients had a significantly lower CD4 count than the controls. According to the results of the present study, mean vitamin D was lower in a group of CD4 < 200 cells/mm³, but the correlation was not statistically significant. In HIV patients in the HAART era, 9.2% patients of CD4 < 200/mm³ had a low serum calcium compared with 0.5% of CD4 > 200/mm³ (P < 0.002) (26). As reported in (1), in HIV seropositive women (age 18 - 25 years), serum iron levels were higher at low CD4 levels.

### 4.1. Conclusions

Vitamin D, calcium, Iron, and TIBC levels need to be checked regularly in all HIV-infected patients and vitamin
D supplementation should be given when needed; however, calcium and iron supplementations are not necessary for these patients.

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