PREVALENCE OF 25-HYDROXYVITAMIN D DEFICIENCY AMONG A COHORT OF OMANI AIDS PATIENTS

ABSTRACT

Objective: To determine the prevalence of hypovitaminosis D in a cohort of Omani AIDS patients who are presented at the Sultan Qaboos University Hospital (SQUH) for treatment with highly active antiretroviral therapy (HAART) and to investigate if there is a significant correlation between vitamin D deficiency and age, gender, type of treatment and the CD4+ T cells count.

Methods: Data of 63 Omani AIDS patients (31 males and 32 females) including subjects’ age, sex, type of drug used and CD4+ T cells were collected using SQU-Hospital Information System. The subjects’ ages were between the ranges of 18-70 years, with a mean age of 38 years. Serum level of 25-hydroxyvitamin D [25(OH)D] was measured using standard ELISA. Vitamin D deficiency was defined as the level of 25(OH)D less than 30 nmol/L. Data analysis and the prevalence of vitamin D deficiency was calculated using SPSS software.

Results: Out of the 63 Omani AIDS patients, 44 (70%) were 25(OH)D deficient. Most of these deficient patients were females with percentage of 78.1% versus 61.3% for males. The frequency of 25(OH)D deficiency was detected more in patients younger than 30 years of age (11/12, 92%). Patients who received HAART treatment had higher odd of vitamin D deficiency than untreated patients. A total of 29 and 21 patients received non-nucleoside reverse transcriptase inhibitors (NNRTI) and protease inhibitors (PI), respectively, and were found to be vitamin D deficient (P = 0.870). No significant correlation between the serum concentration of 25(OH)D and CD4+T cells count was observed.

Conclusion: There was a high prevalence of 25(OH)D deficiency amongst Omani AIDS patients. The deficiency of 25(OH)D was more prevalent among females than males. No correlation between vitamin D level and the use of HAART was detected.

Key words: Oman, AIDS, Vitamin D, HAART
INTRODUCTION

Vitamin D is a fat-soluble organic substance, which has key roles in some metabolic functions. It is synthesized in the skin as vitamin D3, also called cholecalciferol, and ingested through diet as 7-dehydrocholesterol, vitamin D2. After exposure to the sunlight, 7-dehydrocholesterol is converted into cholecalciferol which is transported in plasma, and then converted into 25-hydroxyvitamin D [25(OH) D] by 25-hydroxylase enzyme. Low concentration of vitamin D has an impact on the body homeostasis as vitamin D has a key role in calcium and phosphate regulation through controlling calcium deposition into the bone and enhancing the absorption of calcium from gastrointestinal tract. Consequently, inadequate vitamin D concentration is attributed with a number of co morbidities, including hypertension, cardiovascular disease, insulin resistance, diabetes, dyslipidemia, impaired immune function, decreased neuro-cognitive function, and malignancies.

Several studies reported that acquired immunodeficiency (AIDS) patients were more likely to suffer from insufficient amount of vitamin D. This may be due to increase consumption of vitamin D for body processes in comparison to healthy individuals. Furthermore, HIV patients seem to be more vulnerable to have defect in renal hydroxylation due to pro-inflammatory cytokines such as TNF-α. As a result, less 1,25(OH)2 D is available in their bodies. Other studies indicated that vitamin D is linked with highly active antiretroviral treatment (HAART) amongst AIDS patients.

Currently no data is available on vitamin D status in AIDS patients in the Arab world. This study aims to investigate the prevalence of hypovitaminosis D in a longitudinal cohort of Omani patients with AIDS who are on HAART treatment and to determine the influence of gender, age, type of HAART and CD4+ T cells on vitamin D status.

Materials and Methods

Patients

A total of 63 adult Omani AIDS patients (31 males and 32 females) treated with HAART at Sultan Qaboos University Hospital (SQUH), a referral hospital for the whole of Oman, were studied. The age range was 18-70 years, with a mean age of 38 years. Demographic data, type of drug and CD4+ T cells count were obtained from patients’ medical record using the Hospital Information System (HIS). There were 12 patients who were below 30 years of age (female =7, male = 5). A total of 51 patients were more than 60 years (female = 27, male = 24). The AIDS patients were classified into two groups according to their age as ≤ 30 year (n=12), > 30 year (n=51). After completing the database, the 63 patients were divided into five main groups in proportional to the type of HARRT treatment at the time of blood sample taken. The first group comprised of 2 patients who did not receive HARRT treatment. The second group comprised of 21 patients who received protease inhibitors (PI). The third group comprised of 29 patients who were treated with non-nucleoside reverse transcriptase (NNRTI). Whereas, the fourth group comprised of 4 patients who received a combination of both PI and NNRTI. The fifth group comprised of 7 patients, who received other type of reverse transcriptase inhibitors (RTI).
Ethical Clearance

This study was conducted after obtaining the ethical clearance from the Research and Ethics Committee of the College of Medicine and Health Sciences, Sultan Qaboos University. All the patients were consented before being accepted to participate in this study and before blood was extracted.

Blood collection and measurement of Vitamin D level

A total of 10 ml blood sample was collected into a sterile vacuum container from each participant. Sera were separated after centrifuging at 4000 rpm in a cooling centrifuge. Serum samples were stored at -80ºC until tested. All sera were tested in duplicate for 25 (OH) D levels using ELISA tests (EurolImmune, Germany) and in accordance with the manufacturer instructions. Vitamin D deficiency was defined as total serum 25(OH) D of less than 30nmol/L.

Statistical analysis

SPSS software (version 19) was used for the data analysis. In order to investigate the relations between the type of drug and the level of vitamin D, the Fisher’s exact test were used. The differences in such levels was considered significant when p< 0.05.

Results

There was a high prevalence of vitamin D deficiency among the Omani AIDS patients. Approximately 70% (n=44) had 25(OH)D concentration below 30nmol/L compared with 54.1% of the Omani healthy adults population. Only 19 patients (30.1%) had 25(OH)D concentration within the normal range.

There was no significant correlation between 25(OH) D deficiency and age group in AIDS patients (P = 0.067). However, the prevalence of 25(OH)D deficiency was more among patients younger than 30 years. Figure 3 illustrate that 92% (n= 11) of patients who were younger than 30 years of age had 25(OH)D below 30 nmol/L, whereas, 65% (n= 33) of patients whore more than 30 years of age were Vitamin D deficient.

Patients who received HARRT treatment had higher percentage of 25(OH)D deficiency compared to untreated patients. Half (50%) of patients who had not received HARRT treatment were 25(OH)D deficient. On the other hand, the prevalence of 25(OH)D deficiency among patients who have received NNRTI was 72.4% and 71.4% of those who received PI were found to be 25(OH)D deficient. These findings were similar to those who received other types of HARRT. It was clear that the prevalence of 25(OH)D deficiency between different drug groups did not differ significantly from each other (Figures 1-3). The only different we observed is that there was slightly lower percentage of 25(OH)D deficiency among patients who received the combination of both PI and NNRTI compared to the other group who received only one type of HARRT as illustrated in Table 1.
Table 2 illustrates the correlation of the CD4+ T cells count and vitamin D level among Omani HIV/AIDS patients. Only 37 of HIV/AIDS patients had CD4+ T cells count less than 500 cell/ml, whereas, the others (i.e., 26 of HIV/AIDS patients) were found to have CD4+ T cell count above 500 cell/ml. However, we did not observe any significant correlation between CD4+ T cell count and vitamin D concentration (P = 0.639). Moreover, the prevalence of 25(OH)D deficiency among those who had CD4+ T cells count of less than 500 cell/ml was found to be 68%. In addition, 73% of these patients, i.e., who had CD4+ T cells count more than 500 cell/ml, were also found to have a high prevalence of 25(OH)D deficiency as shown in Figure 4.

DISCUSSION

It is well documented that vitamin D deficiency is common among HIV/AIDS patients. Our investigation confirmed this by demonstrating the high prevalence of vitamin D deficiency among Omani adult AIDS patients. Although our measurements occurred in summer when nadir vitamin D is expected to be high, seven out of ten patients had inadequate 25(OH)D level. This finding of high prevalence of 25(OH)D deficiency may be attributed to a defect in renal hydroxylation through the direct effect of proinflamatory cytokines such as Tumor necrosis factor-α (TNF-α). Conrado et al., (2010) demonstrated that as the disease progresses, there is increase in the utilization of vitamin D by machrophages and lymphocytes for cell proliferation and differentiation. Moreover, 25(OH)D deficiency is highly prevalent in female patients (Figure 2). There are many factors that may contribute to inadequate 25(OH)D level, such as, sedentary life style, poor nutrition, aging, skin pigmentation and inadequate sun exposure especially in elderly patients. However, we did not observe any correlation between 25(OH)D level and age (Figure 2).

Because exposure to sun light is the importance source of vitamin D, 25(OH)D had been more prevalent in female than in male. This may be attributed to limit exposure to sunlight for cultural reason among Arab Muslim women.

Vitamin D has been suggested to influence immunity and play a vital role in the proliferation of CD4+ T cells, where such deficiency have been contributed to low CD4+ T cells count. It has been documented that many cells especially the immune cells express vitamin D receptor which modulates their function. For instance, studies show the ability of vitamin D to control CD4+ T cells by modulating the production of interleukin-10. For that reason it is believed that the concentration of vitamin D may be affected by immune diseases. For example, lower vitamin D level has recently been associated with increased mortality in the general population as well as with HIV disease progression and overall mortality in a cohort of Tanzanian pregnant women with HIV infection. However, our findings did not confirm any influence of low 25(OH)D concentration on CD4+ T cells count as illustrated in Figure 4.

It has been well documented that the frequent of hypovitaminosis D is more in patients on HAART than untreated patients. In such documentation some studies showed there is a correlation between which type of HAART being prescribed to patient and vitamin D deficiency. For example, Studies from Spain and Japan have found that the early bone demineralization in HIV sufferers is attributed to the use of HAART especially protease inhibitors (PI). Other study aimed to prospectively studied vitamin D status in HIV individuals on HAART in Belgium, has reported that using non-nucleoside reverse transcriptase inhibitors (NNRTI) therapy causes a significant decrease in plasma 1,25(OH)2 D concentration but not in patient treated with protease inhibitors. Although, the role of HAART in vitamin D deficiency is not clear, a number of studies have suggested that HAART may affect and

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influence the pathway of vitamin D metabolism. For instance, PI and NNRTI induce cytochrome P450 in the liver. The cytochrome P450 attribute in catabolizing both 25(OH)D and 1,25(OH)\textsubscript{2}D and decrease their serum concentration. Therefore, it has been recommended vitamin D supplementation to be administered to the HIV/AIDS patients when needed.

Previous studies have observed association between 25(OH)D deficiency and NNRTI. In our analysis, we demonstrated that NNRTI recipients have greater decrement in serum 25(OH)D concentration. Many studies concluded that NNRTIs, for example efavirenz, have the potential to alter vitamin D metabolism by inducing 24-hydroxylase (cytochrome P450 enzyme) which convert 25(OH)D to its inactive form, 24,25(OH)\textsubscript{2}D. This effect help to explain the attribution of NNRTIs in 25(OH)D deficiency.

There are a number of mechanisms known to explain this deficiency; one of these mechanisms is based on the type of antiviral drug used. Our study reached the same conclusion by observing a potential association between vitamin D deficiency and HAART. Consequently, percentage of deficiency in HAART treated patients is extremely high comparing to untreated patients (Figure 3, Table 1).

There is a potential association between PI and the high prevalence of 25(OH)D deficiency. Cozzolino et al., 2003 refer this to the role of PI in altering vitamin D metabolism by inhibiting renal 1\alpha-hydroxylase which converts 25(OH)D to its active form, 1,25(OH)\textsubscript{2}D. In addition, to that, Cozzolino et al., 2003 also suggested that PI is also affecting hepatic hydroxylation of vitamin D by competitive inhibition of CYP3A. In contrast to PI and NNRTI, NRTI are not metabolized by CYP450 in the liver, for this reason NRTIs are less likely to affect 25(OH)D serum concentration.

Surprisingly, the concentration of 25(OH)D is adequate in most patients who received a combination of NNRTI and PI (Figure 3). This might be due to low dose from each drug, which reduces the side effect of each drug on Vitamin D concentration.

Furthermore, evolutions should be made to better understand the effect of HAART particularly NNRTI and PI regimens on enzymes involve in vitamin D metabolism. In addition, the role of vitamin D in preventing HIV/AIDS complications should be adequately investigated.

In conclusion, our data are in concordance with other studies based on the prevalence of vitamin D deficiency amongst AIDS patients treated with HAART. The outcome of this study is the recommendation of screening HIV/AIDS patients for vitamin D concentration periodically so that a supplementation of vitamin D can be given whenever necessarily to avoid immunodependent osteomalacia.

ACKNOWLEDGEMENTS

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REFERENCES


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<table>
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<th>25(OH)D level (nmol/L)</th>
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<tr>
<td></td>
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</tr>
<tr>
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<tr>
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Pl = protease inhibitor; NNRTI = non-nucleoside reverse transcriptase; RTI = reverse transcriptase inhibitor.

Table 1: Frequency of vitamin D level among Omani AIDS patients in relation to types of HARRT

<table>
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<th>CD4+ T cell count (cell/ml)</th>
<th>25(OH)D level (nmol/L)</th>
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<td></td>
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Table 2: Correlation of the CD4+ T cell count and vitamin D level among Omani AIDS patients

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Figure 1. Prevalence of vitamin D deficiency among AIDS male Omani patients (n=31) compare with female patients (n=32). Serum values did not differ significantly between the groups by chi-square ($P = 0.067$).
Figure 2. Level of serum 25-hydroxyvitamin D 25(OH)D in relation to age groups. Group1: patient younger than 30 year (n=12). Group2: patient older than 30 years (n=51). The 25(OH)D deficiency showed more prevalent among those younger than 30 years of age, $P = 0.067$. 
Figure 3. Level of serum 25-hydroxyvitamin D (25(OH)D) in relation to different types of highly active antiretroviral therapy (HAART). Serum values did not differ significantly between the groups by chi-square. **Group 1**: patient never on HAART. **Group 2**: patient on protease inhibitor (PI). **Group 3**: non-nucleoside reverse transcriptase inhibitor (NNRTI). **Group 4**: protease inhibitor and non-nucleoside reverse transcriptase inhibitor (PI+ NNRTI). **Group 5**: other reverse transcriptase inhibitor (RTI).
**Figure 4.** Level of serum 25-hydroxyvitamin D 25(OH)D in relation to CD$_4^+$ T cell count. **Group 1:** patients having CD$_4^+$ T cell count lesser than 500 cell/ml. **Group 2:** patients having CD$_4^+$ T cell count more than 500 cell/ml.