ISSN: 0976-3376

Available Online at http://www.journalajst.com

ASIAN JOURNAL OF SCIENCE AND TECHNOLOGY

Asian Journal of Science and Technology
Vol. 08, Issue, 08, pp.5236-5240, August, 2017

RESEARCH ARTICLE

SERUM VITAMIN D IN PATIENTS OF HIV/AIDS & ITS COORELATION WITH CD_4 COUNT& ART TREATMENT

***Dr. Nabeel Mushtaque Ahmed, Zaheer, M.S. Dr. Siddiqui, S.S. and Dr. Aiman Fatima**

Department of Medicine, Jawaharlal Nehru Medical College, Aligarh, India

ARTICLE INFO

Article History:
Received 17th May, 2017
Received in revised form
26th June, 2017
Accepted 14th July 2017
Published online 31st August, 2017

Key words:
Water quality,
Microbiological parameters,
Physical parameters,
Health education.
HIV, AIDS,
HAART, Vitamin D.

ABSTRACT

Vitamin D is important for cell growth, immunity, and metabolism. Vitamin D deficiency has classically been associated with rickets and decreased bone density and more recently with increased risk and severity of autoimmune diseases, cancers, myocardial infarction, diabetes, and infectious diseases. The active form of vitamin D (vitamin D3) has been implicated recently in an intracellular process known as autophagy. HIV-1 reduces autophagy during permissive infection and that agents that induce autophagy, including vitamin D3, can inhibit HIV-1 replication. These findings help provide a biological explanation for the increased risk of more rapid disease progression observed in HIV-infected persons with low levels of vitamin D or with genetic variants within the vitamin D receptor that alter binding to vitamin D. HIV itself can also affect levels of vitamin D. Furthermore the vitamin is metabolized by the body in the same way as many anti-HIV drugs, using the P450 pathway, and some earlier research had suggested that protease inhibitors can inhibit the body's ability to metabolize vitamin D_2. Persons with HIV infection frequently have low vitamin D $levels_3$. Moreover, patients treated with non-nucleoside reverse transcriptase inhibitors and protease inhibitors are at increased risk of vitamin D $deficiency_4$. Thus, vitamin D deficiency is common in HIV-infected persons regardless of treatment status, viral load, or D4+ lymphocyte count.

INTRODUCTION

Vitamin D is important for cell growth, immunity, and metabolism. Vitamin D deficiency has classically been associated with rickets and decreased bone density and more recently with increased risk and severity of autoimmune diseases, cancers, myocardial infarction, diabetes, and infectious diseases. The active form of vitamin D (vitamin D3) has been implicated recently in an intracellular process known as autophagy (Holick *et al.*, 1971). HIV-1 reduces autophagy during permissive infection and that agents that induce autophagy, including vitamin D3, can inhibit HIV-1 replication. These findings help provide a biological explanation for the increased risk of more rapid disease progression observed in HIV-infected persons with low levels of vitamin D or with genetic variants within the vitamin D receptor that alter binding to vitamin D. HIV itself can also affect levels of vitamin D. Furthermore the vitamin is metabolized by the body in the same way as many anti-HIV drugs, using the P450 pathway, and some earlier research had suggested that protease inhibitors can inhibit the body's ability to metabolize vitamin D (Mueller *et al.*, 2010).

****Corresponding author:*** **Dr. Nabeel Mushtaque Ahmed,**
Department of Medicine, Jawaharlal Nehru Medical College, Aligarh, India.

Persons with HIV infection frequently have low vitamin D levels (Rodriguez *et al.*, 2009). Moreover, patients treated with non-nucleoside reverse transcriptase inhibitors and protease inhibitors are at increased risk of vitamin D deficiency (Bouvier, 2009).Thus, vitamin D deficiency is common in HIV-infected persons regardless of treatment status, viral load, or CD4+ lymphocyte count.

AIMS AND OBJECTIVES

- To determine the mean serum levels of vitamin D among HIV/AIDS patients.
- Study the association between vitamin D status and CD4 cell counts in HIV patients.
- Study the association between HAART and Vitamin D status in HIV patients.

MATERIALS AND METHODS

We carried out this study within the framework of the Revised National Tuberculosis Control Programme (RNTCP) and National Aids Control Programme (NACP) with diagnosis and treatment of HIV and TB patients conducted in accordance with recommended procedures. Diagnosis of HIV infection was done by confirmatory Western Blot Assay and diagnosis

of pulmonary tuberculosis (PTB) was established using sputum smear microscopy and chest x-ray. Patients suspected to have TB from in and out patients department were asked to submit 3 sputum (spot - morning – spot) for examination of acid fast bacilli (AFB) using Ziehl-Neelsen staining. A definitive diagnosis of PTB was made when patients met the following criteria; (a) at least two positive AFB smears from the two different sputum smears; and (b) One positive AFB smears and typical results of lung infection on chest X-ray.

HIV test was done by confirmatory Western Blot Assay.

Inclusion and exclusion criteria

The present study included all HIV positive and HIV/Pulmonary Tuberculosis patients who attended ART Centre of Jawaharlal Nehru Medical College Hospital, Aligarh between January 2012 to September 2014. Subjects were excluded from the study if they had diseases deemed to affect vitamin D metabolism such as:

- Diabetes Mellitus,
- Hypertension,
- Chronic Liver Disease (AST>200, or ALT>225 IU/liter)
- Renal Failure (Creatinine>2.0 mg %)
- Patients on drugs that might affect serum vitamin D, such as ketoconazole and anticonvulsants.
- Patients with prolonged immobility (over one month) were not recruited to the study.
- AIDS related Neoplasia
- HIV positive women who were pregnant at the time of recruitment were excluded from the study, as were lactating mothers in WHO stage I or II, who started ART exclusively to prevent vertical transmission

Sampling and Study Design

A simple randomized sampling selection of HIV patients who attended ART Centre during the study period was done, which included a total of 100 HIV patients of which 49 were male, 51 female, 80 on ARV, 20 pre-ARV and 14HIV/TB co-infected making up 100 of the total sample size required. This study was an open- labelled, randomized, cross-sectional study.

Data Collection

Participants Recruitment

Patients attending Anti-Retroviral Treatment (ART) Centre at Jawaharlal Nehru Medical College and Hospital were seen on a first come first serve basis. An estimate of more than 250 patients attended the ART Centre during the study period. These include patients who come three monthly for laboratory checkup, drugs collection, and newly HIV/TB diagnosed. Each person was given an ART number and a patient card, which then waits until their number was called to be seen by the clinician. A purposive sampling method meeting the criteria during the data collection period was applied. Due to the fact that HIV/TB co-infected patients were relatively few, a roaster list of patient planned to visit the clinic the day after was used to identify patients with co-infection (HIV/TB). To every HIV/TB patient identified, two HIV patients were then selected randomly.

The list (ID numbers) of the patients selected was then handled to the clinician on duty the day after. When the potential patient arrived, the examining clinician referred him/her to the researcher. The researcher queried their interest in participating in this study. If interested, the researcher reviewed eligibility criteria and explained the purpose of the study, method which included free viral loads, vitamin D, duration, benefits and risks as part of the informed consent. Adequate time was given for the participant to consider participation and asking questions if any. A written informed consent was obtained from all individuals who agreed to participate. A study ID number was then allocated.

Abstraction of medical records

A standard data abstraction tool relating to clinical history was developed and used to abstract required information from the patients files/charts and HIV/AIDS treatment and monitoring database. The following data were recorded: duration and kind of antiretroviral/ Anti- TB therapy, CD4 T cell counts, Complete Blood Cells count (CBC) and blood chemistries (Creatinine, AST and ALT,), demographic data such as (age, sex), duration and stage of HIV infection [according to WHO CD4 criteria], HIV and ARV duration were calculated from the date confirmed HIV positive and date started ARV respectively to the date when blood sample for vitamin D analysis was taken and age was calculated from the date of birth to the date when blood taken for vitamin D.

Specimen collection & Processing

Under aseptic techniques, we performed routine venous puncture. Using a serum separator tube (SST; Becton Dickinson, Franklin Lakes, NJ, USA), 7mls non fasting whole blood was drawn and left to clot for about 30 minutes, followed by centrifugation for 12-15 minutesneeded for analysis of vitamin D 25-(OH)-D. Other laboratory measurements that we checked at Jawaharlal Nehru Hospital included Liver function enzymes (Aspartate & Alanine transaminases), serum Creatinine, absolute CD4 cells count, and Complete Blood Cells counts (CBC).

Laboratory analysis

Hematological and Chemistry analysis

Complete Blood Count: Complete blood cell counts were done using Sysmex Kx-21 (Sysmex Corporation; Kobe Japan). The machine automatically dilutes a whole-blood sample, lyses, counts and gives a printout result of absolute numbers of leucocytes (expressed as number of cells × [109] per liter), erythrocytes(number of cells × [1012] per liter), platelets (number of cells × [109] per liter), lymphocytes(number of cells × [109] per liter), mononuclear cells (number of cells × [109] per liter), granulocytes (number of cells × [109] per liter) and hemoglobin (grams per deciliter). The quality and accuracy of the technique and the machine was assessed every six months.

Blood Urea**:** This was performed by Nessler's method.

Serum Creatinine**:** Serum creatinine was estimated by Jaffe's Manner's method.

***Liver function test*:**

a) ***Total serum bilirubin*:** estimated by Van den Bergh method of calorimetry.
b) ***AST/ALT*:** Serum aspartate aminotransaminase (AST) and alanine aminotransaminase (ALT) were estimated by simplified calorimetric test at 505nm.
c) ***Alkaline phosphatase*:** Estimated calorimetrically by the test described by E.J. King.

CD4 T cell counts analysis

Cluster differential cells (CD4 T cells) were analyzed using a FACS Count Flow cytometer (Becton Dickinson Immunocytometry Systems, San Jose, Calif.). In brief, 50 µl of whole blood was mixed and incubated at room temperature for 20 min with 20 µl of aCD4. Red blood cells were then lysed by adding 450 µl of fluorescence-activated cell sorter lysing solution (Becton Dickinson Immunocytometry Systems). The tubes were incubated at room temperature for 10 min, and then analyzed with the FACS Count's Cell Quest software (Becton Dickinson Immunocytometry Systems) within six hours. By using quality control (Multicheck; Becton Dickinson Immunocytometry Systems), the accuracy of the technique was assessed every 6 months.

Serum Vitamin D Analysis

We measured total serum 25-(OH)-D by Diasorin competitive radioimmunoassay (RIA) (AID Diagnostika, GmbH, Strasburg, Germany). Prior to analysis, the serum samples were precipitated and extracted with acetonitrile. After centrifugation, an aliquot of the supernatants were incubated with specific antibodies against 25(OH)D for 90 minutes and 2hours respectively at an ambient temperature. After centrifugation, the supernatants were decanted and counted.

Definition of Vitamin D Status

Vitamin D status was defined as:
Serum 25(OH)D levels ≤ 75 nmol/l was used to define hypovitaminosis, and serum 25(OH)D ≤ 25, 25 – 50, and 50 – 75 nmol/l defined severe vitamin deficiency, mild vitamin D deficiency and vitamin D insufficiency, respectively (Gordon *et al.*, 2004).

Data Management

The principle investigator abstracted and filled the information obtained from the patients file to the abstraction forms. Immediately following completion of abstraction form, the researcher double checked the instruments for completeness and consistency of answers. Completed abstraction forms were then coded by numbers and entered in Microsoft excel sheet version 2010. Cross-checking and data cleaning was done. During data cleaning and cross checking missing information were obtained by going back to the abstraction form, HIV treatment and monitoring database and when necessary reviewing the patients on the next visit to the clinics. The data were then transferred to SPSS version 21 software for analysis.

Statistical analysis

All analyses were performed with SPSS software for Windows, version 21 (SPSS Inc., IBM, Version 21.0, USA).

RESULTS

In our study group, there were 14 patients (14%) having HIV/TB co-infection. Of which 7 were male (50%) and 7 were female (50%).86 patients (86%) were HIV-1 mono-infected patients of which 43 were male (50%) and 43 were female (50%). 20 (20%) patients were not HAART of which 10 were females (50%) and 10 were males (50%). The average CD4 count in our study group was 314.28 ± 178.8.30% of the patients had CD4 counts below 200/mm^3; 57% between 200-500 cells/mm^3 while only 13% of patients had CD4 counts above 500 cells/mm3.The mean Serum Vitamin 25-(OH)D was 44.996 nmol/l ±12.43.64% of the patients had a Vitamin D level between 25-50 nmol/L, 35% of patients had a Vitamin D level between 50-75 nmol/L while only 1 patient was having a Vitamin D level above 75 nmol/L and none below 25 nmol/L. Subjects with CD4 counts <200 cells/mm^3 had a mean 25(OH)D level of 44.68 nmol/l while subjects with CD4 counts between 200-500 cells/mm^3 had a mean 25(OH)D level of 44.99 nmol/l; subjects having a CD4 count > 500 cells/mm^3 had a significantly greater mean 25(OH)D levels of 59.88nmol/l.

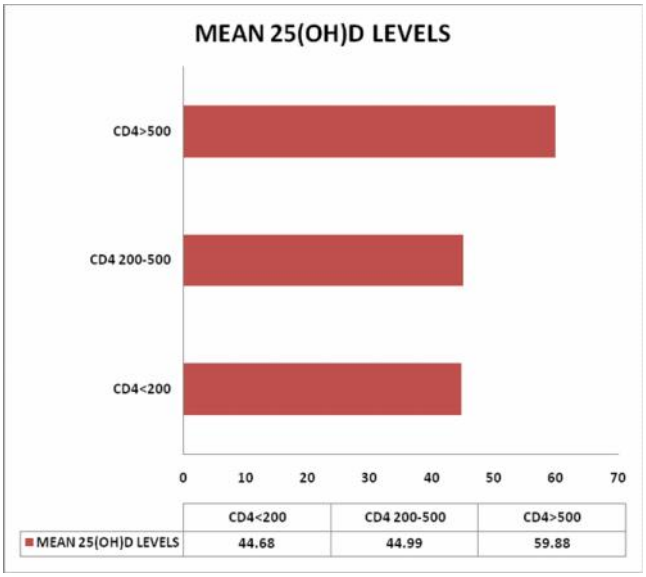

Figure 1. Immune Stage and Serum Vitamin D levels

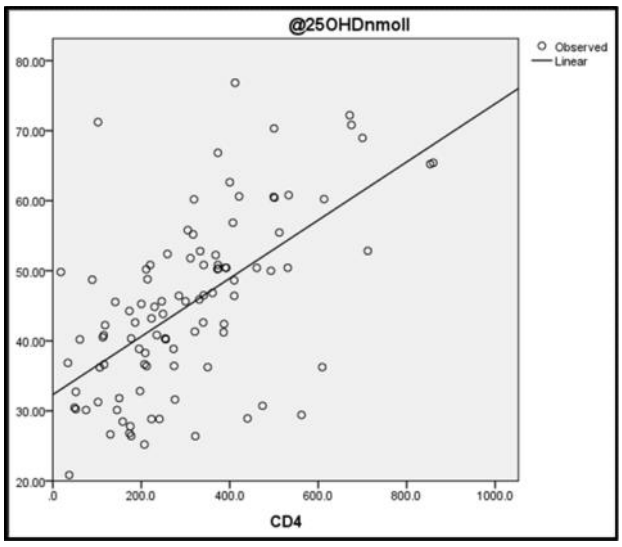

Figure 2. Linear Regression between Dependent Variable 25 (OH) D and Independent Variable CD4

Using Spearman's Correlation Coefficient; the Correlation coefficient between CD4 count and Serum 25(OH)D level in patients of HIV was found to be 0.596 with a statistically significant p value of <0.001. The mean 25(OH)D levels in patients of HIV was 47.11±11.8 nmol/l while in patients of HIV/TB Co-infection was found to be 31.99±7.0 nmol/l.It was found out in our study that 58.1 % of HIV patients had Vitamin D levels less than 50 nmol/l while 41.9 % of them had a Vitamin D level between 50-75 nmol/l.On the contrary, only 7.2 % of HIV/TB co-infection patients had a Vitamin D level of 50-75 nmol/l while a highly significant 92.8 % of them had a Vitamin D level less than 50 nmol/l. Of all; 80 (80 %) HIV patients of the 100 were on antiretroviral treatment of which 40 were male and 40 were female.20 (20 %) patients were not on antiretroviral treatment of which 10 were male and 10 were female.HAART has been documented to have a decreasing effect on hepatocyte serum 25-(OH)-D and macrophage 1,25-(OH)2D levels.In our study, the mean serum 25-(OH)-D levels in patients on HAART was found to be 44.07 ± 11.8 nmol/l while the mean serum 25-(OH)-D levels in patients not on HAART was found to be 48.68 ± 14.4 nmol/l.Thus in our study the mean serum 25-(OH)-D levels in HIV patients not on HAART was found out to be higher than patients on HAART.Moreover, it was found out that 66.2 % of patients on HAART were having serum Vitamin D deficiency (25-OH-D levels < 50 nmol/l)as compared to 50 % of patients not on HAART.

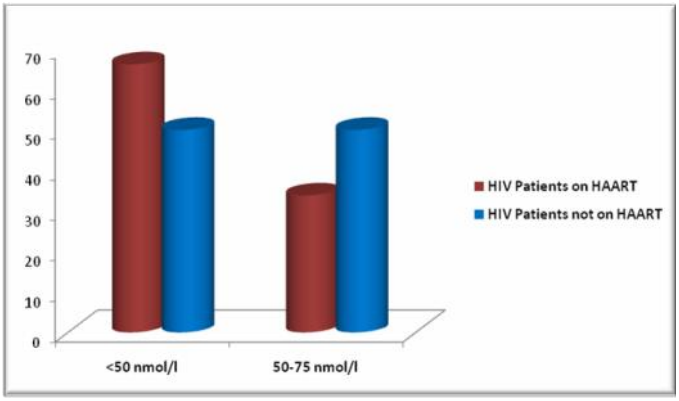

Figure 3. Comparison between Serum Vitamin D levels in patients on HAART and not on HAART

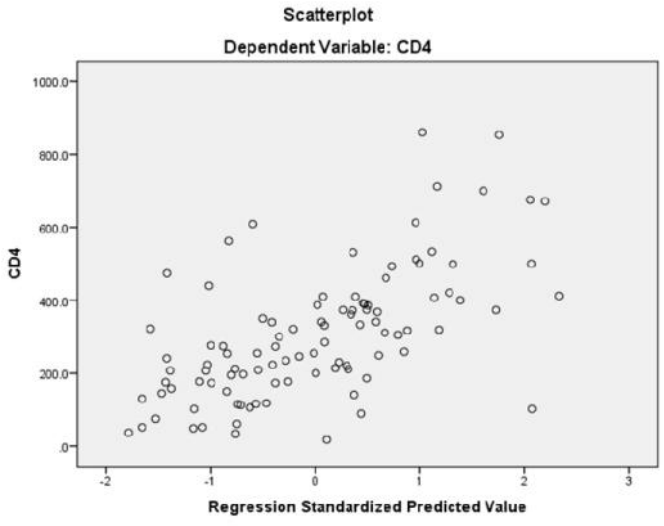

Figure 4. Scatter Plot Depicting Dependent Variable CD4 with respect to the Regression Standardized Predicted Value

It was thus found that 25(OH)D was a highly significant parameter (p value <0.001) contributing to low CD4 counts.

DISCUSSION

This study was an open-labelled, randomized, cross-sectional study to observe the serum levels of vitamin D among HIV-1 mono-infected and HIV/TB co-infected patients enrolled in ART Centre JNMC, AMU, Aligarh. Serum 25-(OH)-D associations with antiretroviral therapy werealso determined. In our study 100 HIV Positive patients were included. Out of which, 49 were male (49%) and 51 were female (51%) of the study group respectively. This study was a cross sectional study, and the design should be appropriate for the study questions, because we set out to determine serum vitamin D levels among HIV-1 positive and HIV-1 co-infected with pulmonary tuberculosis. The mean Serum Vitamin 25-(OH) D in our study was 44.996 nmol/l ±12.43. 64% of the patients had a Vitamin D level between 25-50 nmol/L, 35% of patients had a Vitamin D level between 50-75 nmol/L while only 1 patient was having a Vitamin D level above 75 nmol/L and none below 25 nmol/L. Thus in our study 99 patients (99 %) of the study group had Hypovitaminosis D; 64 (64 %) were having Vitamin D insufficiency. HIV infection was significantly associated with vitamin D status among HIV subjects in this study.Our findings are consistent with the study done in Tanzania urban, where they found low vitamin D status (serum 25-hydroxyvitamin D < 32 ng/ml) was significantly associated with HIV progression (WHO HIV disease stage III) (Mehta *et al.*, 2011). In our study we found that there was as a significant correlation between the Immune Stage i.e. CD4 counts and levels of Vitamin D ; with patients having a CD4 count less than 200 cells/mm^3 having a mean 25-(OH)-D level of 44.68 nmol/l; patients having a CD4 count between 200-500 cellls/mm^3 having a mean 25-(OH)-D level of 44.99 nmol/l and patients having a CD4 count above 500 cells/mm^3 having a mean 25-(OH)-D level of 59.88 nmol/l with a statistically significant p value of <0.001. Furthermore, it was seen in our study that besides low Total Leukocyte Counts, serum 25(OH)D levels is a significant predictor of low CD4 cell counts in the study group (p value <0.0001).

The mean 25(OH)D levels in patients of HIV was 47.11±11.8 nmol/l while in patients of HIV/TB co-infection was found to be 31.99±7.0 nmol/l. It was found out in our study that 58.1 % of HIV patients had Vitamin D levels less than 50 nmol/l while 41.9 % of them had a Vitamin D level between 50-75 nmol/l. On the contrary, only 7.2 % of HIV/TB co-infection patients had a Vitamin D level of 50-75 nmol/l while a highly significant 92.8 % of them had a Vitamin D level less than 50 nmol/l with a highly significant p value of <0.001. These findings add to the growing evidence that vitamin D plays a role in the regulation of *Mycobacterium tuberculosis*. In our study it was further found that CD4 count (p value 0.004), patients not on HAART (p value 0.005) and remarkably patients having a low 25(OH)D have a highly significant contribution to the development of HIV/TB co-infection with a p value of <0.001 which is highly significant. In our study, HIV/TB co-infected patient had 4.8 standard deviation lower serum 25(OH) D concentrations than in the HIV mono-infected patients which was in agreement with the observations found in the meta-analysis. Thus, overall, our finding is consistent with most previous studies. Although low 25-(OH)-D and calcium levels in HIV/TB patients could be a

consequence of the disease, it is highly possible that vitamin D is antecedent risk factors for tuberculosis. In our study there was a difference between HIV patients receiving HAART and HIV patients naïve of HAART. The mean serum 25-(OH)-D levels in patients on HAART was found to be 44.07 ± 11.8 nmol/l while the mean serum 25-(OH)-D levels in patients not on HAART was found to be 48.68 ± 14.4 nmol/l. Thus in our study the mean serum 25-(OH)-D levels in HIV patients not on HAART was found out to be higher than patients on HAART. Moreover, it was found out that 66.2 % of patients on HAART were having serum Vitamin D deficiency (25-OH-D levels < 50 nmol/l) as compared to 50 % of patients not on HAART. However, the p value in our study for the above observation came out to be 0.138.This is explained by the small sample size of HAART naïve HIV patients in our study group. Our finding of low serum 25-(OH)2D following use of HAART in HIV infected patients may have some explanation and implications; Use of HAART especially protease inhibitors (PI) and non-nucleoside reverse transcriptase inhibitors (NNRTIs) have been found to inhibit the function of hepatic-25-hydrolylase and macrophage-1,25-hydroxylase which are critical for active vitamin D synthesis. The net effect is a reduced production of 1, 25-(OH)2 D that could influence immunity (Cozzolino *et al.*, 2003).

Conclusion

This cross-sectional study showed that

- Hypovitaminosis D was highly prevalent among HIV, HIV/TB co-infection. (p value < 0.001)
- Advanced stage of HIV disease was associated with lower serum 25(OH) D concentrations.
- CD4 count has a significant association with serum 25(OH)D concentrations in HIV. (p value <0.001)
- Serum 25(OH) D levels is a highly significant predictor of low CD4 cell counts in the study group. (p value < 0.001)
- Serum 25-(OH)-D concentration was significantly higher in HIV mono-infected than HIV/TB co-infected patients, hence hypovitaminosis D was more common among HIV/TB co-infected compared to HIV-1 mono-infected. (p value < 0.001)
- Hypovitaminosis D was higher in HIV patients on antiretroviral therapy compared with patients not on ART but the difference was not statistically significant due to small sample size of HAART naïve patients. (p value = 0.138)

REFERENCES

Bouvier, G. 2009. Protease inhibitors can interfere with vitamin D metabolism. *HIV Clin.*, 21:9-10.

Cozzolino, M., Vidal, M., Arcidiacono, M.V., Tebas, P., Yarasheski Ke, Dusso As, 2003. HIV protease inhibitors impair vitamin D bioactivation to 1,25-dihydroxyvitamin D. AIDS, 17: 513-520.

Gordon, C.M., DePeter, K.C., Feldman, H.A., Grace, E., Emans, SJ. 2004. Prevalence of vitamin D deficiency among healthy adolescents. *Arch Pediatr Adolesc Med.*, Jun, 158(6):531-537.

Holick, M.F., Schnoes, H.K., Deluca, H.F., Suda, T., Cousins R.J. 1971. "Isolation and identification of 1, 25-dihydroxycholecalciferol. A metabolite of vitamin D active in intestine". *Biochemistry*, 10, (14): 2799–804.

Mehta, S., Mugusi, F.M., Spiegelman, D., Villamor, E., Finkelstein, J.l., Hertzmark, E., Giovannucci, E.l., msamanga , G.I., Fawzi, W.W. 2011. Vitamin D status and its association with morbidity including wasting and opportunistic illnesses in HIV-infected women in Tanzania. AIDS Patient Care STDS, 25: 579-585

Mueller, N.J., Fux, C.A., Ledergerber, B. et al. 2010. High prevalence of severe vitamin D deficiency in combined antiretroviral therapy–naive and successfully treated Swiss HIV patients. AIDS, 24:1127-1134

Rodriguez, M., Daniels, B., Gunawardene, S., Robbins, G.K. 2009. High frequency of vitamin D deficiency in ambulatory HIV-positive patients. AIDS Res Hum Retroviruses, 25:9-14.

****