**Full title:** Prevalence of vitamin D deficiency in HIV positive, antiretroviral treatment

naïve patients in a single centre study.

**Short title:** Vitamin D deficiency in ART naïve patients

**Key words:** HIV; Vitamin D; Antiretroviral treatment.

**Word Count:** 1296 (excluding table, abstract and references)

**Abstract**

*Objective***:**  To describe the prevalence of vitamin D deficiency among antiretroviral treatment (ART)-naïve, HIV positive individuals.

*Methods***:** We reviewed records of consecutive ART-naïve patients, registering for care for the first time at a London clinic from 01/01/2008 to 31/12/2009. During this period, serum 25-hydroxycholecalciferol (25-OHD) was measured routinely for all new patients. 25-OHD deficiency and severe deficiency were defined as ≤50 and ≤25 nmol/L, respectively.

*Results***:** Among 253 patients (82% male, median age 36 years, 64% white ethnicity), 148 (58.5%) were 25-OHD deficient, including 32 (12.6%) who were severely deficient. 73.5% (61/83) patients of non-white ethnicity were 25-OHD deficient compared with 50.7% (76/150) of those reporting white ethnicity (p<0.001).  Seven of eight (87.5%) patients with hypocalcaemia (<2.12nmol/L) were 25-OHD deficient. The prevalence of 25-OHD deficiency was higher in winter and spring vs. summer and autumn (89/129 [69.0%] vs. 59/124 [47.6%], p<0.001). Serum 25-OHD deficiency was not associated with gender, CD4 count, HIV viral load or clinical stage.

*Conclusion***:** Serum 25-OHD deficiency was common among ART-naïve patients, with those of non-white ethnicity at highest risk. CD4 count, HIV viral load and HIV clinical staging do not help to identify those at risk, but low serum calcium should prompt investigation of 25-OHD levels.

**Introduction**

In addition to vitamin D's established role in bone metabolism, vitamin D deficiency is reported to be associated with a variety of medical conditions including diabetes, cardiovascular disease [1–5] and infectious diseases particularly tuberculosis and HIV [6–11].

Studies investigating vitamin D's role in HIV infection have been conflicting but associations with an increased risk of HIV acquisition and progression to advanced HIV have been shown [9,12,13] . Vitamin D deficiency is also speculated to be associated with an increased risk of cardiovascular disease in non-HIV infected populations [2–5]. One study demonstrated an association between lower vitamin D levels and greater common carotid artery intimal thickness values in HIV positive individuals [14]. Severe vitamin D deficiency (serum 25-hydroxycholecalciferol (25-OHD) concentration <10μg/ml or <25nmol/L) has also been associated with increased levels of serum inflammatory markers including IL-6 and hsCRP [15] indicating vitamin D deficiency as a risk factor for cardiovascular disease specifically in the HIV population.

Specifically, HIV positive individuals are speculated to be at increased risk of vitamin D deficiency [10,11,13,16–18]. Dark-skinned individuals in the UK have a higher risk of vitamin D deficiency; one study of over eight hundred patients from Birmingham revealed a prevalence of vitamin D deficiency (25-OHD <10 $\mu$g/L) of 26% among Afro-Caribbeans and 31% among Asians compared to 12% of Caucasians [19] . As many HIV positive individuals in the UK are migrants from African countries, this may in part explain the high prevalence observed.

Recent studies in the UK investigating the prevalence of vitamin D deficiency in HIV positive individuals have involved populations where the majority of individuals were of black ethnicity [16,17]. Importantly, previous studies have also investigated the prevalence of vitamin D deficiency predominantly in individuals established on antiretroviral treatment (ART) [10,16–18]. ART itself is associated with vitamin D deficiency, non-nucleoside reverse transcriptase inhibitors (NNRTI's) and protease inhibitors (PI's) appear to interfere with vitamin D metabolism [17,18,20–22] whereas tenofovir may be a culprit by decreasing bone mineralisation and causing secondary hyperparathyroidism through effects on renal phosphate regulation [16,23]. The impact of immune reconstitution on vitamin D levels is less clear.

A recent systematic review of the literature on vitamin D deficiency in HIV infection reported that vitamin D deficiency, secondary hyperparathyroidism and low bone mineral density were common in HIV positive patients but that HIV infection may not be specifically associated with vitamin D deficiency (24). Importantly all the studies reviewed investigating vitamin D deficiency were observational studies that included HIV positive patients on ART or who were ART experienced. Although some adjusted for ART use and individual ART/drug classes, they are still subject to confounding.

The objective of this study was to describe the prevalence of vitamin D deficiency specifically among ART-naïve individuals newly diagnosed with HIV in a central London clinic.

**Methods**

Patients registering for HIV care between 01/01/2008 and 31/12/2009 for the first time at the Mortimer Market centre, a large HIV clinic in central London were included in this retrospective record review. During this period, serum 25-OHD levels were measured routinely for new patients registering for HIV care. We restricted the analysis to ART-naïve patients with an available serum 25-OHD result, all patients included were not taking vitamin D supplements. Vitamin D status was defined as "normal" when 25-OHD was >50nmol/L, "deficient" when 25-OHD ≤50nmol/L and "severely deficient" when 25-OHD was ≤25nmol/L.

We reviewed data fields including demographics, CD4 cell count, HIV RNA viral load; self-reported ethnicity; season when the 25-OHD sample was taken (winter [December – February]; spring [March – May]; summer [June – August]; autumn [September - November]); serum glucose, calcium, phosphate, alkaline phosphatase (ALP) and HIV clinical stage (asymptomatic; symptomatic; AIDS, as recorded by the clinician). Continuous data were grouped into categories and Fisher's exact test was used to assess associations. Associations with vitamin D deficiency were explored by calculating unadjusted odds ratios.

This was a retrospective audit of anonymised data and specific ethical approval was not requested.

**Results**

Among 253 patients fulfilling inclusion criteria, 207 (81.8%) were male and the median

age was 36 years (range 16 - 75 years).  Among 233 with ethnicity data available, 150

(64.4%) were white, and 83 (35.6%) black or other ethnicity. The median CD4 count and

HIV viral load were 450 cells/mm$^3$ (range $10 – 1100$ cells/mm$^3$) and 8,600 copies/mL

(range; less than 50copies/ml $– 11,000,000$ copies/mL) respectively.  236 (93.3%)

presented with asymptomatic HIV infection.


Among 253, 148 (58.5%) patients were 25-OHD deficient (25-OHD ≤50nmol/L),

including 32 (12.6%) who were severely deficient (25-OHD ≤25 nmol/L) (Table 1).

Among 83 patients of non-white ethnicity 61, (73.5%) were 25-OHD deficient compared

with 50.7% (76/150) of those reporting white ethnicity (p<0.001).

Seven of eight (87.5%) patients with hypocalcaemia (<2.12 mmol/L) were 25-OHD

deficient, as were seven of 15 (46.7%) with hypophosphataemia (<0.8mmol/L) and seven

of 11 (63.6%) with high alkaline phosphatase (>130IU/L).  There was no association

between 25-OHD deficiency and hypophosphataemia. The prevalence of 25-OHD

deficiency was higher in winter and spring vs. summer and autumn (89/129 [69.0%] vs.

59/124 [47.6%], p<0.001). Serum 25-OHD deficiency was not associated with age,

gender, CD4 count, HIV viral load, serum glucose or HIV clinical stage.

On univariable analysis, there was trend towards vitamin D deficiency and

hypocalcaemia [p=0.14, odds ratio 5.44 (95% confidence interval $0.7 – 45.0$)] larger

numbers may have produced a significant association and a smaller confidence interval

with these results reflecting small numbers. Season and ethnicity were the only factors

significantly associated with vitamin D deficiency on univariable analysis (where

p<0.05), therefore unadjusted odds ratios were calculated to report measures of

association for season and ethnicity (see table).

**Discussion**

This study found a high prevalence of vitamin D deficiency among newly diagnosed,

ART-naïve patients; which, therefore cannot be attributed to the effect of antiretroviral

agents.  Our study used a vitamin D level of ≤50nmol/L to define deficiency, optimal

vitamin D status is reflected by serum concentrations of 25-OHD of 75 nmol/l (30 μg/l)

and above [5].  We found that 58.5% were deficient including 75% reporting black

ethnicity and 51% of those reporting white ethnicity.  This compares with a study in

South London which found that 57% (131 of 227 HIV positive subjects) were vitamin D

deficient (<50nmol/L), however in their study population only 15% (33/227) were ART-

naïve and over two thirds of the sample were of black ethnicity [16].  Another study in

South London of 1077 HIV positive patients found that 73.5% were vitamin D deficient

(<50nmol/L) but only 12.4% of their study population were ART-naïve and 60% were of

black ethnicity [17].

A recent study investigating vitamin D levels in Swiss HIV-positive patients found a

positive association between vitamin D and time since HIV diagnosis on multivariable

analysis.  They speculated that this may be related to the potential poorer health of

recently diagnosed patients, however the relationship to ART use was unclear [18].

As expected, we found a higher prevalence of vitamin D deficiency in non-white ethnic groups and an association in the winter and spring months [1,8,16–19]. There were no other demographic factors, which identified individuals at higher risk. Among biochemical variables, most of our patients with hypocalcaemia were vitamin D deficient; but a significant association was not observed probably due to relatively small numbers. Other markers of bone metabolism (phosphate, alkaline phosphatase) were not associated with vitamin D deficiency.

Our study population was predominantly white males with asymptomatic HIV infection, however it has demonstrated a high prevalence of vitamin D deficiency in HIV positive, ART-naïve individuals, which thus cannot be attributed to ART. The only clinical factor associated with vitamin D deficiency was non-white ethnicity. This suggests that all HIV positive individuals should be considered for routine screening, particularly individuals of non-white ethnicity. CD4 count, HIV viral load and HIV clinical staging do not help to identify those at risk, but low serum calcium should prompt investigation of 25-OHD levels.

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 **Table: Prevalence of vitamin D deficiency by demographic characteristics and relevant laboratory variables**

| | Total Sample (n=253) | Normal Vitamin D (>50nmol/L) | Deficient Vitamin D (≤50nmol/L) | p-value (Fishers Exact) | Odds ratio (95% confidence interval) |
|---|---|---|---|---|---|
| **Sex, n (%)** | | | | | |
| Male | 207 (81.8%) | 90 (43.5%) | 117 (56.5%) | p=0.19 | |
| Female | 46 (18.2%) | 15 (32.6%) | 31 (67.4%) | | |
| **Age Range** | | | | | |
| <30 | 80 (31.6%) | 48 (60%) | 32 (40%) | p=0.90 | |
| 31 – 40 | 88 (34.8%) | 52 (59.1%) | 36 (40.9%) | | |
| >40 | 85 (33.6%) | 48 (56.5%) | 37 (43.5%) | | |
| **Ethnicity, n (%)** (excl unknown, n=233) | | | | | |
| White | 150 (59.3%) | 74 (49.3%) | 76 (50.7%) | p=0.01 | |
| Black | 63 (24.9%) | 16 (25.4%) | 47 (74.6%) | | |
| Other/non-white (Asian, Chinese, mixed race) | 20 (8.6%) | 6 (30%) | 14 (70%) | | |
| **Ethnicity, n (%)** (excl unknown, n=233) | | | | | |
| White | 150 (64.4%) | 74 (49.3%) | 76 (50.7%) | p<0.001 | 2.7 (1.5 – 4.8) |
| Non white | 83 (35.6%) | 22 (55.4%) | 61 (73.5%) | | |
| **Season, n (%)** | | | | | |
| Summer/Autumn | 124 (49%) | 65 (52.4%) | 59 (47.6%) | p=0.001 | 2.5 (1.5 – 4.1) |
| Winter/Spring | 129 (51%) | 40 (31%) | 89 (69%) | | |
| **KC60\* HIV Stage, n (%)** | | | | | |
| E1 (asymptomatic) | 236 (93.3%) | 135 (57.2%) | 101 (42.8%) | p=0.241 | |
| E2 (symptomatic) | 7 (2.8%) | 6 (85.7%%) | 1 (14.3%) | | |
| E3 (AIDS) | 10 (3.95%) | 7 (70%) | 3 (30%) | | |

| | | | | | |
|---|---|---|---|---|---|
| **CD4 count (cells/mm³ x10)**<br>(missing values)<br>Median<br>IQR | (12/253)<br>0.45<br>0.60 – 0.30 | (9/148)<br>0.43<br>0.59 – 0.25 | (3/105)<br>0.47<br>0.61 – 0.36 | p=0.651 | |
| **HIV viral load (copies/mL)**<br>(missing values)<br>Median<br>IQR | (9/253)<br>8,600<br>66,500 – 335 | (6/148)<br>8,450<br>70,000 – 110 | (3/105)<br>11,500<br>62,000 – 730 | p=0.374 | |
| **Calcium (mmol/L)**<br><br>Normal (2.12 -2.65)<br><br>Hypocalcaemia (<2.12) | <br><br>224(96.5%)<br><br>8 (3.5%) | <br><br>98 (43.7%)<br><br>1 (12.5%) | <br><br>126 (56.3%)<br><br>7 (87.5%) | <br><br>p=0.14 | <br><br>5.4 (0.7 – 45.0) |
| **Phosphate (mmol/L)**<br><br>Normal (0.8-1.4)<br><br>Hypophosphataemia<br>(<0.8) | <br><br>210 (90.5%)<br><br>15 (6.5%) | <br><br>89 (42.4%)<br><br>8 (53.3%) | <br><br>121 (57.6%)<br><br>7 (46.7%) | <br><br>p=0.43 | |
| **ALP (IU/L)**<br><br>Normal (30-130)<br><br>High ALP (>130) | <br><br>233 (95.1%)<br><br>11 (4.5%) | <br><br>98 (42.1%)<br><br>4 (36.4%) | <br><br>135 (57.9%)<br><br>7 (63.6%) | <br><br>p=0.77 | |

* the KC60 code, is a Health Protection Agency (HPA) required 'diagnoses made' and 'services provided' code provided by all genitourinary clinic services in the UK for each patient presentation; for the purpose of internal management, audit and reporting data to the HPA.