Relationship of vitamin D insufficiency to AIDS-associated Kaposi's sarcoma outcomes: retrospective analysis of a prospective clinical trial in Zimbabwe

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Summary

Objectives—The prevalence of vitamin D insufficiency in Africans with AIDS-associated Kaposi sarcoma (AIDS-KS) and the role of vitamin D in AIDS-KS progression are unknown. We hypothesized that a high prevalence of vitamin D deficiency would be found in Zimbabweans with AIDS-KS and that low baseline vitamin D would correlate with progression of AIDS-KS.

Methods—Ninety subjects were enrolled in a prospective pilot study investigation of the effect of antiretroviral therapy in the treatment of AIDS-KS in Harare, Zimbabwe. Co-formulated abacavir, lamivudine, and zidovudine was initiated; chemotherapy was provided at the discretion of the provider. Participants were followed for 96 weeks. 25-Hydroxyvitamin D was measured in stored specimens collected at study entry. The relationship between vitamin D and clinical response was described by odds ratio and 95% confidence interval.

Results—Samples were available for 85 participants; 45 (53%) subjects had inadequate (<75 nmol/l) 25-hydroxyvitamin D. HIV-1 RNA was significantly higher among those with insufficient vitamin D (4.7 vs. 4.5 log, $p = 0.04$). Tumor response, survival, and KS-IRIS were not associated with vitamin D ($p > 0.3$).
Conclusions—Vitamin D insufficiency was common among Zimbabweans with AIDS-KS but not associated with outcomes after initiation of antiretroviral therapy.

Keywords
AIDS-KS; Vitamin D insufficiency; HIV

Introduction
AIDS-associated Kaposi’s sarcoma (AIDS-KS) is the most common tumor in Zimbabwe. Without antiretroviral therapy (ART), AIDS-KS is associated with more than 85% mortality, due to progression of KS or complications associated with advanced AIDS. With effective ART, the mortality associated with AIDS-KS is improved, but the response of clinical AIDS-KS lesions is discouraging, at less than 20%.

Moderate associations of low vitamin D levels with cancer progression have been observed in stomach, esophageal, gallbladder, colon, breast, prostate, and pancreatic cancer. Cancers with the most sensitive vitamin D effect appear to be those of rapidly proliferating tissue, specifically the oral–gastrointestinal tract and the bone marrow. Forms of vitamin D have been shown to inhibit tumor angiogenesis, and calcitriol both inhibits and upregulates vascular endothelial growth factor and angiogenesis in non-KS tumors. Little is known about the effect of vitamin D or the vitamin D receptor on vascular tumors such as KS. In vitro, both the activated form of vitamin D, 1,25-dihydroxycholecalciferol (1,25(OH)2D, calcitriol), and a vitamin D analogue have demonstrated inhibition of KS transformed cell lines. Mice with implanted KS tumors treated with calcitriol or a vitamin D analogue showed significant slowing of tumor growth. Of eight male patients with KS treated with the topical synthetic vitamin D receptor agonist calcipotriene, 50% had improvement in cutaneous lesions.

A high prevalence of vitamin D insufficiency occurs in persons with HIV-1 infection in the USA and in European countries. Persons recently emigrated from Africa to the USA, Europe, or Australia often have vitamin D insufficiency. Existing data on vitamin D among persons with or without HIV-1 residing in Sub-Saharan Africa also suggest a high prevalence of vitamin D insufficiency or deficiency. Based on the existing epidemiological and in vitro and in vivo data described above, we hypothesized that a high prevalence of vitamin D deficiency would be found in Zimbabweans with AIDS-KS and that low baseline vitamin D would be associated with the progression of AIDS-KS.

Methods
Study population
The current study was a retrospective analysis of existing data and stored specimens from 90 subjects enrolled in a prospective investigation of the effect of ART in the treatment of AIDS-KS (Clinicaltrials.gov number NCT00834457) at the Parirenyatwa Hospital Kaposi Sarcoma Clinic in Harare, Zimbabwe between June 2003 and May 2005. The study was reviewed and approved by the Medical Research Council of Zimbabwe and the Colorado
Clinical and immunological/virological outcomes

The stage of KS was assessed using the criteria of both Krigel et al. and the AIDS Clinical Trials Group, as described previously.3,33,34 Clinical response was defined as either complete or partial resolution of clinically apparent KS disease at week 96. Complete resolution was defined as the absence of KS lesions and tumor-related edema for a minimum of 4 weeks, lasting until the end of the follow-up period. Partial resolution was defined as ≥50% improvement in KS lesions without complete resolution. KS-associated immune reconstitution inflammatory syndrome (KS-IRIS) was defined as any progression of KS occurring ≤12 weeks after initiation of ART and was associated with an increased CD4+ lymphocyte count of at least 50 cells/μl above the baseline value, before or at the time of documented KS progression. Virological failure was defined as failure to suppress or failure to maintain suppression of the plasma HIV-1 RNA level to <400 copies/ml after week 24.

CD4 count, plasma HIV-1 RNA, and HHV-8 DNA in peripheral blood mononuclear cells (PBMC) and plasma were measured as described elsewhere.3,35 Plasma samples obtained at study entry were assayed for 25-hydroxyvitamin D (25(OH)D) with the Liaison immunoluminometric direct assay (DiaSorin, Stillwater, MN, USA). Vitamin D deficiency was defined as 25(OH)D ≤50 nmol/l and insufficiency as >50 nmol/l but <75 nmol/l. Inadequate and adequate 25(OH)D were defined as <75 nmol/l and ≥75 nmol/l, respectively.20,36,37 The rainy season was defined as November through April and the dry season as May through October.

Data analyses

Continuous variables are reported as the median and interquartile range (IQR) and categorical variables as the frequency and percentage. Continuous variables were tested with the Mann–Whitney test and categorical variables with the Chi-square test or Fisher’s exact test. The relationship between vitamin D and clinical response was described by odds ratio (OR) and 95% confidence interval (95% CI) and tested with a Chi-square or Fisher’s exact test. Logistic regression models were used to adjust for possible confounding of HIV-1 viral load and CD4 count in predicting clinical outcomes. Other analyses were considered exploratory and were not corrected for multiple comparisons. Statistical analyses were conducted in SAS v. 9.2 (SAS Institute) and GraphPad Prism (GraphPad Software Inc.), and assumed a two-sided significance level of 0.05.
Results

Prevalence of vitamin D insufficiency and study participant characteristics

Baseline serum samples were available for 85 of 90 subjects. Of those with available samples for analysis, the median 25(OH)D was 72.5 nmol/l (IQR 56.3–88.8 nmol/l). Forty-five (53%) subjects had inadequate 25(OH)D and 17 (20%) had 25(OH)D insufficiency. Fourteen subjects died, 11 subjects were lost to follow-up prior to week 96, and 60 subjects completed 96 weeks of observation (Figure 1). Further characteristics of the study population are provided in Table 1. Those enrolling during the rainy season had higher median 25(OH)D levels (78.75 vs. 62.5 nmol/l, p = 0.01).

Relationship of vitamin D status and clinical outcomes

Twenty-nine subjects were diagnosed with stage 4 KS at study entry and 71 with T1 disease; the median vitamin D in these subjects was 66.5 nmol/l (IQR 55.8–88.8) and 73 nmol/l (IQR 58–90), respectively. The proportion of stage 4 or T1 disease was similar between the 25(OH)D groups, and 25(OH)D levels were similar between persons with earlier and later stages of AIDS-KS, as shown in Table 1 and Table 2, respectively. Sixteen subjects (19%) had a partial or complete clinical response, 16 (19%) developed KS-IRIS, 49 (58%) required chemotherapy, and 22 (26%) required radiation therapy by 96 weeks. Clinical response (OR 0.6, 95% CI 0.2, 1.9; p = 0.4), development of KS-IRIS (OR 0.4, 95% CI 0.2, 1.9; p = 0.4), need for chemotherapy (OR 1.2, 95% CI 0.5, 2.8; p = 0.7), and need for radiation therapy (OR 0.6, 95% CI 0.2, 1.5; p = 0.2) were similar between those with inadequate and adequate 25(OH)D. ORs and significance remained unchanged after adjustment for HIV-1 viral load and CD4 count. The median 25(OH)D was not significantly different between those with or without a clinical response, KS-IRIS, chemotherapy, or radiation therapy (Table 2).

Baseline 25(OH)D measurements were available for 12 of 14 participants who died during the study. Those who died during the study had similar 25(OH)D (63 nmol/l, IQR 46–79) as the 60 subjects known to have survived to 96 weeks (69 nmol/l, IQR 55–85; p = 0.27). The odds of death by 96 weeks was not significantly associated with 25(OH)D (OR 0.5, 95% CI 0.1, 1.9; p = 0.3) and remained unchanged when adjusting for plasma HIV-1 RNA and CD4+ lymphocyte count.

Vitamin D and immunological/virological parameters

25(OH)D insufficiency was associated with significantly higher baseline plasma HIV-1 RNA and greater decrease in HIV-1 viral load with ART initiation (p = 0.04, Table 1). Baseline CD4+ count and CD4+ cell increase with ART were slightly lower in persons with vitamin D insufficiency, however this did not reach statistical significance (p ≥0.5). HHV-8 viral load in plasma and PBMC were similar between the 25(OH)D groups (Table 1).

Discussion

Few studies have evaluated the prevalence of vitamin D insufficiency in HIV-infected adults living in Sub-Saharan Africa and no prior published studies have evaluated 25(OH)D insufficiency among Zimbabweans or in persons with KS-AIDS. The limited in vitro data on
vitamin D or vitamin D agonists in KS and in other vascular tumors have been conflicting. Our study provides the first analyses of vitamin D insufficiency among HIV-infected adults in Zimbabwe and is the first to investigate relationships between 25(OH)D insufficiency and clinical and immunological/virological outcomes of persons with KS-AIDS.

The higher baseline HIV-1 RNA observed among subjects with 25(OH)D insufficiency is reported in the literature and may reflect the integral role of vitamin D in innate immunity. Unexpectedly, we observed a greater decrease in HIV-1 RNA in persons with inadequate 25(OH)D and slightly higher baseline 25(OH)D among persons with virological failure. Early in vitro studies of HIV and vitamin D suggested an increase in HIV-1 replication with 1,25(OH)2D partially mediated by inflammatory cytokines, thus, theoretically, higher 25(OH)D may have led to increased HIV-1 expression. Subsequent studies have largely suggested a suppressive effect of 25(OH)D and 1,25(OH)2D. Alternatively, higher levels of immune activation, inflammatory cytokines, or HIV-1 RNA itself may interfere with conversion of 25(OH)D, thus our findings may not accurately represent the levels of biologically active vitamin D, 1,25(OH)2D. The short half-life and transient changes in 1,25(OH)2D make this assay much less useful in determining vitamin D status.

A potential limitation of our study is that we studied only the relationship between baseline 25(OH)D and clinical and immunological/virological outcomes. Our analysis would not have captured the impact of changes in 25(OH)D associated with ART initiation or nutritional status that may have occurred during the course of the study. The small sample population and event number may have limited our power to detect significant differences in outcomes (i.e., survival, clinical response, receipt of adjunctive therapy, development of KS-IRIS). Receipt of chemotherapy or radiation therapy was affected by availability. Regardless of these limitations, we found that more than one half of Zimbabweans with KS-AIDS enrolling in our study had insufficient 25(OH)D. While persons with KS-AIDS may not be representative of the majority of HIV-infected Zimbabweans, the mean 25(OH)D level (75.5 nmol/l) of our population is similar to the weighted mean (84.7 nmol/l) of other African populations with and without HIV infection.

In summary, despite associations between vitamin D deficiency and severe disease, AIDS, and death, we were unable to demonstrate improved KS-AIDS outcomes in clinical response, need for chemotherapy or radiation, development of KS-IRIS, survival rates, or more favorable immunological response to ART in association with higher levels of vitamin D. Larger studies should investigate longitudinal associations with vitamin D state in persons with AIDS-KS and other malignancies prior to routinely recommending vitamin D replacement.

Acknowledgments

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References


18. Gonzalez-Pardo V, D’Elia N, Verstuyf A, Boland R, Russo de Boland A. NFKappaB pathway is down-regulated by 1alpha,25(OH)(2)-vitamin D(3) in endothelial cells transformed by Kaposi


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Figure 1.
Flowchart of study participants.
Table 1
Comparison of demographic and clinical characteristics between persons with insufficient and adequate 25(OH)D.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Inadequate 25(OH)D N=45</th>
<th>Adequate 25(OH)D N=40</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Demographics</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>38 (33–45)</td>
<td>36 (31–43)</td>
<td>0.4</td>
</tr>
<tr>
<td>Gender (female)</td>
<td>14 (31)</td>
<td>12 (30)</td>
<td>0.9</td>
</tr>
<tr>
<td>BMI</td>
<td>21.5 (20.0–22.8)</td>
<td>21.5 (19.0–24.3)</td>
<td>1.0</td>
</tr>
<tr>
<td><strong>Disease stage</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stage 4 KS</td>
<td>23 (51)</td>
<td>17 (43)</td>
<td>0.4</td>
</tr>
<tr>
<td>ACTG T1</td>
<td>38 (84)</td>
<td>33 (83)</td>
<td>0.8</td>
</tr>
<tr>
<td><strong>Baseline laboratory</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hemoglobin</td>
<td>11 (9.4–11.9)</td>
<td>11.3 (10.3–12.6)</td>
<td>0.2</td>
</tr>
<tr>
<td>CD4+ lymphocyte count</td>
<td>117 (76–208)</td>
<td>141 (70–222)</td>
<td>0.5</td>
</tr>
<tr>
<td>HIV-1 VL (log)</td>
<td>4.7 (4.3–5.0)</td>
<td>4.5 (3.9–4.9)</td>
<td>0.04</td>
</tr>
<tr>
<td>HHV-8 plasma VL (log)</td>
<td>2.9 (2.1–3.5)</td>
<td>2.8 (1.8–3.4)</td>
<td>0.4</td>
</tr>
<tr>
<td>HHV-8 PBMC VL (log)</td>
<td>3.2 (2.4–4.4)</td>
<td>3.5 (2.5–4.1)</td>
<td>0.8</td>
</tr>
<tr>
<td><strong>96 week changea</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Increase in CD4+ lymphocyte count</td>
<td>85 (21–291)</td>
<td>105 (30–202)</td>
<td>0.8</td>
</tr>
<tr>
<td>Decrease in HIV-1 VL (log)</td>
<td>1.9 (1.3–2.4)</td>
<td>1.3 (0.8–1.9)</td>
<td>0.04</td>
</tr>
<tr>
<td>Decrease in HHV-8 plasma VL (log)</td>
<td>0.5 (0–1.5)</td>
<td>0.4 (0–1.5)</td>
<td>0.8</td>
</tr>
<tr>
<td>Decrease in HHV-8 PBMC VL (log)</td>
<td>0.9 (−0.4–2.3)</td>
<td>1.0 (0–2.0)</td>
<td>0.9</td>
</tr>
</tbody>
</table>

Median and intraquartile range or number and percentage are reported for each variable; BMI: body mass index; KS: Kaposi Sarcoma; ACTG T1: AIDS Clinical Trials Group Tumor 1 Staging; VL: viral load; HHV-8: Human Herpes Virus 8; PBMC: peripheral blood mononuclear cells.

aweek 96 or last available results
Table 2

Median 25(OH)D levels by baseline disease stage and clinical outcomes

<table>
<thead>
<tr>
<th>Clinical characteristic</th>
<th>Median 25(OH)D</th>
<th>Interquartile range</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>KS Clinical Stage</td>
<td></td>
<td></td>
<td>0.8</td>
</tr>
<tr>
<td>2 or 3</td>
<td>75</td>
<td>57–91</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>72</td>
<td>56–89</td>
<td></td>
</tr>
<tr>
<td>ACTG Stage</td>
<td></td>
<td></td>
<td>0.4</td>
</tr>
<tr>
<td>0</td>
<td>73</td>
<td>40–85</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>73</td>
<td>58–90</td>
<td></td>
</tr>
<tr>
<td>Survival Status</td>
<td></td>
<td></td>
<td>0.3</td>
</tr>
<tr>
<td>Alive at 96 weeks</td>
<td>69</td>
<td>55–85</td>
<td></td>
</tr>
<tr>
<td>Died by 96 weeks</td>
<td>63</td>
<td>46–79</td>
<td></td>
</tr>
<tr>
<td>Clinical Response</td>
<td></td>
<td></td>
<td>0.7</td>
</tr>
<tr>
<td>Partial or complete</td>
<td>64</td>
<td>56–88</td>
<td></td>
</tr>
<tr>
<td>No response</td>
<td>73</td>
<td>58–92</td>
<td></td>
</tr>
<tr>
<td>Immune Response</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No KS-IRIS</td>
<td>73</td>
<td>57–89</td>
<td>0.7</td>
</tr>
<tr>
<td>Developed KS-IRIS</td>
<td>64</td>
<td>56–88</td>
<td></td>
</tr>
<tr>
<td>HIV-1 virological response</td>
<td>66</td>
<td>55–86</td>
<td>0.3</td>
</tr>
<tr>
<td>HIV-1 virological failure</td>
<td>83</td>
<td>58–93</td>
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<tr>
<td>Adjunctive therapy</td>
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<tr>
<td>No chemotherapy</td>
<td>66</td>
<td>52–93</td>
<td>0.8</td>
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<tr>
<td>Chemotherapy</td>
<td>73</td>
<td>57–88</td>
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<tr>
<td>No radiation</td>
<td>75</td>
<td>58–90</td>
<td>0.5</td>
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<tr>
<td>Radiation therapy</td>
<td>67</td>
<td>55–86</td>
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