Association of Serum Vitamin D Levels With Inflammatory Response Following Hip Fracture: The Baltimore Hip Studies

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Background. Vitamin D, known for its role in calcium homeostasis, may also regulate immune function. Whether vitamin D deficiency at the time of hip fracture is associated with the inflammatory response postfracture is not known.

Methods. In a cohort from the Baltimore Hip Studies, women aged ≥65 years were evaluated at baseline and 2, 6, and 12 months after hip fracture repair. Serum at baseline was analyzed for 25-hydroxyvitamin D (25(OH)D), and serum from all time points was analyzed for interleukin-6 (IL-6). Participants were divided into two groups based on their baseline 25(OH)D levels. Vitamin D deficiency was defined as a 25(OH)D level of ≤15 ng/mL (<37.5 nmol/L). We examined IL-6 level as a function of vitamin D status using generalized estimating equations, adjusting for covariates.

Results. Women deficient in vitamin D at baseline had higher IL-6 levels in the year postfracture (p = .02). On average, participants with low 25(OH)D levels had adjusted serum IL-6 levels that were 6.0 pg/mL (95% confidence interval [CI]: 0.6, 11.9, 13.1 pg/mL (95% CI: 4.6, 21.6), and 13.4 pg/mL (95% CI: 2.3, 24.5) higher at baseline, 2, 6, and 12 months after hip fracture, respectively.

Conclusions. Women with vitamin D deficiency at the time of hip fracture had higher serum IL-6 levels in the year after hip fracture. Whether the proinflammatory state of vitamin D deficiency explains the association of this deficiency with adverse outcomes in older adults warrants further study.

Vitamin D has long been known to play a key role in the maintenance of calcium homeostasis and in the maintenance of bone strength. Vitamin D is also believed to have important immunomodulatory effects. It causes a relative shift away from T-helper cell subtype 1-like profile (Th1) and toward a T-helper cell subtype 2-like profile (Th2) (1). The end result of these changes is a switch away from a proinflammatory cytokine profile to a more anti-inflammatory profile. Vitamin D deficiency has been associated with an increased risk of Th-1 cytokine–mediated autoimmune diseases such as inflammatory bowel disease, rheumatoid arthritis, systemic lupus erythematosus, multiple sclerosis, and type 1 diabetes mellitus (2).

We have found that in the year following hip fracture, levels of the inflammatory cytokine interleukin-6 (IL-6) are elevated, with median serum levels of 7.4 pg/mL at 12 months postfracture (3). In hip fracture patients, vitamin D levels have been found to be low (4), but whether vitamin D levels at the time of hip fracture are associated with the inflammatory response postfracture is not known.

We examined a cohort of women admitted to two Baltimore area hospitals between 1992 and 1995 who were enrolled in the Baltimore Hip Studies (BHS). Women 65 years old or older were followed for 1 year after hip fracture, to evaluate whether vitamin D levels measured shortly after fracture are associated with serum levels of the inflammatory cytokine IL-6 in the year postfracture.

METHODS

Participants were drawn from the third cohort of the BHS. Between 1992 and 1995, 205 Caucasian women, at least 65 years old, admitted to one of two hospitals in Baltimore with a new fracture of the proximal femur agreed to participate in a prospective study. The study was reviewed and approved by the Institutional Review Boards of the University of Maryland and the individual study hospitals, and informed consent was obtained from each participant prior to enrollment. Women were evaluated at 3 or 10 days (baseline) and at 2, 6, and 12 months posthospitalization for fracture (follow-up). A more detailed description of this cohort may be found elsewhere (5).

Vitamin D Measurement

Serum samples were obtained within 10 days postfracture and stored at −70°C. Samples from 79 women were analyzed for 25(OH)D levels using radioimmunoassay (DiaSorin, Stillwater, MN). The inter-assay coefficient of
Variation (CV) and the intra-assay CV for this assay were 12%–14% and 8.7%–10%, respectively (6).

**IL-6 Measurement**

The methods for the collection and storage of the serum samples have been described in detail elsewhere (7). Briefly, sera were obtained at baseline and at 2-, 6-, and 12-month time points, and were stored at −70°C. When stored at these temperatures, IL-6 is believed to remain stable in serum for many years (8). IL-6 levels were measured in serum that had not been previously thawed by a two-antibody enzyme-linked immunosorbent assay (ELISA) using biotin–streptavidin–peroxidase detection and commercially available human antibodies (Pierce/Endogen, Rockford, IL). Samples from all time points were analyzed in the same batch to minimize laboratory variability. The linear range for the IL-6 assay was 1.5–100 pg/mL. The inter-assay CV for this assay was 8%. Cytokine measurements above the detection limit for the assay were converted to the highest level of the assay, and measurements below the detection limit were scored as zero. Seventy-nine participants provided serum samples at baseline; 48, 49, and 37 women provided serum samples at 2, 6, and 12 months, respectively.

**Covariates**

Age, type of surgical repair (internal fixation vs arthroplasty), and presence or absence of 20 noninfectious and 3 infectious in-hospital complications were collected from the hospital record. At the baseline evaluation, women were asked “Have you ever taken vitamin D or a multivitamin containing vitamin D at least once a week?” The presence, prior to the fracture, of osteoarthritis, coronary artery disease, congestive heart failure, stroke, dementia, diabetes, chronic obstructive pulmonary disease (COPD), and peripheral vascular disease was collected from the hospital record. These conditions were chosen because they have been observed to be associated with an adverse effect on functional recovery in elderly persons (9). Serum creatinine, as measured on the initial hospital admission laboratory analyses, was recorded. Renal dysfunction was defined as an admitting serum creatinine of >1.1 mg/dL. Fat tissue mass (FTM) and lean body mass (LBM) were estimated using a method described in detail elsewhere using dual-energy x-ray absorptiometry (DXA; Hologic, Inc., Waltham, MA) (5). Percent body fat at baseline was calculated as FTM/(FTM + LBM). Proxy data were used whenever patient data were unavailable or the patient was cognitively impaired (Mini-Mental State Examination [MMSE]; score < 17) (10).

**Statistical Analyses**

Women were divided into two groups based on their serum 25(OH)D levels at the baseline evaluation. As has been done previously, vitamin D deficiency was defined as a 25(OH)D level of ≤15 ng/mL (<37.5 nmol/L) (11,12). Baseline characteristics were compared between participants deficient in vitamin D and the rest of the cohort using the Fisher exact test or chi-square test for dichotomous and categorical variables, and two-sample t tests for continuous variables. Generalized estimating equations (GEE) (13) were used to model the longitudinal relationship between 25(OH)D category and serum IL-6 level using a normal working model and robust standard errors to account for correlations across time within individuals. Generalized F tests were used to compare IL-6 trajectories between women deficient in vitamin D and women with higher vitamin D levels, and confidence intervals were calculated when a significant difference (p < .05) between serum IL-6 trajectories was observed. The models were adjusted for age, number of comorbidities, presence of renal dysfunction, postsurgical complications, percent body fat, and time postfracture.

The validity of GEE results is contingent on missing data being missing completely at random (MCAR) as defined by Rubin (14). A sensitivity analysis of the MCAR assumption using weighted estimating equations (WEE) was performed (15). The weights were calculated by performing a logistic regression of observing both IL-6 and vitamin D (yes/no) on the covariates used in the analysis model for each visit.

**RESULTS**

Characteristics of the study sample appear in Table 1. Mean age (standard deviation [SD]) was 79.7 (8.0) years. All participants were white. Mean (SD) serum 25(OH)D levels were 8.7 (3.5) ng/mL and 21.4 ng/mL in the vitamin D-deficient and nondeficient groups, respectively. Fewer of the women deficient in vitamin D reported taking vitamin D supplements prefracture. Seven women, six of whom were from the low vitamin D group, died during the follow-up period. Women who provided vitamin D data were younger, had fewer comorbidities, and had less prefracture disability than those who did not provide vitamin D data (p < .05). Among participants who provided vitamin D data, those with complete data at 12-month follow-up had higher vitamin D levels at baseline (14.5 vs 10.5 ng/mL, p = .04) and were younger (mean age 77.9 vs 81.2 years, p = .07) than those with incomplete 12-month data for the analyses presented here. In addition, participants with missing data at 12 months tended to have greater comorbidity at baseline, lower MMSE scores, and worse lower extremity function at 12 months, although these differences were not statistically significant (data not shown).

The mean (± 95% confidence interval [CI]) serum IL-6 levels at each time point appear in Figure 1. Adjusted for age, comorbidity, renal dysfunction, postsurgical complications, percent body fat, and time postfracture, women deficient in vitamin D at baseline had higher IL-6 levels throughout the year postfracture (p = .02). On average, women with low 25(OH)D levels had adjusted serum IL-6 levels that were 6.0 pg/mL (95% CI: −6.7, 18.7 pg/mL), 11.9 pg/mL (95% CI: 3.5, 20.4), 13.1 pg/mL (95% CI: 4.6, 21.6), and 13.4 pg/mL (95% CI: 2.3, 24.5) higher at baseline and 2, 6, and 12 months after hip fracture, respectively. The sensitivity analysis yielded similar results and the same conclusions as the GEE analysis (data not shown).

**DISCUSSION**

We found that the serum level of 25(OH)D measured shortly after hip fracture is associated with IL-6 postfracture,
with women deficient in vitamin D at the time of the fracture displaying higher serum levels of IL-6 at all follow-up time points in the year postfracture. Although vitamin D is believed to have important immunoregulatory effects, to our knowledge this is the first study to examine the association of vitamin D deficiency with inflammatory cytokine levels following an acute event such as hip fracture.

Serum levels of both inflammatory cytokines and of vitamin D have been associated with the frailty syndrome (16,17); the associations that we observed may reflect the presence of the frailty syndrome in this cohort of older adults after hip fracture. However, the possible mediating role of vitamin D deficiency on the association of inflammation with the frailty syndrome should also be considered.

The majority of the biologic effects of vitamin D are mediated through binding of the activated form, 1,25 dihydroxyvitamin D [1,25(OH)₂D₃] to the vitamin D receptor (VDR), which has been identified in almost every tissue in the body including most cells of the immune system (1,18). The end result of 1,25(OH)₂D₃ stimulation is a relative shift away from a proinflammatory state as well as immune modulation. 1,25(OH)₂D₃ has been shown to inhibit the maturation of dendritic cells (DC) in vitro as well as the secretion by DCs and other antigen-presenting cells of the immunostimulatory cytokine IL-12. IL-12 stimulates the development of CD4⁺ Th-1 cells and inhibits the development of CD4⁺ Th-2 cells, which results in a shift away from a Th-1-like profile and toward a Th-2-like type (1). In addition, the transcription of several key Th-1 cytokines such as interferon-γ (IFN-γ) and IL-2 is also inhibited by vitamin D (1). Vitamin D deficiency has been associated with an increased risk of Th-1 cytokine-mediated auto-immune diseases such as inflammatory bowel disease, rheumatoid arthritis, systemic lupus erythematosus, multiple sclerosis, and type 1 diabetes mellitus (2). Furthermore, although vitamin D attenuates the antigen-presenting function of monocytes and macrophages, the chemotactic and phagocytic function of these cells is enhanced by exposure to 1,25(OH)₂D₃, suggesting an immune modulating effect rather than merely an immunosuppressive one (1).

Chronic inflammatory processes are believed to play a role in aging, and serum markers of inflammation have been associated with functional decline, sarcopenia, and the frailty syndrome (16,19–22). More recently, vitamin D has been associated with many of these same outcomes in elderly persons, with studies indicating that vitamin D deficiency increases risk for decreased muscle strength, sarcopenia, falls, and frailty (17,23,24). Although specific information on the frailty syndrome was not collected in the cohort studied here, many older women who suffer hip fracture are frail. A hallmark of the frailty syndrome is a limited ability to recover from physiologic stressors (25).

### Table 1. Characteristics of the Study Population

<table>
<thead>
<tr>
<th>Variable</th>
<th>Overall Cohort</th>
<th>Low Vitamin D</th>
<th>Vitamin D Sufficient</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Baseline age, y, mean (SD)</strong></td>
<td>79.7 (8.0)</td>
<td>80.0 (7.6)</td>
<td>78.9 (9.1)</td>
<td>.58</td>
</tr>
<tr>
<td><strong>Baseline IL-6, pg/mL, mean (SD)</strong></td>
<td>12.5 (7.2)</td>
<td>8.7 (3.5)</td>
<td>21.4 (10.5)</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td><strong>Prefracture use of vitamin D supplements or multiple vitamins containing vitamin D, n (%)</strong></td>
<td>40 (59.7)</td>
<td>25 (52.1)</td>
<td>15 (79.0)</td>
<td>.06</td>
</tr>
<tr>
<td><strong>No. of baseline comorbidities (%)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Coronary artery disease</td>
<td>28 (35.4)</td>
<td>22 (39.3)</td>
<td>6 (26.1)</td>
<td>.31</td>
</tr>
<tr>
<td>Stroke</td>
<td>5 (6.3)</td>
<td>4 (7.1)</td>
<td>1 (4.4)</td>
<td>1.0</td>
</tr>
<tr>
<td>Congestive heart failure</td>
<td>12 (15.2)</td>
<td>7 (12.5)</td>
<td>5 (21.7)</td>
<td>.32</td>
</tr>
<tr>
<td>Peripheral vascular disease</td>
<td>4 (5.1)</td>
<td>3 (5.4)</td>
<td>1 (4.4)</td>
<td>1.0</td>
</tr>
<tr>
<td>Dementia</td>
<td>7 (8.9)</td>
<td>5 (8.9)</td>
<td>2 (8.7)</td>
<td>1.0</td>
</tr>
<tr>
<td>Diabetes</td>
<td>11 (13.9)</td>
<td>9 (16.1)</td>
<td>2 (8.7)</td>
<td>.49</td>
</tr>
<tr>
<td>Osteoarthritis</td>
<td>30 (38.0)</td>
<td>22 (39.3)</td>
<td>8 (34.8)</td>
<td>.80</td>
</tr>
<tr>
<td>COPD</td>
<td>11 (13.9)</td>
<td>7 (12.5)</td>
<td>4 (17.4)</td>
<td>.72</td>
</tr>
<tr>
<td>Serum creatinine &gt; 1.1 mg/dL on admission, n (%)</td>
<td>11 (13.9)</td>
<td>2 (8.7)</td>
<td>9 (16.1)</td>
<td>.21</td>
</tr>
<tr>
<td><strong>In-hospital complications, n (%)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>59 (77)</td>
<td>43 (78)</td>
<td>16 (73)</td>
<td>.56</td>
</tr>
<tr>
<td>1</td>
<td>11 (14)</td>
<td>7 (13)</td>
<td>4 (18)</td>
<td></td>
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<tr>
<td>2</td>
<td>5 (7)</td>
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<td>3</td>
<td>1 (1)</td>
<td>1 (2)</td>
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<tr>
<td>≥4</td>
<td>1 (1)</td>
<td>0 (0)</td>
<td>1 (5)</td>
<td></td>
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<tr>
<td><strong>Baseline BMI, kg/m², n (%)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>&lt; .18.5</td>
<td>7 (10.1)</td>
<td>6 (12.0)</td>
<td>1 (5.3)</td>
<td>.81</td>
</tr>
<tr>
<td>18.5–24.9</td>
<td>38 (55.1)</td>
<td>27 (54.0)</td>
<td>11 (57.9)</td>
<td></td>
</tr>
<tr>
<td>25.0–29.9</td>
<td>19 (27.5)</td>
<td>13 (26.0)</td>
<td>6 (31.6)</td>
<td></td>
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<tr>
<td>≥30.0</td>
<td>5 (7.3)</td>
<td>4 (8.0)</td>
<td>1 (5.3)</td>
<td></td>
</tr>
<tr>
<td><strong>Baseline IL-6, pg/mL, mean (SD)</strong></td>
<td>49.0 (28.1) (N=76)</td>
<td>50.5 (30.4) (N=53)</td>
<td>45.6 (22.2) (N=23)</td>
<td>.49</td>
</tr>
</tbody>
</table>

Note: SD = standard deviation; 25(OH)D = 25-hydroxyvitamin D; COPD = chronic obstructive pulmonary disease; BMI = body mass index.
older adults (26). In the cohort studied here, individuals with low vitamin D levels had higher levels of IL-6 at all time points postfracture. If vitamin D deficiency is associated with frailty in older adults, then the proinflammatory effects of this deficiency may explain, in part, an increased cytokine response in frail elderly persons. Because inflammatory cytokines are believed to have an adverse effect on muscle strength and function (19,20,22,27), an increased inflammatory response following acute medical events, such as hip fracture, may explain, in part, the observed decreased functional resiliency to physiologic stress in frail older adults (3,25,28).

Although these results are interesting, they must be viewed with the following limitations in mind. The baseline measures of serum 25(OH)D and IL-6 were collected within 10 days of the fracture. Because serum 25(OH)D has a half-life of approximately 2 weeks (29), this measurement is likely to reflect vitamin D status at the time of the fracture. However, we are unable to determine whether the levels reported here are representative of the individual’s long-term vitamin D status. Furthermore, we are unable to assess whether the trauma of hip fracture, and subsequent inflammatory response, might influence serum 25(OH)D levels. The effect of inflammation on serum vitamin D levels in this context is not entirely understood. Although activated macrophages associated with inflammatory states such as sarcoidosis, tuberculosis, and rheumatoid arthritis can produce 1,25(OH)_{2}D_{3} (30); typically in chronic inflammatory states, however, 1α-hydroxylase activity is downregulated by the transcription factor nuclear factor-κB, resulting in lower 1,25(OH)_{2}D_{3} levels (31). The effect of inflammation on 25(OH)D levels is less clear. In studies of severe injury, such as in burn patients, both 25(OH)D and 1,25(OH)_{2}D_{3} levels are inversely correlated with burn severity, although this association was only seen beginning at 3 weeks after the burn injury (32).

In addition, eight study participants had cytokine levels beyond the range of the assays used, and due to selective forces that resulted in loss to follow-up, serum samples at later evaluations were obtained from younger women with less comorbidity compared to the cohort as a whole at baseline. Furthermore, we were unable to examine the association of vitamin D levels with the specific Th1-like (e.g., tumor necrosis factor-α, IL-1, IL-17, IL-18) or Th2-like (e.g., IL-4, IL-10, IL-13) cytokines as serum from this cohort was not analyzed for these substances; therefore, this association needs to be explored in future studies. Also, all of the participants included in these analyses were white women, thus we are unable to assess whether similar associations exist in men or in women of other ethnicities.

Perhaps due to the advanced age and frail nature of hip fracture patients, many of our participants did not complete all of the study measures needed for the analyses that we presented. Our data would suggest, however, that individuals with incomplete data at 12 months postfracture had lower 25(OH)D levels at baseline, were older, and had greater comorbidity burden and poorer function in the year postfracture. It is unlikely, therefore, that loss to follow-up would explain the results presented here; however, these losses and the small sample size of this study make it difficult to draw strong conclusions from the analyses presented.
Conclusion

We have found that, in this cohort of older women after hip fracture, those individuals with low serum levels of vitamin D at baseline had higher serum levels of IL-6 in the year postfracture. Whether the relative proinflammatory state of vitamin D deficiency explains the association of this deficiency with adverse outcomes in older adults warrants further study.

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REFERENCES


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