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ORIGINAL ARTICLE

Non-linear relationship between serum 25-hydroxyvitamin D concentration and subsequent hip fracture

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Abstract
Summary Serum 25-OH vitamin D levels were compared in 254 hip fracture subjects and 2,402 matched control subjects. There was a significant inverse association between 25-OH vitamin D and hip fracture only between 0 and 70 nmol/L.
Introduction Vitamin D is integral to bone metabolism, however the utility of serum 25-OH vitamin D as a risk marker for hip fractures is controversial.
Methods We conducted a case–control study of patients admitted to the hospitals with hip fractures in Calgary, Alberta, (catchment population 1.4 million) between January 1, 2007 and August 31, 2011. We searched the laboratory information system of Calgary Laboratory Services for serum 25-OH vitamin D levels within 6 months prior to admission on patients admitted to hospital with hip fractures. Cases were identified through the Calgary Laboratory Services laboratory information system and were matched to controls for age, sex, and month of testing. The hip fracture–25-OH vitamin D association was examined using multiple linear and spline regression.
Results Of 305 subjects initially identified with hip fractures, serum 25-OH vitamin D levels were available for 254 (83 %). These were matched to 2,402 control subjects. We observed a significant ($p<0.01$) non-linear relationship such that 25-OH vitamin D was inversely associated with hip fracture only below 70 nmol/L (odds ratio=0.81 per 10 nmol/L increase; 95 % CI 0.86–0.93).
Conclusions The utility of 25-OH vitamin D level as a risk marker for hip fracture depends on the cut-off level used and was of potential use only for lower levels of 25-OH vitamin D.

Keywords Case–control study · Data matching · Hip fracture · Serum 25-OH vitamin D

Introduction

Considerable clinical interest exists in the potential utility of serum 25-OH vitamin D (25-hydroxyvitamin D) levels as a risk factor for hip and other fractures. As a consequence of this as well as other potential health benefits of adequate 25-OH vitamin D levels, clinical laboratories in North America have seen a marked increase in requests for 25-OH vitamin D testing [1, 2].

Evidence for the utility of 25-OH vitamin D as a risk marker has come from large case series which have consistently reported low levels of 25-OH vitamin D in hip fracture patients [3–8]. Likewise, case–control studies have generally shown lower 25-OH vitamin D levels in hip fracture cases compared to controls [7, 9–15]. However, a recent case–control study of women by Cauley et al. [16] has yielded some contradictory results. In this study, 25-OH vitamin D was inversely associated with hip fracture among whites, but positively associated with hip fracture among African Americans and Asians. No relationship was observed among Hispanics or Native Americans.

The effect of vitamin D supplementation on fracture risk has also been controversial, with a number of clinical trials

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yielding negative results [17–19]. However, in a recent pooled analysis of seven randomized trials of vitamin D supplementation, the risk of hip and total fractures was lower among individuals taking vitamin D supplements provided that calcium supplements were also taken [20]. This protective effect was present regardless of the subject's age, sex, or prior fractures.

Thus, while supplement use appears to be beneficial in primary and secondary prevention of fractures, the utility of serum 25-OH vitamin D levels as a risk marker for fracture is uncertain. We performed a case–control study on hip fractures and 25-OH vitamin D status using hospital admission data on hip fractures in a large, mostly urban population (1.4 million people) merged with pre-fracture 25-OH vitamin D data from our hospital laboratory information system. Our objective was to assess the utility of serum 25-OH vitamin D level using both linear and non-linear models as a risk marker for hip fractures. Furthermore, we explored the possibility of a non-linear relationship between 25-OH vitamin D level and hip fracture risk.

Methods

Ethics statement

The study protocol was approved by the University of Calgary Conjoint Health Review Ethics Board (Ethics ID 23919).

Data acquisition

Cases of hip fracture were identified by searching the Alberta Health Services Data Integration, Measurement & Reporting database for the city of Calgary, Alberta, for hospital discharges with the following associated ICD-10 codes: S72.0 (fracture of neck of femur), S72.1 (pertrochanteric fracture), and S72.2 (subtrochanteric fracture). Only discharge records from January 1, 2007 to August 31, 2011 were considered due to the limited availability of 25-OH vitamin D levels from our laboratory information system before January 1, 2007. For each case, we retrieved age, sex, date of admission, and provincial health card number.

Table 1 Baseline characteristics of cases and controls

Variable	Cases	Controls	*p* value
Age, mean (SD)	79.11 (11.4)	78.17 (11.0)	0.194
Female, % (*n*)	29 (74)	29 (701)	1.00
25-Hydroxyvitamin D level, mean (SD) (nmol/L)	86.5 (38.2)	85.3 (40.5)	0.64

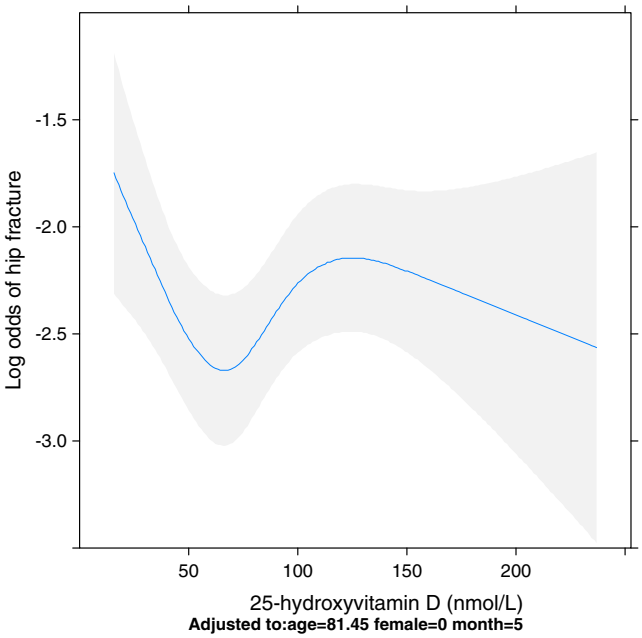

Fig. 1 Restricted cubic spline function of the relationship between serum 25-OH vitamin D level and risk of hip fracture. The relationship is adjusted for age (set at a median of 81 years), sex (set as male), and testing month (set at May) and is modeled as a four-knot cubic spline. Log odds is calculated as the natural logarithm of the odds of hip fracture. The *shaded area* represents the 95 % confidence interval

We included cases due to any cause, including trauma, in order to capture those due to osteoporotic fractures [7, 21].

The provincial health card number was used as a linking variable to search for a pre-fracture serum 25-OH vitamin D measurement in the laboratory information system of Calgary Laboratory Services (CLS). CLS is the sole provider of laboratory testing for the city of Calgary and surrounding areas, and therefore the laboratory information system contains essentially a complete record of laboratory test results

Table 2 Significance tests for linear and non-linear terms and their associations with hip fracture risk in the cubic spline model

Factor	Chi-square	Degrees of freedom	*p* value
Age	9.63	3	0.02
Non-linear	7.34	2	0.03
Female sex	0.07	1	0.80
Serum 25-hydroxyvitamin D	10.85	3	0.01
Non-linear	10.76	2	<0.01
Month	0.19	1	0.66
Total non-linear	18.13	4	<0.01
Total	20.62	8	<0.01

Both age and serum 25-hydroxyvitamin D level had significant non-linear relationships with hip fracture risk

Table 3 Piecewise linear regression for varying 25-OH vitamin D ranges

25-OH vitamin D range	*N*	Odds ratio for hip fracture a 10 nmol/L increase in 25-OH vitamin D (95 % CI)	*p* value	*p* value for difference
Overall	2,654	1.01 (0.97–1.04)	0.75	
<70 nmol/L	954	0.81(0.86–0.93)	<0.01	
≥70 nmol/L	1,700	1.01 (0.96–1.05)	0.61	<0.01 (vs. <70 nmol/L)
70 to 120 nmol/L	1,314	1.12 (0.88–1.27)	0.11	<0.01 (vs. <70 nmol/L)
≥120 nmol/L	386	0.98 (0.92–1.06)	0.61	0.09 (vs. 70 to 120 nmol/L)

The relationship was significant only for vitamin D levels below 70 nmol/L and was significantly different only for the values between 70 and 120 nmol/L

for the entire city. In matching 25-OH vitamin D levels to individual patients, we considered only the most recent result prior to hip fracture (to eliminate any effect of trauma on serum 25-hydroxyvitamin D level) [8, 22, 23], with a maximum time interval of 12 months prior to admission. Cases were matched to control subjects from our laboratory information system who were tested during the same period (January 1, 2007–August 31, 2011) without a diagnosis of hip fracture. Initially, this group consisted of approximately 200,000 subjects. As 25-OH vitamin D level is associated with age, sex, and date of testing, we randomly matched cases by age (±90 days), sex, and testing month. We attempted to match 10 controls for each case; however, it was difficult to identify adequate numbers of controls for subjects over 90 years of age, so we relaxed the age matching in this subgroup to ±180 days. All records were de-identified by removing health card number, hospital admission date, and 25-OH vitamin D testing date from the data set.

Statistical analysis

We used Student's *t* test to evaluate differences in continuous variables between cases and controls and the chi-squared test for dichotomous variables. Several different 25-OH vitamin D "exposure" levels have been used to evaluate fracture risk: 75 [24], 50 [25, 26], 37.5 [27], and 25 nmol/L [25, 26]. However, because 25-OH vitamin D level is best defined as a continuum [31], we first evaluated the continuous association of 25-OH vitamin D with hip fracture using logistic regression. We then employed a restricted cubic spline function to graphically explore the possibility of a non-linear relationship between varying 25-OH vitamin D levels and log odds of hip fracture. In this analysis, four knots were placed at the quartile midpoints of 25-OH vitamin D and we simultaneously adjusted for sex and age (also modeled as a four-knot cubic spline). We verified non-linear associations identified in the spline analysis using piecewise linear regression and interaction terms (25-OH vitamin D × cut point). Statistical analyses were performed using SPSS v. 19 for Windows, SAS v. 9.3, and R v. 2.25.1 (with rms package).

Results

The hospital discharge database contained 305 hip fracture cases during the study period. A serum 25-OH vitamin D level within 12 months prior to the hip fracture admission date was available for a total of 254 subjects (180 females and 74 males). The mean age of included cases was 78.3 years (range 44.1–99.7 years). For 235 of these subjects (93 %), 10 control subjects were identified. For the remaining 18 cases, between 1 and 9 control subjects were identified. This yielded a total of 2,402 control subjects matched for sex, age, and month of testing (mean of 9.5 controls per case). Baseline characteristics of cases and controls are given in Table 1. Overall, the linear association between 25-OH vitamin D and hip fracture after being adjusted for age, sex, and testing month was not significant (odds ratio per 10 nmol/L increase=1.01; 95 % CI 0.97–1.04; p=0.75). However, we observed a statistically significant (p<0.01) complex non-linear association characterized by changes in the direction of the relationship at 70 and 120 nmol/L when using a cubic spline (Fig. 1, Table 2). We then used piecewise linear regression to verify that the inverse association between 25-OH vitamin D and hip fracture was significant only for 25-OH vitamin D levels below 70 nmol/L. Moreover, when we tested for differences in the piecewise associations below 70 nmol/L, between 70 and 120 nmol/L, and above 120 nmol/L, we saw a significant difference only between associations in the ranges of <70 nmol/L and 70–120 nmol/L (Table 3).

Discussion

In a case–control study conducted in a major Canadian city and surrounding catchment area, we found an inverse association between pre-fracture 25-OH vitamin D levels and hip fracture only for 25-OH vitamin D levels of less than 70 nmol/L. For individuals in this range, a 10 nmol/L increase in 25-OH vitamin D was associated with 19 % lower odds of fracture.

Numerous randomized controlled trials suggest that vitamin D and calcium supplementation lead to lower fracture risk, but there is ongoing confusion as to the role of serum 25-hydroxyvitamin D as a risk marker. Our findings suggest that at the lower end of the spectrum of observed 25-OH vitamin D

levels, there may be a modest role for the use of 25-OH vitamin D as a risk marker, but the presence or absence of a relationship is influenced by the cut-off level used.

Our results combined with other studies suggest that caution should be exercised in the interpretation of serum 25-OH vitamin D levels as a risk marker for hip fracture. In contrast, vitamin D supplementation has been shown to reduce fracture risk in multiple studies. Therefore, a strategy of supplementation without testing may be reasonable in some instances and has the benefit of reducing healthcare expenditures. Calcium and vitamin D supplementation are frequently not prescribed to post-hip fracture patients [28–32], and even when vitamin D supplements are prescribed, there is poor patient compliance [29, 30]. It would appear that this is an area where interest in vitamin D could be usefully applied, especially given the possible additional benefits of vitamin D supplementation [24, 31].

Our study has several strengths. The first is the relatively large sample size and catchment area, which suggests that our estimates are relatively stable and generalizable to a large geographic area. Second, our use of pre-fracture 25-OH vitamin D levels eliminated any possible reverse causality, as hip fracture is known to lower 25-OH vitamin D level. It also avoids capturing subjects who began supplementation following a fracture, which would produce a bias that would make it appear as if higher 25-OH vitamin D level is associated to hip fracture risk.

There are several limitations to this study. First, although our control subjects did not suffer a hip fracture in our jurisdiction within the study period, we did not contact the control subjects to ensure that they did not suffer a hip fracture in another jurisdiction during this time period or in our jurisdiction prior January 1, 2007 or after August 31, 2011. However, the small number of subjects with hip fractures during this period (305) compared to the large number of potential control subjects (200,000) makes it statistically unlikely that a significant number of control subjects would have suffered a hip fracture that did not appear in our hospital discharge database. We also do not know if the cases suffered from a previous fracture. Second, we looked only for an association between 25-OH vitamin D status and hip fracture and did not consider other fractures. This is a question for future study. Finally, an important limitation is that we do not know whether the cases or controls were being supplemented with vitamin D. It is possible that the lack of a significant relationship at higher 25-OH vitamin D levels may have been due in part to individuals with poor bone health (and therefore at higher risk for fractures) being more likely to take vitamin D supplements and therefore exhibiting higher serum 25-hydroxyvitamin D levels. In conclusion, we found that the utility of 25-OH vitamin D level as a risk marker for hip fracture depends on the cut-off level used and was of potential use only for lower serum levels of 25-OH vitamin D.

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Conflicts of interest None.

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