Original Communication

Estimation of the 25(OH) Vitamin D Threshold below which Secondary Hyperparathyroidism may Occur Among African Migrant Women in Paris

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Abstract: Purpose: It has previously been shown that there is a threshold of serum 25-hydroxy vitamin D below which secondary hyperparathyroidism may occur. Our purpose was to estimate this threshold in a population of migrant African women living in Paris.

Methods: Between February 2008 and December 2009, measurements of 25-hydroxy vitamin D and parathormone were performed in 165 African migrant women living in Paris. The estimation of 25-hydroxy vitamin D and parathormone marginal distributions as Gaussian mixtures, combined with a nonparametric kernel estimation method of the regression function of parathormone on 25-hydroxy vitamin D, provided the desired estimate of serum 25-hydroxy vitamin D threshold.

Results: In our sample of African women, our method has shown that serum 25-hydroxy vitamin D levels below 48 ± 2 nmol/L may induce an increase in parathormone concentrations.

Conclusion: In this sample, our method provided a reliable estimate (95% confidence interval) of the serum 25-hydroxy vitamin D level below which an increase in parathormone concentrations can be observed.

Key words: African migrant women, 25-hydroxyvitamin D, Gaussian mixture, kernel regression, secondary hyperparathyroidism
Introduction

Vitamin D is essential for the maintenance of good health. There are two ways to obtain vitamin D: orally from the diet or vitamin D supplements and dermally, through synthesis in the skin, in response to solar ultraviolet B radiation. In humans, dermal production is the major source of vitamin D. Vitamin D is hydroxylated in the liver into 25-hydroxyvitamin [25(OH) D] and subsequently in the kidney to 1,25-dihydroxyvitamin D [1,25(OH)₂D]. This is the active metabolite, which stimulates intestinal calcium absorption. The production of 1,25(OH)₂D is stimulated by parathyroid hormone, or parathormone (PTH). There is a negative feedback through calcium, which decreases PTH, and a direct negative feedback from 1,25(OH)₂D to PTH. PTH increases the reabsorption of calcium in the kidney and stimulates bone resorption, resulting in an increase in serum calcium. When 25(OH)D availability declines, serum 1,25(OH)₂D declines, and this results in reduced calcium absorption, a transient decline in serum calcium concentration, and stimulation of PTH secretion known as secondary hyperparathyroidism.

Vitamin D status is defined by serum 25(OH)D concentration [1, 2], which is influenced in turn by various factors including latitude, skin pigmentation, and dietary calcium intake [3]. Many investigators have shown that there is a threshold for serum 25(OH)D below which secondary hyperparathyroidism occurs [4] and this threshold has been estimated in the region of 40–50 nmol/L [5]. Our purpose was to estimate this threshold, using a nonparametric regression method, in a migrant population of African women living in Paris.

Methods

Patients

The study was conducted in a Community Clinic located in eastern Paris at latitude 48.5N.

The inclusion criteria specified adult African migrant women. Patients were excluded if younger than 18 years old, had a body mass index (BMI) > 30, or if their medical history might influence their serum PTH concentration (hyper- or hypocalcemia, renal insufficiency, medical treatment: bisphosphonates, anti-convulsants, lithium).

In France, a migrant is defined as someone who was born in a foreign country as a non-French citizen. One hundred sixty-five migrant adult women with a mean age of 38.6 ± 9.9 years were included in the study between February 2008 and November 2009. This study was approved by the Independent Ethics Committee of Paris IV and all participants provided written informed consent.

Calcium intake

We used a food-frequency type self-assessment questionnaire to estimate the daily calcium intake of each patient [6].

Measurements

Serum 25(OH)D was measured using chemiluminescence methodology (DiaSorin, LIAISON®) [7] by the Pasteur Cerba laboratory. The test interval measure was between 10 and 350 nmol/L. Over all seasons of the year and for both genders, 25(OH)D norms [8] were as follows:

- Recommended levels: 75–200 nmol/L (30–80 µg/L),
- Insufficiency: 25–75 nmol/L (10–30 µg/L),
- Deficiency: < 25 nmol/L (<10 µg/L).

Functional sensitivity was 17.5 nmol/L with interassay coefficients of variation (CVs) of 12.9%. PTH was measured by chemiluminescence using a LIAISON® N-tact™ PTH test. The test interval was between 1 and 2,000 cm/L and normal PTH levels were < 51 cm/L [9].

Serum calcium was measured using the o-Cresolphthalein Complexone® method and the normal ranges were 2.2–2.55 mmol/L. Renal function was estimated by creatinine clearance, calculated using the Cockcroft and Gault equation and serum creatinine. After an overnight fast, blood samples were drawn from 8.00 to 10.00 a.m. for measurement of 25(OH) D, PTH, and calcium. Samples were collected in dry tubes for serum 25(OH)D, in tubes with EDTA for PTH, and in tubes with heparin for serum calcium.

Statistical analysis

The Pearson correlation test performed on patients with 25(OH)D concentrations >17.5 nmol/L established the influence of age, body mass index (BMI), creatinine clearance, daily calcium intake, serum calcium, and PTH on 25(OH)D. The Expectation-Maximization (EM) algorithm [10] was used to estimate PTH distribution as a Gaussian mixture. The Stochastic EM
algorithm for censored data (SEMc) [11] was used to estimate the 25(OH)D distribution since 25(OH)D ≤ 17.5 nmol/L was coded as 17.5 nmol/L.

The 25(OH)D level below which levels of PTH may increase was defined as the first value below which the regression function of PTH on 25(OH)D was strictly increasing. We used a recent data-driven, nonparametric kernel regression method implemented in the ‘npreg’ function of the Hayfield et al. ‘np’ package [12] for the R software version 2.10.0. The authors suggested that outlier data should be excluded when the data-driven option is chosen. Our outliers were defined using the above PTH and 25(OH)D distributions.

Various types of kernels were tested and their antialiasing parameters selected with penalty criteria [13]. The bootstrap option [14] was chosen to obtain a 95% confidence interval for the regression function.

Results

Basic statistics

In the sample, mean age was 38.6 ± 9.9 years old (Table I). Among the 158 subjects having a non-censored 25(OH)D, there were significant negative correlations between creatinine clearance and 25(OH)D, and between PTH and 25(OH)D (p < 0.01). However, age, BMI, daily calcium intake, and serum calcium did not explain the variance of 25(OH)D levels (Table II). There was no relationship between serum PTH values and serum creatinine. In our sample, the 25(OH)D level was < 80 nmol/L in 93% of the patients, and < 50 nmol/L in 70.3% of the patients. The highest PTH concentration was 196 cm/L and the mean PTH was

| Table I: Sample characteristics and dietary daily intake of calcium |
|----------------------|------------------|------------------|
| Age                  | 38.6 ± 9.9       | BMI (kg/m²)      | 24.45 ± 3.65     |
| Creatinine clearance (mL/min) | 119.32 ± 36 | Skin Pigmentation | 6               |
| Serum calcium (mmol/L) | 2.31 ± 0.11     | Daily calcium intake (mg/d) | 800 ± 303 |
| Dietary calcium sources | 50 %            | Milk products    | 50 %            |
| Mineral water        | 14 %            | Other foods      | 36 %            |

<table>
<thead>
<tr>
<th>Table II: Pearson correlation tests for 25(OH)D</th>
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<tbody>
<tr>
<td>Data (n = 158)</td>
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<tr>
<td>PTH</td>
</tr>
<tr>
<td>Age</td>
</tr>
<tr>
<td>BMI</td>
</tr>
<tr>
<td>Calcium intake</td>
</tr>
<tr>
<td>Serum calcium</td>
</tr>
<tr>
<td>Creatinine clearance</td>
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65.44 ± 31.07 cm/L. We noted that 40% of the patients had PTH levels > 65 cm/L.

Double Gaussian mixture fitted to 25(OH)D data

In our sample, the SEMc algorithm provided the estimates of two Gaussian classes: class 1 (weight p = 0.62 ± 0.05) with lower levels of 25(OH)D and class 2 (weight 1-p) with higher levels of 25(OH)D (Table III).

<table>
<thead>
<tr>
<th>Table III: 25(OH)D distribution estimated as a mixture of two Gaussians</th>
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<tbody>
<tr>
<td>Gaussian Mixture</td>
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<tr>
<td>Class 1</td>
</tr>
<tr>
<td>Threshold of minimal density</td>
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<tr>
<td>Class 2</td>
</tr>
</tbody>
</table>

Double Gaussian mixture fitted to PTH data

The EM algorithm provided the estimates of two Gaussian classes: class A (weight: p = 0.60 ± 0.04) with lower PTH levels, and class B (weight 1-p) with higher levels of PTH (Table IV).

25(OH)D threshold which may induce a rise of PTH

In our sample, seven patients with a serum 25(OH)D level censored at 17.5 nmol/L were necessarily excluded from the estimation of the regression function. The best regression $R^2$ was 16.80% when choosing the following options [13]: data-driven method, Epachenikov kernel
Table IV: PTH distribution estimated as a mixture of two Gaussians

<table>
<thead>
<tr>
<th>Gaussian Mixture</th>
<th>Mean PTH (ng/L)</th>
<th>Standard deviation</th>
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</thead>
<tbody>
<tr>
<td>Class A</td>
<td>43.7 ± 1.81</td>
<td>6.8</td>
</tr>
<tr>
<td>Threshold of minimal density</td>
<td>51</td>
<td></td>
</tr>
<tr>
<td>Class B</td>
<td>64.8</td>
<td>5.7</td>
</tr>
</tbody>
</table>

of order 4, least square (ls) criteria for selecting the best bandwidth parameter, and when excluding 41 outliers patients having a serum concentration of 25(OH)D > 35 nmol/L and a serum concentration of PTH > 65 ng/L. Note that the values 35 nmol/L and 65 ng/L appeared in the Gaussian mixture estimates of the 25(OH)D and PTH distributions (Tables III and IV).

The regression function estimated from 117 patients showed a PTH plateau when 25(OH)D was between 48 nmol/L and 59 nmol/L, followed by a large rise in PTH when 25(OH)D decreased. The first 25(OH)D threshold below which there was a strict increase in PTH levels was found to be 48 nmol/L (Figure 1). Confidence intervals of 95% provided by Bootstrap (Figure 1) showed that a 25(OH)D level < 48 ± 2 nmol/L might induce a rise of PTH levels.

Figure 1: Estimation of the regression function of PTH on 25(OH)D among 117 African women and 25(OH)D threshold below which PTH may rise

Discussion

We chose to examine this population as it was characterized by low dermal synthesis of vitamin D due to high melanin pigmentation [15], low 25(OH)D levels, and reactive increases in PTH, which are highly prevalent in African migrants living in Northern latitudes [16]. On the other hand, recent studies have shown that elevated PTH levels are associated with increased cardiovascular risk in the general population [17–19]. Thus, identifying a 25(OH)D threshold might be of use in estimating cardiovascular risk due to high PTH concentrations. The daily dietary intake of calcium was also taken into account as it influences the 25(OH)D threshold [5], and as calcium metabolism is impaired when daily calcium intake is < 400 mg (10 mmol) [20]. In our study, the daily calcium intake of African women, estimated with 20% precision [6], was higher than 400 mg/day. This estimate was higher than the 300 mg/day reported by Prentice et al. among African women living in Africa [21], and close to the 900 mg/day recommended for French women younger than 55 years old [22]. However, Heaney et al. estimated that African American women require 300 mg less calcium per day compared to white women [23], which suggests that the average calcium daily intake of African women in our sample was sufficient.

Our correlations agreed with previous studies. Aloia et al. reviewed 25 studies that reported a correlation between PTH and 25(OH)D, and the average correlation was –0.30 [5]. Our results were similar. Vieth et al. studied 1741 adults ranging in age from 19 to 97 years, and found that increasing creatinine (indicative of diminishing renal function) correlated with a higher 25(OH)D level only in the youngest age group (age < 51 years, p < 0.001) [24]. This result was similar to our findings. It suggests, since 1,25(OH)2D is negatively correlated with creatinine as mentioned by Vieth et al. [24], that in our sample the mechanism by which 25(OH)D can adequately suppress PTH secretion was not due solely to the circulating 1,25(OH)2D produced in the kidney. This agrees with Vieth et al. [24], who concluded that circulating 25(OH)D can affect PTH secretion and parathyroid growth via 1,25(OH)2D generated within parathyroid tissue.

In our method the estimated Gaussian mixtures highlighted some interesting values. In a cross-sectional study of 379 black and white adults from Washington, D.C. [25], Yanoff et al. defined low 25(OH)D status as serum concentrations of 25(OH)D < 37.5 nmol/L, a value close to the minimum 25(OH)D density, suggesting that class 1 was the 25(OH)D low-level group while class 2 was the high-level one. Aloia et al. [26] showed that the upper limit for serum PTH using the Allegro radioimmunometric assay is 65 cm/L: this value was the mean of our high-level class of PTH.
The estimated Gaussian mixtures contributed to the detection of outlier data: this appears to be a major difference compared to some previously used methods.

We found 41 outliers. Thirty-four patients had a 25(OH)D level of 50–60 nmol/L with PTH levels abnormally higher than 80 ng/L. With such data we could not detect any rising threshold of the regression function. For the remaining 7 outliers with a 25(OH)D level of 40–50 nmol/L and PTH level of 66–79 ng/L, the bootstrap option did not provide any stable regression function.

Using the Loess regression model, Alloa et al. [5], in a study of 208 African American women, found a 25(OH)D threshold of 44 nmol/L, below which PTH concentrations increase with 60% of their sample to get an R² of 69.9%. In our study, we found a threshold with 70% of the sample and a greater R² value. This suggests that our method is equally applicable as the one used by Alloa et al.

Various reports in the literature have established a serum 25(OH)D threshold in the range of 30–50 nmol/L [27–30]. Our outlier correction procedure gave a more precise range. Note that there are noncancer effects of vitamin D in other biological metabolisms which likely need higher 25(OH)D concentrations than those required to maintain PTH secretion [31, 32]. Therefore, our 25(OH)D threshold should not be interpreted as an optimal vitamin D status.

There were two limitations in our study: the weak proportion of African American women with high 25(OH)D concentrations (> 75 nmol/L) and the proportion of outliers, even though this latter value was lower than in the above procedure.

In conclusion, from our sample of 165 calcium-sufficient African migrant women living in Paris, we found a 25(OH)D threshold of 48 ± 2 nmol/L below which PTH concentrations may increase. Estimating such a 25(OH)D threshold and the proportion of outliers using our method with other populations might be of interest.

References


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