Targeting the hepcidin-ferroportin axis may open the way to new interventions for the management of anemia of CKD [4].

Meguid El Nahas

References


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Abstract

Background. The role of vitamin D in kidney stone disease is controversial. Current evidence is inconsistent and existing studies are limited by small sample populations.

Methods. We used the third National Health and Nutrition Examination Survey (NHANES III), a large US population-based cross-sectional study, to determine the independent association between serum 25-hydroxyvitamin D [25(OH)D] concentration and prevalent kidney stone disease in a sample of 16 286 men and women aged 18 years or older. A prevalent kidney stone was defined as self-report of any previous episode of kidney stones.

Results. Among 16 286 adult participants, 759 subjects reported a history of previous kidney stones. Concentrations of serum 25(OH)D were not different between stone formers and non-stone formers (mean 29.28 versus 29.55 ng/mL, P = 0.57). Higher 25(OH)D concentration was not associated with increased odds ratio (OR) for previous kidney stones [OR = 0.99; 95% confidence interval (CI) 0.99–1.01] after adjustment for age, sex, race, history of hypertension, diabetes, body mass index, diuretic use and serum calcium. Furthermore, after we divided 25(OH)D concentrations into quartiles, or into groups using clinically significant cut-offs (e.g. 40 and 50 ng/mL), still no significant differences were found in stone formation in group comparisons.

Conclusions. High serum 25(OH)D concentrations are not associated with prevalent kidney stone disease in NHANES III participants. Prospective studies are needed to clarify the relationship between vitamin D and kidney stone formation, and whether nutritional vitamin D supplementation will increase risk of stone recurrence.

Keywords: 25-hydroxyvitamin D; kidney stone disease; Third National Health and Nutrition Examination Survey

Introduction

Kidney stone disease is common in the general population with an estimated prevalence of around 10–15% in males and 3–5% in females [1]. The calcium-based kidney stone is the most common type (>80%), and high urine calcium excretion is a strong risk factor for stone formation [1, 2]. Prior studies have shown that a higher concentration of the active vitamin D metabolite, 1,25-dihydroxyvitamin D [1,25(OH)2D], is associated with increased urinary calcium excretion [3, 4], which can lead to increased risk of stone formation.

However, less is known about the relationship between 25-hydroxyvitamin D [25(OH)D] concentrations and kidney stones. Existing studies among stone formers have failed to show a correlation between serum 25(OH)D and 1,25(OH)2D concentrations [5, 6]. In a small study involving 160 stone formers and 217 controls, Netelenbos et al. [7] failed to show any significant difference in serum 25
(OH)D concentrations between stone and non-stone formers. Berlin et al. [6] examined the serum vitamin D levels among stone formers and found that high 25(OH)D concentrations were associated with increased intestinal calcium absorption and urinary calcium excretion. This implies that 25(OH)D may have a direct impact on urine calcium excretion. However, in their study, the correlation between urinary calcium excretion and serum 25(OH)D level was low, and the analysis did not adjust for potential confounders. More recently, Leaf et al. examined the effect of vitamin D repletion on 24-h urinary calcium excretion among 29 stone formers. Overall, they failed to show any significant change in urinary calcium excretion after 8 weeks of vitamin D repletion [8]. At this time, there is no consensus in the management of nutritional vitamin D supplementation in patients with kidney stone disease. In clinical practice, physicians often withhold calcium and vitamin D supplementation in stone formers [9]. However, this practice has become a health-care challenge because of the overwhelming epidemiological evidence of health benefits from nutritional vitamin D supplementation in the general population. It is especially problematic with regard to bone health, as reduced bone mineral density is more prevalent in patients with kidney stone disease [10–12], and nutritional vitamin D supplementation is important in maintaining a dynamic calcium and bone balance [13–16].

Here, we analyzed a large US population database, the third United States National Health and Nutritional Examination Survey (NHANES III), for the independent association of high serum 25(OH)D concentration with prevalent kidney stone disease.

Materials and methods

Study population

The third National Health and Nutrition Examination Survey (NHANES III) is a national probability sample of the total non-institutionalized civilian population 2 months of age or over in the USA. The survey collected demographic, socioeconomic, dietary and health-related information, in addition to the examination and laboratory data obtained by highly trained medical personnel.

There were a total of 33,994 participants in NHANES III, and our analyses were limited to 20,050 adult participants of 18 years or older. Among those, 19,597 responded yes or no to the question regarding the history of kidney stones. Responders who had missing body mass index (BMI), serum 25(OH)D or serum calcium measurements, or had incomplete data on the history of hypertension, diabetes and diuretics use were excluded (n = 3311). Thus, the final sample used in this study included 16,286 adult participants.

Primary predictor and outcome

The primary predictor or independent variable was serum 25(OH)D concentration, which was obtained from the laboratory results data file. Briefly, at the mobile examination center, blood samples were obtained, processed and frozen to −70°C. The samples were then shipped to central laboratories for testing. Serum 25(OH)D concentrations were measured using a radioimmunoassay kit (DiaSorin Inc., Stillwater, MN) [17].

The outcome or dependent variable of interest was prevalent kidney stone disease. It was extracted from the interview data file. ‘Have you ever had a kidney stone?’ was the question asked during the standardized home interview. The adult participants who responded ‘yes’ to the question were considered to have a history of kidney stones. The participants were also asked the number of different occasions they passed a stone and whether they were taking medication or underwent procedures to treat kidney stone disease. This outcome variable has been used by prior NHANES III analyses on prevalent kidney stone disease [1].

Table 1. Characteristics of the study population with and without history of kidney stone disease

<table>
<thead>
<tr>
<th>Covariates</th>
<th>Stone formers (n = 757)</th>
<th>Non-stone formers (n = 15,529)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>54 ± 0.81</td>
<td>43 ± 0.19</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Men (%)</td>
<td>453 (59.84%)</td>
<td>7715 (46.20%)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Race: non-Hispanic white</td>
<td>645 (85.20%)</td>
<td>10,441 (67.24%)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Hispanic white</td>
<td>303 (40.03%)</td>
<td>4043 (26.04%)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>History of hypertension</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>27.84 ± 0.31</td>
<td>26.38 ± 0.07</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>25(OH)D (ng/mL)</td>
<td>29.28 ± 0.58</td>
<td>29.55 ± 0.15</td>
<td>0.6</td>
</tr>
<tr>
<td>Diuretic (HCTZ)</td>
<td>21 (2.77%)</td>
<td>260 (1.67%)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Serum calcium (mg/dL)</td>
<td>9.28 ± 0.02</td>
<td>9.27 ± 0.01</td>
<td>0.6</td>
</tr>
</tbody>
</table>

Values were expressed as mean ± SE or frequency in percentage.

Statistical analysis

We hypothesized that a higher serum 25(OH)D concentration would not be associated with a history of kidney stones in NHANES III participants ≥18 years of age. To be included in the study, participants had to have data on the kidney stone, serum 25(OH)D, age, sex, race, responses regarding history of diabetes and hypertension, BMI, serum calcium and usage of thiazide diuretics. Owing to the complex sample strategy of NHANES III, appropriate weights and strata were applied. SAS (9.2) PROC SURVEYMEANS and SURVEYFREQ were used to obtain descriptive statistics for the population. Serum 25(OH)D was analyzed as a categorical variable (quartiles as follows: Q1: ≤17.6 ng/mL, Q2: 17.7–24.2 ng/mL, Q3: 24.3–32.0 ng/mL and Q4: >32.0 ng/mL), as a continuous variable and as a binary variable (concentrations ≥40 versus <40 mg/dL and ≥50 versus <50 mg/dL). Characteristics of the population were compared using the Rao-Scott χ² for categorical variables and analysis of variance with pre-planned contrasts of the second, third and fourth quartiles compared with the first quartile of vitamin D. SAS (9.2) PROC SURVEYLOGISTIC was used to perform logistic regression to determine whether vitamin D was associated with a history of kidney stone. Results are presented as odds ratio (OR) and 95% confidence interval (CI).

Results

Among 16,286 adult participants for whom self-reported history of kidney stone disease was available, there were 757 (4.6%) subjects with prevalent kidney stone disease. As shown in Table 1, stone formers tended to be older, male, non-Hispanic white and had a higher BMI compared with non-stone formers. They were also more likely to have a history of hypertension and diabetes, and had a higher usage of HCTZ diuretics. Stone formers had a
mean 25(OH)D concentration of 29.28 ng/mL, compared with 29.55 ng/mL in non-stone formers (Table 1; Figure 1). The difference was not statistically significant. Among stone formers, serum 25(OH)D concentrations decreased with advancing age in women, but not in men (Table 2). Overall, men tended to have a higher 25(OH)D concentration than women (Table 2). Of note, only 1% of the cohort was receiving nutritional vitamin D (i.e. cholecalciferol or ergocalciferol).

Among 757 participants who reported a history of kidney stone, ~44% were taking medications for treatment of kidney stone disease, 8% received lithotripsy and 21% underwent surgery for stone removal. The mean stone occurrences were three episodes per each participant (Table 3).

Table 4 depicts that higher 25(OH)D concentration was not associated with prevalent kidney stone disease in multivariate logistic regression analyses. After adjusting for age, sex, race, history of diabetes and hypertension, BMI, usage of HCTZ and serum calcium, 25(OH)D again was not associated with kidney stone formation when modeled either as a continuous variable (OR = 0.99, 95% CI 0.99–1.01, P = 0.5) or as a categorical variable in quartiles (Table 4). In addition, we did not observe any significant relationship between clinically significant cut-offs of 25(OH)D concentration (i.e. 25(OH)D ≥40 ng/mL or 25(OH)D ≥50 ng/mL) with prevalent kidney stones.

When we included dietary protein intake, salt, water and vitamin D intake into our final analysis, the results were similar (data not shown). Furthermore, there was no two-way interaction of Age × 25(OH)D or of Sex × 25(OH)D on kidney stone formation. Also, no three-way interaction of Age × Sex × 25(OH)D on kidney stone formation was noted (data not shown). Finally, in our regression analyses, the following variables were found to have a significant association with prevalent kidney stone disease: older age (OR = 1.03, 95% CI 1.02–1.03, P < 0.0001); male sex (OR = 1.77, 95% CI 1.42–2.21, P < 0.0001); non-Hispanic white (OR = 3.08, 95% CI 2.34–4.06, P < 0.0001); history of hypertension (OR = 1.37, 95% CI 1.09–1.71, P < 0.01) and increasing BMI (OR = 1.03, 95% CI 1.01–1.05, P < 0.01) (Table 5).

Discussion

In this study, we showed that age, gender and racial background are significant determinants of kidney stone formation (Tables 1 and 5), as previously reported [1]. Stone formers also had a higher BMI and were more likely to have a history of hypertension (Tables 1 and 5), consistent with what has been reported from the Health Professional

Table 4. OR of prevalent kidney stone according to serum 25(OH)D concentrations as continuous, categorical or binary variables using a multivariate regression model

<table>
<thead>
<tr>
<th>25(OH)D</th>
<th>OR (95% CI)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Continuous variable</td>
<td>0.99 (0.99–1.01)</td>
<td>0.5</td>
</tr>
<tr>
<td>Categorical variable</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Quartile 2 versus 1</td>
<td>0.93 (0.65–1.32)</td>
<td>0.7</td>
</tr>
<tr>
<td>Quartile 3 versus 1</td>
<td>0.92 (0.64–1.30)</td>
<td>0.6</td>
</tr>
<tr>
<td>Quartile 4 versus 1</td>
<td>0.92 (0.64–1.31)</td>
<td>0.6</td>
</tr>
<tr>
<td>≥40 versus &lt;40 ng/mL</td>
<td>0.85 (0.62–1.18)</td>
<td>0.3</td>
</tr>
<tr>
<td>≥50 versus &lt;50 ng/mL</td>
<td>1.13 (0.67–1.91)</td>
<td>0.7</td>
</tr>
</tbody>
</table>

Adjusted for age, sex, race, history of diabetes and hypertension, BMI, usage of HCTZ and serum calcium

Table 5. Multivariate-adjusted ORs of covariates from the model with 25(OH)D as a continuous variable

<table>
<thead>
<tr>
<th>Multivariate adjusted OR (95% CI)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>1.03 (1.02–1.03)</td>
</tr>
<tr>
<td>Men</td>
<td>1.77 (1.42–2.21)</td>
</tr>
<tr>
<td>Non-Hispanic white</td>
<td>3.08 (2.34–4.06)</td>
</tr>
<tr>
<td>Other race</td>
<td>1.27 (0.44–3.66)</td>
</tr>
<tr>
<td>Diabetes</td>
<td>1.10 (0.79–1.55)</td>
</tr>
<tr>
<td>History of hypertension</td>
<td>1.37 (1.09–1.71)</td>
</tr>
<tr>
<td>BMI</td>
<td>1.03 (1.01–1.05)</td>
</tr>
<tr>
<td>Diuretic (HCTZ) use</td>
<td>1.34 (0.69–2.60)</td>
</tr>
<tr>
<td>Serum calcium</td>
<td>1.06 (0.85–1.32)</td>
</tr>
</tbody>
</table>
Follow-up Study, the Nurses Health Study and a large population-based survey study in Europe [18, 19]. Overall, men tended to have higher 25(OH)D concentrations than women, consistent with the trend in the general population [20]. Serum 25(OH)D concentrations were not significantly different between stone formers and non-stone formers (Table 1; Figure 1). The difference was not significant even after adjusting for important covariates such as age, gender, race, hypertension, diabetes, BMI, diuretic use and serum calcium (Table 5).

Vitamin D is important for a variety of body actions, and it is now a common practice in the general population to actively improve vitamin D store with the use of nutritional vitamin D supplementation. However, in patients with kidney stone disease, physicians are hesitant to replete vitamin D even in those with low bone mineral density because of the concern of worsening urinary calcium excretion. This prompted us to examine the NHANES III data and to our knowledge, this is the first study examining the independent association of serum vitamin D concentrations with risk of kidney stone disease using a large US population database.

Our study does not provide an explanation for the finding. The concern regarding using vitamin D in stone formers is the possible worsening of urinary calcium excretion, which is a major risk factor for stone formation. However, the direct role of vitamin D in renal calcium handling is far from being established [5, 8]. It is possible that despite lack of differences in vitamin D level between stone formers (as a whole) and non-stone formers, vitamin D may still affect stone formation in selected patients with certain genetic or clinical characteristics.

The expression and function of vitamin D receptor (VDR) might be different in stone formers, rendering them with either increased or reduced tissue response to vitamin D. Two independent groups have reported an association of VDR gene Taq I polymorphism with recurrent kidney stone formation in both Japanese adults and Turkish children [21, 22]. Other VDR polymorphisms have been reported in hypercalciuric stone formers [23, 24]. However, those findings are still controversial at this time [25].

It is also likely that vitamin D administration does not affect urinary calcium excretion until a higher threshold level is reached, considering high rates of bone loss in a stone-forming population, especially in those with high urinary calcium excretion. Most of the positive calcium balance from vitamin D (if any) will likely be deposited into the bone, instead of being excreted in the urine. In fact, short-term administration of nutritional vitamin D did not appear to alter mean urinary calcium excretion in a small group of stone formers [8]. In this study, we divided the serum vitamin D levels into quartiles, and compared the study participants with the highest 25(OH)D levels (>32 ng/mL) with those in the lowest quartiles. We found no significant increased risk of stone formation (Table 4). Furthermore, we compared different groups using higher cut-offs of 40 and 50 ng/mL, and there were no significant differences in risk of stone formation (Table 4).

Our study has several limitations. First, because this study is cross-sectional, the present analysis is limited in its ability to establish causal or temporal relationships between 25(OH)D and kidney stone formation. Second, the prevalent stone cases were self-reported, and some participants may have kidney stone disease without self-awareness or clinical diagnosis. This will lead to potential misclassification and is likely non-selective regarding vitamin D status. Therefore, it may bias results toward null. Third, initiation of medical treatment after the initial stone event (i.e. use of thiazide diuretics) might be successful in reducing urinary calcium excretion, thus attenuating the association. Fourth, we were unable to evaluate urinary calcium excretion or any possible effects of 1,25(OH)2D3 and parathyroid hormone, since measurements of these variables were not performed in this study. Finally, we do not have information on stone composition although >80% of kidney stones in the general population like NHANES are calcium based.

In summary, we demonstrated that higher serum 25(OH)D concentrations were not associated with previous kidney stone formation based on the NHANES III. We speculate that nutritional vitamin D administration does not affect urine calcium excretion and stone formation, however its use may affect a selective subpopulation of stone formers with underlying absorptive hypercalcuria or with certain genetic characteristics. Studies are needed to answer these important clinical questions.

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Conflict of interest statement. None declared.

References

Increased S261 phosphorylation in AQP2-P262L


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Vasopressin increases S261 phosphorylation in AQP2-P262L, a mutant in recessive nephrogenic diabetes insipidus

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Abstract

Background. Mutations in the aquaporin-2 (AQP2) gene cause nephrogenic diabetes insipidus (NDI), a renal disorder characterized by polyuria due to a lacking antidiuretic response to vasopressin. While most AQP2 mutants in recessive NDI are misfolded and retained in the endoplasmic reticulum, AQP2-P262L in NDI was impaired in its vasopressin-dependent translocation from vesicles to the plasma membrane.

Methods. Vasopressin-induced translocation of AQP2 coincides with AQP2 phosphorylation at S256, S264 and T269 and dephosphorylation at S261. Since P262 lies adjacent to S261, we tested whether a changed phosphorylation could underlie AQP2-P262L missorting in NDI.

Results. In polarized cells, AQP2-P262L expressed as a double 29/30 kDa band, whereas wt-AQP2 expressed only as a 29 kDa band. Phosphatase treatment revealed that the 30 kDa AQP2-P262L band was due to changed phosphorylation. The use of newly developed phospho-specific antibodies showed that forskolin not only increased pS256 and pT269, but, in contrast to wt-AQP2, also pS261 in AQP2-P262L. The expression of AQP2-P262L proteins in which S261 phosphorylation was prevented (S261A), however, was still missorted to vesicles/basolateral membrane, despite the absence of the 30 kDa band.

Conclusions. Together, our data reveal that vasopressin induces instead of reduces the phosphorylation of S261 in AQP2-P262L, but it remains to be established whether the changed phosphorylation causes its missorting in NDI.

Keywords: AQP2 water channel; nephrogenic diabetes insipidus; phosphorylation; water transport