Unsuppressed parathyroid hormone in patients with autoimmune/inflammatory rheumatic diseases: implications for vitamin D supplementation

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Abstract

Objectives. To verify if autoimmune/inflammatory rheumatic disease (ARD) patients were more refractory to PTH suppression by 25(OH) vitamin D (VITD).

Methods. Data from 105 consecutive ARD patients (including RA, PMR, spondyloarthritis and other CTDs) attending a tertiary-level immuno-rheumatology clinic and 1542 subjects tested at our central laboratory from 2008 to 2010 (controls) were collected. After exclusion of patients with renal failure, primary hyperparathyroidism and hypercalcaemia (n = 522), plasma VITD, PTH, calcium and phosphate concentrations were compared between these two groups.

Results. Plasma VITD concentrations were < 25 nmol/l in 257 patients (severe deficit, 22.8%), ≥ 25 nmol/l but < 75 nmol/l in 661 (mild deficit, 58.8%) and ≥ 75 nmol/l in 207 (normal, 18.4%). Despite similar median age, plasma VITD, calcium and phosphate values (P = 0.96, 0.30, 0.94, respectively), PTH was higher in ARD (73.0 [interquartile range (IQR) 54.2–93.7] pg/ml) than in controls [61.4 (46.9–80.3), P < 0.0002], also in all above-defined VITD categories (P < 0.05). Suppressed PTH was observed in 96.9% (95% CI 95.8%, 98.0%) of controls with VITD ≥ 75 nmol/l. However, PTH was increased more frequently in ARD vs controls. At multiple linear regression analysis, plasma VITD, age and the presence of an ARD (partial correlation coefficients −0.21, 0.15, 0.12, respectively, P < 0.0001) were independent predictors for increased PTH.

Conclusions. Patients with ARD had, on average, an increased PTH concentration for any plasma VITD range, suggesting an impaired vitamin D metabolism. Therefore, vitamin D supplementation to ARD patients may be targeted to reach PTH suppression and not simply to obtain VITD concentrations considered optimal in other categories of patients.

Key words: Autoimmune/inflammatory rheumatic disease, Parathyroid hormone, 25(OH) vitamin D.

Introduction

Hypovitaminosis D is a highly prevalent condition, with a well-known detrimental impact on bone health. In fact, low plasma 25(OH) vitamin D (VITD) concentrations determine a compensatory hyperparathyroidism that in turn leads to increased plasma calcium concentration by modulation of bone turnover [1]. The desirable plasma level of VITD in normal individuals is not precisely defined; however, maintaining a VITD plasma concentration ≥ 75–80 nmol/l (30–32 ng/ml) is widely believed to grant bone health [2], since PTH is nearly always suppressed above this threshold [3]. Recent reports suggest an additional role for the active metabolite 1,25(OH)₂ vitamin D in immune regulation; in fact, in vitro, 1,25(OH)₂ vitamin D promotes differentiation of monocytes into antigen-presenting cells, reduces inflammatory cytokine production and favours CD4
T-lymphocyte activation with a Th2 phenotype [4–7]. Additionally, epidemiological studies suggest an inverse relationship between hypovitaminosis D and autoimmune diseases, including multiple sclerosis (MS) [8], RA [9] and type I diabetes mellitus [10]. Consequently, plasma VITD concentrations have been investigated in relation to occurrence and/or severity of autoimmune/inflammatory rheumatic diseases (ARDs), although with conflicting results. Low plasma VITD levels have been observed in patients affected by SLE, RA, SS, PM/DM [11, 12], but these observations have not been confirmed in other studies [13, 14]. Part of the discrepancy between different studies may be due to the fact that VITD was studied in isolation, without taking into consideration other important parameters of calcium homeostasis, such as PTH. Hypothetically, in fact, ARD patients might be more refractory to PTH suppression by VITD, and may require higher VITD concentrations to obtain it. In this study, our aim was to test this hypothesis, evaluating the relationship between plasma VITD and PTH concentrations in patients affected by ARD with respect to which is observed in a large number of control subjects not affected by an autoimmune/inflammatory disease (NARD).

Patients and methods

Design

We retrospectively evaluated clinical and laboratory data from consecutive adult ARD patients (including RA, PMR, spondyloarthritis and other CTDs) attending a tertiary-level immuno-rheumatology clinic and adult NARD patients tested at the central laboratory of our university hospital for any medical reason from January 2008 to June 2010. We included in the study all patients who had a simultaneous measurement of plasma VITD, PTH, calcium and phosphate. We excluded all patients affected by conditions known to alter PTH concentration, such as renal failure (estimated creatinine clearance <50 ml/min from the Cockcroft–Gault formula applied on plasma creatinine) and known primary hyperparathyroidism or hypercalcaemia (total plasma calcium >10 mg/dl). Since the present study was not designed to assess the prevalence of hypovitaminosis D, we also considered in the study patients who were receiving cholecalciferol supplementation at any dose. Applying these criteria, out of 1647 patients, we included 105 ARD (21 males) and 1020 NARD (231 males) patients; 522 patients were excluded. Median age in the entire population (n = 1125) was 66.5 (interquartile range (IQR) 54.0–75.8) years, being similar in ARD and NARD patients [64.8 (IQR 54.6–71.9) vs 66.5 (IQR 54.0–76.3) years, respectively; P = 0.07].

Analytical studies

All assays were performed at the same central laboratory. VITD and PTH were both measured with a chemiluminescence method [Liaison, Diasorin, Saluggia (Vercelli), Italy]. VITD assay had a lower limit of detection (LLD) of 10 nmol/l, and PTH had an LLD of 1 pg/ml. Plasma PTH concentration was considered normal when <72.9 pg/ml, according to the cut-off for normality applied at our laboratory (this reference value has been established by modifying the normality range proposed by the manufacturer identifying the 95% CI for healthy subjects locally). Calcium and phosphate were measured with a spectrophotometric method (ADVIA 2400 Chemistry System, Siemens, Munchen, Germany). The retrospective analysis was performed according to the local code of conduct for clinical study and data protection rules. Local ethical committee approval was not required.

Statistical analysis

Data were collected and stored in an electronic database to be analysed with the Statistica statistical software program, release 7 (StatSoft, Tulsa, OK, USA). The Shapiro–Wilk test was performed to assess normality for any continuous variable analysed. The measures of central tendency and dispersion used throughout the article were means and standard deviations for continuous variables with normal distribution, and medians (IQR) for non-normally distributed variables. Accordingly, normally distributed variables were compared among groups by means of the Student’s t-test, and non-normally distributed by means of the Mann–Whitney and Kruskal–Wallis tests. Categorical variables were analysed with Pearson’s chi-square test. Multiple linear regression analysis was used to identify independent predictive factors of normal PTH concentration. A level of 0.05 (two-sided) was chosen to indicate statistical significance.

Results

The main demographic and clinical features of the studied population are presented in Table 1 and the main parameters of calcium homeostasis in Table 2. While plasma VITD, calcium and phosphate values were not significantly different among groups, PTH was higher in ARD [73.0 (IQR 54.2–93.7) pg/ml] than in NARD [61.4 (46.9–80.3) pg/ml, P < 0.0002] patients. We also observed that ARD patients showed a higher prevalence of increased PTH (>72.9 pg/ml) than controls (50.5 vs 33.4%, χ² = 12.1, P < 0.0005).

To better assess VITD effect on plasma PTH values, we categorized patients based on VITD concentrations according to previous epidemiological studies [2, 15]: severe deficit (<25 nmol/l) was present in 257 (22.8%) patients, mild deficit (≥25 but <75 nmol/l) in 661 (58.8%) and normal values (≥75 nmol/l) in 207 (18.4%), with similar distributions in ARD and NARD patients (23.3, 58.3 and 18.4% in NARD and 19.1, 62.8 and 18.1% in ARD, χ² = 1.1, P = 0.59). As shown in Fig. 1, plasma PTH concentration was always significantly higher in ARD vs NARD patients for each of the VITD categories defined above (P < 0.05 for each category).

As expected, plasma PTH concentration was inversely related to plasma VITD (Fig. 2). However, as shown in Table 3, PTH was increased more frequently in ARD vs controls. Data on PTH relation to VITD in ARD vs controls are also reported in Fig. 3.
Considering ARD patients, 37 were not receiving either DMARDs, such as MTX, SSZ, LEF and HCQ, or biologics or glucocorticoids [ARD not in treatment (ARD-N)] when calcium homeostasis parameters were measured; as expected ARD-N had a higher median plasma CRP concentration [1.1 (0.7–2.0) mg/dl] with respect to ARD patients receiving immunosuppressive drugs [ARD in treatment (ARD-T), 0.5 (0.1–1.5) mg/dl, \(P<0.002\)].

Immunosuppressive treatments did not alter PTH concentration: in fact, ARD-N had similar PTH, VITD, calcium and phosphate values (\(P=0.24, 0.12, 0.12\) and 0.91, respectively). Both ARD-N and ARD-T had plasma PTH concentrations higher than NARD [Kruskal-Wallis test: \(H=17.7, P<0.0005\); post hoc analysis: ARD-N 81.9 (55.5–94.2) pg/ml vs NARD 61.4 (46.9–80.3) pg/ml, \(P<0.003\) and ARD-T 67.5 (52.5–90.0) vs NARD 61.4 (46.9–80.3) pg/ml, \(P=0.05\)]. At multiple linear regression analysis, plasma VITD, age and the presence of an ARD were independent predictors for hyperparathyroidism, as displayed in Table 4.

### Discussion

Vitamin D is a hormone with a well-defined role in bone homeostasis: it enhances calcium and phosphate absorption and regulates bone turnover, inducing the expression of receptor activator of nuclear factor kappa-B ligand (RANKL) and down-regulating osteoprotegerin [16]. Vitamin D causes the suppression of PTH synthesis indirectly, by increasing plasma calcium concentration, and directly, by acting on parathyroid cells; in this latter case, PTH synthesis is down-regulated by the complex of 1,25(OH)\(_2\) vitamin D with its receptor (VDR), which binds the regulatory elements of the \(PTH\) gene [17, 18]. Many clinical reports confirmed the inverse relationship between plasma VITD and PTH concentrations; indeed, in the presence of hypovitaminosis D, secondary hyperparathyroidism, which is detrimental for bone health, is frequently observed [19, 20]. In spite of the lack of consensus about the plasma cut-off for normality for VITD, many authors consider 75–80 nmol/l the minimum concentration that guarantees a normal bone metabolism [21]; in fact plasma VITD concentrations above this threshold efficiently avoid secondary hyperparathyroidism [3].

Recently, new pathophysiological functions of vitamin D have been explored. In particular, \textit{in vitro} studies evidenced suppressive and regulatory effects on cells of the immune system, macrophages and dendritic cells [22]. Moreover, several epidemiological studies correlated low VITD values with the occurrence or the severity of an ARD.
however, without definitive conclusions [23]. In a recent contribution from our group we reported (i) similar plasma VITD values in patients affected by rheumatic autoimmune/inflammatory or not autoimmune/inflammatory disease, and (ii) a strikingly high prevalence of hypovitaminosis D in rheumatic patients of both kinds [13]. However, since vitamin D is important in calcium homeostasis and since it is the principal determinant of secondary hyperparathyroidism, it is possible that the evaluation of plasma VITD concentration in isolation, without considering PTH values, is not the best way to establish a link with autoimmune/inflammatory diseases. Consequently, in this study we evaluated if ARD patients could have an altered metabolism of vitamin D expressed as PTH refractoriness to suppression induced by VITD.

Our results showed that patients with an ARD had higher plasma PTH values also when stratified for plasma VITD concentration with respect to controls. This occurs notwithstanding the fact that ARD and NARD patients are similar with regard to age and plasma concentrations of VITD, calcium and phosphate. Moreover, ARD patients more frequently displayed increased plasma PTH values than controls. So what our study demonstrates is that ARD patients are more frequently prone to hyperparathyroidism for any given plasma VITD concentration; in other words, in these patients the mechanisms that regulate PTH synthesis seem to be more refractory to plasma VITD. These results are novel and suggest that patients with autoimmune/inflammatory diseases may actually have a failure of vitamin D metabolism, which is poorly evidenced by isolated plasma VITD measurement. One possible explanation for these findings is that chronic inflammatory processes may reduce parathyroid cells sensitivity to VITD. Alternatively, since the active vitamin D metabolite [1,25(OH)₂ vitamin D] has immunosuppressive and immunoregulatory effects on immune cells expressing the VDR [23], when activated in chronic inflammation, immune cells may consume higher quantities of 1,25(OH)₂ vitamin D; this event may lead to lower availability of 1,25(OH)₂ vitamin D to parathyroid cells with consequent PTH hyperproduction to restore this deficit. The 100 log lower concentration of the active vitamin D metabolite (measured in picomoles per litre instead of nanomoles per litre as happens for VITD) may explain why this deficit could barely affect plasma VITD concentration. 1,25(OH)₂ vitamin D measurement in patients with ARD may help to disclose this hypothesis. It must be pointed out, however, that recent reports indicate that the measurement of 25(OH) vitamin D is a better indicator of vitamin D status than 1,25(OH) vitamin D. In fact, 25(OH) vitamin D has a
much longer half-life (~3 weeks) than 1,25(OH) vitamin D (4 h); furthermore, 1-hydroxylation is tightly influenced by calcium concentration, while 25(OH) vitamin D synthesis is substantially dependent on substrate availability [24].

ARD patients exhibit disorder in PTH, but not in calcium and phosphate levels, which are normal. Hence, the PTH elevation is compensatory and sufficient to maintain normal calcium values without influencing phosphate levels too much. This is the calcium-phosphate profile typical of secondary hyperparathyroidism due to hypovitaminosis D, where calcium is normal or slightly reduced but never increased [2–3]. This fact supports again our hypothesis that ARD patients have an impairment of vitamin D metabolism better disclosed by assaying simultaneously VITD and PTH rather than by measuring VITD in isolation.

Our results indicate that PTH elevation in patients with ARD may be a useful and sensible indicator of an altered vitamin D status in spite of an apparently normal calcium homeostasis. On the other hand, there is no evidence to support the hypothesis that PTH elevation plays a direct role in inflammatory/autoimmune processes, nor that inflammation, per se, may directly increase PTH concentration. So we interpreted PTH elevation in ARD patients as an indicator of vitamin D impairment.

The relationship between the presence of an ARD, inflammation and PTH increase is confirmed by stratifying patients for immunosuppressive or steroid treatments; in fact, treatment did not alter either PTH or VITD concentration. Moreover, both categories of ARD patients maintained a higher refractoriness to PTH suppression in comparison with NARD, suggesting a possible disease-associated impairment of PTH/vitamin D metabolism. In our population of ARD patients, glucocorticoids were nearly always used at low doses in association with DMARDs or biologics (Table 1), suggesting that the slight PTH reduction among ARD patients under

**Table 3** Proportion of patients with unsuppressed PTH

<table>
<thead>
<tr>
<th>VITD, nmol/l</th>
<th>Percentage of patients with unsuppressed PTH</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NARD, % (95% CI)</td>
</tr>
<tr>
<td>≥ 25</td>
<td>28.0 (24.9, 31.1)</td>
</tr>
<tr>
<td>≥ 50</td>
<td>23.0 (19.0, 27.0)</td>
</tr>
<tr>
<td>≥ 75</td>
<td>17.0 (11.6, 22.4)</td>
</tr>
</tbody>
</table>

Plasma PTH > 72.9 pg/ml in ARD vs NARD patients for different plasma VITD cut-offs.

**Table 4 Risk factors for an increased PTH**

<table>
<thead>
<tr>
<th>Risk factors</th>
<th>β</th>
<th>t</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Log VITD, nmol/l</td>
<td>-0.25</td>
<td>-8.0</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Age, years</td>
<td>0.15</td>
<td>5.2</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>ARD</td>
<td>0.12</td>
<td>4.3</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Specimen obtained in summer/autumn vs spring/winter</td>
<td>0.003</td>
<td>0.1</td>
<td>0.91</td>
</tr>
<tr>
<td>Gender: female</td>
<td>0.003</td>
<td>0.1</td>
<td>0.90</td>
</tr>
</tbody>
</table>

Multiple linear regression analysis model with five variables. Plasma PTH and VITD have been log transformed to better fit the linear model. Residuals had normal distribution (Shapiro-Wilk test).

**Fig. 3** Scatter plot of individual plasma VITD (x-axis) and PTH (y-axis) concentrations after logarithmic (log 10) transformation of NARD vs ARD. The regression that correlates the two variables is represented: plasma log (PTH) = −0.15 × plasma log (VITD) + 4.53; R² = 0.068, P < 0.001 for NARD and plasma log (PTH) = −0.15 × plasma log (VITD) + 4.68; R² = 0.068, P < 0.001 for NARD.
immunosuppressive treatment might be due to suppression of inflammation rather than a glucocorticoid effect on bone metabolism; this interpretation is supported by recent data showing that glucocorticoids influence spontaneous PTH secretory dynamics but not its total plasma concentration [25].

Our results may apply to several issues important for clinical practice. First, assaying VITD concentration in isolation is not accurate enough to establish if a patient has a normal vitamin metabolism and should or should not receive cholecalciferol supplementation; to this aim, VITD assay should be better associated with PTH, calcium and phosphate measurement, especially in ARD patients. Secondly, we found that ~30% of ARD patients still displayed out-of-range plasma PTH values in spite of plasma VITD >75 nmol/l. This fact leads to the conclusion that vitamin D supplementation should be targeted to obtain complete PTH suppression and not simply to reach a plasma concentration that may be optimal in the general population. Thirdly, >60% of patients in the control group had normal calcium, phosphate and well within range PTH concentrations in spite of suboptimal plasma VITD concentrations. This finding means that subjects not affected by an ARD may have a balanced vitamin D metabolism with plasma VITD values <75 nmol/l, a fact that strengthens the need to combine VITD and PTH assay. Since PTH and VITD simultaneous assay may be expensive for population screening, we identified the at-risk population in those subjects with higher age and suffering from an ARD.

In our study, ARD and NARD patients had similar plasma VITD concentrations, as previously demonstrated by our group and others [13, 14]. These results need to be interpreted with caution, however, since the present study was not designed to assess the prevalence of hypovitaminosis D; in fact, we also considered patients receiving cholecalciferol supplementation, since our objective was to assess bone metabolism and PTH response to plasma VITD.

A limitation of our study is that we considered as controls subjects who underwent VITD and PTH measurement for any clinical reason other than ARD rather than healthy volunteers; this may have led to an overestimation rather than an underestimation of hyperparathyroidism in controls. A second important limitation is that we analysed a statistical association between VITD and PTH in the two groups instead of evaluating plasma PTH according to VITD variations in the same patient; however, the number of patients studied in the two groups allowed us to reach statistically reasonable conclusions.

In conclusion, patients with ARD had, on average, an increased PTH concentration for any plasma VITD range, suggesting an impaired vitamin D metabolism and a higher proportion of secondary hyperparathyroidism. Therefore, in these patients, vitamin D metabolism may be better studied combining plasma VITD and PTH assays. Consequently, since VITD assay alone is not accurate in the assessment of vitamin D metabolism, cholecalciferol supplementation may be better targeted to reach full PTH suppression and not simply to obtain those VITD concentrations considered optimal in other categories of patients. However, prospective studies are needed to support this conclusion.

### Rheumatology key messages

- For any plasma VITD range, ARD patients showed higher PTH values, suggesting impaired VITD metabolism.
- VITD and PTH assays should be combined to study vitamin D metabolism in ARD patients.
- VITD concentration may be not accurate enough in driving cholecalciferol supplementation for ARD patients.

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### Disclosure statement

The authors have declared no conflicts of interest.

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