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Clinical Report

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Undetectable serum calcidiol: not everything that glitters is gold

Gioconda Gallegos-Bayas[1], José Francisco Pascual-Pareja[2], Maria D. Sanchez-Niño[3], Felix Manzarbeitia[4] and Alberto Ortiz[3]

[1]Department of Family and Community Medicine, Centro de Salud Barrio del Pilar, Area 5, Madrid, Spain, [2]Department of Internal Medicine, Hospital Cantoblanco-La Paz, Universidad Autonoma de Madrid, Madrid, (IdiPAZ), Spain, [3]Department of Nephrology, IIS-Fundacion Jimenez Diaz, Universidad Autonoma de Madrid, Madrid, Spain and [4]Department of Pathology, IIS-Fundacion Jimenez Diaz, Universidad Autonoma de Madrid, Madrid, Spain

Correspondence and offprint requests to: Alberto Ortiz; E-mail: Aortiz@fjd.es

Abstract
There is an increased awareness of the adverse consequences of nutritional vitamin D deficiency. We report a patient with chronic tophaceous gout, chronic kidney disease (CKD) Stage 3/4 and undetectable serum calcidiol who developed severe hypercalcaemia upon vitamin D supplementation despite serum 25(OH) vitamin D within the normal range. Upon recovery, serum $1,25(OH)_2$ vitamin D remained in the normal range despite CKD and serum 25(OH) vitamin D 6 ng/mL. Gout tophi biopsies from additional patients showed macrophage expression of 25(OH) vitamin D 1α-hydroxylase. This case illustrates the dangers of supplementing vitamin D in patients with low serum 25(OH) vitamin D and increased 1α-hydroxylase activity due to granulomatous disease.

Keywords: 25-hydroxyvitamin D-1α hydroxylase; 1,25(OH)2 vitamin D; CYP24; chronic tophaceous gout; vitamin D deficiency

Background

Increased awareness of the adverse consequences of nutritional vitamin D deficiency has led to guideline recommendations on monitoring serum 25(OH) vitamin D and correction of any deficiency [1]. We report a case in which correction of vitamin D deficiency led to hypercalcaemia in a patient with granulomatous disease caused by chronic tophaceous gout.

Case report

A 75-year-old male was admitted for constitutional syndrome, nausea, diarrhoea and vomiting. He had hypertension, chronic kidney disease (CKD) Stage 3/4 (estimated glomerular filtration rate 26–32 mL/min/1.73 m^2) and chronic tophaceous gout. Prescribed treatment included colchicine 2 mg q.d, allopurinol 200 mg q.d., sodium bicarbonate 2 g q.d. and weekly calcifediol (25 OHD3: 266 mcg = 16 000 U). Calcifediol was prescribed 2 months before admission due to undetectable serum 25(OH) vitamin D levels, following guidelines by the Spanish Society of Nephrology [2]. At the time of calcifediol prescription, Intact parathyroid hormone (iPTH) was 98 ng/L and total serum calcium (Ca) 2.22 mmol/L (8.9 mg/dL) [ionized Ca 1.15 mmol/L (4.6 mg/dL)].

Physical findings included disorientation and extensive, severe tophi in hands, elbows, arms, thighs and gluteus (Figure 1A and B). Key analytical values were normocytic normochromic anaemia, serum creatinine (sCr) 185 μmol/L (2.1 mg/dL), uric acid 618 μmol/L (10.4 mg/dL), calcaemia 3.5 mmol/L (14 mg/dL) [ionized Ca 1.87 mmol/L (7.52 mg/dL)], iPTH $<$3 ng/L), 25(OH) vitamin D 187 nmol/L (75 ng/mL) [normal range 37–250 nmol/L (15–100 ng/mL)], angiotensin-converting enzyme (ACE) 110.4 U/L (normal range 20–60), normal thyroid hormones and absence of anti-nuclear antibodies. There was no evidence of cancer in serum tumour markers, proteinogram, axillary lymph node biopsy, gastroscopy, colonoscopy, bone marrow biopsy, abdominal sonography, bone gammagraphy and neck, thorax, abdomen and pelvis CT scan. The CT scan showed vascular enhancement in the subcutaneous tissue in both buttocks (Figure 1C). Fine needle aspiration biopsy of the buttock lesions showed uric acid crystals.

Initial treatment at the emergency room included intravenous alendronate and fluids for suspected neoplastic hypercalcaemia. Calcifediol was stopped. Calcaemia was corrected within days. Four weeks later analytical values were Ca 2.52 mmol/L (10.1 mg/dL), uric acid 618 μmol/L (10.4 mg/dL). With the diagnosis of granulomatous disease-related hypercalcaemia, the allopurinol dose was increased and prednisone 10 mg q.d. was prescribed for 1 months. At last follow-up, 10 weeks after admission, he was asymptomatic, sCr 168 μmol/L (1.9 mg/dL), Ca 2.12 mmol/L (8.5 mg/dL), P 1.07 mmol/L (3.3 mg/dL), uric acid 398 μmol/L (6.7 mg/dL), PTH 63 gg/L, 25(OH) vitamin D 15 nmol/L

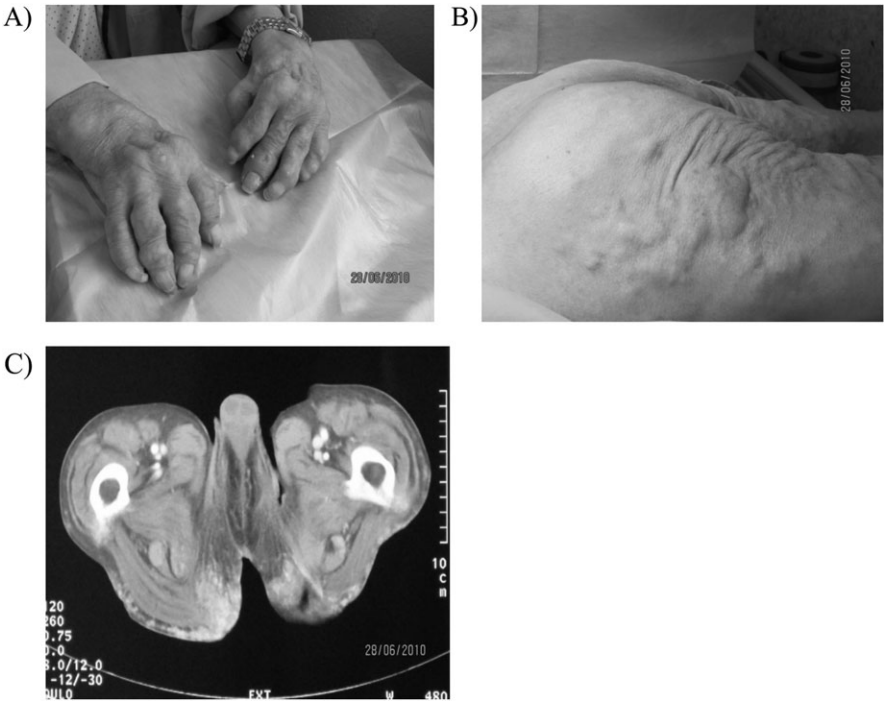

Fig. 1. Extensive severe tophaceous gout. (**A**) Extensive tophi in both hands. (**B**) Extensive gluteal tophi. (**C**) CT scan imaging of hypervascularized gouty tophi in buttocks. The nature of the lesions was confirmed by fine needle aspiration biopsy.

(6 ng/mL) and 1,25(OH)$_2$ vitamin D 114 pmol/L (44 pg/mL) [normal 65–172 pmol/L (25–66 pg/mL)]. The size of gluteal lesions had decreased.

Additional studies

Based on the presence of a chronic granulomatous disease, increased ACE levels [3], hypercalcaemia despite normal 25(OH) vitamin D levels and, later in the disease, normal 1,25(OH)$_2$ vitamin D levels despite CKD and severe 25(OH) vitamin D deficiency, it was hypothesized that the patient had increased extrarenal 1α-hydroxylase activity. However, there were no prior reports of 1α-hydroxylase expression in tophi. Thus, we performed immunohistochemistry for 1α-hydroxylase in biopsies from three additional tophaceous gout patients, which showed intense 1α-hydroxylase expression by macrophages and multinucleated cells surrounding tophi (Figure 2B). Furthermore, these cells stained for 24-hydroxylase (Figure 2C) and were confirmed to be CD68-positive macrophages (Figure 2D).

Discussion

Hypercalcaemia associated to granulomatous diseases is due to increased 25(OH) vitamin D to 1,25(OH)$_2$ vitamin D extrarrenal conversion by activated macrophages from granulomas. Over 30 diseases have been associated with 1,25(OH)$_2$ vitamin D excess [4–6]. Tophaceus gout is characterized by foreign body granulomas formed by mononuclear and multinuclear macrophages surrounding microcrystal deposits of monosodic urate. Nevertheless, only one case of symptomatic hypercalcaemia related to chronic tophaceous gout has been described and this entity is not usually thought of as a cause of hypercalcaemia [7].

Granulomatous chronic tophaceous gout is the most plausible reason for the severe hypercalcaemia in our patient. We hypothesize that high doses of vitamin D supplements in the context of granulomatous disease facilitated hypercalcaemia. Granulomatous hypercalcaemia is particularly sensitive to vitamin D administration even though toxic 25(OH) vitamin D levels are not reached [5]. This has been attributed to avid 25(OH) vitamin D metabolism into 1,25(OH)$_2$ vitamin D by macrophage 1α-hydroxylase. Availability of 25(OH) vitamin D becomes the main regulator of 1,25(OH)$_2$ vitamin D synthesis. Under these circumstances, treatment of vitamin D deficiency will increase the availability of 25(OH) vitamin D and lead to high 1,25(OH)$_2$ vitamin D levels and hypercalcaemia [8, 9]. In our patient extensive subcutaneous tophi, evidenced in the CT scan and confirmed by fine needle aspiration biopsy, would behave as a granulomatous disease. Indeed, gout tophi were observed as hypervascularized masses through magnetic resonance or CT scans. Unfortunately 1,25(OH)$_2$ vitamin D levels were not verified at admission.

In normal subjects, 25(OH) vitamin D is synthesized in the liver and is converted to 1,25(OH)$_2$ vitamin D by kidney proximal tubular 1α-hydroxylase. CKD patients have 1,25(OH)$_2$ vitamin D deficiency due to loss of renal mass and negative regulation of 1α-hydroxylase by fibroblast growth factor 23. Activated macrophages in granuloma express 1α-hydroxylase and synthesize 1,25(OH)$_2$ vitamin D. Contrary to the tubular cell enzyme, granulomatous macrophages 1α-hydroxylase is not under the regulation of PTH or hypercalcaemia and may lead to inappropriate high 1,25(OH)$_2$ vitamin D levels. Abnormal 1,25(OH)$_2$ vitamin D synthesis is present even in normocalcaemic and normocalciuric patients with granulomatosis [5]. However, hypercalcaemia is not frequent in granulomatous diseases (20% in sarcoidosis).

In gout, there are apparently two opposing forces regulating serum 1,25(OH)$_2$ vitamin D at the level of renal and

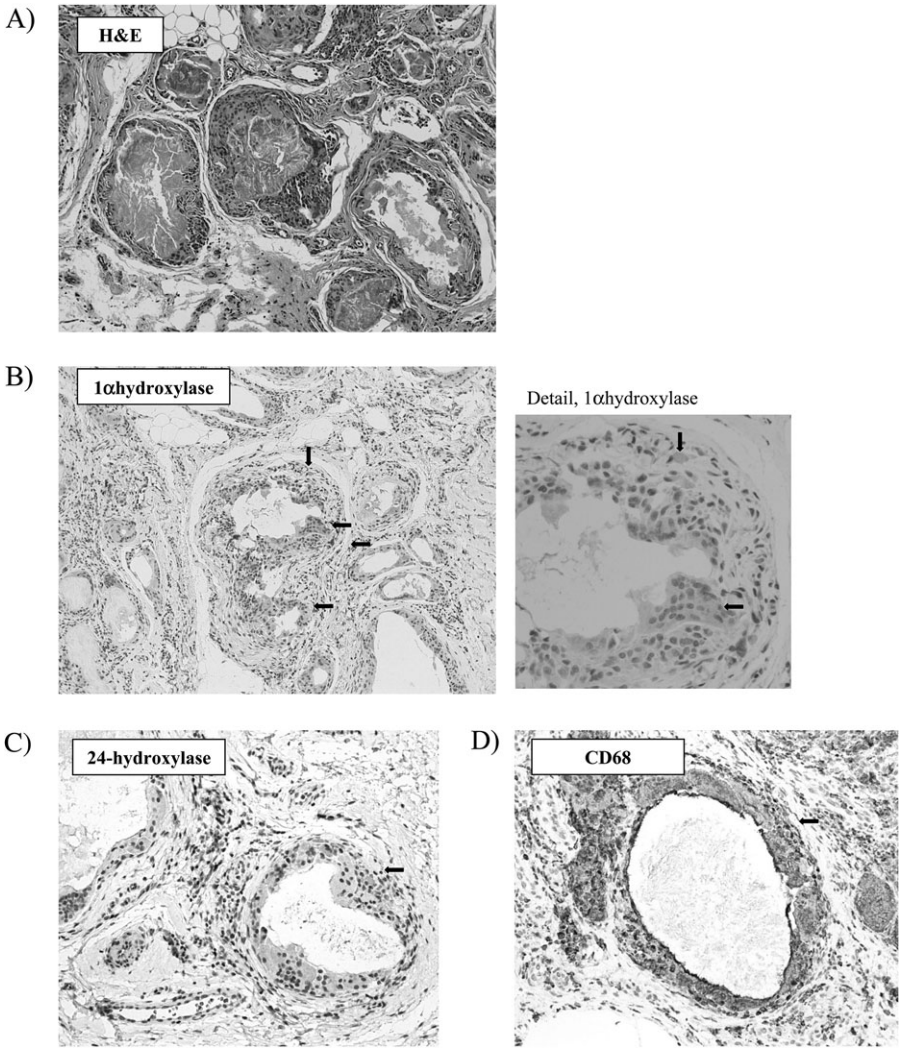

Fig. 2. Macrophage expression of 1α-hydroxylase. (**A**) Gouty tophi stained with H&E. Granulomatous inflammation is observed surrounding the matrix in which urate crystals, dissolved during the deprocessing procedure, were originally embedded. Original magnification ×100. (**B**) Representative image of 1α-hydroxylase-expressing histiocytic mononucleated and multinucleated giant cells (arrows) in gouty tophi, as assessed by immunohistochemistry. Original magnification ×100, detail ×400. (**C**) 24-Hydroxylase-expressing 'macrophagic' mononucleated and multinucleated giant cells (arrows) in gouty tophi, as assessed by immunohistochemistry. Original magnification ×100. (**D**) CD68 staining confirmed the macrophagic nature of cells surrounding the urate core (arrows) in gouty tophi. Original magnification ×100.

macrophage 1α-hydroxylase, respectively. On one hand, urate may directly inhibit renal 1α-hydroxylase activity [10]. Probably as a result, serum $1,25(OH)_2$ vitamin D was significantly lower in patients with gout compared with controls, whereas no differences were observed for serum 25(OH) vitamin D and allopurinol did not change serum 25(OH) vitamin D levels but decreased plasma uric acid and increased $1,25(OH)_2$ vitamin D [11]. On the other hand, synthesis of $1,25(OH)_2$ vitamin D from 25(OH) vitamin D was shown in cells from knee joint synovial fluid in gout [12]. We have now observed that tophi macrophage express 1α-hydroxylase. This supports the notion that extensive tophi may lead to excess $1,25(OH)_2$ vitamin D and hypercalcaemia. $1,25(OH)_2$ vitamin D promotes its own catabolism by inducing the transcription of the CYP24A1 gene encoding the vitamin D catabolizing enzyme 24-hydroxylase. This mechanism is also active in macrophages and tophi macrophages expressed 24-hydroxylase. However, in macrophages, an alternative splicing yields a 24-hydroxylase protein that binds to vitamin D but does not promote its 24-hydroxylation due to a defect in mitochondrial targeting [13]. Additionally CKD either through difficulty in excreting calcium or through loss of renal 24-hydroxylase deficiency secondary to loss of renal mass may have contributed to the severity of the manifestations.

In summary, despite the clinical guidelines recommendation to treat 25(OH) vitamin D deficiency, caution should be exercised when treating patients with granulomatous diseases, where increased 1α-hydroxylase activity could be contributing to decreased 25(OH) vitamin D levels. These patients may be at increased risk of vitamin D intoxication, even with serum 25(OH) vitamin D levels within the normal range.

Supplementary data

Colour versions of figures are available as supplementary material online. Supplementary data are available online at http://ckj.oxfordjournals.org.

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Conflict of interest statement. None declared.

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