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Serum calcitriol concentrations measured with a new direct automated assay in a large population of adult healthy subjects and in various clinical situations

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ABSTRACT

The measurement of calcitriol [1,25(OH_2)D], is important for the differential diagnosis of several disorders of calcium/phosphorus metabolism but is time-consuming and tricky. We measured serum calcitriol with a new automated direct assay on the Liaison XL platform in 888 healthy French Caucasian subjects aged 18–89 years, 32 patients with a surgically-proven PHPT, 32 pregnant women at the end of the first and at the end of the third trimester, and 24 dialysis patients before and after one year of supplementation with vitamin D3 or placebo. The mean calcitriol concentration (±SD) in the healthy population was 52.9 ± 14.5 ng/L with a 95% CI interval of 29–83.6 ng/L. In PHPT patients, calcitriol concentration was 81.6 ± 29.0 ng/L, 15 of them (46.9%) having a concentration >83.6 ng/L. In pregnant women, calcitriol was 80.4 ± 26.4 ng/L at the end of the first trimester, and 113.1 ± 33.0 ng/L at the end of the third trimester, 12 (37.5%) and 26 (81.3%) of them having a calcitriol concentration >83.6 ng/L at the first and third trimesters respectively. In 14 dialysis patients, calcitriol was 9.5 ± 7.7 ng/L and rose to 19.3 ng/L after one year of supplementation with 50,000 IU vitamin D3/month. In 10 other dialysis patients, calcitriol was 9.9 ± 2.9 ng/L and remained stable (12.4 ± 3.7 ng/L) after one year of placebo. In conclusion, this new automated calcitriol assay, in addition to presenting excellent analytical performances, gives the expected variations in patients compared to "normal" values obtained in an extensive reference population.

1. Introduction

The active vitamin D metabolite, 1,25 dihydroxyvitamin D [1,25(OH_2)D], also called calcitriol, is secreted into the bloodstream by the cells of the renal proximal tubule and binds to the vitamin D receptor (VDR) in several distant tissues to exert genomic effects. It must thus be considered as a real hormone [1]. Renal 1α-hydroxylation of 25-hydroxyvitamin D (25OHD), the precursor of calcitriol produced by the liver, is tightly regulated, mainly stimulated by parathyroid hormone (PTH) and inhibited by Fibroblast Growth Factor 23 (FGF23). Calcitriol is also produced by numerous tissues where it acts in an intracrine manner [2]. The measurement of calcitriol in serum must not be used to evaluate the vitamin D status which is consensually assessed through the measurement of 25OHD [3]. However, it is important for the differential diagnosis of several disorders of calcium/phosphorus metabolism, especially in the case of hypercalcaemia, hypercalciuria, and low PTH level [4], or in the case of rickets/osteomalacia which persists after vitamin D supplementation [5]. Calcitriol serum levels are modified in many clinical situations, increased for example during pregnancy [6], or primary hyperparathyroidism (PHPT) [7], and decreased in chronic kidney disease (CKD) [8] or hypoparathyroidism [9].

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The measurement of $1,25(OH)_2D$ concentration in serum is not an easy task due to its hydrophobic nature and because it circulates at picomolar levels compared to 25OHD, which has one less hydroxyl residue and circulates at a 1000-fold higher concentration. Currently available $1,25(OH)_2D$ assays are either immunoassays [10,11] or HPLC/LC–MSMS methods [12,13] that require quite a large amount of serum (most often 0.5 mL) and a complicated and time-consuming extraction step to eliminate potentially interfering compounds. Very recently, a new automated rapid (65 min to obtain the first result), and with a high throughput (90 tests/h) $1,25(OH)_2D$ assay that does not require an extraction step became available on the LIAISON XL automated platform [14]. Another potential advantage of this new assay is that it requires a much smaller sample volume (75 μL + adjustable dead volume of 50 μL at least) than the other $1,25(OH)_2D$ assays, a point that may be especially interesting in paediatric settings. A recently published evaluation of this new kit reported excellent analytical performances [14].

The aim of the present study was to use a large, well-defined cohort of French healthy subjects to establish serum $1,25(OH)_2D$ reference values with this new assay. We also measured $1,25(OH)_2D$ in several well-characterized groups of patients.

2. Subjects and methods

2.1. Healthy subjects and patients

We enrolled healthy volunteers who participated in the VARIETE study, a population-based cross-sectional study designed to recruit a reference population in order to harmonize normal serum IGF-I values in adults (ClinicalTrials.gov identifier: NCT01831648). They were recruited between January 2011 and February 2012 by the clinical research units of 10 university hospitals distributed throughout France. To be included in the study, subjects had to have a normal physical work-up (weight, height, blood pressure, nutritional status and gonadal/sexual status), normal laboratory values determined after an overnight fast (plasma sodium, potassium, calcium, phosphate, creatinine, glycaemia, total cholesterol, liver enzymes, TSH, blood cell counts, albuminaemia, prothrombin time, and HIV and HCV serology), age 18–89 years and BMI between 19 and 28 kg/m^2, and to give their written informed consent to participate in the study. The exclusion criteria were a medical history of thyroid, renal, hepatic, cardiovascular, pulmonary, intestinal or psychiatric disorders, cancer, epilepsy, intercurrent illness occurring during the week preceding inclusion, current consumption of tobacco or other toxics, and treatment potentially modifying IGF-I or calcium/phosphorus metabolism (antiandrogens or antiestrogens, loop diuretics, hydrochlorothiazide, CYP-inducing drugs). In addition to the blood samples necessary for the screening biological evaluation, 50 mL of whole blood and 30 mL of EDTA blood were obtained from each subject. Blood was promptly centrifuged (3000 *g* at 4 °C), and serum or plasma was aliquoted in polypropylene tubes that were immediately stored at −80 °C. This study was funded by Programme Hospitalier de Recherche Clinique, French Ministry of Health, N° P081216/IDRCB 2009-A00892-55, and was approved by the Paris-Sud Ethics Committee in November 2009.

We also studied serum samples from 32 patients with PHPT before parathyroid surgery. In these patients, the diagnosis of PHPT was ascertained by a calcium load test showing an insufficient fall in serum PTH when ionized calcaemia rose well above the upper normal limit. In those with either normal total calcaemia or normal ionized calcaemia (n = 13), an *IV* calcium load test was performed as described in [15]. These patients were osteoporotic (low bone mineral density [BMD] and/or low-trauma fracture) and were initially referred to our tertiary care centre for etiological diagnosis of abnormal calcium/phosphorus or related hormone levels detected during a screening biological evaluation aimed at ruling out secondary causes of osteoporosis. We requested that the physician who referred the patient prescribe vitamin D if the 25OHD concentration was low, before investigations in our unit. All patients in this group underwent successful parathyroidectomy, as confirmed by pathological examination of parathyroid tissue removed during surgery.

We also obtained serum samples of 32 pregnant women participating in the FEPED study, an ongoing prospective observational study of the association between serum 25OHD levels and the risk of preeclampsia (ClinicalTrials.gov identifier: NCT01648842). In these 32 women, blood was drawn at the end of the first trimester and at the end of the third trimester of pregnancy. They received a 100,000 IU vitamin D3 dose at the beginning of the third trimester of pregnancy as it is the rule in France for the prevention of neonatal hypocalcaemia. The FEPED study was funded by Programme Hospitalier de Recherche Clinique, French Ministry of Health, N° NI10024-ID RCB 2011-A00355-36 and was approved by the CPP Ile de France IV Ethics Committee in April 2011.

Finally, we included sera from 24 haemodialysis patients who participated in a pilot randomized study of vitamin D supplementation, registered with a Belgian clinical trial number (B70720084117) [16]. In these patients, blood was drawn before and after one year of vitamin D supplementation (50,000 IU vitamin D3 per month; n = 14) or placebo (n = 10) just before a dialysis session.

2.2. Laboratory methods

The biological parameters of the healthy volunteer screening evaluation were determined locally by the laboratories attached to the clinical research units, using standard chemistry. The CKDepi formula was used to estimate glomerular filtration rate (eGFR). PTH, 25OHD, and $1,25(OH)_2D$ measurements were centralized and done in batches by means of immunochemiluminescent assays on the LIAISON XL automated platform (DiaSorin, Stillwater, MN, USA), using serum samples that had never been thawed. The new $1,25(OH)_2D$ assay is a sandwich assay that uses the ligand binding domain (LBD) of the VDR as a $1,25(OH)_2D$ capture molecule. Reaction conditions were chosen at which $1,25(OH)_2D$ binds the VDR with an approximately 200-fold higher affinity than 25OHD, while the vitamin D binding protein binds $1,25(OH)_2D$ with a 10 to 100-fold lower affinity than 25OHD, leaving thus a 2000–20,000-fold differential favouring the transfer of $1,25(OH)_2D$ from the VDBP with binding to the recombinant VDR-LBD. The assay then exploits the conformational change of the LBD induced by $1,25(OH)_2D$ binding with the use of paramagnetic microparticles coated with a specific monoclonal antibody which recognizes this induced $1,25(OH)_2D$-bound LBD conformation. After a washing step aimed at eliminating unbound LBD, a second isoluminol-labelled anti-LBD antibody is added and binds to the $1,25(OH)_2D$–LBD complex already bound to the solid-phase. After eliminating the excess (unbound) second antibody, luminescence is counted and is proportional to the $1,25(OH)_2D$ present in the serum sample. Analytical performances of the new $1,25(OH_2)D$ assay have been reported in [14].

Table 1
Characteristics of the healthy subjects participating in the VARIETE study.

	Mean ± SD (range)
Gender: men/women	466/432
Age (years)	39.7 ± 18.6 (18–89)
BMI (kg/m^2)	23.0 ± 2.4 (18.5–28)
Serum 25OHD (ng/mL)	23.8 ± 8.1 (5.2–59.4)
Serum PTH (ng/L)	20.6 ± 8.0 (7.4–79.0)
Serum calcium (mmol/L)	2.30 ± 0.10 (2.10–2.60)
Serum phosphate (mmol/L)	1.09 ± 0.18 (0.75–1.51)
Serum albumin (g/L)	43.0 ± 3.9 (32.6–50)
eGFR (CKDepi) (mL/min/1.73 m^2)	100 ± 7 (60–144)