Clinical Biochemistry 49 (2016) 850–853

Contents lists available at ScienceDirect

Clinical Biochemistry

journal homepage: www.elsevier.com/locate/clinbiochem

Clinical

The impact of seasonal variation of 25-hydroxyvitamin D and parathyroid hormone on calcium levels

Robert Piirainen [a], Erling Englund [b], Anders E. Henriksson [a,c,*]

[a] Department of Laboratory Medicine, Sundsvall County Hospital, Sweden
[b] Department of Research and development, Sundsvall County Hospital, Sweden
[c] Department of Natural Sciences, Mid Sweden University, Sundsvall, Sweden

ARTICLE INFO

Article history:
Received 4 May 2016
Received in revised form 15 June 2016
Accepted 17 June 2016
Available online 24 June 2016

Keywords:
Vitamin D
Parathyroid hormone
Parathormone
Calcium

ABSTRACT

Background: Primary hyperparathyroidism is often diagnosed by high calcium levels in blood. It is well known that calcium levels are dependent on vitamin D and Parathyroid hormone (PTH). Since vitamin D has a seasonal variation the calcium levels might also be influenced by seasonal variation. If a seasonal variation in calcium levels exists, this must be considered in the investigation of suspected hyperparathyroidism. The aim of the present study was to investigate the possible influence and magnitude of the seasonal variation of vitamin D and PTH on calcium levels.

Method: In the present study the individual seasonal variation of 25-hydroxyvitamin D [25(OH)D], PTH and calcium in 69 healthy volunteers living at latitudes with extremely variable seasonal exposure to sunlight have been investigated.

Results: As expected the 25(OH)D levels were significantly higher (42%) in summer compared to winter. PTH levels were significantly lower (7%) in summer than in winter. The mean serum concentration of calcium was 1% higher in August than in February, however not statistically significant. A good agreement between summer and winter calcium values was confirmed by Bland-Altman analysis.

Conclusion: This study did not show any clinically important influence of seasonal variation of 25(OH)D and PTH on calcium that may influence a clinician's decision to investigate suspected hyperparathyroidism.

1. Introduction

Calcium homeostasis is essential for human health and performance [1,2]. Hypercalcemia is a condition commonly encountered by primary care physicians. The diagnosis is often made incidentally in asymptomatic patients. The most common causes of hypercalcemia are primary hyperparathyroidism and malignancy. It is well known that calcium levels depend on vitamin D and Parathyroid hormone (PTH). The major source of vitamin D for most humans comes from exposure of the skin to sunlight [3]. Many studies have reported a seasonal variation for both vitamin D and PTH with an inverse relationship between vitamin D and PTH concentrations [1]. This raises the question of the influence of seasonal variation on calcium levels. There is a recent study, investigating healthy volunteers in United States, that indicates seasonal calcium variation [4]. However, the study is small and does not use the newest method for serum analysis of calcium, which is the starting point for our research project. Are calcium levels, like vitamin D, higher during the summer compared to winter [1]? If this is true for calcium in serum it may constitute a source of error, for both false positive and false negative test results. Clearly, this source of error ought to be large in countries like Sweden, with extreme differences in temperature, hours of sunlight and UVB irradiation in winter compared to summer.

The present study is expected to provide additional knowledge of whether or not there is a clinically significant seasonal calcium variation. If we consider possible seasonal variation, we can do a risk analysis of false positive or negative calcium test results and thus have the opportunity to eliminate this potential problem. The results of the present study will then hopefully assist physicians with the interpretation of analytical results relating to calcium. To answer the question we have investigated the individual seasonal variation of calcium in healthy volunteers.

* Corresponding author at: Department of Laboratory Medicine, Sundsvall County Hospital, SE-851 86 Sundsvall, Sweden.
E-mail address: anders.henriksson@lvn.se (A.E. Henriksson).

http://dx.doi.org/10.1016/j.clinbiochem.2016.06.009
0009-9120/

2. Methods

2.1. Subjects

Healthy employees and trainees at a hospital laboratory were invited to participate in the present study. The study was performed in accordance with the principles of the Declaration of Helsinki. Participants signed an informed consent form approved by the regional ethics committee. Only participants who completed blood samplings during both winter and summer were included in the study. All participants stated that they were in full health and answered questionnaires regarding sunny holidays abroad, tanning bed use and vitamin D supplement use.

2.2. Blood sampling and assays

Non-fasting peripheral venous blood samples were taken from each participant on two occasions; August 2012 and February 2013. There is a seasonal variation for vitamin D, with the highest serum concentrations at the end of summer and the lowest serum concentrations at the end of the winter [5]. Therefore, August and February were selected for blood sampling. Serum samples were frozen in plastic tubes and stored at −70 °C until analysis. All samples were analyzed at the same time using the same batch of reagents by board-certified laboratory technicians who were blinded to clinical information.

In the present study we used LC–MS/MS for total 25-hydroxyvitamin D [25(OH)D] which is currently considered the gold standard for 25(OH)D measurement for assessing and monitoring vitamin D status [6]. In summary, protein was precipitated, and through supported liquid extraction, interfering components were removed and the analytes concentrated. Isolute SLE+ (supported liquid extraction) plates from Biotage (Biotage AB, Stockholm, Sweden) were used for the extraction procedure. The LC–MS/MS analysis was performed using an Acquity UPLC (ultraperformance liquid chromatography) connected in-line with an Acquity TQD (tandem quadruple mass detector) both from Waters (Waters Corporation, Milford, MA, USA). The chromatographic separation was performed on a reversed phase column (Acqity UPLC BEH Phenyl 1.7 μm 2.1 × 50 mm, Waters Corporation, Milford, MA, USA) with a mobile phase consisting of 2 mM ammonium acetate and 0.1% formic acid in water and methanol. Under these conditions no derivatization of the analytes was required. Commercially available standard reference material (MassCheck 25-OH-Vitamin D3/D2 Serum Control Bi-Level I + II, no 0221) and calibration solution (3PLUS1® Multilevel Serum Cal Set 25-OH-Vitamin D3/D2, no 62,028) both from Chromsystems (Chromsystems GmbH, Munich, Germany) were used. 25(OH)D_3 levels < 50 nmol/L, 50 to < 75 nmol/L and ≥ 75 nmol/L were defined as deficient, insufficient, and sufficient, respectively [3].

An electrochemiluminescence immunoassay (ECLIA), Cobas® PTH (Roche Diagnostics GmbH, Mannheim, Germany), was used for the quantitative determination of intact Parathyroid hormone in serum on a fully-automatic Cobas e601 analyser (Roche Diagnostics). For quantitative determination of calcium levels in serum the assays Cobas® CA2 (Roche Diagnostics) were used on a fully-automatic Cobas c501 analyzer (Roche Diagnostics). The intraassay CVs were <3% for both the PTH and calcium assays.

2.3. Sunlight and temperature during the study period

Monthly sums of sunlight hours and mean temperature, in Sundsvall (Sweden) for August 2012 and February 2013 were obtained from the webpage of Swedish Meteorological and Hydrological Institute (www.smhi.se).

2.4. Statistics

Mean ± standard deviation (SD) values were calculated for continuous variables and categorical data were expressed as absolute numbers. Differences in findings between summer (August 2012) and winter (February 2013) values were assessed by paired *t*-test (two-tailed). Bland-Altman plot [7] was used to analyze the intra-individual agreement between summer and winter values. The bias (defined as the mean differences between both measurement) and the 95% limits of agreements [defined as ±1.96 SD of mean difference] were calculated. A power analysis using G*Power version 3.1 (www.gpower.hhu.de) [8] was performed retrospectively to determine if our sample size was adequate to find seasonal differences in serum calcium levels. Remaining statistical analyses were performed with IBM SPSS 23.0 (IBM, Armonk, NY, USA). The level of significance was set at $p < 0.05$.

3. Results

3.1. Subject included

Seventy-five healthy volunteers agreed to participate in this study. Six participants were excluded due to incomplete blood samplings on two occasions. A total of 69 healthy volunteers (59 women and 10 men) with a mean age of 48 ± 13 were included in the study. All included participants were Caucasian and residents of Sundsvall, Sweden (Latitude: 62°23′N and Longitude: 17°18′E). The included subjects were healthy without previous or current diseases that might influence calcium homeostasis. All subjects denied sunny holidays abroad or tanning bed use during the month before blood sampling in February 2013. Nine of the included subjects had a regular daily use of vitamin D supplements during the month before blood sampling. The performed power analysis with a significance level of 0.05 showed that our sample size (n = 69) had an adequate power (0.99) to find a difference of 0.1 mmol/L (SD = 0.19 mmol/L) between summer and winter serum calcium using a paired *t*-test.

3.2. Sunlight and temperature during the study period

Monthly sums of sunlight hours in Sundsvall were approximately 200 h in August 2012 and approximately 50 h in February 2013. Mean temperature in Sundsvall during August 2012 was 16 °C and during February 2013 was −3 °C.

3.3. Seasonal variation in serum vitamin D, PTH and calcium

The mean serum concentration of total 25(OH)D was 42% higher in summer (71 ± 20 nmol/L) than in winter (50 ± 24 nmol/L) with a

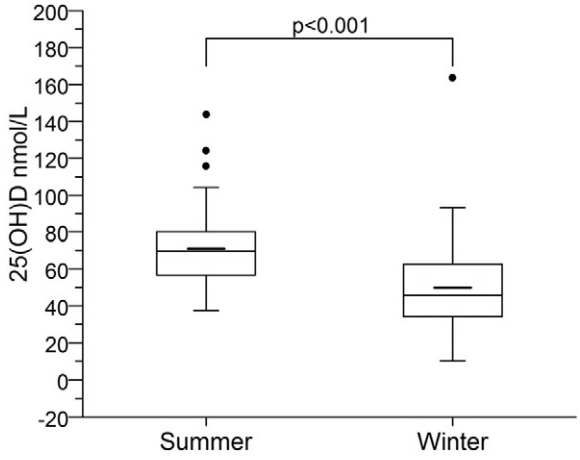

Fig. 1. Box and whisker plot for the seasonal comparison of serum concentration of total 25(OH)D in healthy volunteers (n = 69. The box represents the interquartile range, the line through the box the median level and the short line within the box the mean level. The whisker represents the values from first and third quartile up to 1.5 time the interquartile length, while outliers (•) are the values above that limit.