Abstract. Background/Aim: Vitamin D (vitD) levels are positively associated with bone health and seasonality affects serum vit D. The aim of the study was to investigate the degree of seasonal variation on 25-hydroxyvitamin D (25(OH)D) serum levels in a population-based cohort of post-menopausal women with osteoporosis. Patients and Methods: Serum levels of 25(OH)D were assessed in 596 patients (mean age=65.3 years; standard deviation (SD)=9.4) in different time points over a period of 2.5 years. Results: The minimum 25(OH)D serum levels were observed in March (13.4±9.5 ng/ml) and the maximum levels in August, September and October (29.1±16.1, 28.9±12 and 28.4±8.9 ng/ml, respectively). The prevalence of vitD deficiency, insufficiency and sufficiency in March was 76.5, 15.7 and 7.8%, respectively. On the contrary, the highest prevalence of vitD sufficiency was observed in August, September and October (38.1%, 45.3% and 46.5%, respectively). Conclusion: Seasonal variations should be considered when measuring for 25(OH)D serum levels and treating vitD deficiency.

25 hydroxy vitamin D (25(OH)D) vitamin levels are positively associated with bone mineral density (1) and several studies have reported a link with muscle mass and strength (2). Additionally, probable associations with type-2 diabetes mellitus (3, 4), certain types of cancer (5, 6), infections (7), autoimmune diseases (8) and cardiovascular disease (9) have been described. 25(OH)D levels vary with age (10), obesity (11), skin phototype (12), liver and kidney disease, medication, nutritional habits and others (13). Solar radiation is closely related to serum 25(OH)D levels. Variations in daylight throughout the year and zenith angle, depending on the latitude of residence, influence ultraviolet solar radiation (14). Accordingly, season (14-16), time of day, sun exposure duration, atmospheric pollution (17), type of clothing and sunscreens’ interference (SFP and way of application) influence its synthesis (12, 13).

The aim of the study was to examine the degree of seasonal variation of 25(OH)D serum levels, which represent the vitamin D (vitD) status in a population-based cohort of female patients evaluated at the Endocrinology Department for diagnosis and treatment of osteoporosis of the Anticancer Hospital of Piraeus “Metaxa”, Piraeus, Greece.

Patients and Methods

Between August 2012 and December 2014, a total of 596 Greek Caucasian female patients were admitted to the Endocrinology Department for osteoporosis management. All women, living in Athens for at least 5 years, were postmenopausal and over 46 years old. Total hip bone mineral density was measured by dual-energy X-ray absorptiometry and all women had a T-score of -2.5 or less. Each subject contributed one blood sample during the observation period. All women were on osteoporosis medication prescribed already by external physicians, including antiresorptive drugs in the form of biphosphonates or denosumab and vitD supplements as multivitamin tablet or as Calcium carbonate with vitD chewable tablet. They had been also advised to expose parts of their bodies without sunscreen to sunlight for five to ten minutes every day between 10 am and 3 pm in spring, summer and autumn (18).

The prevalence of 25(OH)D deficiency (<20 ng/ml), insufficiency (20-30 ng/ml) and sufficiency (≥30 ng/ml), as described by the Endocrine Society Clinical Practice guidelines (18), was estimated for each month. The analytical assay for the estimation of total 25(OH)D was obtained by Roche electrochemiluminescence immuno assay (ECL) on Elecsys and Cobas immunoassay analyzers. Data were analyzed using the SPSS analytical software 22 (SPSS Inc – IBM Corporation, New York, United States). The study was approved by the scientific committee of our hospital.

Results

The study population consisted of 596 osteoporotic post-menopausal women, (mean age=65.3 years (standard deviation (SD)=9.4, range=46-92 years) with measurements

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of 25(OH)D serum levels. The mean age and number of patients assessed every month is shown in Table I. The average 25(OH)D serum level was 21.7 ng/ml (SD=11.67 ng/ml). The monthly mean values of 25(OH)D are presented in Figure 1 and the monthly prevalence of 25(OH)D deficiency (serum 25(OH)D <20 ng/ml), insufficiency (serum 25(OH)D=20-30 ng/ml) and sufficiency (serum 25(OH)D ≥30 ng/ml) are presented in histograms in Figure 2.

The lowest value of 25(OH)D was in March (13.4±9.5 ng/ml) and the highest values in August, September and October (29.1±16.1, 28.9±12 and 28.4±8.9 ng/ml, respectively). The prevalence of 25(OH)D deficiency, insufficiency and sufficiency in March was 76.5, 15.7 and 7.8%, respectively. On the other hand, the highest percentage of patients with 25(OH)D sufficiency (38.1%, 45.3% and 46.5%, respectively) were observed in August, September and October.

There was no significant difference in 25(OH)D levels between patients younger than 65 years old and older than 65 years old. In detail, patients ≤65 years old (n=302) had a mean 25(OH)D=22.3 ng/ml (SD=11.3) and older patients (n=294) had a mean 25(OH)D=21.0 ng/ml (SD=11.9); p=0.47.

<table>
<thead>
<tr>
<th>Month</th>
<th>N</th>
<th>Mean</th>
<th>Std. deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jan</td>
<td>28</td>
<td>65.36</td>
<td>8.425</td>
</tr>
<tr>
<td>Feb</td>
<td>51</td>
<td>65.61</td>
<td>8.766</td>
</tr>
<tr>
<td>Mar</td>
<td>51</td>
<td>64.04</td>
<td>9.232</td>
</tr>
<tr>
<td>Apr</td>
<td>43</td>
<td>67.44</td>
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<tr>
<td>May</td>
<td>55</td>
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<td>8.595</td>
</tr>
<tr>
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<td>74</td>
<td>65.27</td>
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</tr>
<tr>
<td>Jul</td>
<td>60</td>
<td>65.10</td>
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<tr>
<td>Aug</td>
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<tr>
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</tr>
<tr>
<td>Dec</td>
<td>49</td>
<td>64.22</td>
<td>10.664</td>
</tr>
<tr>
<td>Total</td>
<td>596</td>
<td>65.34</td>
<td>9.416</td>
</tr>
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</table>

**Discussion**

VitD is absolutely essential for the maintenance of healthy skeleton during our lives. There exists evidence that vitD insufficiency and vitD deficiency in elderly people, and...
especially women, leads to secondary hyperparathyroidism and, consequently, to increased bone turnover and finally bone loss (19). It is the casual exposure to sunlight that provides most humans with VitD requirements. Seasonal changes can substantially influence the cutaneous production of VitD. Season at latitudes above or below approximately 33˚ has to be considered as UVB (280-320 nm) intensity decreases during the winter months affecting skin VitD synthesis (20-22). The results of the study show a strong relation between season for a given latitude higher than the tropics (Athens, Greece: 37.9˚ north of the equator) and 25(OH)D serum levels. This is why we observe the lowest levels during winter and the first months of spring. It is possible that a patient with sufficient 25(OH)D serum levels at the end of summer may be deficient at the end of winter. In our study, there was a decrease in 25(OH)D serum levels of almost 54% from August to March. These monthly patterns are similar to the results of other studies conducted in Germany and Ireland (15, 16), countries located at higher latitudes than Greece.

Exposure to sunlight results in more sustained levels of available 25(OH)D in comparison to the orally-administrated VitD (23). Any excess of VitD produced can be stored in the body fat and used during the winter when little VitD is produced in the skin (22). This is why the levels are higher in October when there is still significant sunlight and the fat stores are replete after the summer months. On the contrary, the levels are minimal in March, after wintertime, when it may be preferred to assess serum 25(OH)D levels. This could be attributed to sub-optimal medication adherence and/or inadequate therapy in combination with the minimal cutaneous production and depleted adipose tissue stores.

Greece extends between the 34˚ and 42˚ latitudes and the climate is generally typical of the Mediterranean region with mild and rainy winters, relatively warm and dry summers and plenty of sunshine throughout most of the year (24). In climatological terms, the year can be divided into two main seasons: The cold and rainy period lasting from mid-October until the end of March and the warm and dry season lasting from April to October. Rainfall, even during the winter

Figure 2. Monthly prevalence of 25(OH)D status (deficiency, insufficiency and sufficiency).
season, does not last for many days and the sky does not remain cloudy for too long. Winter storms are often interrupted during January and the first days of February by sunny days, known since antiquity as "halcyon days". Accordingly, the weak effect of sunlight for the given latitude of Athens could be a possible explanation why serum 25(OH)D levels are higher in February than in January and March in our study. The minimal exposure of face and hands -without sunscreen- due to increased outdoor activities might promote some vitD synthesis during winter. The warmest period is between the last ten days of July and the first of August when the average maximum temperature ranges from 29°C to 35°C.

Irrespective of the month measured, the majority of post-menopausal women studied had 25(OH)D serum levels below the recommended threshold of 30 ng/ml. Many older women avoid sunlight exposure because they are not interested in tanning and follow the recommendations of media and Dermatologic Societies concerning skin cancer prevention (25), (26). It is generally accepted that UV radiation is the major etiologic agent in the development of skin cancer, melanoma or non-melanoma (27).

The sample of our study represents Greek women receiving osteoporosis treatment. The data are aggregated and do not permit for analysis by personal medical history, complete medication of each patient or other covariates, such as type of vitD regimen, multivitamin use, body mass index, skin pigmentation, clothing style, place, duration of outdoor stay. Use of calcium and vitD supplements is very common among Greek post-menopausal women. VitD supplements are contained in calcium chewing tablets at a usual dose of 400 IU. Medicines containing vitD at higher doses and supplementation of vitamin D with multi-vitamin preparations (1,200 IU and 2,000 IU) that are usually prescribed for a couple of months are expensive and not public healthcare insurance-covered. The same is also valid regarding measurement of serum 25(OH)D levels.

Additionally, it was difficult to collect information regarding the dietary habits of the patients. Vitamin D status can be improved by ingestion of fatty fish and intake of vitD-fortified milk or orange juice. A high dietary calcium intake has a vitamin D-sparing effect because it increases the half-life of 25(OH)D (28). In Greece, foods are not fortified with vitamin D and diet seems to be constant throughout the year.

Finally, we only had one spot measurement for each participant. Over-the-year consecutive measurements would be important and future studies may also consider seasonal range of vitD as a confounding factor, from a clinical and public health perspective. However, our study has an important strength: we included a large sample of post-menopausal women with osteoporosis, a special population with high medical needs. It is important to measure serum vitD levels in women with osteoporosis as vitD is related to this disease (29). This is by far the largest sample-size study to date for examining the seasonality of vitD levels in Greek post-menopausal women with osteoporosis.

Greece is considered a country of high sunlight levels. Based on the important contribution of sunlight exposure to the production and maintenance of serum 25(OH)D levels, one might consider that 25(OH)D deficiency may be a problem limited to countries located at higher latitudes. Nevertheless, numerous studies from countries with high sunlight levels have shown that vitD deficiency is a common occurrence and a global health problem (30, 31). This research shows that the majority of women treated for osteoporosis have vitD insufficiency and deficiency throughout most of the year. Although a daily dose of at least 600-800 IU of Vit D is recommended for persons aged more than 50 years old, the dose should be individualized depending on the vitD status and month measured (18).

Conclusion

Seasonal variations should be considered when measuring and correcting vitamin D serum levels. The halcyon days and the lower latitude of Athens, compared to studies conducted in other parts of the world, could augment some vitD synthesis even in the winter. However, the majority of Greek post-menopausal women with osteoporosis could not reach sufficient 25(OH)D levels, regardless of type and dose of vitD supplementation and degree of sun exposure.

References


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