Vitamin D status in adults and children in Transcarpathia, Ukraine

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Research article

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Abstract

**Background:** Vitamin D deficiency is a global health problem, it is assessed by measuring serum 25(OH)D, nevertheless epidemiological data for many countries remains underreported.

**Objectives:** To study the prevalence of vitamin D deficiency throughout the calendar year in a large cohort recruited in a multiethnic Transcarpathian region of Ukraine.

**Methods:** In this retrospective study 25(OH)D serum concentration was measured during all 12 months of the years 2019 by electrochemoluminescent assay “Elecsys Vitamin D total”, Roche® (Germany) on the automatic analyzer Cobas e411 in 1,823 subjects, including both children and adults (1,551 females (85.03%) and 273 males (14.97%)).

**Results:** Mean 25(OH) D concentration in adults shows significantly lower levels compared to children (22.67±8.63 ng/ml vs. 26.00±10.72 ng/ml respectively, \(p=0.00001\)). Adult women expressed significantly lower mean year serum 25(OH) D concentration in comparison to men (22.29±8.46 ng/ml vs. 25.75±9.38 ng/ml respectively, \(p=0.0000003\)). On the other hand, children did not show a significant difference between sexes (girls 24.98±10.38 ng/ml vs. boys 27.01±11.01 ng/ml, \(p=0.2003\)). In the winter months, 25(OH)D levels fell below 20 ng/ml in 51.74% of adult population of Transcarpathia, and in 12.91%, below 12 ng/ml.

**Conclusions:** The results of this study contradict the previously reported evaluations of the vitamin D levels in Ukraine assessed by measuring serum 25(OH)D. Specifically, only around half of the studied population is vitamin D deficient during “low” winter season. This study features the most representative sample size in Ukraine to date.

Introduction

Vitamin D is an important fat-soluble vitamin that acts as a steroid prohormone playing a key role in bone mineralization. It is synthesized in skin under UV light exposure and is ingested in food [1]. To date, the effects of vitamin D have been associated not only with musculoskeletal disorders, but also with such common conditions as cancer, autoimmune, inflammatory, infectious and cardiovascular diseases, as well as diabetes[2]. Vitamin D levels in the population depend on UV light exposure, consequently geographic location, skin type, clothing culture, testing methods, nutritional habits including supplements consumption, as well as genetic factors[3].

Vitamin D status is generally evaluated by measuring serum 25-hydroxyvitamin D (25(OH)D), and the gold standard method for this test is considered to be the liquid chromatography-tandem mass spectrometry (LC-MS/MS)[4]. However, despite the vast knowledge amassed on the topic, the concentration of 25(OH)D that indicates vitamin D deficiency in the human body still remains controversial. The National Academy of Medicine (USA) [5] considers levels of 25(OH)D below 12 ng/ml
to be a sign of vitamin D deficiency, while the Endocrine Society (www.endocrine.org)[6] contends that the threshold level is at 20 ng/ml.

Indisputably, vitamin D deficiency is a global health problem, and it was recorded in many countries, especially among institutionalized elderly, postmenopausal women, and immigrants. Reportedly, from 2 to 30% of Europeans may have 25(OH)D below 10 ng/ml[3]. At the same time, there is a large regional variation, as population of Nordic countries seem to have higher vitamin D levels, that may be attributed to the traditionally high consumption of fish liver oil[7].

Ukraine is the largest territory fully located in Europe that spreads over various climate zones, from temporal to subtropical. Previous studies have reported that as much as 37.3% of the Ukrainian population could be vitamin D deficient (< 10 ng/ml), which is very high proportion, especially when compared to other European countries[8],[9]. In this study we attempted to validate the previous studies, and to reevaluate the prevalence of vitamin D deficiency using the largest and the most representative sample collected in a multiethnic Transcarpathian region (Transcarpathia) of Ukraine, bordering four other European countries (Poland, Slovakia, Hungary and Romania).

**Methods**

This retrospective study included 1,823 randomly selected subjects from Transcarpathia, whose serum concentration of 25(OH)D was recorded in 2019 at the medical laboratory center “Astra Dia” (www.astradia.ua). The recorded data were depersonalized and included also the following information: sex, age, and month of blood collection. The 25(OH)D serum concentration was measured on the automatic analyzer Cobas e411 using the “Elecsys Vitamin D total” electrochemoluminescent assay (Roche®, Germany). The studied individuals self-stated as not consuming any dietary supplements at the time of blood draw. The data was analyzed taking into account two established 25(OH)D concentration cut-offs indicating deficiency: <12 ng/ml by National Academy of Medicine, USA and < 20 ng/ml by Endocrine Society, USA. All the statistical data analysis including normality of the distribution (Shapiro–Wilk test), the differences between groups (2-sample independent T-test) and ANOVA (Analysis of variance) were performed using STATISTICA® software (StatSoftInc., Tulsa, OK, USA). P values less than 0.05 were regarded as statistically significant.

**Results**

Among the 1,823 studied individuals, 1,551 were females (85.03 %) and 273 were males (14.97 %). The majority (1,639 or89.9%) were adults and only 184 (10.1 %) were either children or adolescents (aged <18 years). The average age (mean ± SD) among all the adults was 40.13±13.41 years (range 18-82), while the males were slightly older (42.21±14.66, range 18-75 years) than the females (39.87±13.22, range 18-82 years). Among the children and adolescents (<18 years) the average age was 10.67±4.83 years, with males (9.3±4.75 years) younger than females (12.05±4.52 years). Both age and 25(OH)D concentration data were normally distributed (Shapiro–Wilk test, p>0.05). We analyzed crude 25(OH)D levels every
month of the calendar 2019 year among adults and children, and observed significant seasonal variability in 25-hydroxyvitamin D expression levels (ANOVA test, p=2.55298009662856E-24). Among the adults the lowest levels were observed in February (19.44 ng/ml), and the highest - in September and July (26.97 ng/ml and 26.83 ng/ml, respectively). Similarly, children and the adolescents expressed the lowest levels of 25(OH)D in February (16.60 ng/ml), and the highest in August (32.53 ng/ml). Monthly 25(OH)D concentrations in different age categories are presented in Table 1. In comparison to children and the adolescents, the adults had significantly lower mean year serum 25(OH) D concentration (26.00±10.72 ng/ml vs.22.67±8.63 ng/ml respectively, p=0.00001). The adult women had significantly lower mean annual serum 25(OH) D concentration in comparison to the men (22.29±8.46 ng/ml vs.25.75±9.38 ng/ml respectively, p= 0.0000003). Monthly fluctuations of 25(OH)D concentration levels among the adults are shown in Figure 1. Children and adolescents did not show any significant differences in 25(OH)D between sexes (girls 24,98±10.38 ng/ml vs. boys 27.01±11.01 ng/ml, p=0.2003). The concentration of 25(OH)D negatively correlated with age both among adult males (r= -0.16, p<0.05) and females(r=-0.12, p<0.05). Interestingly, even stronger negative correlation of 25(OH)D with age was observed in children: among males (r= -0.42, p<0.05) and females (r=-0.43, p<0.05). Since many studies calculate and report the prevalence of vitamin D deficiency in winter months[7] we also analyzed and presented the winter data for comparison. In our sample, 51.74 % of adult population of Transcarpathia have 25(OH)D levels below 20 ng/ml and 12.91% - below 12 ng/ml in the winter (December through February of 2019). The prevalence of vitamin D deficiency in different sex and age groups in our sample is shown in Table 2. The prevalence of vitamin D deficiency in different age groups throughout the years is shown in Table 3. Across the age groups, the highest level of vitamin D deficiency is observed in adults over 60 years (58.7% of people with levels below 20 ng/ml, and 9.9% of people with levels below12 ng/ml). Among the children and adolescents, the highest prevalence of vitamin D deficiency is observed in the age range of 8-12 years old (41.86% below 20 ng/ml and 4.65% below 12 ng/ml). Vitamin D deficiency was completely absent in the infants and toddlers (0-3 years age group).

**Discussion**

To the best of our knowledge, this is the largest study of the vitamin D deficiency in Ukraine to date and the first study of this condition in the Transcarpathian region. Previously, the only countrywide study of vitamin D levels in Ukraine conducted and published in 2010 by Povoroznyuk et al.[9] reported that 81.8% of the Ukrainian population was deficient with levels of 25 (OH)D below 20 ng/ml, while 37.3% had levels below 10 ng/ml. In addition, Povoroznyuk et al.[9] reported that the average level of 25(OH)D in Ukraine was13.87 ng/ml, and the lowest level of 12.61 ng/ml was in western Ukraine, which geographically includes Transcarpathia as the westernmost region of the country. Unfortunately, the times of the year when the blood samples were drawn have not been reported for this study[9].

Overall, our data significantly differs from the results obtained by the other study discussed here; our results suggest the lesser prevalence of vitamin D deficiency than was thought previously. In contrast to the earlier study[9], our results indicate that only 51.74% of the studied population have a deficiency of vitamin D (below 20 ng /ml) in the winter months, and only 12.91% of individuals have levels below
12 ng/ml in Transcarpathia. Moreover, in our sample the mean 25(OH)D concentrations during the winter months was at 20.84 ng/ml, while the annual average was at 22.67 ng/ml, almost twice the level previously reported [9].

There may be several reasons for the stark discrepancies in results between the two studies. First, Povoroznyuk et al. [9] reported using the electrochemoluminescent method, polyclonal Vitamin D assay (Roche Diagnostics®, Germany) on Elecsys 2010 analyzer. There were reports in 2011 that Roche Diagnostics® withdrew several lots of this particular assay from use referring to the deterioration of the results conformity to the reference method (liquid chromatography - tandem mass spectrometry; LCMSMS), the same studies reported those polyclonal assays tend to lower the actual serum 25(OH)D levels [10]. In contrast, in the current study the samples were tested using the Elecsys assay with a newer generation kit one a different analyzer (Cobas® e411, Roche Diagnostics®, Germany). Second, the season of sample collection can significantly contribute to the difference in our results. The exact difference between seasons cannot be established, because the earlier study did not specify the timing of samples collection [9]. It is unlikely that the climate could account for a large difference, as Transcarpathia has similar average solar activity compared to other regions of Ukraine. The total annual amount of sun exposure in Transcarpathia low lands is around 2,000 hours, while in mountainous areas it is approximately 1,700 hours, which is very similar to the total sun exposure in Ukraine overall (1,700 to 2,400 hours annually) [11].

Given the close geographic proximity of Transcarpathia to other European countries it seems reasonable to compare our findings to those from neighboring countries. The similar values reported for these countries give additional credence to our results. For instance, a recent study in Poland that involved 5,775 adults with a mean age of 54.0 ± 15.9 years reported the mean level of 25(OH)D at 18.0 ± 9.6 ng/ml [12]. This study also indicates that 65.8% of the population had a level of 25(OH)D below 20 ng/ml, which is closer to our results (51.74%) than to the earlier study of the Ukrainian population overall (81.8%). In addition, while our results showed lower levels in females in all age groups of adults in almost all months of the year, the study conducted in Poland showed lower levels of vitamin D in men compared to women. Although the authors believe the geographic location plays a minor role in vitamin D status in Poland, this may in fact account for the relatively higher prevalence of the deficiency in Poland (14.06% higher compared to Transcarpathia) because the territory of Poland is located more to the north (52.13° N, 21.02° E) compared to Transcarpathia (48.41°N, 23.29°E). It should be noted that yet a different method of measurement of 25(OH)D was used in this Polish study (Liaison XL system (DiaSorin; CLIA method). The collection of material in this study was conducted from February 14 to March, and from April 28 to May 2, which is considered to be a “low” solar activity season. In the neighboring Slovakia, a study of healthy women aged 25–40 years showed an average level of 25(OH)D at 32.6 ng/ml [13], which is 10.3 ng/ml above 22.3 ng/ml 25(OH)D of during the year reported for women in Transcarpathia (mean age 39.87 ± 13.22 years). Unfortunately, this study did not account for the season. Similarly, the prevalence of severe vitamin D deficiency in Transcarpathia (12.91% below 12 ng/ml in the winter months) is lower than in Germany [14] and Great Britain [15], but higher than that reported in Spain [16], Italy [17], and France, where a study of 2,007 individuals, in the age ranges 30–54 reported only 5.2% prevalence of vitamin D
deficiency below 10 ng/ml [18]. The average level of vitamin 25(OH)D in adult population of Transcarpathia (20.84 ng/ml in the winter months) is the closest to the levels reported in Austria (20.88 ng/ml, adults, age range 21–76 years) and Belgium (19.28 ng/ml, adults, age range 21–69)[19, 20].

The above comparison supports the notion that Ukrainian population has similar levels of vitamin D deficiency compared to its geographical neighbors, and that Transcarpathia may be part of the presumable north-south trend in serum vitamin D, where southern European countries have lower levels than northern European countries, though this trend does not follow the exact pattern (Fig. 2)[7, 12, 14–25].

**Limitations**

While our study had several limitations that prevented the direct comparisons to others (the method of measurement we used in our study is not the gold standard method for this type of tests, though it shows the correlation with tandem mass-spectrometry of $r = 0.89$ according to the manufacturer), and can be considered a rough population-based study on vitamin D status in the region, it highlights an important geographical trend that should be further explored and could be useful to aid the development of health care strategies in the region. Unfortunately, while we collected the seasonality data, we did not obtain data on the exact place of residence, and the origin of the people who were included in the study, as well as the information on supplements consumption was self-reported, D, all these could be useful covariates in the future investigations.

**Conclusions**

This study showed a noticeable prevalence of deficiency of vitamin D in the Transcarpathian population in Ukraine, documented by a moderate decrease in 25(OH)D levels (<20 ng/ml) in up to 51.74 % of the society in winter months. Only a limited part of the studied population (12.91%) expressed severely decreased 25(OH)D levels (<12 ng/ml). Taking together our findings contradict the previous study conducted in Ukraine, which showed much higher prevalence of vitamin D deficiency, but are consistent with the majority of the regional European reports on vitamin D status, and fit in the hypothetical south to north trend in deficiency across Europe. Adult women have significantly lower vitamin D levels than men, and children have higher levels than adults in our cohort ($p < 0.001$). Serum 25(OH)D concentration negatively correlates with age both in children ($r = -0.42, p < 0.05$) and adults (males: $r = -0.16$; females: $r = -0.12, p < 0.05$).

**Abbreviations**

25(OH)D - 25-hydroxy-vitamin D

ANOVA-analysis of variance
Declarations

Ethics approval and consent to participate

This study was approved by IRB of SU “Uzhhorod National University” with the waiver of consent as it is a retrospective anonymous study with the minimal possible risk to participants

Consent for publication

Waiver of consent by IRB decicision

Availability of data and material

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The author declares that there are no competing interests.

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Authors' contributions

The author solely designed the study, performed the analysis, data interpretation, and preparation of the manuscript.

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Tables

**Table 1.** Monthly levels of 25 (OH) D in Transcarpathia, Ukraine

| Month**  | Adults                          | Children and adolescents                  |
|----------|--------------------------------|-------------------------------------------|
|          | Mean±SD 25(OH)D (ng/ml) | Number of individuals | Mean±SD 25(OH)D (ng/ml) | Number of individuals |
| January  | 21.09±8.74      | 125                           | 22.56±7.72             | 11                           |
| February | 19.45±7.67      | 174                           | 16.60±6.41             | 12                           |
| March    | 21.57±9.18      | 130                           | 25.43±12.06            | 16                           |
| April    | 21.03±8.44      | 127                           | 23.56±10.44            | 14                           |
| May      | 20.75±7.59      | 151                           | 19.80±6.08             | 7                            |
| June     | 23.12±8.18      | 137                           | 26.02±7.55             | 17                           |
| July     | 26.83±9.56      | 139                           | 29.59±6.63             | 16                           |
| August   | 23.59±7.71      | 125                           | 32.53±15.43            | 24                           |
| September| 26.98±8.00      | 129                           | 27.66±11.78            | 14                           |
| October  | 23.87±8.59      | 134                           | 27.48±10.41            | 18                           |
| November | 23.61±8.83      | 136                           | 25.86±7.39             | 15                           |
| December | 21.17±7.58      | 132                           | 24.76±9.82             | 20                           |
| Year mean| 22.67±8.63      | 1639                          | 26.00±10.72            | 184                          |

**Measurements were conducted during 2019 and summarized for each month across the years**

**Table 2.** Prevalence of vitamin D deficiency during winter months (December–February 2019).
| Indicator                               | Adult population | Adult males  | Adult females | Children |
|----------------------------------------|------------------|--------------|---------------|-----------|
| Mean 25(OH)D (ng/ml)                   | 20.45±7.99       | 23.91±8.33   | 20.09±7.85    | 21.92±8.98 |
| Prevalence of 25(OH)D below 20 ng/ml   | 51.74%           | 30.18%       | 54.64%        | 46.5%     |
| Prevalence of 25(OH)D below 12 ng/ml   | 12.91%           | 9.25%        | 13.52%        | 4.6%      |

Table 3. Mean 25(OH)D concentration and the prevalence of vitamin D deficiency in different age groups in the calendar year

| Indicator                               | 0-3  (n=23) | 4-7  (n=37) | 8-12 (n=43) | 13-17 (n=81) | 18-25 (n=232) | 26-35 (n=470) | 36-45 (n=394) | 46-60 (n=412) | > 60 (n=131) |
|----------------------------------------|-------------|-------------|-------------|--------------|---------------|---------------|---------------|---------------|-------------|
| Mean±SD 25(OH)D ng/ml                 | 38.55±11.25 | 29.55±12.93 | 23.1±8.01   | 23.33±8.47   | 23.23±8.13    | 24.18±8.51    | 22.45±9.21   | 21.76±7.87   | 19.81±9.35   |
| Prevalence of 25(OH)D below 20 ng/ml  | 0%          | 21.62%      | 41.86%      | 35.8%        | 36.20%        | 35.10%        | 45.43%        | 44.1%         | 58.7%        |
| Prevalence of 25(OH)D below 12 ng/ml  | 0%          | 2.7%        | 4.65%       | 0%           | 3.01%         | 3.40%         | 4.82%         | 5.8%          | 9.9%         |

Figures

Figure 1

Monthly distribution of 25(OH)D concentration among adults
Figure 2

Mean 25(OH)D reported among adults in different European countries. This map is drawn by the author, using the software Tableau, data is taken from the cited studies, data for Ukraine is taken from our study.