

*Original Article***Vitamin D status in healthy postmenopausal Iranian women**

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**Abstract**

**BACKGROUND:** There are few epidemiologic studies on vitamin D status of postmenopausal women in the Middle East countries. This study aimed to investigate the 25-hydroxyvitamin D levels in postmenopausal women living in the north-west of Iran.

**METHODS:** Using the records of the local household registry, 300 cases were enrolled by simple random sampling. Serum 25-hydroxyvitamin D levels were determined by fully automated chemiluminescent immunoassay. In addition, the study included survey questions regarding age, body weight and height, use of supplements and skin protection agents and clinical and reproductive histories.

**RESULTS:** Our cases had the mean age of  $63.41 \pm 4.64$  years with menopause duration of  $16.79 \pm 6.15$  years. Median and interquartile 25-75 range of vitamin D were 14.20 ng/ml and 7-37.2 ng/ml respectively. We found hypovitaminosis D [25(OH) D < 10 ng/ml] in 38.3% of our cases. Serum 25(OH) D concentrations were not significantly correlated with age or BMI.

**CONCLUSIONS:** These findings indicate that 25(OH) D levels in postmenopausal women of north-west Iran are low. Studies to elucidate and assess the dietary intake of vitamin D in elderly women of this region can be of further benefit.

**KEYWORDS:** Vitamin D Deficiency, Dietary Supplement, Postmenopausal Period, Middle East, Iran.

JRMS 2009; 14(3): 171-177

**A**ge-related decline of vitamin D reserves, low exposure to sunlight and low intake of vitamin D are major factors contributing to hypovitaminosis D. Vitamin D plays an important role in bone growth and maintenance by enhancing intestinal absorption of calcium and influencing bone metabolism in other ways. In the mid-to-late 20th century, it became evident that vitamin D inadequacy was very common among the elderly, and was implicated in the development of osteopenia and osteoporosis.<sup>1-3</sup> Vitamin D inadequacy has also been implicated as a contributing factor to muscle weakness and falls.<sup>4-8</sup> Many investigators have pointed out that low level of serum 25-hydroxyvitamin D, a good nutritional indicator of vitamin D stores in the

body, is prevalent in in-patients<sup>9,10</sup> and in institutionalized elderly subjects.<sup>11,12</sup> Vitamin D status is highly different between various countries.<sup>13,14</sup> This is caused by different exposure to sun light, dietary intake of vitamin D and the use of supplements. The prevalence of hypovitaminosis D has been reported to be greater than 35% in such high-risk populations. Even in independent-living elderly populations, the prevalence has been reported to be 6-16%.<sup>15-17</sup>

The majority of studies reported a high prevalence of serum 25(OH) D values below specified cut-off points among healthy postmenopausal women. Rahman SA et al showed that vitamin D insufficiency (< 20 ng/ml) among postmenopausal Malaysian women

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was 70%.<sup>18</sup> In the study of Khoja SO in Saudi Arabia the prevalence of vitamin D deficiency (< 12 ng/ml) in postmenopausal women was 74%.<sup>19</sup> In Iran a few studies of vitamin D status in general population have been published.<sup>20-23</sup> In these studies prevalence of vitamin D deficiency (< 10 ng/ml) has been reported to be 30-67%. Prevalence of vitamin D deficiency in postmenopausal women has been reported 5.3% in only one study in Tehran.<sup>24</sup>

As sunlight plays an essential role in vitamin D synthesis and Iran is a geographically heterogeneous area, mean daily sun exposure will be different in various areas. In this way, the results of studies conducted in Tehran cannot be applicable to other regions. This study investigated the 25(OH) D levels and prevalence of hypovitaminosis D in healthy postmenopausal women in Tabriz.

## Methods

### *Subjects and Methods*

The subjects of this study were enrolled from the participants of a community-based survey which was carried out from January 2008 to April 2008 in Tabriz, the capital city of East Azerbaijan, a province in north-west Iran. We targeted healthy postmenopausal women and selected our sample by simple random method using a sampling frame constructed from the records of the local household registry. Exclusion criteria were history of diseases known to alter serum levels of vitamin D metabolites such as hepatic dysfunction, renal disease, diabetes mellitus, metabolic bone disorders, current steroid therapy or use of vitamin D supplements. Finally, 300 subjects aged between 53 to 80 y were included in our survey.

Vitamin D status is defined according to the serum concentration of 25-hydroxyvitamin D (25(OH) D). For this study, vitamin D deficiency is defined as serum 25(OH) D of lower than 25 nmol/l (10 ng/ml); and vitamin D insufficiency is considered as serum 25(OH) D of 25 to 50 nmol/l (10-20 ng/ml), as was suggested previously.<sup>25</sup>

After the cases signed the informed consent, they underwent a clinical examination. Demographic data collection, anthropometric examinations and medical history taking were made by trained physicians. Body height and weight were measured to the nearest 1.0 cm and 0.1 kg, respectively. Body mass index (BMI) was calculated as weight (kg) divided by the square of height (m<sup>2</sup>).

The history taking included questions about diet (daily consumption of dairy products) and supplements, use of skin protection agents, clinical and reproductive histories and age of menopause.

### *25(OH) D Measurements*

After blood specimens were drawn from subjects, samples were centrifuged and extracted serum stored at -70°C until the 25(OH) D measurements was performed. Serum 25-hydroxyvitamin D [25(OH) D] concentrations were determined by the DiaSorin "25-OH Vitamin D TOTAL" competitive chemiluminescence's immunoassay on the automated LIAISON® analyzer (Stillwater, MN). This method has 100% specificity for both 25(OH) vitamin D<sub>2</sub> and 25(OH) vitamin D<sub>3</sub>. This assay has a limit of detection of 4ng/ml, an intra-assay coefficient of variation (CV) of 5%, and an inter-assay CV of 7%. Samples were analyzed in one continuous batch with quality control samples inserted at periodic intervals.

### *Statistical Analysis*

Data were expressed as Mean ± SD and n (%). The Kolmogorov-Smirnov statistic was used for testing normality for continuous variables. As the distribution of serum 25(OH) D was highly skewed, we applied nonparametric statistical measures (median and interquartile 25%-75%). Vitamin D status was analyzed with the Kruskal-Wallis nonparametric test and Spearman coefficient correlation. A p value less than 0.05 was considered statistically significant. The Statistics Package for Social Science (SPSS 14.0) was used for statistical analyses.

**Table 1.** Characteristics of the 300 postmenopausal women

Variable	Mean $\pm$ SD	Median	Interquartile 25-75	Range
Age	63.41 $\pm$ 4.64	62	60-65	53-80
Duration of menopause (year)	16.79 $\pm$ 6.14	16	13-20.75	1-35
Number of delivery	6.11 $\pm$ 2.58	6	4-8	1-14
Body weight (kg)	67 $\pm$ 12.15	67	60-75	32-125
Body mass index (kg/m <sup>2</sup> )	28.08 $\pm$ 5.08	27.99	24.44-31.22	14.41-47.94
Serum 25(OH) vitamin D	-----	14.20	7-34.1	4-144

## Results

The subjects' age ranged from 53 to 80 years, with a mean age of 63.41  $\pm$  4.64 years. One hundred five subjects (35%) were overweight and 101 (33.6%) were obese. The mean BMI was 28.08  $\pm$  5.08 kg/m<sup>2</sup>. Other characteristics of study subjects are shown in table 1. Median and interquartile range of vitamin D were 14.20 ng/ml and 7-37.2 ng/ml, respectively. The prevalence of 25(OH) D < 10 ng/ml and 10 to 20 ng/ml were 38.3% and 22.6%, respectively. Therefore, 183 (61%) of subjects had 25(OH) D less than 20 ng/ml (median serum vitamin D, 7.5ng/ml). Furthermore, 25(OH) D  $\geq$  20 ng/ml was seen only in 117 (39%) of the women in our study.

Using some skin protection agents was reported by less than (1.6%) of the subjects. The study subjects did not take calcium or vitamin D supplements for a month prior to the study.

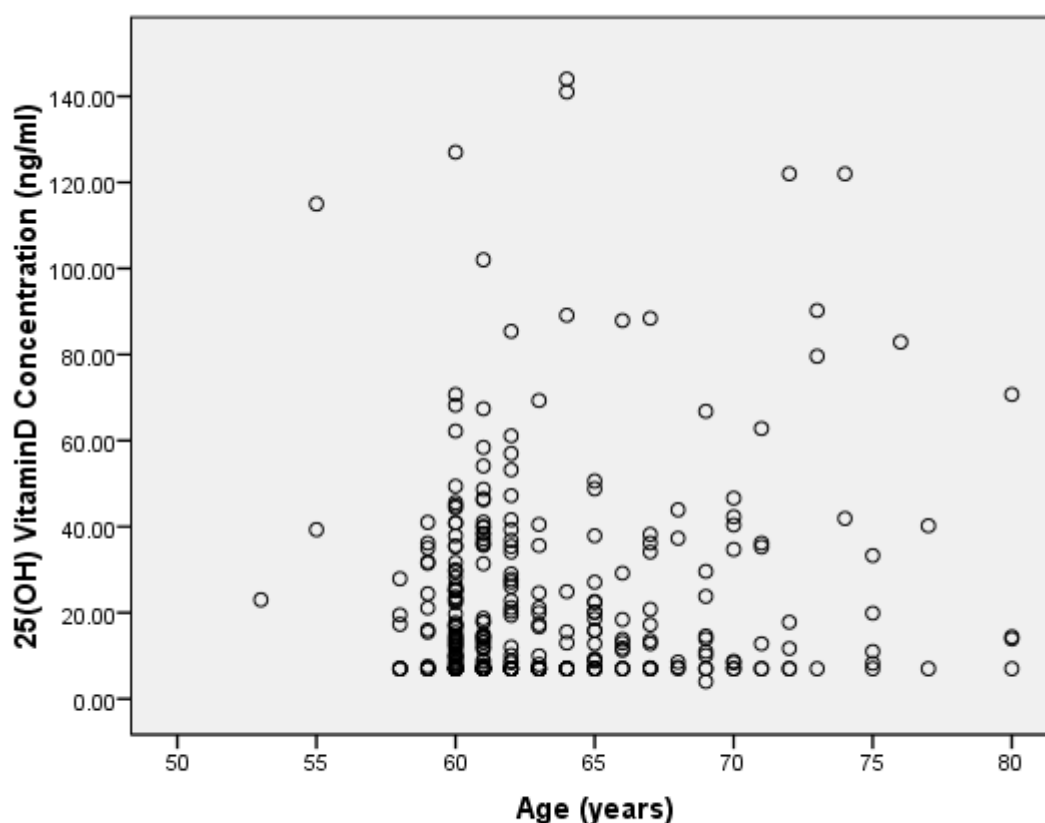
Out of 300 participants of this study, 231 (77%) were taking one glass (250 cc) of milk or yogurt in daily dietary program (median of 25(OH) D, 13.4ng/ml) and 69 (23%) of them took no milk or yogurt (median of 25(OH) D, 16ng/ml), but the difference between median 25(OH) D in these two groups was not statistically significant ( $p = 0.58$ ).

The median of 25-hydroxyvitamin D concentration for each age group is shown in table 2 and scatter plots of serum 25(OH) D levels versus age is shown in figure 1. Results showed that the difference between median 25(OH) D in various age groups was not statistically significant ( $p = 0.425$ ).

The relation of delivery and BMI with serum 25(OH) D levels was not statistically significant [( $r_s = 0.07$ ,  $n = 254$ ,  $p = 0.12$ ) and ( $r_s = 0.02$ ,  $n = 296$ ,  $p = 0.35$ ), respectively].

**Table 2.** Distribution of 25(OH) vit D values by age in a sample (300) of postmenopausal women.

Age Group	n	25(OH)vitD Median (ng/ml)	Interquartile 25-75	Range
50-60	94	15.45	7.48-28.52	7-127
60-70	178	13.35	7-34.87	4-144
70-80	28	16.10	7-57.57	7-122



**Figure 1.** Scatterplots of serum 25(OH) vitamin D concentrations versus age.

## Discussion

This population-based study on healthy postmenopausal women residing in an urban area of the north-west Iran showed that the prevalence of vitamin D inadequacy in the study population was very high. Based on the results, 61% of subjects had vitamin D levels lower than 20ng/ml. Our result was in agreement with other reports of Europe, Asia and Middle East countries.<sup>18,19,26-33</sup>

In another cross-sectional population-based study on 245 healthy menopausal women (mean age =  $57.7 \pm 7.7$  and duration of menopause =  $9.4 \pm 6.8$  years) living in Tehran, 42.9% of subjects had 25(OH) vitamin D below 20 ng/ml.<sup>24</sup>

The difference between prevalence of vitamin D inadequacy in these two studies may be related to higher age range and longer duration of menopause in our sample and also exposure to sunlight in different geographical areas. Tabriz is situated in the north-west of

Iran, at an elevation of about 1,400 meters above sea level and its geographical coordinates are  $38^{\circ} 4' 48''$  North. Tehran is located in  $35^{\circ} 44'N$  and has a mean sun exposure of 8 hours per day. But in Tabriz, this time decreases to 4 hours in winter. Furthermore, low consumption of vitamin D enriched foods, such as sea products in East Azerbaijan could explain our results.

It is generally believed that 25(OH) vitamin D levels decrease with age due to decreased capacity for vitamin D production in the skin.<sup>34,35</sup>

In a study in Belgium, a significant inverse correlation was found between age and serum 25 hydroxyvitamin D levels in elderly women.<sup>36</sup> In contrast, our study did not found such association and this result was compatible with the findings of other studies.<sup>24,37,38</sup>

Moreover, Hashemipour et al reported that serum 25(OH) D concentrations in young and middle aged women were significantly lower

than older ones.<sup>20</sup> In another study in Tabriz, Ostad Rahimi et al showed that vitamin D status improves with the age increase.<sup>22</sup> The Holvik et al study on five immigrant groups living in Oslo showed that serum level of vitamin D tended to increase with age, but this was only significant for those born in Turkey.<sup>39</sup> Looker reported that vitamin D level increases until the age of 65 years and then declines.<sup>40</sup> Therefore, it seems that the relationship between vitamin D and age is relatively complicated, and is influenced by lifestyle factors. There are several limitations to our study. First, our study was performed during winter months, when serum 25(OH) D concentrations are typically near the lowest levels of the year. Very little vitamin D is made in the skin after November at latitudes above 35° north.<sup>41</sup> Even with adequate sun exposure during the

summer and fall, subjects are at risk of low serum 25(OH)D concentrations because of the relatively short half life of 25(OH)D (~2 wk). Second, our study population was confined to urban north-west women only; thus, we could not fully assess the prevalence of vitamin D inadequacy in the area.

### Conclusion

Our findings emphasize the importance of increasing vitamin D intake in postmenopausal women.

### Acknowledgment

This study was supported by a grant from Tabriz University of Medical sciences. The authors thank the research deputy of Tabriz University of Medical Sciences for providing financial support.

### Conflict of Interest

Authors have no conflict of interests.

### Authors' Contribution

MN carried out the design of the study and coordinated the research, participated in most of the experiments and prepared the manuscript. AB provided assistance in the design of the study. AA provided assistance for preparing the manuscript and participated in sampling. NA, FN and MM participated in sampling.

All authors have read and approved the content of the manuscript.

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