Vitamin D Deficiency among Pregnant Women and Their Newborns in Isfahan, Iran

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Key words
- vitamin D deficiency
- sun light
- mothers
- newborns
- Iran

Abstract

Background and aims: Vitamin D deficiency is one of the major health problems and unexpectedly has a high prevalence in sunny countries (e.g. Middle East). In this study we determined the prevalence of vitamin D deficiency in pregnant women and their newborns in Isfahan, a sunny city in Iran.
Methods: In a cross-sectional study, 88 newborns born in Beheshty hospital, affiliated to Isfahan University of Medical Sciences (August–September, 2005) and their mothers were studied. Their data were collected by questionnaires and blood sampling was done to measure serum alkaline phosphatase (ALP), calcium, phosphorus, 25(OH) vitamin D and parathormone (PTH). Vitamin D deficiency defined as levels of 25(OH) D < 20 and < 12.5 ng/ml for mothers and newborns, respectively and local cut-offs defined as levels in which mean serum PTH started to increase.
Results: The prevalence of vitamin D deficiency according to 25(OH) D < 20ng/ml in mothers and < 12.5 ng/ml in newborns was 5.7% and 4.5%, respectively. According to local cut-offs (35 ng/ml for mothers and 26 ng/ml for newborns) 26.1% of mothers and 53.4% of newborns were vitamin D deficient.
Conclusion: According to local definition, vitamin D deficiency is a health problem in pregnant women and their newborns in this sunny city.

received 29.08.2007
first decision 25.10.2007
accepted 20.12.2007

Bibliography
DOI 10.1055/s-2008-1042403
Published online: March 25, 2008
Exp Clin Endocrinol Diabetes 2008; 116: 352–356

ISSN 0947-7349

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Introduction

Reappearance of vitamin D deficiency rickets in many countries has made vitamin D deficiency as one of the major health problems in children [1–4]. Vitamin D is a fat-soluble vitamin, supplied from food and produced in the skin by sunlight exposure [5].
Although vitamin D deficiency is unexpected in sunny regions, it is reported to be highly prevalent in such countries, e.g. India, Pakistan, Kuwait and Iran. This paradox can be explained by many factors including type of clothing, skin pigmentation and inadequate dietary intake of vitamin D [1,6–8].
In some countries where dairy products are not supplemented and have insufficient sunshine exposure, vitamin D supplementation in the third trimester of pregnancy has been recommended [9–10].
Previous studies in Iran have shown high prevalence of vitamin D deficiency among different age groups such as high-school students of Isfahan (lat 32° 39′ N), young females of Urmia (lat 37° 32′ N) and general population of Tehran (lat 36° 21′ N) [8, 11–14].
A study on pregnant women in 2002–2003 in Tehran has found that 60% of them in the first trimester, 48% in the second and 47% in the third trimester had either severe(25(OH)D level < 10 ng/ml) or moderate(25(OH)D level < 20 ng/ml) vitamn D deficiency and another study in 1997 in Tehran found a prevalence of 80% for vitamin D deficiency(25(OH)D level < 10 ng/ml) in women at delivery time [15–16].
Considering the lack of data about vitamin D deficiency in Isfahani pregnant women and their newborns, we conducted this study to investigate the status of vitamin D in pregnant women and their newborns at labor time in the largest obstetric center in Isfahan. The other goal was to study the correlation between vitamin D deficiency and some serum markers as well as social and demographic factors.

Methods

Study population

This cross-sectional study was conducted on 88 newborns and their mothers who delivered between August and September 2005 at Beheshty hospital, the main center for obstetric and gynecology department of Isfahan University of Medical Sciences. The hospital serves the urban and rural population. Participants were selected by convenient method. Informed written consent was obtained from mothers. Women with a gestational age of less than 37 weeks, history of any disease including renal, bone and gastrointestinal disorders and those under anticonvulsant medications were excluded. All women were healthy, Iranian, between 18 and 44 years of age and their body mass index (BMI) was 22.7(3.5) kg/m^2.

Data collection

For each mother, 3 questionnaires were completed by pediatric residents. The first one was used to obtain demographic information and data to assess the socioeconomic state of subjects. The second one was a validated food-frequency questionnaire based on native foods to assess the dietary intake of vitamin D, and the last, was filled to estimate the exposure of participants to ultraviolet light during pregnancy. Face validity of questionnaires was approved by a pilot study among 20 individuals.

At labor time 10 ml of cord blood and 10 ml of maternal blood were collected. The blood samples were centrifuged and sera were stored at −20° C. Alkaline phosphatase (ALP), calcium, phosphorus, 25(OH)D and parathormone (PTH) were measured in the laboratory of Isfahan Endocrine and Metabolism Research Center that is under quality control of the National Reference Laboratory (WHO-Collaborating center) in Tehran.

Labratory measurements

Serum 25OHD level was assayed by radioimmunoassay (RIA) using Biosource 25OH_vit.D3-Ria-CT kit (Biosource Europe SA, Belgium).

Serum PTH was measured using RIA kit(CIS Biointernational, France)with a normal range 8–76 pg/ml. Intra- and Inter- assay coefficients of variation(CV) for 25(OH)D were 3.3 and 5.2%, respectively and 7.5% and 6.8% for PTH, respectively. Serum calcium and phosphorus were analyzed using Pars Azmoon kits (Pars Azmoon co, Iran) by cresolphthalein complexion and UV methods, respectively (normal ranges of calcium and phosphorus was 8.6–10.3 mg/dl and 2.5–5 mg/dl, respectively). ALP was measured by electrophotometry (normal range: 70–300 IU/L).

We defined vitamin D deficiency as levels of 25(OH)D < 20 ng/ml for mothers [8] and < 12.5 ng/ml for newborns [10].

There is no universal definition for vitamin D deficiency and it seems that it is not same in different populations [17]. Most scientists have defined vitamin D deficiency as levels of 25(OH)D < 20 ng/ml, while studies that have examined PTH levels have suggested that the inflection point is closer to 30 ng/ml [8, 17]. So in addition to the international definitions [8, 10] we determined a cut-off point for vitamin D deficiency in our population (mothers and newborns, separately). We analyzed the data to determine cut-off point of serum 25(OH)D in which the mean serum PTH starts to increase. We plotted serum 25(OH)D levels of less than 19,21,23, …, 57 ng/ml against mean PTH concentrations either mothers (**Fig. 1**) and less than 12,14,16, …, 44 ng/ml for their newborns (**Fig. 2**). Then we defined 25(OH)D levels of 33 ng/ml and 26 ng/ml for mothers and newborns, respectively. At serum 25(OH)D levels above these points mean PTH appeared to reach a plateau.

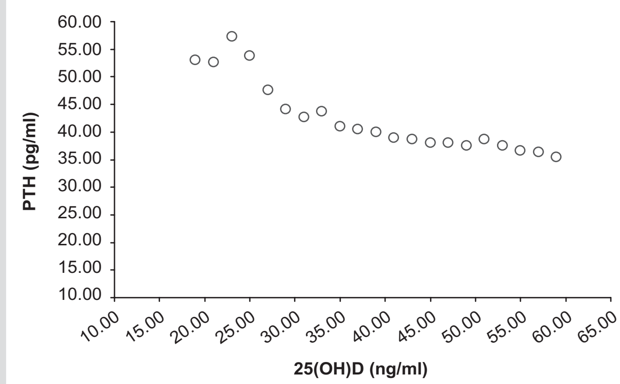

Fig. 1 Mean serum PTH concentrations at different cut pints of mothers' 25(OH)D.

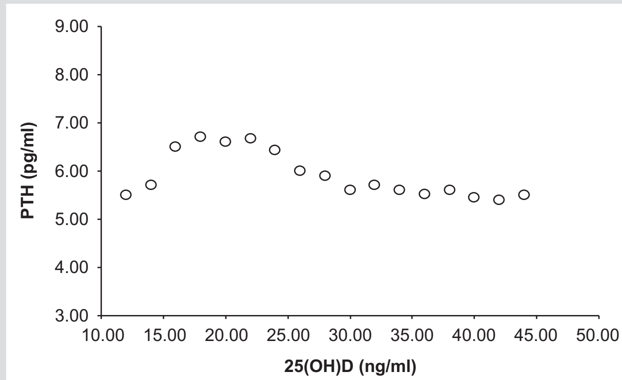

Fig. 2 Mean serum PTH concentrations at different cut pints of newborns' 25(OH)D.

Statistical analysis

Normality of data distribution was assessed with Kolmogrov-Smirnov. Descriptive data were expressed as mean ± 1 SD. Correlations were tested by computing Pearson's correlation coefficients. ANOVA and sample t test were used for comparison of mean values, and chi-square was used for comparison of frequencies. Differences were considered statistically significant at $p < 0.05$. Statistical analysis was conducted using SPSS software version 11.5 (SPSS.Chicago, Inc).

Results

The mean age of mothers was 25.5 ± 5.3 years (range 18–44 years). The mean serum 25(OH)D of mothers and newborns was 52.2 ± 35.64 ng/ml and 27.42 ± 11.4 ng/ml, respectively. The prevalence of vitamin D deficiency (the serum 25(OH)D level < 20ng/ml for mothers and < 12.5 ng/ml for newborns) was 5.7% (n = 5) in mothers and 4.5%(n = 4) in newborns. In addition, as it is mentioned earlier some studies have suggested a value of 30 ng/dl for 25(OH)D in all ages [8, 17] and in our study 70.5% of mothers and 21.6% of newborns had a serum 25(OH)D level of less than 30 ng/ml. A positive correlation approaching statistical significant was observed between levels of serum 25(OH)D in mothers and newborns($r = 0.79$, $p < 0.001$). The mean serum 25(OH)D

Table 1 Social and demographic factors related to mean of 25(OH)D of mothers and newborns in Isfahan

Factor	Patients n(%)	Serum 25(OH)D of mother ng/ml	p- Value	Serum 25(OH)D of newborn ng/ml	p- Value
maternal age					
≤25 yrs	47 (53)	52.3 ± 24.4	0.18	28.8 ± 10.5	0.97
>25 yrs	41 (47)	52 ± 45.5		25.7 ± 12.2	
maternal parity					
≤3	84 (95.5)	53.3 ± 36	0.35	27.7 ± 11.5	0.16
≥4	4 (4.5)	27.6 ± 11.6		20.2 ± 4.1	
living area					
urban area	51 (57)	47.89 ± 24.6	0.2	25.59 ± 9.8	0.07
rural area	37 (43)	58.2 ± 46.6		29.94 ± 12.9	
maternal education					
<5 yrs	33 (37.5)	40.05 ± 10.1	0.4	26.88 ± 8.4	0.47
6–11 yrs	33 (37.5)	61.09 ± 50.6		29.56 ± 13.1	
12 yrs	16 (18)	42.78 ± 20.2		24.18 ± 11.5	
>12 yrs	6 (6)	49.2 ± 22.3		27.21 ± 15.4	
socioeconomic class					
upper	8 (9)	43.3 ± 15.8	0.54	25.61 ± 6.6	0.8
intermediate	64 (73)	54.8 ± 14		27.86 ± 12.4	
lower	16 (18)	46.35 ± 19.4		26.55 ± 8.9	
residence					
apartment	36 (24)	39 ± 19.2	0.03	23.8 ± 9.7	0.01
house	52(76)	61.3 ± 41.3		29.9 ± 11.8	
sunscreen use					
yes	13(14.8)	38.5 ± 20.4	0.32	23.43 ± 10.3	0.99
no	75(85.2)	54.6 ± 10.3		28.11 ± 10	

Table 2 Biochemical data of mothers and newborns in Isfahan

Groups	Calcium (mg/dl)	Phosphorus (mg/dl)	Alkaline phosphatase (IU/l)	Parathormone (pg/ml)	Serum 25(OH)D (ng/ml)
newborns	8.95(6.6–10.4)	4.65(4.1–5.7)	473(40.1–2224)	2.7(0.8–43.3)	27.42 ± 11.4
mothers	9.1(6.4–18.6)	4.4(3.2–5.1)	499(149–1218)	29.4(0.8–153.5)	52.2 ± 35.64

level of mothers was not significantly related to maternal age or parity (**Table 1**), but it was significantly higher in those who lived in houses with yard ($p = 0.03$). Furthermore, the mean serum 25(OH)D level was higher in newborns of these mothers ($p = 0.01$), but was not related to other factors (**Table 1**).

The mean daily intake of dietary vitamin D of mothers was 27.95 ± 11.26 IU/day and was not correlated to the level of 25(OH)D in newborns($p = 0.2$, $r = 0.13$).

The biochemical data (level of 25(OH)D, calcium, phosphorus, alkaline phosphatase and parathormone) of mothers and newborns are shown in **Table 2**.

We assessed the correlation between serum calcium and serum parathormone either in mothers ($r = 0.11$, $p = 0.28$) or in their newborns($r = -0.34$, $p < 0.001$) which showed no significant correlation in mothers and a negative correlation in neonates. There was no significant relationship between vitamin D deficiency of mothers and level of calcium ($p = 0.9$), phosphorus (($p = 0.2$) or parathormone ($p = 0.1$) in them but the mean serum alkaline phosphatase of the mothers with vitamin D deficiency was significantly higher than that of other group ($p = 0.006$). There was no relationship between vitamin D deficiency of mothers and level of calcium ($p = 0.9$), phosphorus ($p = 0.1$), parathormone ($p = 0.7$) or alkaline phosphatase ($p = 0.1$) of newborns.

Serum 25(OH)D of mothers showed a correlation with the number of days per week which they had sunlight exposure (5.53 ± 1.9 days/week) ($p = 0.042$, $r = 0.217$).

Having considered the local cut-off points 26.1 % of mothers and 53.4 % of newborns had vitamin D deficiency.

Discussion

In our study the prevalence of vitamin D deficiency in mothers and newborns (according to levels of 25(OH)D <20 ng/ml for mothers and <12.5 ng/ml for newborns) was 5.7 and 4.5 %, respectively, while, according to defined local cut-off points, its prevalence was as high as 26.1 and 53.4 %. To our knowledge this is the first data on prevalence of vitamin D deficiency in pregnant women and newborns in central part of Iran.

In some of countries in the Middle East vitamin D deficiency rickets causes significant morbidity in children [19,20] and Vitamin D deficiency in nursing mothers has been reported from tropical countries like Saudi Arabia, Pakistan, Kuwait and India [1,3,6,7]. The preference of the women to avoid direct sunlight, cultural and social obligations for the women to cover their skin when going outside their homes decrease the exposure [7] and there is consistency in published data that the vitamin D deficiency in veiled women is the result of a combination of limitation in sunlight exposure and a low intake of vitamin D [21]. By comparing veiled and unveiled Turkish women, Guzel R et al. have found that veiled women have low 25(OH)D status [22].

Having considered other studies in our region (Middle East) and because of the dress style of women in this region and also regarding the two studies conducted in Tehran, we expected to find a higher rate of vitamin D deficiency. Also, comparing with a study in The US the prevalence of vitamin D deficiency was lower in mothers and newborns of our study. This may be because of the fact that 65 % of the subjects in the US study were black, and melanin inhibited vitamin D production in the skin [18]. In the study by Ainy et al. on 48 tehrani pregnant women 47 % had vitamin D deficiency in the third trimester, which is higher than this prevalence in Isfahan and also is another study by Bassir et al. in Tehran 80 % of mothers were vitamin D deficient (vitamin D < 10 ng/ml). It is possible that these differences are partly because of the more air pollution that exists in Tehran which is known as an effective factor in preventing enough UV exposure [12]. Another reason can be the considerable group of our subjects who lived in rural areas, however the difference was not significant.

As in some previous studies, in our study cord blood 25(OH)D level was correlated with maternal values [12–16, 18–26]; also, mean level of 25(OH)D in newborns was significantly lower than that of their mothers, averaging 52 % of mothers' level, which is in agreement with other researches showed that most of neonates had lower serum 25(OH)D than their mothers[10, 23].

In our study, women who lived in apartments had a lower level of 25(OH)D than those who lived in houses probably because of inadequate sunlight exposure. This result is consistent with the study in Pakistan by Atiq et al. [7]; furthermore in our study newborns of these mothers had a significant lower level of vitamin D. Also, as it was expected there was a significant correlation between levels of 25(OH) D in mothers and the number of days/week which they were exposed to sunlight. But considering other factors which seem to be effective in sunlight exposure including use of sunscreen, no significant relationship was found in contrast to previous studies. It maybe due to the rare usage of sunscreen by mothers of our study (**Table 1**) [8]. And living area (rural or urban area) had no relationship with their 25(OH)D level too, the same result as the result of Indian study by Sachan et al. [6].

Our results showed a lower dietary vitamin D intake in Isfahani women comparing with other studies in Japan, Pakistan, England [7, 27] and there was no significant relationship between the daily dietary vitamin D intake of mothers and the level of vitamin D in their newborns. The facts that foods in Iran are not fortified with vitamin D and the biases in estimating daily intake are probable causes of this result.

We also assessed the relationship between level of 25(OH)D and many other factors such as maternal age, parity, education and socioeconomic class. The same as the study in Pakistan by Atiq et al. [7], maternal vitamin D was not related to their age and parity; but in contrast to our results they had found a significantly higher level in uneducated mothers and those of low socioeconomic class. In another study in Norway on five immigrant groups in Oslo, education length was positively associated with vitamin D level in women born in Turkey and Iran, and it was suspected that education level influences sun exposure such as clothing habits [28].

In present study mothers' vitamin D status was not related to their and their newborn calcium and phosphorus concentration which is in constant with the study by Hashemipour et al. [29]. Having considered these results, it seems that usual biochemical parameters (Ca, P and ALP) do not have sufficient sensitivity to detect vitamin D deficiency especially its mild form.

One of the limitations of this study was that we evaluated the prevalence in summer time while there is a general agreement that 25(OH)D level varies in different months [14, 30]. Another limitation is that number of subjects in some groups was too small, so no significant relationship was expected and interpretation about these factors should be made with caution.

Conclusions

The US national Academy of Sciences suggests providing of 400 IU as the dietary reference intake of vitamin D in pregnancy [31]; and most of the authors recommend pregnant mothers should receive 2000 iu of 25(OH)D daily during the third trimester [32]. Vitamin D supplementation is not a part of current prenatal care program in Iran. On the basis of our results, we recommend such supplementations in Iranian women especially those who live in apartments and do not have enough exposure to sunlight. Also, health education regarding sunshine exposure and modification of dietary habits is highly recommended.

Further studies are needed to evaluate rickets in Iranian newborns and to clarify the significance of different etiologic factors.

Acknowledgements

This project was funded by the Bureau of Research, Isfahan University of Medical Sciences. We thank the staff of Endocrine and Metabolism Research Center of Isfahan University of Medical Sciences for their support.

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