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ORIGINAL ARTICLE

Investigation of multiple factors which may contribute to vitamin D levels of bedridden pregnant women and their preterm neonates

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Abstract

Objective: 25-Hydroxyvitamin D (25-OH-D) is the marker, which indicates vitamin D levels. The aim of this study was to investigate the possible factors, which contribute to serum 25-OH-D levels in bedridden mothers and their preterm neonates.
Methods: Twenty-six preterm neonates born during the period of 24–33 weeks of gestational age and 20 mothers (who experienced pregnancy complications) were recruited to the study.
Results: Five major results were obtained. (i) The 25-OH-D serum levels for preterm neonates and their mothers were found to possess strong correlation (ii) and both differed significantly in comparison with the optimal levels. (iii) An increase of mothers' 25-OH-D serum levels was associated with an increased possibility that the neonates would be measured to have normal 25-OH-D levels. (iv) Sex was not a key factor to neonates' 25-OH-D levels. (v) No correlation was found between mothers' 25-OH-D levels and their vitamin D_3 supplement (400 IU/d during pregnancy).
Conclusions: Due to insufficient exposure to sunlight and a diet not enriched with vitamin D, bedridden pregnant women suffer from vitamin D deficiency and pregnancy complications lead often to birth of preterm neonates with the same deficiency. Mothers should increase the total amount of vitamin D intake (food and supplement).

Keywords

25-Hydroxyvitamin D, neonates: 24–33 weeks of gestational age, pregnancy complications, supplement of vitamin D_3, vitamin D deficiency

History

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Introduction

The term vitamin D refers mainly to two fat-soluble molecules, D_2 (ergocalciferol) and D_3 (cholecalciferol). Human cholecalciferol is synthesized in the skin in the presence of UV-B from the precursor compound 7-dehydrocholesterol. Ergocalciferol is the product of the effect of ultraviolet light on ergosterol in plants [1]. A study in 2006 reached to the conclusion that vitamin D_3 is more effective in comparison with vitamin D_2 [2]. 25-Hydroxyvitamin D (25-OH-D) is produced into the liver and its serum concentration is considered as the appropriate marker of vitamin D [1,3].

The benefits of vitamin D are particularly significant for human. The major benefit is that vitamin D increases calcium absorption [4,5]. Other important functions include regulation of the phosphorous absorption and parathyroid hormone synthesis, reduced risk of diabetes mellitus, colon cancer, multiple sclerosis, normal development of bones and teeth, reduction of the chance of developing heart disease as well as flu [4–9].

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Only few foods contain vitamin D. Specific fish are considered to be the sources with the highest levels of vitamin D: (i) salmon, (ii) mackerel, (iii) tuna, (iv) sardines, (v) fish liver oils, and (vi) swordfish [10,11]. Beef liver, cheese, egg yolks, and some mushrooms contain also vitamin D_2 or D_3 [10]. The fact that in many countries there is no adequate sunshine exposure for humans in order to synthesize the appropriate amount of vitamin D, the policy for prevention of vitamin D deficiency led to commercial D_3-fortified food [12–14].

Different dietary guidelines for vitamin D have been suggested for all age groups as well as for special categories (e.g. women during lactation). Recommended daily intake of vitamin D varies from country to country, because each population is characterized by its distinct mean sunshine exposure. Moreover, other factors interfere to the vitamin D level. Melanin's concentration (people with dark skin have reduced ability to synthesize vitamin D), air pollution, winter, amount of cloud cover, medication, malabsorption, national or regional clothing, aging, and obesity are the main reasons related to low levels of vitamin D [1,15–18]. In addition, nowadays people spend more time indoors [e.g. people (a) work indoors, (b) surf the internet a lot of hours, (c) watch TV].

The institute of Medicine published in 2011 guidelines about vitamin D dietary reference intake [19].

The recommended dietary allowance of vitamin D was 600 IU (International Units) for male and female (also for women during pregnancy and lactation) 1–70 years old and 800 IU for older adults. These quantities are supposed to provide serum 25-OH-D concentrations of 20 ng/ml (50 nmol/L). In the same publication, the adequate intake of vitamin D for infants 0–12 months was 400 IU [19]. Moreover, a study in 2011 (for full-term infants) indicated an important interrelationship between maternal and infant cord blood 25-OH-D concentrations [20]. Usually, a newborn infant's 25-OH-D levels are 60% of maternal levels [21]. Additionally a study for preterm infants correlated their very low 25-OH-D concentrations with relevant low maternal vitamin D levels [22]. European recommendation for enteral supply of vitamin D refers to a daily intake of 800–1000 IU [23]. Steven et al. proposed in 2013 for breastfed preterm infants a daily intake of 400 IU. Furthermore, Polish guidelines recommend for women a daily intake of 800–1000 IU from the second trimester of pregnancy in order to obviate vitamin D deficiency [24].

The aim of this study was to investigate the possible factors, which contribute to serum 25-OH-D levels in bedridden mothers and their preterm neonates, as well as to evaluate the correlation between these two levels.

Methods

Design of the study

There were four inclusion criteria: (i) preterm neonates should be aged from 24 to 33 weeks, (ii) mothers should be motionless within their home or bedridden at least for 2 months (according to doctor's instructions), (iii) mothers should not suffer from inflammatory bowel disease [IBD – e.g. Crohn's disease and ulcerative colitis], nephropathy, liver disease, or parathyroid diseases, (iv) mothers should not take medications that could affect vitamin D levels. The study was conducted after obtaining hospital's Scientific and Ethical Committee approval (Iaso Hospital). A schematic overview of the design of the study is presented in Figure 1.

Patients and data collection

Twenty-six preterm neonates (12 males and 14 females) born during the period of 24–33 weeks of gestational age and 20 mothers of whom four had multiple gestation pregnancy (one case of triplet and four cases of twins) were recruited to this study. All mothers experienced pregnancy complications (mainly bleeding and placental abruption) and as a consequence, they were motionless within their home or bedridden (at least for 2 months). Four mothers reported that they were smokers (smoking of cigarettes). All preterm neonates were born in 2014 (April–July) at ''*IASO*'' maternity hospital.

Anthropometric data concerning gestational age, birth weight, and sex were collected from the patient's medical records. An accurate electronic scale was used in order to measure the weight of neonates. Mothers' demographics concerning ethnicity, place of residence and economic status were collected through personal interviews. All mothers were Greek. They lived in an urban area (province: Attiki) and they stated that they belong to middle class.

Parenteral nutrition and nasogastric milk feeding

The preterm neonates received parenteral nutrition (PN) on average for 19 d and they were fed exclusively with PN the first 3 d of life. Then, they continued PN and simultaneously were fed with milk [18, exclusively with breast milk, five, breast milk and a substitute (Almiron Premature, Neocate, Alfare, Almiron Pepti Alfare, and Frisolac Premature) and only three received substitute]. Nasogastric milk feeding was applied in order to administer the substitute.

Personal interviews

A nutritionist and dietitian gave instructions to mothers in order to complete the questionnaires. In this study, mothers were asked to answer a Food Frequency Questionnaire (Supplementary material). The questionnaire was adapted to their dietary habits (and based on guidelines published by Greek Ministry of Health), in order to be possible for the nutritionist to make an assessment of the vitamin D levels received from food. During the interviews, portion food models were shown to mothers to assist them to estimate the quantities. The assessment of vitamin D levels was based on the United States Department of Agriculture, National Nutrient Database (USDA database). An additional questionnaire, which included factors affecting vitamin D levels, was answered by mothers (Supplementary material).

25-OH-D analysis

Measurements for 25-OH-D in the serum of neonates were performed at the first day of life and at the same day for mothers (day of childbirth). After blood sampling, 1 mL of sample was centrifuged and serum 25-OH D was measured by electrochemiluminescence immunoassay ''ELCIA'' (Roche Diagnostics GmbA, Mannheim, Germany). The analyzer used, was COBAS e 601 by Roche Diagnostics GmbA, Mannheim, Germany. Vitamin D Total assay was applied. The vitamin D kits were stored at 2 °C. Serum was collected using standard sampling tubes. Vitamin D total calibration (calset) was used for calibration. Controls were applied in order to check out the curve.

Statistical analysis

Statistical analysis was performed using the SPSS software for Windows (version 21.0; SPSS, Inc, an IBM Company, Chicago, IL). The Shapiro–Wilk test was run for all continues variables. All variables followed the normal distribution except mothers' vitamin D intake from food sources and overall (food sources and supplement). Descriptive statistics were conducted and values are shown as mean, standard deviation, minimum and maximum (Table 1). A logistic regression was performed to investigate the impact of (i) gestational age, (ii) birth weight, (iii) mother's body mass index [BMI (kg/m^2)] at the beginning of the pregnancy, (iv) weight gain during pregnancy, and (v) mothers' 25-OH-D serum levels on the possibility that neonates have normal 25-OH-D serum levels. Pearson's (r) and Spearman's (r_s) correlation were run to determine the relationship between mothers' 25-OH-D serum levels and neonates' 25-OH-D serum levels, as well as monthly vitamin D intake (total

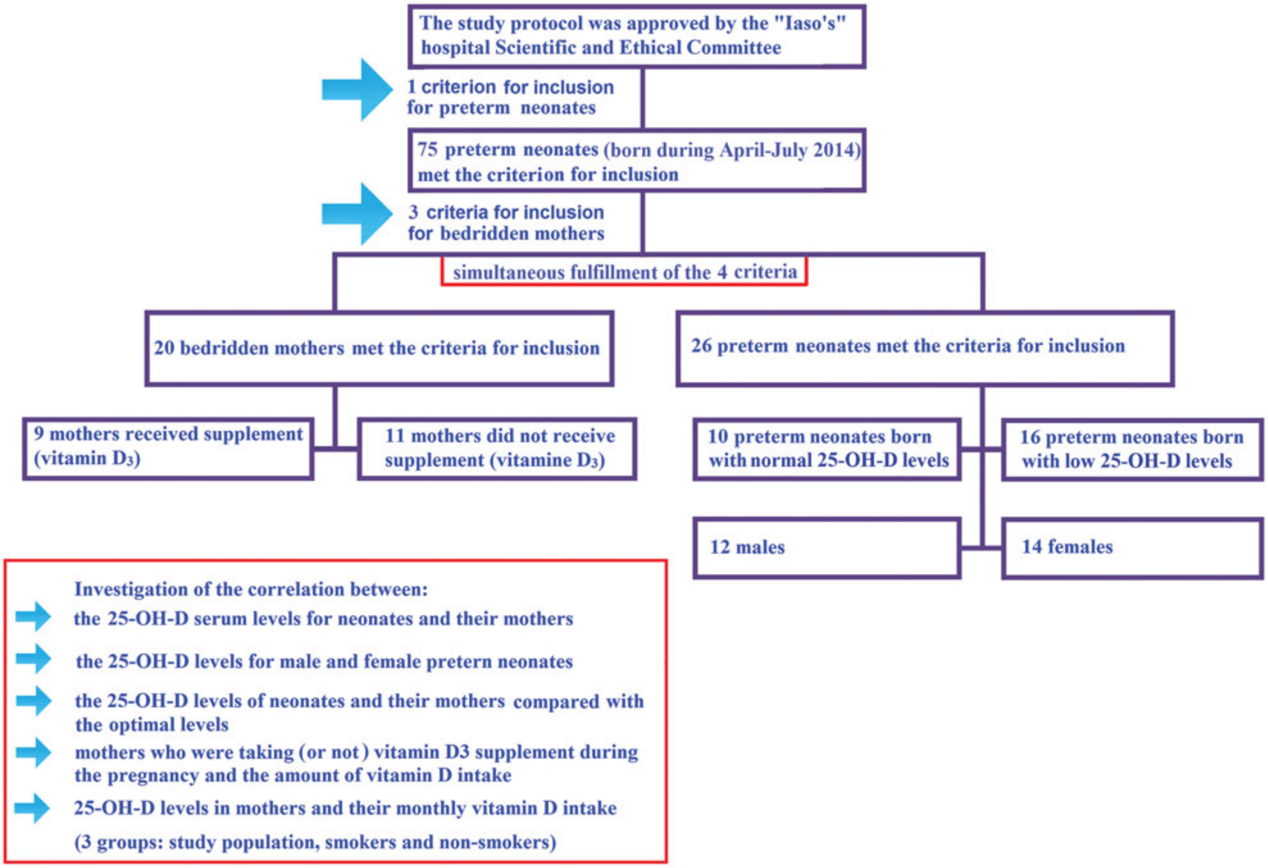

Figure 1. Schematic overview of the design of the study.

Table 1. Descriptive statistics.

	N	Minimum	Maximum	Mean	Standard deviation	Standard error of the mean
Gestation age (week)	26	24	33	29.54	1.86	0.89
Birth weight (kg)	26	0.71	2.22	1.30	0.36	0.15
Neonate's serum 25-OH-D [1st day of life] (ng/mL)	26	3.44	35.56	17.93	8.44	3.89
Parenteral nutrition (d)	26	6*	43	18.62	7.82	2.82
Mother's serum 25-OH-D (ng/mL)	20	4.09	34.69	17.44	10.75	2.26
Mother's age (years)	20	29	45	34.33	4.76	1.14
Mother's total vitamin D intake† (IU/month)	20	1228.00	13 959.00	7293.88	6083.46	1360.00
Mother's BMI (kg/m^2) at the beginning of the pregnancy	20	19	35	23.98	1.24	2.27
Normal weight BMI = 18.5–24.9 (kg/m^2) 66.7%‡						
Overweight						
BMI = 25.0–29.9 (kg/m^2) 19%						
Obese BMI ≥ 30 (kg/m^2) 14.3%						
Weight gain during pregnancy (kg)	20	-7	14	7.52	1.09	1.65
Mother's BMI (kg/m^2) in the day of gestation	20	21	40	26.70	1.02	2.51

*The preterm neonates received exclusively parenteral nutrition (PN) during the first 3 d.

†Total vitamin D intake – from supplement and from food. 9 mothers received supplement (vitamin D_3) according to doctor's and nutritionist's instructions.

‡Relative frequency.

monthly vitamin D intake – from supplement and from food) and 25-OH-D levels (for smokers and non-smokers). A comparison among 25-OH-D serum levels, BMI at the beginning of the pregnancy, and weight gain during pregnancy in mothers taking a vitamin D_3 supplementation or not, was conducted with Student's *t*–test, while the comparison of monthly total vitamin D intake in the same groups was conducted with the Mann–Whitney *U*-test. Finally, a one sample *t* test was performed in order to compare 25-OH-D serum levels in neonates and mothers with the normal ranges as they are established from the bibliography [25,26].

Results

The descriptive statistics for preterm neonates and for their mothers are presented in Table 1. The 25-OH-D serum levels for neonates and their mothers were found to possess strong

Figure 2. Correlation between 25-OH-D levels of bedridden mothers and their preterm neonates.

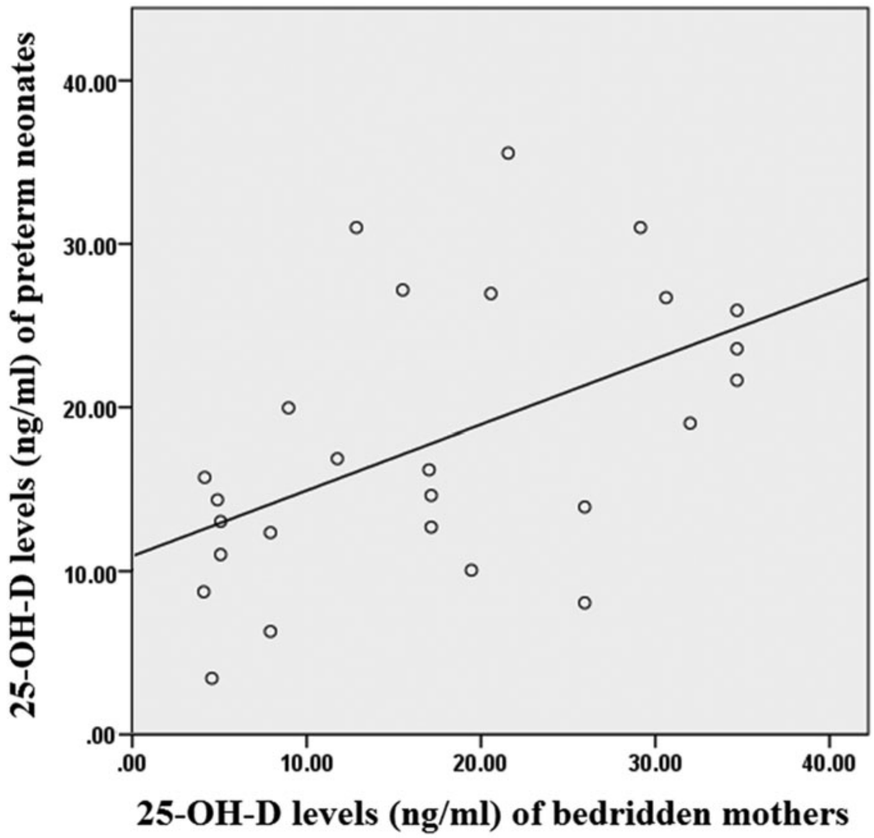

Table 2. Logistic regression to investigate the impact of gestational age, birth weight, mothers' 25-OH-D serum levels, BMI at the beginning of the pregnancy, and weight gain during pregnancy on the possibility that neonates have normal 25-OH-D serum levels.

	p Values	Odds ratio	95% confidence interval for odds ratio	
			Lower	Upper
Gestational age	0.969	0.970	0.440	2.190
Birth weight	0.276	0.087	0.001	8.614
Mothers' 25-OH-D serum levels	0.017	1.198	1.031	1.394
Mother's BMI (kg/m^2) at the beginning of the pregnancy	0.322	0.170	0.002	8.333
Weight gain during pregnancy	0.502	0.377	0.077	6.460

correlation (Pearson's correlation $-r = 0.511$, $p = 0.008$). Figure 2 demonstrates this result.

A logistic regression with the major available risk factors for vitamin D deficiency (gestational age, birth weight, mothers' 25-OH-D serum levels [27], BMI at the beginning of the pregnancy and weight gain during pregnancy), was performed and showed that increase of mothers' 25-OH-D serum levels was associated with an increased likelihood that the neonates would be measured to have normal 25-OH-D levels (Table 2). Logistic regression's dichotomous-dependent variable (neonates 25-OH-D levels) falls into two categories: (a) preterm neonates with normal 25-OH-D levels (10 neonates) and (b) preterm neonates born with low 25-OH-D levels (16 neonates).

According to Table 2, logistic regression model was statistical significant only for the impact of mothers' 25-OH-D serum levels [concerning the possibility that neonates have normal 25-OH-D serum levels ($p = 0.017$)]. Moreover, a different grouping according to sex was made (12 males and 14 females). The two groups did not differ concerning the serum 25-OH-D levels ($p = 0.447$).

One sample t test was performed in order to compare the serum 25-OH-D levels of neonates and their mothers with the optimal levels (30 ng/mL and 32 ng/mL, respectively) [25,26] and they differed significantly ($p \approx 0.000$ for both cases).

After the third day of life, the preterm neonates received PN and simultaneously were fed with milk. Overall, the total vitamin D intake for preterm neonates in hospital results by the following possible means: (i) breast milk, (ii) substitute milk (contains vitamin D_3), and (iii) PN (contains vitamin D_2). In order to ameliorate vitamin D levels, it is necessary for preterm neonates (with vitamin D deficiency) to receive substitute milk or/and appropriate individualized PN.

In addition, the sample of mothers was divided in two subgroups (Table 3) depending on whether they were taking supplement of vitamin D_3. For the first subgroup, the daily dose of vitamin D_3 was 400 IU/d during pregnancy.

Table 3. Group statistics for mothers depending on whether they were taking supplement of vitamin D_3.

	Supplement	N	Mean (IU)	Std. deviation	Std. error mean
Mothers' total vitamin D intake (IU/month)	No supplements*	11	1969.17	995.55	300.20
	Supplements†	9	13801.86	152.49	50.83
Mothers' vitamin D intake derived from food sources (IU/month)	No supplements	11	1969.17	995.55	300.20
	Supplements	9	1801.86	152.49	50.83
Mother's BMI (kg/m^2) at the beginning of the pregnancy	No supplements	11	23.31	4.48	1.24
	Supplements	9	25.09	6.41	2.26
Weight gain (kg)	No supplements	11	6.77	6.87	1.90
	Supplements	9	8.75	4.65	1.65

*This group did not take any supplements containing vitamin D_3 (or other prenatal vitamins)

†For this group, the daily dose of vitamin D_3 was 400 IU/d during pregnancy (one tablet: 500 mg Ca + 400 IU vitamin D_3).

The pharmaceutical formulations (tablets) provided were Calcioral D_3 [one tablet: 500 mg Ca + 10 μg (400 IU) vitamin D_3], Ideos [one tablet: 500 mg Ca + 400 IU vitamin D_3], or calcium Plus D_3 [500 mg Ca + 400 IU vitamin D_3]. The second subgroup did not take these supplements (or other prenatal vitamins) during pregnancy. The two subgroups differed significantly ($p \approx 0.000$) in monthly intake.

On the contrary, it was shown that there is no correlation between 25-OH-D levels in mothers and their monthly vitamin D intake [(i) for the whole sample $r_s = 0.158$, $p = 0.289$; (ii) for smokers $r_s = 0.205$, $p = 0.396$; (iii) for non-smokers $r_s = 0.019$, $p = 0.546$]. Additionally, it was not observed any significant difference for 25-OH-D levels between smokers and non-smokers (a mean value for smokers: 19.38, a mean value for non-smokers 17.15, $p = 0.697$).

Moreover, it was demonstrated that there was not any significant difference concerning vitamin D intake derived from food between the subgroup which received supplement and the subgroup which did not receive ($p = 0.625$). In addition, there was no remarkable difference in mother's BMI and weight gain between the two groups ($p = 0.462$ and $p = 0.483$ respectively).

Discussion

The study has some limitations. The number of participants (both, for mothers and preterm neonates) is small. In addition, for the first time, a questionnaire concerning vitamin D was used for bedridden pregnant women. The questionnaire's reliability and internal consistency should be verified again, in case it is applied to a greater study population. Then, some additions or/and clarifications could lead to the design of a more appropriate questionnaire. The study revealed that in Greece people rely on the fact that in their country, the sunny weather lasts for a long period (8–9 months per year and so they rely on the fact that cholecalciferol is synthesized in the skin). Furthermore, Greek (national) guidelines do not recommend to pregnant women to consume frozen fishes with high levels of vitamin D (e.g. tuna). In addition, Greek women prefer olive oil (0 IU of vitamin D) instead of margarine enriched with vitamin D and they declare also that they do not consume juices and cereals enriched with vitamin D.

In this study, the mothers experienced pregnancy complications and were motionless within their home or bedridden. Due to lack of physical activity and insufficient exposure to sunlight (exposure to the sun can lead to the synthesis of 10 000–20 000 IU/day of vitamin D from the skin [28]), these mothers may suffer from vitamin D deficiency. (In general, there is also the possibility that vitamin D deficiency may exist prior to pregnancy for various behavioral or genetic reasons and it may be one of the risk factors in the etiology of pregnancy problems). The preterm neonates were measured to have serum 25-OH-D < 30 ng/mL and their mothers < 32 ng/mL, which are the optimal levels. One sample t test indicated that serum 25-OH-D levels differed significantly compared with the ideal levels. Also, serum 25-OH-D levels of preterm neonates and that of their mothers were found to have strong correlation. Since mothers with pregnancy complications are at increased risk of giving birth to preterm neonates and additionally because of their probable vitamin D deficiency, these facts underscore that mothers may receive appropriate supplements. Mothers who had received supplement of vitamin D_3 during pregnancy differed significantly ($p = 0.002$) in monthly intake compared to those who had not received. In a recent publication [29], the debate if all women should receive vitamin D supplementation was pointed out.

Furthermore, in this study, it was not found strong correlation between 25-OH-D levels in mothers and their monthly vitamin D intake. Furthermore, increasing mothers' 25-OH-D serum levels was associated with an increased likelihood of exhibiting the neonates normal 25-OH-D levels. Taking into account that mothers' population had insufficient exposure to sunlight, it seems that mothers should increase the total amount of vitamin D, which they receive (from food and from supplement). Therefore, (i) the dose of supplement of vitamin D_3 (400 IU/Day during pregnancy) should be considered inadequate, (ii) doctors and nutritionists should collaborate in order to adjust an enriched diet with vitamin D to this special study population. A national policy concerning enrichment of foods with vitamin D would probably ameliorate the current situation.

Finally, it was observed that there was no significant difference either for maternal 25-OH-D levels between smokers and non-smokers or 25-OH-D levels between male and female newborns.

A future research suggestion is to design a similar study in Greece with a greater number of bedridden mothers and preterm neonates in order to confirm conclusions. The most challenging idea is to compare results (i) with those of other countries and (ii) between bedridden and not bedridden mothers (in order to detect quantitative differences).

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Declaration of interest

The authors declare that there are no conflicts of interest and that there was no funding or support for the project titled ‘‘Investigation of multiple factors which may contribute to vitamin D levels of bedridden pregnant women and their preterm neonates.’’

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