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Original Article

High prevalence of maternal vitamin D deficiency in preterm births in northeast China, Shenyang

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Abstract: Introduction: Maternal vitamin D deficiency has been associated with a number of fetal and neonatal health problems. Preterm birth is one of the most detrimental, and the role of maternal vitamin D deficiency in preterm births has not been universally acknowledged. There had been limited epidemiological studies of vitamin D deficiency on the Chinese population. Subjects and methods: 1103 women delivered in Shengjing Hospital, China Medical University from January 1st, 2012 to January 1st, 2013. Finally, 821 mother-newborn pairs which contained 143 mother-newborn pairs who were preterm delivery were recruited for analysis. Results: There was significant difference between spring and summer (P<0.0001) as well as spring and autumn (P<0.01). Compared to those in summer and autumn, the 25 (OH) D level was significantly lower in winter (summer vs winter P<0.0001, autumn vs winter P<0.0001). Maternal vitamin D level showed obvious variation with months and seasons, with higher level in summer months and lower level in winter months. There were significant difference between the vitamin D level of the very preterm group and the mildly preterm groups (P<0.01), as well as the very preterm group and the in-term groups (P<0.001). Prevalence of Vitamin D deficiency occurred in 63.04% of pregnant women in very preterm group, compared with 36.61% in in-term group. Conclusion: Vitamin D nutritional status of pregnant women and their newborns in Shenyang were relatively good compared to cities in similar latitudes. Vitamin D deficiency was most severe in late spring and least in summer. Severe preterm births before 31 weeks of gestation was associated with maternal vitamin D deficiency.

Keywords: Serum 25-hydroxyvitamin D, vitamin D deficiency, pregnancy, season, preterm

Introduction

Maternal vitamin D deficiency has been associated with a number of fetal and neonatal health problems, such as impaired bone development, multiple sclerosis, cancer, insulin-dependent diabetes mellitus, asthma, preeclampsia, low birth weight and gestational diabetes [1, 2]. It can be influenced by all kinds of ethnic, geographical, seasonal, dietary and physiological/pathological factors [1, 3, 4]. In northern area at latitudes above 42° north, endogenous production of vitamin D essentially ceases from November until March [5], which makes season an important influential factor for northern people.

Premature birth is defined either as the same as preterm birth or the birth of a baby before the developing organs are mature enough to allow normal postnatal survival. Premature infants are at greater risk of short and long term complications, including disabilities and impediments to growth and mental development. Among all adverse outcomes of maternal vitamin D deficiency, preterm birth is one of the most detrimental. Compared to healthy newborns, preterm infants have low plasma concentration of 25 (OH) D at birth [6], which might be in part caused by high prevalence of vitamin D deficiency in pregnancy [7]. However the role of maternal vitamin D deficiency in preterm births has not been universally acknowledged, as some studies reported no difference in the third trimester 25 (OH) D levels between mothers delivering preterm vs. normal gestational length babies [8].

Despite the worldwide attention to vitamin D deficiency recently, there had been limited epi-

Table 1. Results of regular physical examination and blood tests in mothers

	Mean ± SD	Reference range
Pre-pregnancy Bodyweight (kg)	57.8±9.86	-
Pre-pregnancy BMI	21.9±3.47	-
Bodyweight before Delivery (kg)	75.5±11.36	-
Bodyweight Increase (kg)	17.6±6.29	-
Red Blood Cell Count	4.0±1.84	3.7~5 (×10^{12}/L)
Hemaglobin (g/L)	116.4±20.66	110~150 (g/L)
Hematocrit (%)	34.3±6.05	37~47 (%)
Platelet Count	202.7±66.55	135~350 (×10^{9}/L)
Prothrombin Time (s)	10.9±0.66	10.5~13.5 (s)
Activated Partial Thromboplastin Time (s)	26.5±2.78	21~37 (s)
25 (OH) D (nmol/L)	60.9±32.00	-

Table 2. Vitamin D Status in 821 pregnant women in this study

Vitamin D status	Range	Cases	Percentage
Severe Deficiency	≤25 nmol/L	97	11.5
Mild Deficiency	25-50 nmol/L	216	25.7
Insufficency	50-75 nmol/L	270	32.1
Sufficency	≥75 nmol/L	258	30.7

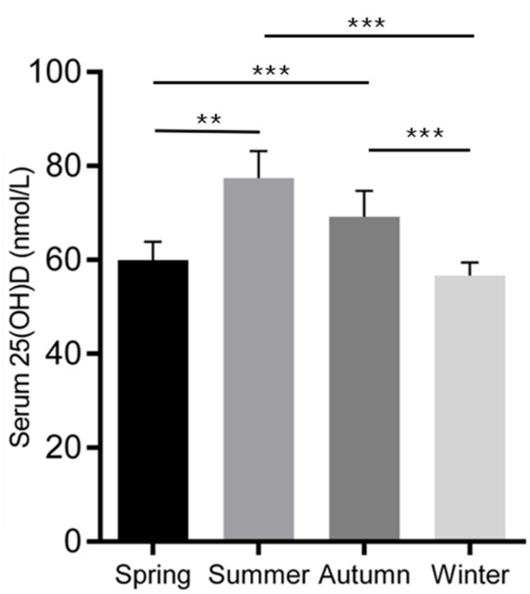

Figure 1. The relationships between serum 25 (OH) D (nmol/l) concentration and different season. **P<0.01: Spring vs Summer. ***P<0.001: Spring vs Autumn, Summer vs Winter, Autumn vs Winter.

demiological studies on the Chinese population [9, 10]. This study was executed in Shenyang, a typical northern city in China. The geographical position of Shenyang is from 41° 11' to 43° 02' N in latitude and from 122° 25' to 123° 48' E in longitude. The frost-free period is 183 days and the city receives 2,468 hours of bright sunshine annually. We aimed at exploring the maternal vitamin D level, as represented by serum 25 (OH) D, and its association with premature birth.

Material and method

All research procedures were approved by the Ethics Committee of China Medical University, China on Human Studies and conducted in accordance with the Declaration of Helsinki. All participants signed written informed consent before participation. Because newborns had compromised capacity to consent, we gave all research procedures to their next of kins, care takers or guardians, and they consented on behalf of participants whose capacity to consent was compromised.

Subjects and data collection

1103 women delivered in Shengjing Hospital, China Medical University from January 1st, 2012 to January 1st, 2013. The inclusion criteria of this study were: (1) no diagnosed major illness before and during pregnancy; (2) neither of the parents had smoking, alcohol or drug abuse before and during pregnancy; (3) no medication intake during pregnancy. The exclusion criteria were: (1) either of the parents had been diagnosed congenital deformities or illnesses; (2) history of abnormal pregnancy; (3) multiple pregnancy, stillbirth or other complications during this delivery; (4) unwillingness to join this study.

Sample collection

The participants were asked to complete a questionnaire concerning their general information, pregnancy nutrition status and behavior and vitamin D fortification (all pregnant mothers were suggested to take vitamin D_3 200 U/d and calcium 600 mg/d during the last month of pregnancy period). Regular blood tests were routinely done at the time of admittance.

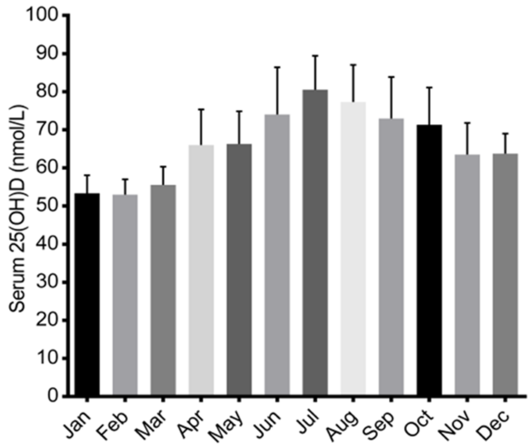

Figure 2. Distribution of vitamin D levels from January to December (Mean + 95% CI).

Table 3. Maternal 25 (OH) D level in every season (mean ± SD)

Season	Month	25 (OH) D
Spring	March-May	59.9±32.22
Summer	June-August	73.1±28.51
Autumn	September-November	66.0±32.94
winter	December-February	59.5±27.87

Blood samples were collected from all women prior to labor and all samples were separated and stored in -80°C freezer until use. Serum 25 (OH) D was measured with ELISA kits according to the manufacturer's instruction (Advantage, Nichols Institute Diagnostics, San JuanCapistrano, CA). Vitamin D deficiency was defined as serum 25 (OH) D ≤50 nmol/L, and was further categorized into severe deficiency (≤25 nmol/L) and mild deficiency (25-50 nmol/L). Serum 25 (OH) D between 50 and 75 nmol/L was defined as vitamin D insufficiency. Serum 25 (OH) D above 75 nmol/L was defined as vitamin D sufficiency.

Anthropometric data of the neonates, including body weight, body length, head and chest circumference were collected at the time of childbirth. Neonates born before 31 weeks of gestation were classified as very preterm. Those born between 32 and 37 weeks of gestation were classified as mildly preterm, and those born after 37 weeks of gestation were in-term.

Data analysis

All data were collected and analyzed with Statistical Package for Social Science (SPSS 17.0). Data were shown as mean ± standard deviation. Independent-sample *t* test was used to explore differences between groups. $P<0.05$ was regarded as statistically significant.

Results

Sample description

821 subjects were finally included in the study. The average age on delivery was 31, ranging from 17 to 45. 88.3% (725) of the mothers lived in urban area and 11.7% (96) lived in rural area. 15.3% (126 cases) received primary education (elementary and middle school), 46.2% (379 cases) received secondary education (academic or vocational high school), 30.1 (247 cases) received university education and 8.4% (69 cases) went on to post-graduate education. 50.4% (414 cases) were first-time pregnant, 30.2% (248 cases) were second-time pregnant, 11.3% (93 cases) were third-time pregnant and the other 8.1% (66 cases) had been pregnant for four or more times. 79.8% (655 cases) were having their first baby, 17.7% (145 cases) were having their second baby and 2.5% (21 cases) were having their third baby or more.

During pregnancy 63.8% of the mothers exposed to sunlight for the average of less than one hour a day, 22.7% exposed to sunlight for one to two hours a day, 9.1% exposed for two to four hours and 4.4% received sunlight for more than four hours a day. 13.5% of the mothers wore sunscreen routinely when they were to expose under sunshine; 66.5% of them used sunscreen occasionally and 20% never used sunscreen. 48.2% of the mothers did not consume sea fish or other vitamin D-rich food regularly. 24.2% of the mothers consume vitamin D-rich food once a week. 14.6% of them consume vitamin D-rich food two to three times a week. 13.0% of them consume vitamin D-rich food more than three times a week. They were unable to further quantify the food amount they took every time. 42.4% of the mothers took regular vitamin D supplement during pregnancy, ranging from 200 to 600 IU per day.

Regular physical examination and blood tests did not reveal any further abnormalities. The results of the tests were listed in **Table 1**. Serum 25 (OH) D status was further categorized according to the severity of deficiency, as shown in **Table 2**.

Table 4. Anthropometric parameters and maternal vitamin D status in different gestation groups (mean ± SD)

	Very Preterm	Mildly Preterm	In-term
Birth Bodyweight (g)	1535±328.2	2395±562.0	3358±512.2
Body length (cm)	40.5±2.81	45.8±3.65	50.3±2.95
Head Circumference (cm)	28.7±2.36	32.5±2.26	35.0±1.72
Chest Circumference (cm)	26.0±2.23	30.3±3.09	34.2±2.05
Mean Vitamin D (nmol/L)	47.1±24.90	61.6±30.57	63.7±30.87
Deficiency (percent)	63.04%	31.34%	36.61%
Insufficiency (percent)	21.74%	36.57%	31.47%
Sufficiency (percent)	15.22%	32.09%	31.92%

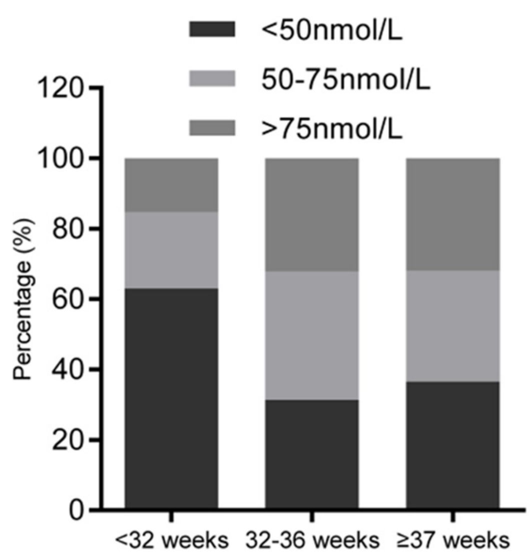

Figure 3. Prevalence of vitamin D deficiency, insufficiency and sufficiency among different pregnant age groups. Vitamin D deficiency [25 (OH) D<50 nmol/L), insufficiency [25 (OH) D 50-75 nmol/L] and sufficiency [25 (OH) D>75 nmol/L].

Comparisons of the maternal serum 25 (OH) D concentration among different seasons

The maternal serum 25 (OH) D concentrations in different season were shown in **Figures 1**, **2** and **Table 3**. Independent-sample *t* test revealed that there was significant difference between spring and summer (P<0.0001) as well as spring and autumn (P<0.01). Compared to those in summer and autumn, the 25 (OH) D level was significantly lower in winter (summer vs winter P<0.0001, autumn vs winter P<0.0001). There was no differences between spring and winter (P=0.761) as well as summer and autumn (P=0.0503). The maternal concentrations of 25 (OH) D showed obvious variation with months and seasons, with higher level in summer months and lower level in winter months.

Comparisons of the serum 25 (OH) D concentration among the very preterm group, mildly preterm group and in-term group

Of the 821 newborn babies 438 (53.3%) were boys and 383 (46.7%) were girls. 46 neonates were born very preterm (ranging from 26 to 31 weeks), 134 neonates were born mildly preterm and 641 neonates were born in-term (ranging from 37 to 40 weeks). Their anthropometric data, as well as corresponding maternal vitamin D status were shown in **Table 4**; **Figure 3**, which revealed that prevalence of Vitamin D deficiency occurred in 63.04% of pregnant women in very preterm group, compared to 36.61% in in-term group. There was significant difference between the vitamin D level of the very preterm group and the other two groups (very preterm vs mildly preterm P<0.01, very preterm vs in-term group P<0.001), but there was no significant difference between the mildly preterm and in-term group (P=0.47) (**Figure 4**). In each group the vitamin D level showed similar seasonal pattern (**Figure 5**). Although we did see less preterm births in summer, the statistical significance was limited because of the low birth rate in summer.

Discussion

This study had been conducted in Shenyang, a city in northeast China (41.8°N). Based on a large sample of 821 cases, we aimed at finding out the vitamin D status in pregnant women in north China and its influence on preterm birth. Vitamin D deficiency was prevalent in pregnant women, accounting for 37.2% of the total cases included. This result, however, was more optimistic than that reported in urban Beijing (39.9°N), where the mean serum 25 (OH) D concentration in pregnant women was 28.64 nmol/L and severe vitamin D deficiency was detected in 54.5% of the pregnant mothers [9]. Another study from Chengdu urban area (37.7°N; south-west China) found that the concentration of serum 25 (OH) D was 48.5 nmol/L in pregnant mothers [10].

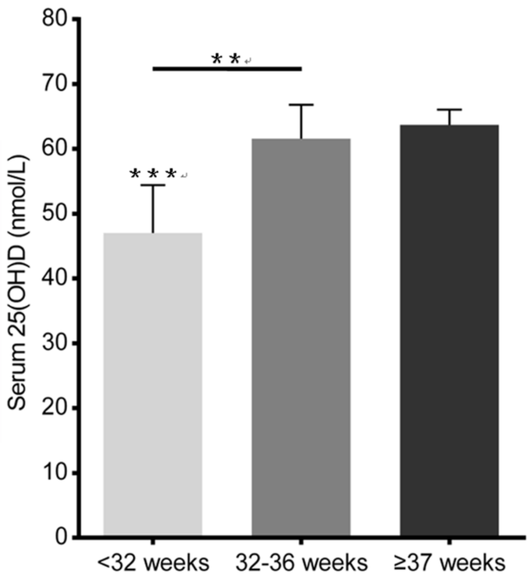

Figure 4. The relationships between serum 25 (OH) D concentration and preterm. **P<0.01: Very preterm group compared with mildly preterm group. ***P<0.001: Very preterm group compared with interm group.

Vitamin D deficiency seemed typical in high-latitude area, as reported in studies carried out in Pittsburgh, northern USA (40°N) [11] and Hague, Netherlands (52°N) [12]. Although vitamin D status in Shenyang seemed optimistic compared to the studies mentioned above, the pregnant mothers in Shenyang were still at high risk for vitamin D deficiency. First, although regular pre-labor healthcare system had been established, it covered mostly urban citizens, leaving a great part of rural mothers poorly guided. Second, relative low education level of the pregnant mothers had prevented them from being sufficiently informed or educated on nutrition. Third, vitamin D-rich food, including seafood and dairy product, were not a regular part in typical Chinese diet, and vitamin D fortified food was not as easily available as in western countries.

The major two sources of vitamin D are exposure of the skin to solar ultraviolet B radiation and dietary intake. Cutaneous synthesis of vitamin D is under considerable influence of latitude, season, and time of day [13]. In this study we found that maternal vitamin D level markedly increased from the beginning of summer, peaked in July, declined through autumn and remained low in winter and spring. This seasonal variation had also been reported in other studies. Walsh reported that concentrations of serum 25 (OH) D were the lowest at the beginning of spring and highest in mid-summer [14]. However, our study found that the lowest concentration of serum 25 (OH) D presented at the end of winter, possibly because the cold, windy and cloudy winter in Shenyang actually diminished outdoor activities and sunshine exposure.

This study also explored the relationship between maternal vitamin D level and the incidence of preterm delivery. Mothers who delivered their babies before 31 weeks of gestation had significantly lower vitamin D level than those who delivered after 31 weeks of gestation, while there was no difference between those who delivered mildly preterm babies and those who delivered in term. 63.04% of the mothers that delivered very preterm babies were vitamin D deficient, almost twice the percentage in normal deliveries (36.61%). High prevalence and increased severity of maternal vitamin D deficiency had been reported in preterm infants [15]. A study investigated the association between maternal 25 (OH) D concentrations at 24-28 weeks of gestation and preterm birth in a multicenter U.S. cohort of twin pregnancies, and results displayed that the mean serum 25 (OH) D concentration was 82.7 nmol/L; 40.3 percent women had concentrations less than 75 nmol/L. pregnant mothers with serum 25 (OH) D above 75 nmol/L were associated with a 60% reduction in the odds of preterm birth compared to those less than 75 nmol/L [16]. Considering the strong relevance of maternal and neonatal vitamin D levels, low maternal vitamin D level may lead to neonatal vitamin D deficiency [16] and add to the adverse effect of preterm birth. In view of the high prevalence of vitamin D deficiency in the mothers at delivery and its possible adverse effects on the fetus and the contribution to low vitamin D in infancy [17], it is important to supplement vitamin D during pregnancy as part of the strategies to maintain vitamin D sufficiency in mothers and their preterm infants.

Our study has some limitations. Firstly, because of the diversity in sunshine exposure, sunscreen usage, food intake, vitamin D supplement and vitamin D-rich food intake, we were unable to draw any conclusion as to their influence on maternity vitamin D level. Secondly,

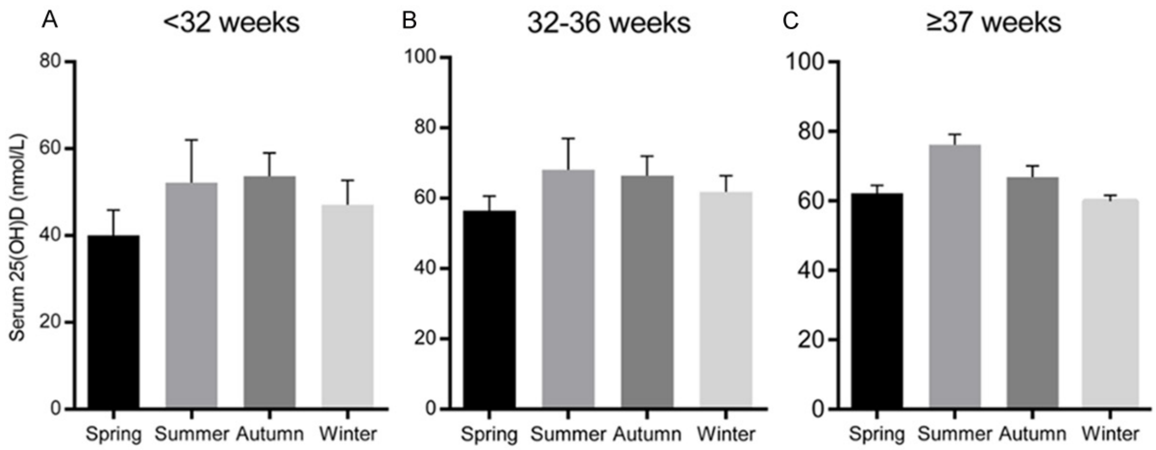

Figure 5. Seasonal distribution of vitamin D in different gestational age groups including very preterm group (A: <32 weeks), mildly preterm group (B: 32-36 weeks) and in-term group (C: ≥37 weeks).

maternal 25 (OH) D levels were not measured serially during pregnancy, therefore it was impossible to identify the crucial stage when maternal vitamin D level showed the most influence on fetal development. Further studies that adjust further potential confounders may clarify the effect of vitamin d on preterm delivery.

Conclusions

In conclusion, we found the vitamin D nutritional status of pregnant women in Shenyang was relatively good compared to cities in similar latitudes. Vitamin D deficiency was most severe in late spring and least in summer. Severe preterm births before 31 weeks of gestation were associated with maternal vitamin D deficiency.

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Disclosure of conflict of interest

None.

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