

# Serum 25 hydroxyvitamin D levels and affecting factors among preconception fertile women

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## Research article

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# Abstract

**Background** Recent study found that vitamin D before conception was considered as a potential additional determinant for achieving pregnancy and live births. The study aimed to evaluate the serum 25 hydroxyvitamin D (25(OH)D) levels and its affecting factors among preconception fertile women.

**Methods** This cross-sectional study enrolled 410 women aged 22-44 years who attended a preconception genetic counseling clinic from January 2018 to May 2019. Sociodemographic characteristics and reproductive history of women were collected, and height and weight were measured. Serum 25(OH)D concentration was assayed with chemiluminescence immunoassay. Descriptive statistics were used to examine serum 25(OH)D concentration, and socio-demographic characteristics and reproductive history among preconception women. Determinants of vitamin D deficiency and its affecting factors were assessed using  $\chi^2$  test.

**Results** Findings showed 84.4% of women had serum 25(OH)D concentration below 20 ng/mL. Women working indoors, without a history of childbirth, and young women aged 22-30 years had significantly lower 25(OH)D levels compared with those non-working individuals, giving birth to a live infant, and between 31-44 years (all  $P < 0.05$ ). The 25(OH)D levels were the lowest in winter than in spring, summer, and autumn (all  $P < 0.001$ ). Women had a RR of 3.472 (95% CI: 1.319 to 9.141) in winter to develop vitamin D deficiency. Seasonal variation in serum 25(OH)D levels was not present in non-working individuals and women aged 31-44 years.

**Conclusions** Vitamin D deficiency is common among preconception women especially young women and working women, which propose to screen serum 25(OH)D on preconception evaluation and emphasize need vitamin D supplements and get sunshine exposure.

## Introduction

Vitamin D<sub>3</sub> is a lipid-soluble hormone that has well-established classical physiological function in maintaining calcium and phosphate homeostasis and promoting bone mineralization [1]. However, vitamin D<sub>3</sub> is a pleiotropic signaling molecule, which plays numerous physiological roles ranging from regulating cellular proliferation and intracellular metabolism, and modulating innate and adaptive immune responses [2,3,4]. Indeed, current considerable amount of literature has been published on the association of vitamin D insufficiency with a number of diseases [5,6,7,8,9,10]. Especially, the inadequacy vitamin D during pregnancy is associated with adverse outcomes for mothers and the fetus or consequences on the offspring [11,12]. Recently, a study showed that increased preconception vitamin D concentrations, not in early pregnancy, are associated with reduced pregnancy loss. Vitamin D before conception was considered as a potential additional determinant for achieving pregnancy and live births [13]. Moreover, childhood vitamin D insufficiency is strongly associated with maternal vitamin D insufficiency [14,15,16,17]. Women usually neglect their vitamin D inadequacy due not to feel uncomfortable. Consequently, all the information and maternal/fetal health related issues associated with vitamin D inadequacy have highlighted the need to investigate the vitamin D level in fertile women who will become pregnant in following 3–6 months. So, the objective of study was to determine the levels of vitamin D and its association with possible determinant factors (age, [body mass index](#),

employment status, gravidity and parity, seasons) among fertile women who received preconception evaluation in Xi'an, China. We believe this information is valuable for women's health care providers to provide better counseling for women planning recent pregnancy concerning their need of vitamin D supplementation.

## **Materials And Methods**

### **Research subjects**

Fertile women who attended a preconception genetic counseling clinic in First Affiliated Hospital of Xi'an Jiaotong University in China were recruited the cross-sectional study from January 2018 till May 2019. All the participant women aged 22–44 years received health counseling regarding preparing for a healthy pregnancy and infant at 3–6 months before they became pregnant with their new infant. The participants who were pregnant, complained muscular weakness and fatigue, diagnosed metabolic bone disease, taking drugs affecting bone physiology, were not included in the study. Data on the age, nationality, profession, gravidity, and parity of the subjects were collected, followed by clinical examination (height and weight were measured, body mass index (BMI) was calculated) and laboratory results (serum 25-hydroxyvitamin D3 concentration). The study was approved by the Ethics Committee of the First Affiliated Hospital of Xi'an Jiaotong University, and written informed consent was provided from all participants.

March-May was constituted "spring", June-August "summer", September-November "autumn", and December-February "winter" according to the climate in China. According to classification criteria of BMI in China, the BMI <18.5 kg/m<sup>2</sup> is as underweight, 18.5–23.9 kg/m<sup>2</sup> normal weight, 24–27.9 kg/m<sup>2</sup> overweight, ≥28 kg/m<sup>2</sup> obese.

### **Measurement of serum vitamin D**

25-hydroxyvitamin D3 (25(OH)D) is the primary circulating form of vitamin D, which is the most abundant vitamin D metabolite and considered the best indicator of an individual's vitamin D status within the human body [18]. Therefore, serum 25(OH)D was measured to determine vitamin D status with chemiluminescence immunoassay. Blood samples from fasting women were collected at times of usual blood draws. Serum 25(OH)D levels were detected using the "Elecsys 25(OH)D" kit (cobas® 25 Hydroxyvitamin D3 03314847 190, Roche) with a functional sensitivity of 4.0ng/mL (to convert from ng/mL to nmol/L, multiply by 2.5) on a Roche Cobas E602 immunoanalyzer. All the assays were performed according to the manufacturer's instruction.

### **Statistical analysis**

Statistical analysis was performed using the SPSS-PC+ software (SPSS Inc., Chicago, IL, USA). Continuous variables were presented as mean  $\pm$  standard deviations (SD) and categorical data as number (%). The independent *t* test or ANOVA were used for normally distributed data of 25(OH)D levels. Data that was normally distributed was analyzed with independent *t* test or analysis. A two-sided *p*-value  $\leq 0.05$  was considered statistically significant.

## Results

### Distributions of general characteristics among participant women

A total of 410 fertile women between the ages 22 and 44 attended the clinics at 3–6 months before they became pregnant were included in this study, of which 100% were Han nationality. The mean BMI of them was 21.4 kg/m<sup>2</sup> with a range of 15.2–34.1 kg/m<sup>2</sup>. Most women (58.5%) reported having a or more histories of pregnancy, and only 19.5% giving birth to a live infant. The 28.5% women underwent voluntary pregnancy termination. 340 (82.9%) subjects were working women who were indoor job holders or office-based workers, while 70 (17.1%) were non-working individuals or housewives. The 128 (31.2%) serum samples were taken in spring, 102 (24.9%) in summer, 88 (21.5%) in autumn, and 92(22.4%) in winter.

### The serum levels of 25(OH)D among preconceptional fertile women

The mean serum 25(OH)D for the cohort was 14.7  $\pm$  5.4 ng/mL. Mean concentrations ranged from a low of 4.5 ng/mL among young age women between 22 to 30 years to a high of 36.0 ng/mL among women aged 31 to 44 years. A large proportion (84.4%) of women had concentrations below 20 ng/mL, indicating that they were deficient in vitamin D, only 15.6% of women had the optimal levels of 20 ng/mL–50 ng/mL. This means that about 84% of the females had inadequate concentrations for multiple health (according to the Institute of Medicine (IOM) and World Health Organization [19,20,21]).

There was no effect of the gravidity, induced abortions, and BMI of the woman on the serum 25(OH)D level. There was however a statistically significant influence of the 25(OH)D level and season, giving birth to a live infant, employment status and age of women (All *p* < 0.05). Table 1 shows the median ranges of 25(OH)D levels for specified subgroups. Women aged 22–30 years had significantly lower 25(OH)D levels compared with those between 31–44 years (14.3  $\pm$  5.0 ng/mL vs 15.6  $\pm$  6.0 ng/mL, *P*<0.05). The mean vitamin D level was significantly lower in working women compared to non-working individuals or housewives (14.3  $\pm$  5.2 ng/mL vs 16.5  $\pm$  5.8 ng/mL, *P*<0.01). Women who have given birth to a live infant had higher 25(OH)D levels in comparison to women without a history of childbirth (15.9  $\pm$  5.7 ng/mL vs 14.4  $\pm$  5.3 ng/mL, *P*<0.05).

## Discussion

There are limited data about vitamin D deficiency in healthy nonpregnant fertile women who will become pregnant in following 3–6 months. This study was focused on comparative affluent and healthy fertile women to receive preconception evaluation but their vitamin D levels were found to be low. Serum 25(OH)D levels reflect body stores of vitamin D [1]. Nearly eighty-four percent of fertile nonpregnant women were 25(OH)D deficient in this study. Especially in young women aged 22–30 years, serum 25(OH)D level was lower in comparison with women between 31–44 years. These findings were nearly consistent with a population-based study by Fang et al [22] reported that serum 25(OH)D level was the lowest in the 18- 29-year-old group ( $46.66 \pm 12.98$  nmol/L) among all participant women aged 18–49 years in Tianjin, China. Our findings are also supported by a recent study from Pakistan on vitamin D deficiency in asymptomatic healthy young students. Almost eighty-nine percent of participants were vitamin D deficient (25(OH)D < 20ng/mL) [23].

This is alarming as these are pregnant in following 3–6 months. Several studies have described an association between insufficiency or deficiency in 25(OH)D levels and adverse pregnancy outcomes including gestational diabetes mellitus, preeclampsia, preterm delivery, recurrent abortion, and intrauterine growth restriction [24,25,26]. More recent data showed that preconception vitamin D status was associated with male live birth [27]. Increased preconception vitamin D concentrations are associated with reduced pregnancy loss [13].

In this study, we also found that working women displayed a lower level of vitamin D relative to non-working individuals. Sowah, et al. also observed a higher prevalence of vitamin D deficiency in all occupational populations examined than the reported population burden of vitamin D deficiency in multiple populations, suggesting that workers may be particularly vulnerable to vitamin D deficiency [28]. Among the occupations considered in the present study, the women almost were office-based workers who spend a considerable amount of time indoors without sunshine exposure. Women in an indoor setting would be expected to get their sunshine exposure during mornings and evenings, when sunlight intensity is relatively low. As we all know, vitamin D<sub>3</sub> is needed from not only adequate intake from diet or supplements but also biosynthesis in the skin in response to sufficient exposure to sunlight. Several studies have concerned seasonal variation in serum 25(OH)D concentrations [29,30,31]. This study also found that serum 25(OH)D concentration was the lowest in January and the highest in July and August. Almost ninety-four percent of healthy preconception fertile women had serum 25(OH)D levels <20 ng/mL during winter in Xi'an, China. Similar to the results that traveling to a warmer climate during winter/spring was associated with higher serum 25OHD according to a prevalence of vitamin D deficiency in healthy women of reproductive age in Canada [32]. Statistics Canada found that sunlight exposure more than 1 h per day and month of blood collection were positively associated with higher serum 25OHD levels [33].

It is interesting that when analyzed by season, non-working individuals and women between 31–44 years did not have significant winter decline in serum 25(OH)D levels seen in working individuals and young women aged 22–30 years. This altered pattern may be due to trends of increased multivitamin supplements among women aged 31–44 years. Mitchell et al. study suggests that increased oral intake of vitamin D compensated for decreased ultraviolet radiation B exposure [34]. There may also have been

differences in sun vacation or outdoor time with no sun protection (i.e. no protection from the sun using clothing or sunscreen) between working and non-working women.

In contrast to many studies [35,36], we found no association between BMI and serum 25OHD, which could be accounted for the normal and low BMI of our participants. Indeed, about 84% of women had BMI < 24 kg/m<sup>2</sup>, only one had BMI > 28 kg/m<sup>2</sup> indicating obese.

Our study had some limitations. We did not collect information regarding dietary vitamin D intake, vitamin D/multivitamin supplements, or measures of plasma parathyroid hormone. Regarding occupation, we do not have data on natural ultraviolet B exposure time or amount, which may confound the effects of ultraviolet B in different subgroups. Finally, our data are cross-sectional, thus, we do not have insight on longitudinal changes in 25(OH)D, and we do not provide reproductive outcomes.

## **Conclusions**

Overall, we found a large proportion of vitamin D deficiency in this “lower-risk” cohort. Women working in an institutional setting, young women and living in winter are more prone to vitamin D deficiency due to reduced solar exposure. Decreased serum vitamin D levels among preconception fertile women may predispose to increased risk for adverse pregnancy [13,26]. Therefore, we propose that healthy fertile women who are pregnancy in following 3–6 months may be screened for serum 25(OH)D levels on preconception evaluation and emphasize need vitamin D supplements and get sunshine exposure.

## **Abbreviations**

25(OH)D: 25-hydroxyvitamin D<sub>3</sub>; BMI: body mass index.

## **Declarations**

## **Acknowledgements**

We would like to acknowledge all women who were willing to participate in the research.

## **Authors' contributions**

X. W. Y. conceived and designed the study; H. Y.F, E.B, L. W., and W.H were responsible for the subject recruitment and the clinical data collection; L. Y. H. performed the laboratory analyses; and H. Y. F. and X. T. Y. contributed to data analysis. X. W. Y. obtained funding for the study. All authors read and approved the final manuscript.

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## Availability of data and materials

The data used during the current study are available from the corresponding author on reasonable request.

## Ethics approval and consent to participate

The study was approved by the Ethics Committee of the First Affiliated Hospital of Xi'an Jiaotong University (XJTU1AF2018LSK-2018-131). An informed consent was obtained from all participants.

## Consent for publication

Not applicable.

## Competing interests

The authors declare no conflict of interest.

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## Tables

Table 1. Association of serum vitamin D levels with demographic and reproductive history

Characteristics	Demographic and reproductive history (n)	Serum 25(OH)D (mean± SD ng/mL)	t/F	p
Age	22-30 years (277)	14.3± 5.0	2.203	0.029
	31-44 years (133)	15.6± 6.0		
BMI (kg/m <sup>2</sup> )	Underweight (46)	13.7± 4.5	1.273	0.281
	Normal (293)	14.9 ± 5.4		
	Overweight and Obese (71)	14.4 ± 5.7		
Gravidity	No (170)	14.1± 5.1	1.813	0.071
	One and more (240)	15.1 ± 5.6		
Giving birth to a live infant	Nulliparity(330)	14.4 ± 5.3	2.290	0.023
	One (80)	15.9 ± 5.7		
Numbers of induced abortion	No (293)	14.7 ± 5.7	0.143	0.866
	One (85)	14.7 ± 5.2		
	Two and more (32)	15.2 ± 6.2		
Employment status	Working (340)	14.3 ±5.2	3.127	0.002
	Non-working (70)	16.5 ± 5.8		
Season	Spring (128)	14.5 ± 5.1#	16.417	0.000
	Summer (102)	16.6 ± 5.0		
	Autumn (88)	15.9 ± 5.6		
	Winter (92)	11.8 ± 4.8*		

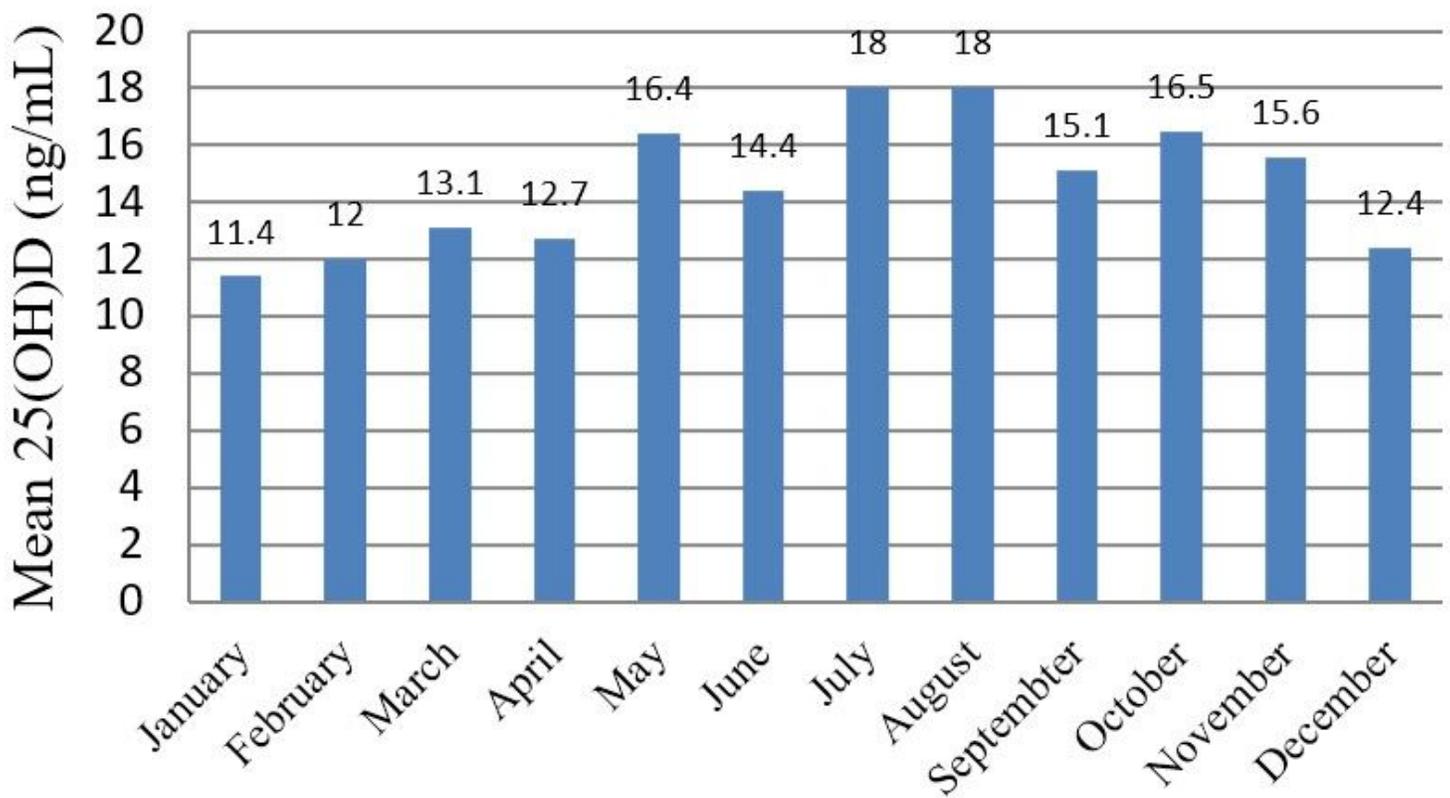
\*Significantly lower compared to spring, summer, and autumn (all  $p < 0.01$ ).

#Significantly lower compared to summer ( $p < 0.01$ ).

Table 2. The mean 25(OH)D levels by season after stratification by age and by women's employment status (mean± SD ng/mL)

Season	Employment status		Age	
	Working (n=285)	No-working (n=57)	22-30 years (n=241)	31-44 years (n=101)
Spring	13.7 ± 4.6	17.3 ± 5.8	14.5 ± 5.0	14.7 ± 5.5
Summer	16.4 ± 4.9	17.7 ± 5.5	16.1 ± 4.9	17.5 ± 5.1
Autumn	15.9 ± 5.6	15.6 ± 5.7	15.7 ± 5.1	16.3 ± 6.7
Winter	11.5 ± 4.6	13.8 ± 6.0	11.0 ± 3.8	13.6 ± 6.5
<i>F</i>	17.013	1.384	15.655	2.664
<i>p</i>	0.000	0.256	0.000	0.051

## Figures



**Figure 1**

Mean serum 25(OH)D concentrations (ng/mL) based on month of serum collection among all participants.