

The associations between serum vitamin D, calcium and uterine fibroids in Chinese women: a case-controlled study

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Abstract

Objective: To investigate the associations between serum vitamin D, calcium and uterine fibroids in a Chinese female population.

Methods: In this case-control study, adult female patients with fibroids (cases) were compared with females without fibroids (controls) in terms of serum 25-hydroxyvitamin D (25OHD) and calcium levels.

Results: Out of 546 total participants (mean age, 41.68 ± 5.99 years; 279 with fibroids and 267 without fibroids), only 232 had serum 25OHD levels above the sufficient threshold (>20 ng/ml). In addition, females with fibroids had lower serum 25OHD levels versus those without fibroids. The prevalence of fibroids in females with deficient (<12 ng/ml) and insufficient (12 – 20 ng/ml) 25OHD levels were significantly higher than in females with sufficient (>20 ng/ml) 25OHD levels. Serum calcium levels were within normal range in both groups.

Conclusion: Hypovitaminosis D was highly prevalent among a population of Chinese females of reproductive-age, and serum 25OHD levels were lower in female patients with fibroids.

Keywords

Asian women, calcium, uterine fibroids, vitamin D

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Introduction

Uterine fibroids are among the most common benign gynaecologic tumours in women of reproductive age,¹ and in 2012, the total annual costs for this disease were estimated to be about \$34.4 billion in the United States alone.² Clinical presentations of uterine fibroids include menorrhagia, prolonged menstrual bleeding, pelvic pressure, pelvic pain and infertility.³ Treatment can differ depending on the location, size and number of uterine fibroids, patient age, interest in fertility preservation, and patient preference.⁴ Since there is no effective way of treating the disease to date, surgery remains the most common choice for patients with symptomatic uterine fibroids.⁵ Oestrogen and progesterone appear to play important roles in the growth of uterine fibroids, however, knowledge regarding the origin and development of uterine fibroids is lacking.^{6–8} Therefore, understanding the molecular events related to this disease may help to develop effective treatments.

Vitamin D is a necessary vitamin for the human body. 25-hydroxyvitamin D (25OHD) is the major circulating form of vitamin D, and serum 25OHD level is regarded as the best assessment of vitamin D status in humans.⁹ A number of studies have reported that vitamin D plays important roles in many biological processes other than calcium homeostasis and bone health.^{10–19} It has also been suggested that vitamin D may be associated with uterine fibroids.²⁰ Epidemiological investigations have shown that vitamin D insufficiency is associated with an increased risk of uterine fibroids in black and white races.^{21,22} Interestingly, serum 25OHD level was found to be significantly lower in people who are black than in those who are white.^{21,23,24} Also, uterine fibroids are at least two to four times more common in black women than in white women.^{1,25} However, the presence of a similar

relationship between uterine fibroids and vitamin D in Asian women has not yet been reported.

In recent years, numerous studies have shown that there may be relationships between serum calcium levels and certain types of tumours, however, the results are inconsistent and inconclusive.^{26–32} In addition, the relationship between serum calcium and uterine fibroids remains unclear and not well studied.

The aim of the present study was to compare serum concentrations of 25OHD and calcium in Chinese women with and without uterine fibroids to investigate the possible correlations among serum vitamin D, calcium levels and uterine fibroids in reproductive-age women.

Patients and methods

Study population and design

This case-control observational study was conducted in premenopausal women (aged ≥ 18 years) who attended routine health check-ups at the Second Affiliated Hospital of Wenzhou Medical University, Wenzhou, China between August 2016 and December 2016. Women who were diagnosed with uterine body fibroids (not cervical fibroids) by gynaecologic ultrasound were enrolled and included in the group with fibroids and healthy women with fibroid-free uterine structure were enrolled during the same period and included in the group without fibroids (control group).

Premenopausal women with the following conditions were excluded from the study: (1) using vitamin D or calcium supplements within 6 months prior to study enrolment; (2) pregnancy or lactation; (3) various serious diseases, including skin diseases (psoriasis), cardiovascular diseases (myocardial infarction, cerebral infarction), endocrine diseases (abnormal parathyroid

gland), infectious diseases (tuberculosis), autoimmune disorders (Type 1 diabetes mellitus, systemic lupus erythematosus), malignancies of any organ, hepatic or renal diseases, digestive system diseases (malabsorption); and (4) a history of prior myomectomy or hysterectomy.

This research did not involve any intervention or treatment, and the study was approved by the Ethical Committee of the Second Affiliated Hospital of Wenzhou Medical University (No. KYLW2016081). Written informed consent was obtained from all study participants prior to involvement in the research.

Ultrasound measurements

All study participants were screened for uterine fibroids by transvaginal ultrasound using GE Voluson™ E6, E8 or E10 ultrasound systems (GE Medical systems, American). For each patient with uterine fibroids, the following parameters were evaluated by ultrasound: the number of uterine fibroid lesions and the size of the largest fibroid. Patients were divided into the following study sub-groups according to the number of uterine fibroids: group without fibroids, single fibroid group (one uterine fibroid), or multiple fibroids group (\geq two uterine fibroids). Ultrasound images were used to measure the three perpendicular planes of the largest uterine fibroid (height, width, depth), and the largest uterine fibroid diameter was recorded for each patient as the index of fibroid size. A fibroid size index of 3 cm was chosen as the cut-off point, and participants were divided into a group without fibroids, a small fibroids group (diameter $<$ 3 cm) and a large fibroids group (diameter \geq 3 cm).

Laboratory analyses

All study participants provided a blood sample that was collected from the cubital

vein during early morning. Serum 25OHD levels were tested in samples from all study participants, and serum calcium levels were analysed in those who had sufficient serum sample remaining for calcium analysis. Blood samples were drawn, processed and stored at -20°C prior to analyses. Electrochemiluminescence immunoassay was used to evaluate serum 25OHD levels with a Cobas e601 fully automated analyser and associated reagent kits (Roche Diagnostics GmbH, Mannheim, Germany), according to the manufacturer's instructions. Serum 25OHD levels were categorized as deficient ($<$ 12 ng/ml), insufficient (12–20 ng/ml), and sufficient ($>$ 20 ng/ml), according to the Institute of Medicine guidelines.³³ Serum calcium levels were quantified using a photometric colorimetric method with a Cobas c501 analyser and associated reagent kits (Roche Diagnostics GmbH, Mannheim, Germany), according to the manufacturer's instructions. As the normal range for serum calcium differs between laboratory and measurement methods, the normal range in the present study was 2.08–2.65 mmol/l.

Statistical analyses

Statistical analyses were performed using SPSS software, version 19.0 for Windows (SPSS Inc., Chicago, IL, USA). Data are presented as mean \pm SD, median (25th percentile, 75th percentile) or n (%) prevalence. One-sample Kolmogorov–Smirnov test was used to analyse the normality of continuous variable distribution. If data were normally distributed, statistically significant differences were determined via independent samples Student's t -test, analysis of variance or Least Significant Difference (LSD), as appropriate. Otherwise, Wilcoxon signed-rank test and Kruskal–Wallis test were used for between-group comparisons, as appropriate. Qualitative variables were

compared using χ^2 -test. A P value < 0.05 was considered to be statistically significant.

Results

A total of 546 participants were included in the study, comprising 279 women with fibroids and 267 healthy women without fibroids. Serum 25OHD levels were analysed in all 546 participants, while serum calcium levels were examined in samples from 226 participants (110 patients with uterine fibroids and 116 participants without uterine fibroids). There was no statistically significant difference in participant age between women with and without uterine fibroids (42.15 ± 5.09 years versus 41.19 ± 6.78 years, respectively; $P > 0.05$).

Among 546 participants, 61 women (11.17%) had deficient 25OHD levels (< 12 ng/ml) and 253 women (46.34%) had insufficient 25OHD levels (12–20 ng/ml). In other words, only 232 women (42.49% of the study population) had sufficient 25OHD levels (> 20 ng/ml). In addition, women with uterine fibroids ($n = 279$) had lower serum 25OHD levels compared with women without uterine fibroids ($n = 267$; $P < 0.05$). The results also showed that women with uterine fibroids ($n = 110$) had lower serum calcium compared with the group of women without fibroids ($n = 116$; $P < 0.05$). However, serum calcium levels

were within normal range in both groups (Table 1).

The prevalence of uterine fibroids in women with deficient 25OHD levels (65.57%) and insufficient 25OHD levels (55.73%) was higher than in women with sufficient 25OHD levels (42.24%; $P < 0.05$; Table 2). There was no statistically significant difference in prevalence of uterine fibroids between women with deficient and insufficient 25OHD levels (Table 2).

To further explore the associations between serum 25OHD, calcium levels and uterine fibroids, subgroup analyses were performed within the fibroids group according to the diameter of the largest uterine fibroid. Statistically significant differences were observed in serum 25OHD levels between participants without fibroids, patients with small (diameter < 3 cm) fibroids and those with large (diameter ≥ 3 cm) fibroids ($P < 0.05$; Table 3). Serum 25OHD levels in the small and large fibroid groups were significantly lower than those in the group without fibroids ($P < 0.05$). There was no statistically significant difference in serum 25OHD level between patients with large fibroids and those with small fibroids ($P > 0.05$). In addition, there were statistically significant differences in serum calcium levels between participants without fibroids, patients with small fibroids and those with large fibroids. The serum calcium levels in each of the two

Table 1. Serum 25-hydroxyvitamin D (25OHD) and calcium levels in female study participants with or without uterine fibroids.

Characteristic	Participant group		Statistical significance
	With fibroids	Without fibroids	
Serum 25OHD level, ng/ml	18.15 ± 6.16 ($n = 279$)	20.73 ± 6.99 ($n = 267$)	$P < 0.05$
Serum calcium level, mmol/l	2.33 ± 0.09 ($n = 110$)	2.39 ± 0.09 ($n = 116$)	$P < 0.05$

Data presented as mean \pm SD.

Statistically significant between-group difference at $P < 0.05$.

Table 2. The prevalence of uterine fibroids in female study participants grouped according to different levels of serum 25-hydroxyvitamin D (25OHD).

Serum 25OHD level, ng/ml	Participant group		Total	χ^2 values	Statistical significance
	With fibroids	Without fibroids			
<12	40 (65.57)	21 (34.43)	61	14.57	$P < 0.05$
12–20	141 (55.73)	112 (44.27)	253		
>20	98 (42.24)* Δ	134 (57.76)	232		
Total, <i>n</i>	279	267	546		

Data presented as *n* (%) prevalence.

$P < 0.05$ was considered statistically significant. * $P < 0.05$ versus prevalence of fibroids in women with deficient 25OHD levels (<12 ng/ml); $\Delta P < 0.05$ versus prevalence of fibroids in women with insufficient 25OHD levels (12–20 ng/ml).

Table 3. Comparison of serum 25-hydroxyvitamin D (25OHD) and calcium levels between female participants without fibroids and patients with fibroids grouped according to fibroid size.

Study group	Characteristic			
	Serum 25OHD level, ng/ml	Statistical significance	Serum calcium level, mmol/l	Statistical significance
Without fibroids	20.73 \pm 6.99 (<i>n</i> = 267)	$P < 0.05$	2.39 \pm 0.09 (<i>n</i> = 116)	$P < 0.05$
With fibroids < 3 cm	18.47 \pm 6.63* (<i>n</i> = 178)		2.36 \pm 0.07* (<i>n</i> = 58)	
With fibroids \geq 3 cm	17.60 \pm 5.22* (<i>n</i> = 101)		2.30 \pm 0.09* Δ (<i>n</i> = 52)	

Data presented as mean \pm SD.

$P < 0.05$ was considered statistically significant. * $P < 0.05$ versus without fibroids. $\Delta P < 0.05$ versus fibroids < 3 cm.

fibroid groups were significantly lower than in participants without fibroids ($P < 0.05$; Table 3). Furthermore, serum calcium levels differed between the small and large fibroid groups ($P < 0.05$). Nevertheless, levels of serum calcium were within normal ranges in all three groups (Table 3).

Serum 25OHD and calcium levels were also compared according to the number of uterine fibroids. Statistically significant differences were found in serum 25OHD levels between participants without fibroids and patients with single or multiple fibroids. In addition, the single fibroid and multiple fibroids groups had significantly lower serum 25OHD levels than participants without fibroids ($P < 0.05$; Table

4). There was no statistically significant difference in serum 25OHD level between patients with multiple fibroids and those with a single fibroid ($P > 0.05$). Significant differences in serum calcium levels were also found between participants without fibroids and patients with single or multiple fibroids (Table 4). Serum calcium levels were significantly lower in the single and multiple fibroids groups compared with patients without fibroids ($P < 0.05$). In addition, patients with multiple fibroids had significantly lower serum calcium levels than those with a single fibroid ($P < 0.05$). However, serum calcium levels were within normal ranges in all three groups (Table 4).

Table 4. Comparison of serum 25-hydroxyvitamin D (25OHD) and calcium levels between female participants without fibroids and patients with fibroids grouped according to presence of single or multiple fibroids.

Study group	Characteristic			
	Serum 25OHD level, ng/ml	Statistical significance	Serum calcium level, mmol/l	Statistical significance
Without fibroids	20.73 ± 6.99 (n = 267)	P < 0.05	2.39 ± 0.09 (n = 116)	P < 0.05
Single fibroid	18.56 ± 6.05* (n = 118)		2.36 ± 0.07* (n = 45)	
Multiple fibroids	17.85 ± 6.25* (n = 161)		2.31 ± 0.09* ^Δ (n = 65)	

Data presented as mean ± SD.

P < 0.05 was considered statistically significant. *P < 0.05 versus without fibroids. ^ΔP < 0.05 versus single fibroid.

Discussion

To the best of our knowledge, the present study is the first to investigate the relationships between serum vitamin D, serum calcium and uterine fibroids among Asian women. The main finding of the study was that within a population of Chinese women, there was an inverse association between uterine fibroids and serum 25OHD levels. Women with uterine fibroids appeared to have lower serum 25OHD levels versus women without uterine fibroids.

To date, several studies have reported an association between vitamin D and uterine fibroids. In a case-control study of reproductive age women referred to two infertility units in Italy, serum 25OHD level was shown to be significantly lower in the fibroids group compared with unaffected controls.³⁴ In addition, another study demonstrated that low serum 25OHD levels were correlated with increased risk of uterine fibroids among white and black women.²¹ Furthermore, each 10 ng/ml increase in 25OHD levels has been shown to relate to a 20% drop in the incidence of uterine fibroids in black and white people, and women with vitamin D sufficiency (25 [OH]D > 20 ng/ml) were shown to have a

32% lower risk of developing uterine fibroids than women with insufficient vitamin D (25[OH]D ≤ 20 ng/ml).²² To date, we have been unable to find similar published studies in Asian women, and during our own research, have discovered that vitamin D insufficiency is universal, even within the normal female Chinese population. Among the 267 individuals without fibroids in the present study, 112 (41.94%) were found to have vitamin D insufficiency and 21 (7.87%) had vitamin D deficiency. Thus, in total, almost 50% of the study population had vitamin D insufficiency or worse. Also, the 279 female patients with uterine fibroids had even lower serum 25OHD levels than 267 participants without fibroids, and the prevalence of uterine fibroids was significantly higher in women with deficient and insufficient 25OHD levels than in women with sufficient 25OHD levels, consistent with previous findings in other ethnic groups.^{22,34}

Previous studies of the relationship between uterine fibroid size and serum 25OHD levels have reported inconsistent and inconclusive results. In a study of 104 women with uterine fibroids and 50 healthy controls, serum 25OHD levels were found to be inversely correlated with the volume

of uterine fibroids.²¹ In contrast, in a study wherein women with uterine fibroids were grouped according to diameter of the largest uterine fibroid (non-fibroids, small fibroids [diameter < 4 cm], large fibroids [diameter ≥ 4 cm]), with a 4-cm diameter as the cut-off, no significant difference in serum 25OHD between different fibroid sizes was found.²² This disagreement may be attributed to different methods for calculating the size of uterine fibroids. In the present study, a 3-cm diameter was chosen as the cut-off point because it classified the patients more evenly into two groups compared with a diameter of 4 cm. With such classification, serum 25OHD levels in women with small fibroids (diameter < 3 cm) and large fibroids (diameter ≥ 3 cm) were significantly lower than in women without uterine fibroids. Although there was no statistically significant difference, there was a numerical trend suggesting that serum 25OHD levels in patients with large fibroids (diameter ≥ 3 cm) may be lower than in patients with small fibroids (diameter < 3 cm). Additionally, patients with a single fibroid and those with multiple fibroids were shown to have significantly lower serum 25OHD levels than subjects without uterine fibroids. Again, although there was no statistically significant difference, there was a numerical trend toward lower serum 25OHD levels in patients with multiple fibroids versus those with a single fibroid. Hence, the combined results suggest that there may be an association between serum 25OHD levels and fibroid number and size.

Possible relationships between serum calcium and certain types of tumours have been reported, however, there is no consensus on the results. A collection of data regarding serum calcium in two nationally representative prospective cohorts showed that high serum calcium levels were markedly positively correlated with the risk of ovarian cancer.²⁶ A nested case-control

study by the same group revealed that women with elevated serum calcium levels had a 2.5-fold enhanced risk of ovarian cancer in early follow-up observations.²⁷ Furthermore, high serum calcium levels have been reported to increase the risk for advanced prostate cancer.²⁸ However, analyses of data from a prospective multicentre study showed that patients with lower serum calcium levels may be more likely to develop prostate cancer.³² An inverse correlation has been demonstrated between serum calcium and percentage of mammographic breast density, which is one of the main risk factors for primary breast cancer,³⁰ and similar results were obtained in another study that indicated the protective effect of serum calcium against breast cancer.³¹ In addition to these discrepancies, little is known about the relationship between serum calcium levels and uterine fibroids. The statistically significant results of the present study suggested that women with uterine fibroids may have slightly lower serum calcium levels compared with women without fibroids. In addition, decreased serum calcium levels were observed in patients with multiple fibroids versus those with a single fibroid, and in patients with large fibroids versus those with small fibroids. Although the differences were statistically significant, it should be noted that serum calcium levels were within the normal range in all study groups.

In the present study, women with uterine fibroids had lower serum 25OHD and calcium levels compared with women without fibroids. However, whether low serum 25OHD or low calcium levels contribute to the occurrence and development of uterine fibroids, or uterine fibroids influence the synthesis and/or distribution of serum 25OHD and calcium, remains unclear. Previous *in vitro* and *in vivo* studies have shown a potential role of vitamin D in the biology of fibroids. For example, an *in vitro* analyses showed that both myometrial and

leiomyoma cells were effectively inhibited by 1,25-dihydroxyvitamin D₃ in a dose-dependent manner.³⁵ In addition, numerous studies have demonstrated that vitamin D has the potential to reduce cell proliferation of human uterine leiomyoma via various mechanisms, including inhibition of Wnt/catenin and mammalian target of rapamycin (mTOR) signalling pathways,³⁶ suppression of expression and activities of matrix metalloproteinase-2 and -9,³⁷ reduction of catechol-O-methyltransferase expression and activity,³⁸ and the down-regulation of transforming growth factor- β -induced fibrosis-related gene expression.³⁹ Furthermore, reduced levels of vitamin D receptor (VDR) protein and up-regulation in sex steroid receptor have been observed in human uterine fibroid cells, and vitamin D₃ treatment may reduce the expression of extracellular matrix-associated protein and sex steroid receptors.^{40,41} A study involving a diet-induced mouse model of vitamin D deficiency showed that vitamin D deficiency caused inflammation, DNA damage, and the promotion of fibrosis in murine myometrium.⁴² In an Eker rat model of leiomyomas, a subcutaneous vitamin D supplement significantly reduced leiomyoma size through the inhibition of proliferation and induction of apoptosis.⁴³ Therefore, based on all of the above studies, we believe that vitamin D deficiency may be a causative factor for uterine fibroids, rather than fibroids leading to low serum vitamin D levels.

Considering the present results and previously published articles,^{20,35-43} it could be speculated that vitamin D supplementation may be beneficial to lower the risk of uterine fibroids, however, to date, clinical trials of the role of vitamin D supplementation in this common disease are rare. The first evaluation of the effect of vitamin D supplementation, in a small-sample clinical trial of patients with leiomyoma, indicated that vitamin D supplementation may play an important role in inhibiting or stabilizing

the growth of uterine fibroids.⁴⁴ Further laboratory and clinical investigations are needed to explore the roles of vitamin D in tumour biology. Therefore, we are conducting a randomized controlled trial, which contains two parts (I and II), to confirm the effectiveness of vitamin D supplementation and the role of vitamin D in the occurrence and development of fibroids. More explicitly, in part I, patients with hypovitaminosis D and without fibroids will be randomly assigned to a vitamin D supplementation group and an observation group, to investigate the effect of vitamin D supplementation on the occurrence of fibroids. In part II, patients with hypovitaminosis D and uterine fibroids will be randomly assigned to a vitamin D supplementation group and an observation group, to evaluate the relationship between vitamin D supplementation and the development/progression of fibroids in reproductive-age women (Trial registration number www.clinicaltrials.gov, NCT03586947 and NCT03584529; pre-results).

A major strength of the present study is that this is the first such attempt to investigate the correlations between serum vitamin D, calcium and uterine fibroids among Asian women. Conversely, the present results may be limited by the relatively small sample size, and the fact that this was a single-centre study. Further studies with multiple clinical centres containing more patients are required.

In summary, the present study suggests that there may be a high prevalence of hypovitaminosis D among women of reproductive age in China. Moreover, women with uterine fibroids were found to have lower serum 25OHD levels, and the prevalence of uterine fibroids in vitamin D deficient and insufficient women was significantly higher than in those with sufficient vitamin D levels. A controlled, prospective, randomized study is ongoing in the Second

Affiliated Hospital of Wenzhou Medical University to determine the effectiveness of vitamin D supplementation and the role of vitamin D in the occurrence and development of uterine fibroids.

Declaration of conflicting interest

The authors declare that there is no conflict of interest.

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