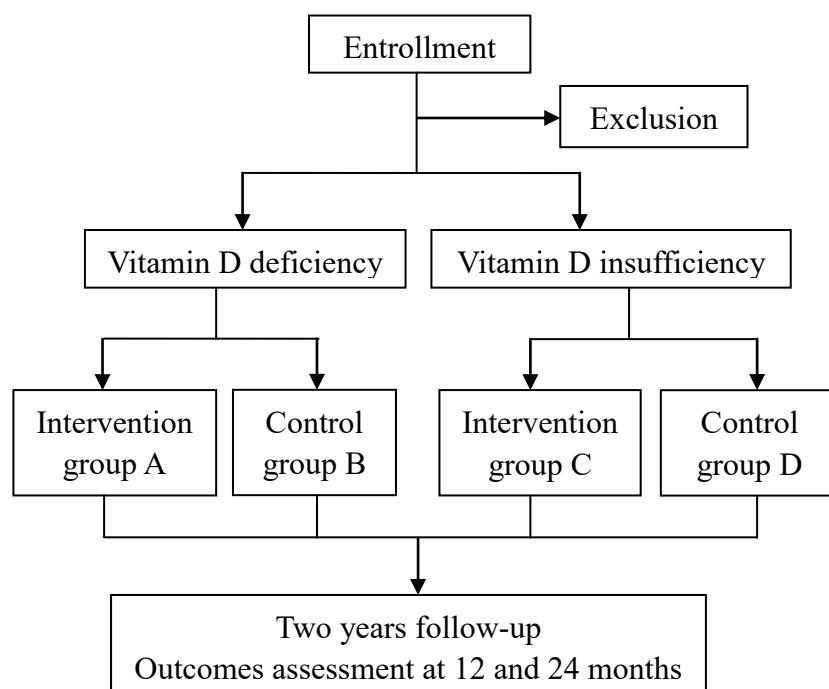


Association between Vitamin D and The Development of Uterine Fibroids

2018.3.15

Study objectives The primary objective of this part is to assess the efficacy of supplementation with vitamin D on inhibiting the development of uterine fibroids in one year and two years. The secondary objective of this study is to evaluate the safety of supplementation with vitamin D in subjects.

Trial design This is an open-label, randomised controlled trial. After signing of informed consent, patients with vitamin D deficiency ($12 \text{ ng/ml} \leq \text{Serum 25-hydroxyvitamin D}_3 < 20 \text{ ng/ml}$) and uterine fibroids will be randomly assigned in a 1:1 ratio to intervention group A or control group B. Intervention group A will accept an oral dose of 1600 IU/day vitamin D₃ for up to 2 years. Control group B will receive 2 years follow-up. Patients with vitamin D insufficiency ($20 \text{ ng/ml} \leq \text{Serum 25-hydroxyvitamin D}_3 < 30 \text{ ng/ml}$) and uterine fibroids will be randomly assigned in a 1:1 ratio to intervention group C or control group D. Intervention group C will accept an oral dose of 800 IU/day vitamin D₃ for up to 2 years. Control group D will receive 2 years follow-up. Gynecologic ultrasound examinations will be performed every three months. The mount, location and size of the uterine fibroids will be documented (the transverse, longitudinal, and antero-posterior diameters of fibroids will be documented at each efficacy ultrasound examination for volume calculation). Percent change in volume of the total fibroids compared to baseline in different groups (baseline = last value obtained before randomization). Three perpendicular diameters (d₁, d₂, and d₃) were collected for each fibroid, and the volume of each fibroid (in cm³) was calculated with the ellipsoid formula: $4/3 * \pi * (d_1 * d_2 * d_3)/8$. For each patient, the total volume of fibroids (defined as the sum of the volumes of all the fibroids detected) was also calculated. The safety of vitamin D in subjects with uterine fibroids will be evaluated, including blood routine examination, electrolyte, hepatic and renal function, liver and urinary system ultrasound, and serum 25-hydroxyvitamin D₃. Vitamin D₃ Drops are purchased from Sinopharm star shark pharmaceutical (xiamen) co., LTD. An overview of the study design is shown in Figure 2 and Table 2.



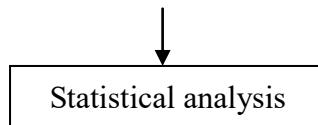


Figure 2 flow diagram.

Table 2:time of date collection

Follow-up	1	2	3	4	5	6	7	8	9
Months	0	3	6	9	12	15	18	21	24
The form of follow-up	Out-patient								
Informed consent	X								
Medical history	X								
Physical examination	X								
Serum 25-hydroxyvitamin D ₃	X	X	X	X	X	X	X	X	X
Urine pregnancy test	X	X	X	X	X	X	X	X	X
Gynecologic ultrasound	X	X	X	X	X	X	X	X	X
Hepatic and renal function	X		X		X		X		X
Electrolyte	X		X		X		X		X
Blood routine examination	X		X		X		X		X
Liver and urinary		X			X				X
System ultrasound			X						
Side-effect assessment		X	X	X	X	X	X	X	X
Changes in menstruation		X	X	X	X	X	X	X	X
Adverse event assessment		X	X	X	X	X	X	X	X
Vitamin D receptor genotype	X								

Sample size According to a previous study, the volume of uterine fibroids was 8.2 (2.1-30.5) cm³ after the supplement of vitamin D 12 months and 11.4 (5.5-22.3) cm³ in the control group, respectively. On the basis of a 0.9 power to detect a significant difference ($\alpha=0.05$, one-sided), 320 participants will be required for the four groups and in a 1:1:1:1 ratio. Allowing for a 10% withdrawal rate, we plan to include 360 patients in the whole trial.

Inclusion criteria

1. Patients are willing to cooperate with the follow-up and sign informed consent;
2. Females aged 35-50 who are diagnosed as having uterine fibroids by transvaginal or abdominal ultrasonography;
3. The maximum average diameter of intramural myoma is $\leq 4\text{cm}$, $\geq 1\text{cm}$; The amount of myoma is less than 4;
4. Serum 25-hydroxyvitamin D₃ $< 30\text{ ng/ml}$, $\geq 12\text{ ng/ml}$.

Exclusion criteria

1. Patients with heavy menstrual bleeding ($>80.0\text{ mL}$), menstrual disorders, pelvic discomfort, infertility, or other indications for operation;

2. Patients complicated with myoma degeneration and adenomyosis that were suspected or diagnosed by ultrasound or gynecologic examination;
3. Allergic to vitamin D₃;
4. Use of sexual hormone, mifepristone, gonadotropin-releasing hormone agonist (GnRHa), or other medication which is likely to interfere with uterine fibroids within 3 months;
5. Pregnancy, lactation, postmenopause, or planned pregnancy within two years;
6. Suspected or identified as other tumors of genital tract;
7. History of osteoporosis or vitamin D deficiency taking vitamin D supplements within previous one month;
8. History of autoimmune diseases, infectious diseases (tuberculosis, AIDS), autoimmune diseases, digestive system diseases (malabsorption, crohn disease, and dysentery);
9. Alanine aminotransferase (ALT) or aspartate transaminase (AST) more than 3 times of the normal upper limit, total bilirubin (TBIL) more than 2 times of the normal upper limit;
10. Creatinine levels ≥ 1.4 mg/dL (123 μ mol/L) or creatinine clearance ≤ 50 mL/min;
11. History of malignant tumors;
12. Simultaneous participation in another clinical study with investigational medicinal product(s) or researcher thinks the subjects are not suitable for this trial.

Outcomes measures Primary outcomes: percent change in volume of the largest fibroid and total fibroids compared to baseline (baseline = last value obtained before randomization; measured by ultrasound examination). Secondary outcomes: percentage of subjects undergoing other medical or surgical treatment, hypercalcemia, urinary calculus, abnormal liver and renal function. Ultrasound examinations will be performed by a physician well experienced in gynecology. If possible, the same examiner should conduct all examinations of a subject throughout the study and the same ultrasound machine (per site) should be used throughout the study. For each subject, the most appropriate ultrasound method (transvaginal or abdominal) should be used depending on fibroids location and this method should be used consistently throughout the study.

Withdrawal Subjects must be withdrawn from the study when one of the following criteria occurs:

1. At their own request. At any time during the study and without giving reasons, a subject may decline to participate further. The subject will not suffer any disadvantages as a result;
2. In the investigator's opinion, continuation of the study treatment would be harmful to the subject's health;
3. Obvious non-compliance;
4. Lost to follow-up;
5. Pregnancy;
6. Other medical or surgical treatments of uterine fibroids.
7. Receive other medical treatments which may affect the level of serum 25-hydroxyvitamin D₃ or other surgical treatments.

8. The level of serum calcium > 3.5 mmol/L or serum 25-hydroxyvitamin D₃ > 100 ng/mL.

Safety assessments Safety will be assessed by renal and liver function test, electrolyte, routine blood test, and serum 25-hydroxyvitamin D₃. Urine pregnancy test and serum 25-hydroxyvitamin D₃ level will be detected every three months. Other indicators are detected during the period of screening and after the treatment of every six months. Liver and urinary system ultrasound will be conducted after the treatment of 12 months and 24 months. The occurrence of any adverse events in trial participants will be recorded in the case report forms during each patient visit. We will withdraw patients who have severe adverse events, as it is unsafe for them to continue the trial. Meanwhile, we will give them relevant medical care and follow them up until the reaction has terminated.

Statistical analysis Statistical analyses will be performed using SPSS version 22.0 for Windows (SPSS Inc., Chicago, IL, USA). The randomisation sequence is generated by the use of the SPSS software. The normality of distribution of continuous variables are tested by one-sample Kolmogorov-Smirnov test. Continuous variables with normal distribution are reported as mean (standard deviation); non-normal variables are presented as median (interquartile range). Means of 2 and 3 or more continuous normally distributed variables, respectively, are compared by independent samples Student's t test or one-way ANOVA test. Mann-Whitney U test and Kruskal-Wallis test are used, respectively, to compare means of 2 and 3 or more groups of variables not normally distributed. The frequencies of categorical variables are compared using Pearson χ^2 or Fisher's exact test, when appropriate. A value of $P < 0.05$ is considered significant.