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Association between vitamin D and uterine fibroids: a study protocol of an open-label, randomised controlled trial

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Abstract

Introduction: Uterine fibroids are the most common pelvic benign tumor with no satisfactory long-term medical treatment. Recent studies have demonstrated that vitamin D significantly inhibited the growth of fibroids in vitro, vivo and a small-sample clinical trial. Therefore, the aim of this randomised clinical trial (RCT) is to evaluate whether supplementation with vitamin D could reduce the risk and inhibit the growth of uterine fibroids in reproductive stage women.

Methods and analysis: The open-label, RCT comprises two parts, including part I and part II. In part I, 2230 vitamin D deficiency or vitamin D insufficiency patients without uterine fibroids will be randomly assigned to two groups: intervention group (according to the level of serum 25-hydroxyvitamin D_3, receive 1600 or 800 IU/d of vitamin D_3 for 2 years) and control group (followed up at the same time points). The incidence of uterine fibroids will be employed to measure the outcome in different groups. In part II, 360 uterine fibroids patients with vitamin D deficiency or vitamin D insufficiency will be randomly assigned to intervention group or control group. According to the level of serum 25-hydroxyvitamin D_3, 180 patients will receive 1600 or 800 IU/d of vitamin D_3 for 2 years. Control group will receive regular follow-up. The outcome measure will be conducted using the growth of uterine fibroids in each group.

Ethics and dissemination: This study has been approved by the institutional review board of the Second Affiliated Hospital of Wenzhou Medical University (No. LCKY2018-35).

Trial registration number: ClinicalTrials.Gov, NCT03586947 and NCT03584529. Pre-results.

Keywords: Vitamin D; uterine fibroids; randomised clinical trial.

Strengths and limitations of this study

1. The results from this RCT will provide new evidences of the efficacy and safety of vitamin D for uterine fibroids patients.

2. One limitation is that the trial is not a double-blind, placebo-controlled trial and implemented in only one hospital.

3. Another limitation is that the trial is implemented in only one hospital in Chinese subjects, which may limits its generalizability.

Introduction

Uterine fibroids (UFs) are the most common benign tumor of the female genital tract, originating from smooth muscle cells. The prevalence of leiomyomas ranges from 70% to 80% in women by the age of 50.[1] Because most patients with UFs remain asymptomatic, the actual prevalence of UFs is assumed to be much higher than that reported. Based on the ultrasound screening, the incidence for UFs is reported to be 1.278% in Asia and 3.745% in African-American women.[2] The common symptoms of UFs include heavy menstrual bleeding, menstrual disorders and pelvic discomfort.[3] Furthermore, UFs are also associated with infertility and early pregnancy loss. The treatment for UFs depends on the size, location, symptoms, age and reproductive plans. Surgery is still the major treatment for UFs, including hysterectomy, myomectomy and uterine arterial embolization therapy.[1] However, it increases the patients' operative complications and generates huge economic impact on healthcare systems. Except invasive surgical procedure, gonadotropin-releasing hormone agonist (GnRHa)[4] and mifepristone[5] are the most common used medical agents for UFs. When these two drugs are stopped, UFs may re-grow rapidly.[6 7] Thus, GnRHa or mifepristone is usually used for the clinically symptomatic patients who are at a perimenopausal period, or who have contraindications of surgery. It is crucial to find a novel nonsurgical alternative for UFs patients and prevent their occurrence.

Vitamin D is one of the essential nutrients for human bodies. Recent studies have considered that vitamin D is involved in the development of UFs.[7 8 9] Low levels of serum 25-hydroxyvitamin D_3 have been demonstrated to be linked with increased risk of UFs.[10 11] Blauer *et al.*[12] found that the growth of both primary myometrial and leiomyoma cells could be significantly inhibited by 1,25-dihydroxyvitamin D_3 in a concentration-dependent way. Moreover, the process of fibrosis induced by the transforming growth factor-β3 (TGF-β3) could be attenuated by vitamin D in immortalized human UF (HuLM) cells. In addition, vitamin D suppressed the protein expression of plasminogen activator inhibitor-1, which is an important TGF-β target in HuLM cells.[13] In vivo studies, Halder *et al.*[14] reported that 1,25-dihydroxyvitamin D_3 decreased fibroid tumor size through the downregulation of proliferation-related genes,

antiapoptotic genes, estrogen and progesterone receptors in Eker rats. Moreover, Halder *et al.*[15] found that the treatment with 1,25-dihydroxyvitamin D_3 or paricalcitol, an analog of 1,25-dihydroxyvitamin D_3 with lower calcemic activity, could significantly reduce tumor size in mouse xenograft models of UFs.

Our group has demonstrated that serum 25-hydroxyvitamin D_3 level was significantly lower in patients with UFs as compared to controls. Patients with vitamin D deficiency had increased risks of UFs (under published). At the same time, an open-label clinical trial indicated that the supplement of vitamin D in women with UFs stabilized the growth of fibroids and prevented the onset of its related symptoms.[16] But it was not a randomised trial and only 108 patients were included in the trail. It is still unclear whether the supplement of vitamin D could decrease the risk of UFs or inhibit the growth of UFs.

Therefore, the objective of our randomised clinical trial is to evaluate the effect and safety of administration with vitamin D on decreasing the risk and inhibiting the development of UFs in reproductive-aged women.

Methods and analysis

Study design

This is an open-label, randomised controlled trial. The study contains two parts (part I and part II) and will be conducted between May 31, 2020 and October 1, 2022 in the Second Affiliated Hospital of Wenzhou Medical University, a hospital in China. Part I is to investigate the effect of supplementation with vitamin D on the risk of UFs. Part II is about the association between vitamin D supplementation and the development of UFs.

Part I: association between vitamin D and the risk of UFs

Study objectives

The primary objective of this part is to assess the efficacy of supplementation with vitamin D on decreasing the risk of incident UFs within 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, vitamin D deficiency patients (12 ng/ml ≤ serum 25-hydroxyvitamin D_3 < 20 ng/ml) without UFs will be randomly assigned in a 1:1 ratio to either the intervention group A or the control group B. Intervention group A will receive an oral dose of 1600 IU/day vitamin D_3 for up to 2 years. Control group B will receive 2 years follow-up. Patients with vitamin D insufficiency (20 ng/ml ≤ Serum 25-hydroxyvitamin D_3 < 30 ng/ml) without UFs 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 D_3 for up to 2 years. Control group D will receive 2 years follow-up. Gynecologic ultrasound examinations will be performed every six months. The number, location and size of the UFs will be documented. The safety of subjects will be evaluated, including blood routine examination, electrolyte, hepatic and renal function, liver and urinary system ultrasound, and serum 25-hydroxyvitamin D_3. Vitamin D receptor genotype of all patients will also be tested. Vitamin D_3 soft capsules (400 IU per capsule) are purchased from Sinopharm star shark pharmaceutical (xiamen) co., LTD and can be preserved for 2 years. An overview of the study design is shown in Figure 1 and Table 1.

Sample size

The planned sample size is based on data from a previous study, in which the UFs incidence was 1.278% per year in Asia and 3.745% per year in African-American women. Women over the age of 40 years are more likely to have UFs.[2] A study also revealed that African-American females had lower level of serum 25-hydroxyvitamin D_3 as compared to Caucasian females.[17] Vitamin D deficiency is shown to increase the risk of UFs in vitro, vivo animal models and a small-sample clinical trial. We assume a one-tailed α error of 0.05 and a power (1-β) of 0.8. If the rates are 3.745% for the control group and 1.278% for the intervention group, we allow for a dropout rate of 10% for an effective sample size of 2108 and propose to enroll 2320 participants (580 randomized to each arm).

Inclusion criteria

1. Volunteers to participate in the study with informed consent;

2. Married females aged 35-50 who are confirmed with a normal, fibroid-free uterine structure, by means of transvaginal ultrasonography;

3. Serum 25-hydroxyvitamin D_3 ≥12ng/ml, <30 ng/ml.

Exclusion criteria

1. Use of sexual hormone, mifepristone, gonadotropin-releasing hormone agonist (GnRHa), or other medications which are likely to interfere with UFs in the past 3 months;

2. Pregnancy, lactation, postmenopause, or planned pregnancy within two years;

3. Allergic to vitamin D_3 soft capsules;

4. Suspected or identified as other tumors of genital tract;

5. History of hysterectomy or myomectomy;

6. History of osteoporosis or vitamin D deficiency taking vitamin D supplements constantly within previous one month;

7. History of hyperparathyroidism, infectious diseases (tuberculosis, Acquired immunodeficiency syndrome), autoimmune diseases, or digestive system diseases (malabsorption, crohn disease and dysentery);

8. 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;

9. Creatinine levels ≥ 1.4 mg/dL (123μmol/L) or creatinine clearance ≤ 50 ml/min;

10. History of malignant tumors;

11. Simultaneous participation in another clinical study with investigational medicinal product(s) or researcher thinks the subjects are not suitable for this trial.

Outcomes measures

The primary outcome is the first diagnosis of UFs in different groups. The secondary outcomes include hypercalcemia, abnormal liver and renal function, and urinary calculus in different groups. Transvaginal ultrasound examinations will be performed by a well-experienced physician in gynecology. If possible, the same examiner should conduct all examinations of a subject and the same ultrasound machine should be used 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. Patients with poor compliance;

4. Lost to follow-up;

5. Pregnancy;

6. Receive other medical treatments which may affect the level of serum 25-hydroxyvitamin D3 or other surgical treatments;

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

Safety assessments

Safety of vitamin D administrated in patients without UFs will be assessed by renal and liver function test, serum electrolyte (sodium, chloride, potassium, calcium, and phosphorus), blood routine 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. Patients will be withdrawn 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.

Part II: Association between vitamin D and the development of UFs.

Study objectives

The primary objective of this part is to assess the efficacy of supplementation with

vitamin D on inhibiting the development of UFs within one year and two years. The secondary objective of part II is to evaluate the safety of supplementation with vitamin D in subjects.

Trial design

After signing of informed consent, patients with vitamin D deficiency (12 ng/ml ≤ Serum 25-hydroxyvitamin D_3 < 20 ng/ml) and UFs 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 D_3 for up to 2 years. Control group B will receive 2 years follow-up. Patients with vitamin D insufficiency (20 ng/ml ≤ Serum 25-hydroxyvitamin D_3 < 30 ng/ml) and UFs 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 D_3 for up to 2 years. Control group D will receive 2 years follow-up. Gynecologic ultrasound examinations will be performed every three months. The number, location and size of the UFs will be documented (the transverse, longitudinal, and antero-posterior diameters of fibroids will be documented at each efficacy ultrasound examination for volume calculation). The safety of vitamin D in subjects with UFs will be evaluated, including blood routine examination, serum electrolyte, hepatic and renal function, liver and urinary system ultrasound, and serum 25-hydroxyvitamin D_3. Vitamin D_3 soft capsules (400IU per capsale) are purchased from Sinopharm star shark pharmaceutical (xiamen) co., LTD. An overview of the study design is shown in Figure 2 and Table 2.

Sample size

According to a previous study, the volume of UFs was 8.2 (2.1-30.5) cm^3 after the supplement of vitamin D 12 months and 11.4 (5.5-22.3) cm^3 in the control group after 12 months follow-up, respectively.[16] On the basis of a 0.9 power to detect a significant difference (α=0.05, one-sided), 320 participants will be required for the four groups in a 1:1:1:1 ratio. Allowing for a 10% withdrawal rate, we plan to include 360 patients in the whole trial (90 randomized to each arm).

Inclusion criteria

1. Patients are willing to cooperate with the follow-up and sign informed consent;

2. Married females aged 35-50 who are diagnosed as UFs by transvaginal ultrasonography;

3. The maximum average diameter of intramural myoma is ≤ 4cm，≥ 1cm;

4. Serum 25-hydroxyvitamin D_3 ≥12ng/ml, <30 ng/ml.

Exclusion criteria

1. Patients with heavy menstrual bleeding (>80.0 mL) per menstrual period, menstrual disorders, pelvic discomfort, infertility, or other indications for operation;

2. Patients complicated with leiomyoma degeneration and adenomyosis that were suspected or diagnosed by transvaginal ultrasound or gynecologic examination;

3. Allergic to vitamin D_3 soft capsules;

4. Use of sexual hormone, mifepristone, gonadotropin-releasing hormone agonist (GnRHa), or other medication which is likely to interfere with UFs in the past 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 (123μmol/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). The volume of the largest uterine leiomyoma (in

cm^3) was calculated with the following formula:

$$volume = \frac{4\pi}{3} \times \frac{a}{2} \times \frac{b}{2} \times \frac{c}{2} = \frac{\pi \cdot a \cdot b \cdot c}{6}$$

Secondary outcomes: percentage of subjects undergoing other medical or surgical treatment, hypercalcemia, urinary calculus, abnormal liver and renal function. Transvaginal ultrasound examinations will be performed by a well-experienced gynecologist. If possible, the same examiner should conduct all examinations for each subject throughout the study and the same ultrasound machine should be used 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. Patients with poor compliance;

4. Lost to follow-up;

5. Pregnancy;

6. Other medical or surgical treatments for UFs;

7. Receive other medical treatments which may affect the level of serum 25-hydroxyvitamin D_3 or other surgical treatments;

8. The level of serum calcium > 3.5 mmol/L or serum 25-hydroxyvitamin D_3 > 100 ng/mL.

Safety assessments

Safety of vitamin D administrated in patients with UFs will be assessed by the same methods as part I.

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 Random Number Table. The normal distribution of continuous variables is 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-Wallistest are used, respectively, to compare means of 2 and 3 or more groups of variables that are not normally distributed. The frequencies of categorical variables are compared using Pearson $\chi 2$ or Fisher's exact test, when appropriate. A value of $P<0.05$ is considered statistical significance.

Ethics and dissemination

The study has been approved by the ethics committee of the Second Affiliated Hospital of Wenzhou Medical University (No. LCKY2018-35) and registered in the United States National Institutes of Health Clinical Trials Registry: NCT03586947 and NCT03584529. The procedure will be performed following the principles described in the declaration of helsinki. We will publish the results of this study in peer-reviewed journals and related websites.

No Patient and Public Involvement

There were no funds or time allocated for patient and public involvement so we were unable to involve patients. We have invited patients to help us develop our dissemination strategy.

Discussion

There is an increasing awareness that vitamin D deficiency is associated with many health outcomes. Up to now, the roles of vitamin D in calcium homeostasis and bone health have been well characterized.[18] In the last decade, it has been recognized that vitamin D also prevented cardiovascular diseases, infections, adverse pregnancy outcomes and tumors.[19 20] Nevertheless, the cut-off thresholds for vitamin D deficiency and optimal levels are still controversial. The guideline of The Endocrine Society suggests vitamin D insufficiency is defined as a serum 25-hydroxyvitamin D_3 of 21–29

ng/ml, and vitamin D deficiency as a serum 25-hydroxyvitamin D_3 below 20 ng/ml. The guideline also recommends that patients with vitamin D deficiency should be treated with 50,000 IU of vitamin D once a week for 8 weeks to achieve a blood level of serum 25-hydroxyvitamin D_3 above 30 ng/ml, followed by maintenance therapy of 1500–2000 IU/day. Patients with vitamin D insufficiency require at least 600 IU/day of vitamin D for the prevention of vitamin D deficiency.[21] In fact, the level of serum 25-hydroxyvitamin D_3 varies from race to race. It is reported that caucasian females have higher serum 25-hydroxyvitamin D_3 concentrations than the others.[22 23] According to the guideline of Institute of Medicine, however, vitamin D deficiency is defined as a serum 25-hydroxyvitamin D_3 below 12 ng/ml, and vitamin D insufficiency as a serum 25-hydroxyvitamin D_3 of 12-20 ng/ml.[24] Specialists from Osteoporosis Committee of China Gerontological Society recommend that patients with high risks (history of osteoporosis, inadequate sun exposure, use of glucocorticoid, etc.) whose blood levels of serum 25-hydroxyvitamin D_3 are between 12 and 20 ng/ml should receive at least 600 IU/day vitamin D supplementation. Patients with blood levels of serum 25-hydroxyvitamin D_3 exceed 20 ng/ml could obtain an adequate amount of vitamin D from dietary sources and sun exposure. It is reported that the single-nucleotide polymorphisms in vitamin D receptor genes modified the efficacy of vitamin D_3 supplementation to increase circulating serum 25-hydroxyvitamin D_3 levels.[25] To determine the potential relationship between them, the vitamin D receptor genotype of all patients will be tested. Females over the age of 35 years are more likely to suffer from UFs. Furthermore, post menopause and pregnancy might affect the development and progression of UFs.[2] So the females aged 35-50 years are chosen in this trial. We will exclude patients who are pregnant, lactant, postmenopausal, or planned pregnancy within two years. In this study, patients with vitamin D deficiency or insufficiency will receive adequate vitamin D supplementation. Dietary vitamin D intake and other supplements of vitamin D will be limited.

In conclusion, this is the first study protocol of an open-label, randomised controlled trial to evaluate the efficacy and safety of vitamin D supplementation in preventing and inhibiting the UFs. However, our study should be interpreted within the

context of two limitations. First, the trial is not a double-blind, placebo-controlled trial. Furthermore, another limitation is that the trial is implemented in only one hospital. Notwithstanding these limitations, the results from this study will provide new evidences about vitamin D preparations in UFs from a well-designed trial. Once our hypothesis is confirmed, this study will provide a more effective, safe, and low-cost therapy in the prevention and treatment of UFs.

Author Contributions

X.Q.Z. is the principal investigator of this study and refined the protocol. B.S. and Y.Z.S. wrote the manuscript and contributed to the design of the study. B.S. will recruit the patients and conduct the trial. Y.Z.S., X.Q.Z. and Y.L. will supervise the trial. C.C.J., the medical statistician for the study, will contribute to the statistical design and analysis of data. All authors revised the protocol critically for important intellectual content and approved the final manuscript.

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Competing interests statement

All authors declare that they have no conflict of interest.

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Figure legends

Figure 1: Flow chart showing the steps of part Ⅰ in participant recruitment, treatment and analysis.

Figure 2: Flow chart showing the steps of part Ⅱ in participant recruitment, treatment and analysis.

Table 1: Flow chart of the study showing timing collection of different variables.

Follow-up (times)	1	2	3	4	5	6	7	8	9
Time points (Months)	0	3	6	9	12	15	18	21	24
Informed consent	X								
Medical history	X								
Physical examination	X	X	X	X	X	X	X	X	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
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 System ultrasound	X				X				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								

Table 2: Flow chart of the study showing timing collection of different variables.

Follow-up	1	2	3	4	5	6	7	8	9
Months	0	3	6	9	12	15	18	21	24
Informed consent	X								
Medical history	X								
Physical examination	X	X	X	X	X	X	X	X	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 System ultrasound	X				X				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								

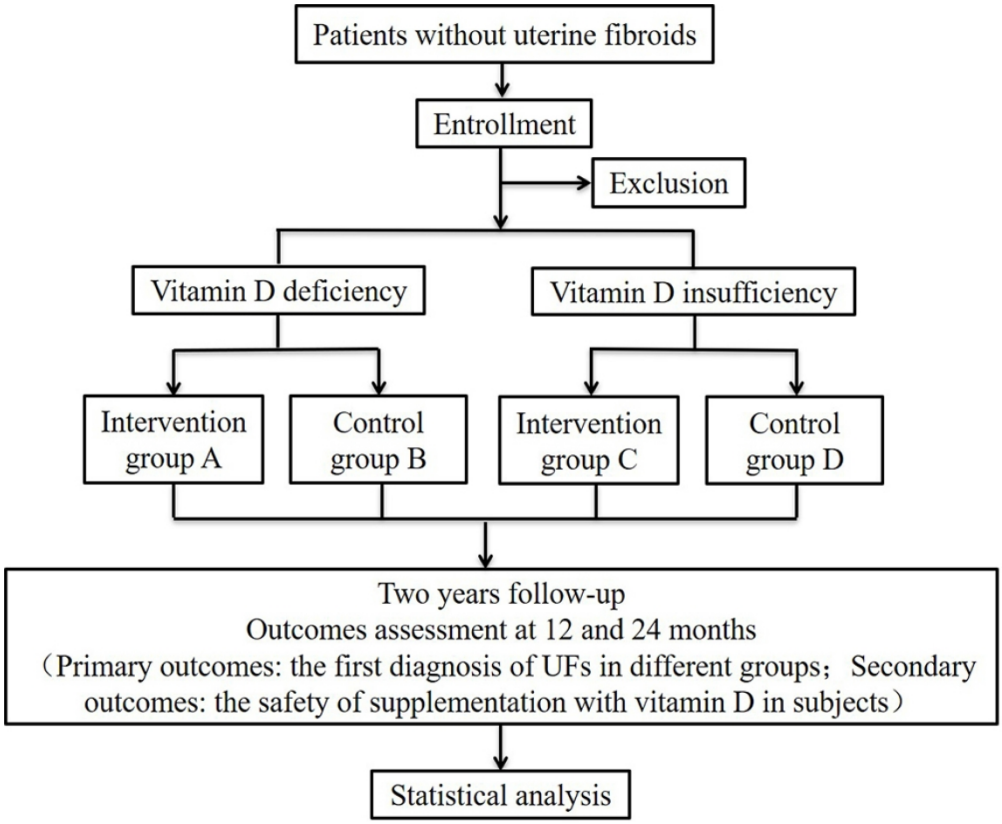

Figure 1: Flow chart showing the steps of part Ⅰ in participant recruitment, treatment and analysis.

352x291mm (300 x 300 DPI)

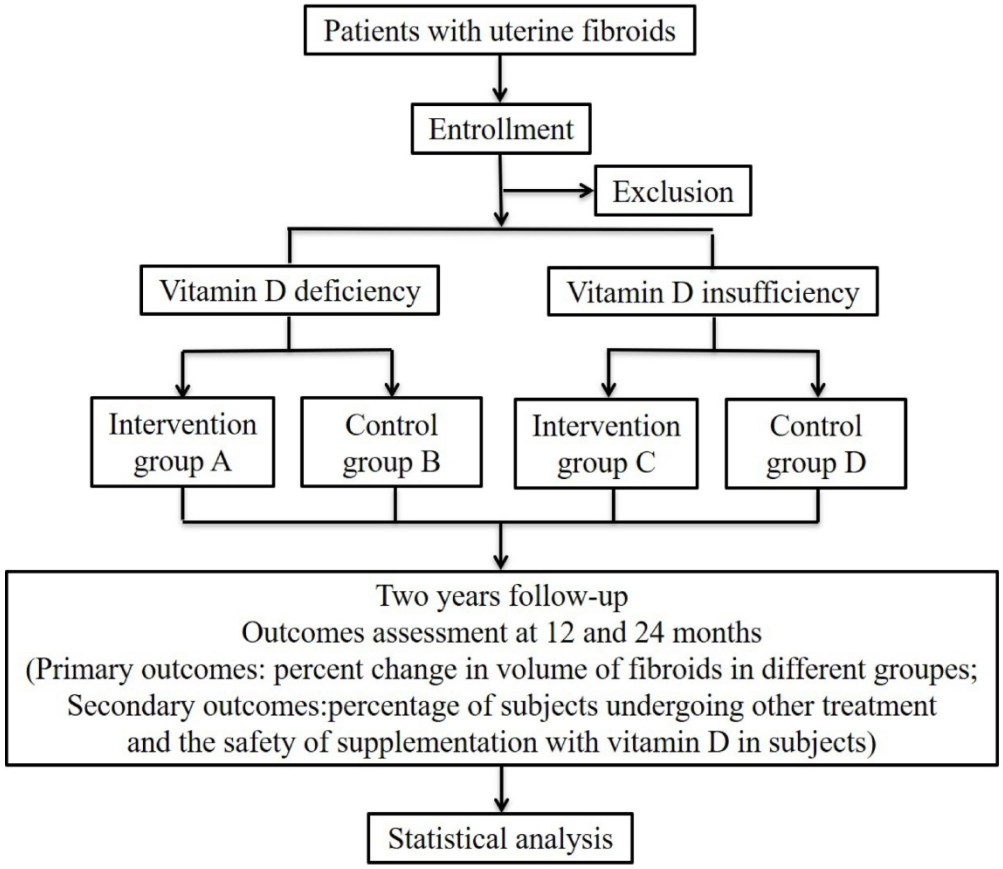

Figure 2: Flow chart showing the steps of part Ⅱ in participant recruitment, treatment and analysis.

208x181mm (300 x 300 DPI)

Reporting checklist for protocol of a clinical trial.

Based on the SPIRIT guidelines.

Instructions to authors

Complete this checklist by entering the page numbers from your manuscript where readers will find each of the items listed below.

Your article may not currently address all the items on the checklist. Please modify your text to include the missing information. If you are certain that an item does not apply, please write "n/a" and provide a short explanation.

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		Reporting Item	Page Number
Administrative information			
Title	#1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1

Trial registration	#2a	Trial identifier and registry name. If not yet registered, name of intended registry	2
Trial registration: data set	#2b	All items from the World Health Organization Trial Registration Data Set	n/a
Protocol version	#3	Date and version identifier	n/a
Funding	#4	Sources and types of financial, material, and other support	14
Roles and responsibilities: contributorship	#5a	Names, affiliations, and roles of protocol contributors	1
Roles and responsibilities: sponsor contact information	#5b	Name and contact information for the trial sponsor	14
Roles and responsibilities: sponsor and funder	#5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	14
Roles and responsibilities: committees	#5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	n/a

Introduction			
Background and rationale	#6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	4
Background and rationale: choice of comparators	#6b	Explanation for choice of comparators	5
Objectives	#7	Specific objectives or hypotheses	5
Trial design	#8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, non-inferiority, exploratory)	5
Methods: Participants, interventions, and outcomes			
Study setting	#9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	5
Eligibility criteria	#10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and	6, 7, 9, 10

		individuals who will perform the interventions (eg, surgeons, psychotherapists)	
Interventions: description	#11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	6, 9
Interventions: modifications	#11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving / worsening disease)	6, 9
Interventions: adherance	#11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return; laboratory tests)	6, 9
Interventions: concomitant care	#11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	6, 9
Outcomes	#12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	7, 10, 11
Participant timeline	#13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended	Figure 1 and 2

		(see Figure)	
Sample size	#14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	6, 9
Recruitment	#15	Strategies for achieving adequate participant enrolment to reach target sample size	6, 9
Methods: Assignment of interventions (for controlled trials)			
Allocation: sequence generation	#16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	12
Allocation concealment mechanism	#16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	12
Allocation: implementation	#16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	12

Blinding (masking)	#17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	n/a
Blinding (masking): emergency unblinding	#17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	n/a
Methods: Data collection, management, and analysis			
Data collection plan	#18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	19, 20
Data collection plan: retention	#18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	19, 20
Data management	#19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values).	19, 20

		Reference to where details of data management procedures can be found, if not in the protocol	
Statistics: outcomes	#20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	11, 12
Statistics: additional analyses	#20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	11, 12
Statistics: analysis population and missing data	#20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	11, 12
Methods: Monitoring			
Data monitoring: formal committee	#21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	n/a
Data monitoring: interim analysis	#21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	n/a
Harms	#22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial	n/a

		conduct	
Auditing	#23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	n/a
Ethics and dissemination			
Research ethics approval	#24	Plans for seeking research ethics committee / institutional review board (REC / IRB) approval	12
Protocol amendments	#25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC / IRBs, trial participants, trial registries, journals, regulators)	n/a
Consent or assent	#26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	6, 9
Consent or assent: ancillary studies	#26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	n/a
Confidentiality	#27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	19, 20
Declaration of	#28	Financial and other competing interests for principal	14

interests		investigators for the overall trial and each study site	
Data access	#29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	14
Ancillary and post trial care	#30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	n/a
Dissemination policy: trial results	#31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	14
Dissemination policy: authorship	#31b	Authorship eligibility guidelines and any intended use of professional writers	14
Dissemination policy: reproducible research	#31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	n/a
Appendices			
Informed consent materials	#32	Model consent form and other related documentation given to participants and authorised surrogates	n/a
Biological specimens	#33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	n/a

Notes:

- 10: 6, 7, 9, 10 The SPIRIT checklist is distributed under the terms of the Creative Commons Attribution License CC-BY-ND 3.0. This checklist was completed on 20. March 2020 using https://www.goodreports.org/, a tool made by the EQUATOR Network in collaboration with Penelope.ai

BMJ Open

Association between vitamin D and uterine fibroids: a study protocol of an open-label, randomised controlled trial

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Association between vitamin D and uterine fibroids: a study protocol of an open-label, randomised controlled trial

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Abstract

Introduction: Uterine fibroids are the most common pelvic benign tumor with no satisfactory long-term medical treatment. Recent studies have demonstrated that vitamin D significantly inhibited the growth of fibroids in vitro, vivo and a small-sample clinical trial. Therefore, the aim of this randomised clinical trial (RCT) is to evaluate whether supplementation with vitamin D could reduce the risk and inhibit the growth of uterine fibroids in reproductive stage women.

Methods and analysis: The open-label, RCT comprises two parts, including part I and part II. In part I, 2230 vitamin D deficiency or vitamin D insufficiency patients without uterine fibroids will be randomly assigned to two groups: intervention group (according to the level of serum 25-hydroxyvitamin D_3, receive 1600 or 800 IU/d of vitamin D_3 for 2 years) and control group (followed up at the same time points). By using gynecologic ultrasound examinations, the incidence of uterine fibroids will be employed to measure the outcome in different groups. In part II, 360 uterine fibroids patients with vitamin D deficiency or vitamin D insufficiency will be randomly assigned to intervention group or control group. According to the level of serum 25-hydroxyvitamin D_3, 180 patients will receive 1600 or 800 IU/d of vitamin D_3 for 2 years. Control group will receive regular follow-up. The outcome measure will be conducted using gynecologic ultrasound examinations to detect the growth of uterine fibroids in each group.

Ethics and dissemination: This study has been approved by the institutional review board of the Second Affiliated Hospital of Wenzhou Medical University (No. LCKY2018-35).

Trial registration number: ClinicalTrials.Gov, NCT03586947 and NCT03584529. Pre-results.

Keywords: Vitamin D; uterine fibroids; randomised clinical trial.

Strengths and limitations of this study

1. The results from this RCT will provide new evidences of the efficacy and safety of vitamin D for uterine fibroids patients.

2. One limitation is that the trial is not a double-blind, placebo-controlled trial and implemented in only one hospital.

3. Another limitation is that the trial is implemented in only one hospital in Chinese subjects, which may limit its generalizability.

Introduction

Uterine fibroids (UFs) are the most common benign tumor of the female genital tract, originating from smooth muscle cells.[1] Due to diverse diagnostic methods and the population source in many epidemiologic studies, the incidence of leiomyomas ranges from 5.4% to 77% of women in their reproductive years.[1] Because most patients with UFs remain asymptomatic, the actual incidence of UFs is assumed to be much higher than that reported. Based on the ultrasound screening, the incidence for UFs is reported to be 1.278% in Asia and 3.745% in African-American women per year.[1] The common symptoms of UFs include heavy menstrual bleeding, menstrual disorders and pelvic discomfort.[2] Furthermore, UFs are also associated with infertility and early pregnancy loss. The treatment for UFs depends on the size, location, symptoms, age and reproductive plans. Surgery is still the major treatment for symptomatic UFs including hysterectomy and myomectomy.[3 4] Uterine arterial embolization (UAE), one of the conservative interventional treatments with the longest track record, has become the major second line option for UFs patients who are properly selected.[3 4] However, these therapies increase the patients' operative complications and generates huge economic impact on healthcare systems. Except invasive surgical procedure, gonadotropin-releasing hormone agonist (GnRHa)[5 6] (eg, leuprorelin) and mifepristone[7 8] are the most commonly used medical agents for UFs in China. When these two drugs are stopped, UFs may re-grow rapidly.[9 10] Thus, GnRHa or mifepristone is usually used for the clinically symptomatic patients who are at a perimenopausal period, or who have contraindications of surgery. Selective progesterone receptor modulators (SPRMs) act by linkage with progesterone receptors in the smooth muscle of UFs, leading to inhibition of cell proliferation.[11] In fact, mifepristone is the first SPRM approved to treat UFs.[12] In addition to mifepristone, four types of SPRMs have been developed including asoprisnil, telapristone acetate, ulipristal acetate and vilaprisan.[13] However, these four SPRMs have not been licensed in China to date and are still being investigated in human clinical trials.[14] Therefore, it is still paramount to finding a novel nonsurgical alternative for UFs patients and prevent their occurrence.

Vitamin D is one of the essential nutrients for human bodies. Recent studies have considered that vitamin D is involved in the development of UFs.[10 15 16] For example, two groups have demonstrated that low levels of serum 25-hydroxyvitamin D_3 are linked with increased risk of UFs.[17 18] Blauer *et al.*[19] found that the growth of both primary myometrial and leiomyoma cells could be inhibited by 1,25-dihydroxyvitamin D_3 in a concentration-dependent way. Moreover, the process of fibrosis induced by the transforming growth factor-β3 (TGF-β3) could be attenuated by vitamin D in immortalized human UFs (HuLM) cells. In addition, vitamin D suppressed the protein expression of plasminogen activator inhibitor-1, which is an important TGF-β target in HuLM cells.[20] Such inhibitory effect of vitamin D on UFs is also verified in several in vivo studies. Halder *et al.*[21] reported that 1,25-dihydroxyvitamin D_3 decreased fibroid tumor size through downregulation of proliferation-related genes, antiapoptotic genes, estrogen and progesterone receptors in Eker rats. Two years later, Halder *et al.*[22] further found that the treatment with 1,25-dihydroxyvitamin D_3 or paricalcitol, an analog of 1,25-dihydroxyvitamin D_3 with lower calcemic activity, could reduce tumor size in mouse xenograft models of UFs. Most recently, one study by Corachan *et al.*[23] demonstrated that vitamin D inhibited the proliferation of human primary uterine leiomyoma cells via cell growth arrest induction and Wnt/β-catenin pathway downregulation. Furthermore, long-term vitamin D treatment significantly decreased the uterine leiomyoma size in a xenograft ovariectomized NOD-SCID mouse model,[24] which confirmed the inhibitory effect of vitamin D on UFs growth *in vivo*.

Our group has demonstrated that serum 25-hydroxyvitamin D_3 level was significantly lower in patients with UFs as compared to controls. In addition, patients with vitamin D deficiency had increased risks of UFs .[25] An open-label clinical trial indicated that the supplement of vitamin D in women with UFs stabilized the growth of fibroids and prevented the onset of its related symptoms.[26] But it was not a randomised trial and only 108 patients were included in the trail. A recently published randomized clinical trial found that vitamin D consumption did not significantly decrease the volume of fibroids in experimental group compared with control group.[27]

However, this new trial was completed shortly with a follow-up period of only 12 weeks. Hence, it is still unclear whether long-term supplementation of vitamin D could decrease the risk or inhibit the growth of UFs.

Therefore, we aim to conduct a randomised clinical trial and evaluate the effect and safety of administration with vitamin D on decreasing the risk and inhibiting the development of UFs in reproductive-aged women.

Methods and analysis

Study design

This is an open-label, randomised controlled trial. The study contains two parts (part I and part II) and will be conducted between May 31, 2020 and October 1, 2022 in the Second Affiliated Hospital of Wenzhou Medical University, a hospital in China. Part I is to investigate the effect of supplementation with vitamin D on the risk of UFs. Part II is about the association between vitamin D supplementation and the development of UFs. The regimen of vitamin D doses from several international guidelines and important published clinical trials are listed in Table 1.

Part I: Efficacy of vitamin D on the risk of UFs

Study objectives

The primary objective of this part is to assess the efficacy of supplementation with vitamin D on decreasing the risk of incident UFs within 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, vitamin D deficiency patients (12 ng/ml ≤ serum 25-hydroxyvitamin D_3 ≤ 20 ng/ml) without UFs will be randomly assigned in a 1:1 ratio to either the intervention group A or the control group B. Intervention group A will receive an oral dose of 1600 IU (4 capsules)/day vitamin D_3 for up to 2 years. Control group B will receive 2 years follow-up. Patients with vitamin D insufficiency (21 ng/ml ≤ Serum 25-hydroxyvitamin D_3 ≤ 29 ng/ml) without UFs 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 (2 capsules)/day vitamin D_3 for up to 2 years. Control group D will receive 2 years follow-up. Gynecologic ultrasound examinations will be performed every six months. The number, location and size of the UFs will be documented. The safety of subjects will be evaluated, including blood routine examination, electrolyte, hepatic and renal function, liver and urinary system ultrasound, and serum 25-hydroxyvitamin D_3. Vitamin D receptor genotype of all patients will also be tested. Vitamin D_3 soft capsules (400 IU per capsule) are purchased from Sinopharm star shark pharmaceutical (Xiamen, China) co., LTD and can be preserved for 2 years. An overview of the study design is shown in Figure 1 and Table 2.

Sample size

The planned sample size is estimated based on the data from a previous study, in which the UFs incidence was 1.278% per year in Asia and 3.745% per year in African-American women. Women over the age of 40 years are more likely to have UFs.[1] A study also revealed that African-American females had lower level of serum 25-hydroxyvitamin D_3 as compared to Caucasian females.[28] Vitamin D deficiency is shown to increase the risk of UFs in vitro, in vivo animal models and in a small-sample clinical trial. We assume a one-tailed α error of 0.05 and a power (1-β) of 0.8. If the rates are 3.745% for the control group and 1.278% for the intervention group, we allow for a dropout rate of 10% for an effective sample size of 2108 and propose to enroll 2320 participants (580 randomized to each arm).

Inclusion criteria

1. Volunteers to participate in the study with an informed consent;

2. Married females aged 35-50 who are confirmed with a normal, fibroid-free uterine structure, by means of transvaginal ultrasonography;

3. Serum 25-hydroxyvitamin D_3 ≥12 ng/ml, ≤ 29 ng/ml.

Exclusion criteria

1. Women with Serum 25-hydroxyvitamin D_3 <12 ng/ml or >29 ng/ml.

2. BMI <18.5 kg/m^2 or BMI >25 kg/m^2.

3. Use of sexual hormone, mifepristone, GnRHa, or other medications which are

likely to interfere with UFs in the past 3 months;

4. Pregnancy, lactation, postmenopause, or planned pregnancy within two years;

5. Allergic to vitamin D_3 soft capsules;

6. Suspected or identified as other tumors of genital tract;

7. History of hysterectomy or myomectomy;

8. History of osteoporosis or vitamin D deficiency taking vitamin D supplements constantly within previous one month;

9. History of hyperparathyroidism, infectious diseases (tuberculosis, Acquired immunodeficiency syndrome), autoimmune diseases, or digestive system diseases (malabsorption, crohn disease and dysentery);

10. 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;

11. Creatinine levels ≥1.4 mg/dl (123 μmol/l) or creatinine clearance ≤ 50 ml/min;

12. History of malignant tumors;

13. Simultaneous participation in another clinical study with investigational medicinal product(s).

Outcomes measures

The primary outcome is the first diagnosis of UFs in different groups. The secondary outcomes include hypercalcemia, abnormal liver and renal function, and urinary calculus in different groups. Transvaginal ultrasound examinations will be performed by a well-experienced physician in gynecology. If possible, the same examiner should conduct all examinations of a subject and the same ultrasound machine should be used 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. Patients with poor compliance;

4. Lost to follow-up;

5. Pregnancy;

6. Receive other medical treatments which may affect the level of serum 25-hydroxyvitamin D3 or other surgical treatments;

7. The level of serum calcium >3.5 mmol/L or serum 25-hydroxyvitamin D3 >150 ng/ml.

Safety assessments

Safety of vitamin D administrated in patients without UFs will be assessed by renal and liver function test, serum electrolyte (sodium, chloride, potassium, calcium, and phosphorus), blood routine test, and serum 25-hydroxyvitamin D_3. Urine pregnancy test and serum 25-hydroxyvitamin D_3 level will be detected every three months. Other indicators will be 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. Patients will be withdrawn 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.

Part II: Association between vitamin D and the development of UFs.

Study objectives

The primary objective of this part is to assess the association of supplementation with vitamin D on inhibiting the development of UFs within one year and two years. The secondary objective of part II is to evaluate the safety of supplementation with vitamin D in UFs subjects.

Trial design

After signing of informed consent, UFs patients with vitamin D deficiency (12 ng/ml ≤ Serum 25-hydroxyvitamin D_3 ≤ 20 ng/ml) 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 (4 capsules)/day vitamin D_3 for up to 2 years. Control group B will receive 2-year follow-up. UFs patients with vitamin D insufficiency (21 ng/ml ≤ Serum 25-hydroxyvitamin D_3 ≤ 29 ng/ml) 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 (2 capsules)/day vitamin D_3 for up to 2 years. Control group D will receive 2-year follow-up. Gynecologic ultrasound examinations will be performed every three months. The number, location and size of the UFs will be documented (the transverse, longitudinal, and antero-posterior diameters of fibroids will be documented at each efficacy ultrasound examination for volume calculation). The safety of vitamin D in subjects with UFs will be evaluated, including blood routine examination, serum electrolyte, hepatic and renal function, liver and urinary system ultrasound, and serum 25-hydroxyvitamin D_3. Vitamin D_3 soft capsules (400 IU per capsale) are purchased from Sinopharm star shark pharmaceutical (Xiamen, China) co., LTD. An overview of the study design is shown in Figure 2 and Table 3.

Sample size

According to a previous study, the volume of UFs was 8.2 (2.1-30.5) cm^3 after the supplement of vitamin D for 12 months and 11.4 (5.5-22.3) cm^3 in the control group after 12 months follow-up, respectively.[26] On the basis of a 0.9 power to detect a significant difference (α=0.05, one-sided), 320 participants will be required for the four groups in a 1:1:1:1 ratio. Allowing for a 10% withdrawal rate, we plan to enroll 360 patients in the whole trial (90 randomized to each arm).

Inclusion criteria

1. Patients are willing to cooperate with the follow-up and sign an informed consent;
2. Married females aged 35-50 who are diagnosed as UFs by transvaginal ultrasonography;
3. The maximum average diameter of intramural myoma is ≤ 4 cm，≥ 1 cm;
4. Serum 25-hydroxyvitamin D_3 ≥12 ng/ml, ≤29 ng/ml.

Exclusion criteria

1. Women with Serum 25-hydroxyvitamin D_3 <12 ng/ml or >29 ng/ml.

2. BMI <18.5 kg/m^2 or BMI >25 kg/m^2.

3. Patients with heavy menstrual bleeding (>80.0 ml) per menstrual period, menstrual disorders, pelvic discomfort, infertility, or other indications for operation;

4. Patients complicated with leiomyoma degeneration and adenomyosis that were suspected or diagnosed by transvaginal ultrasound or gynecologic examination;

5. Allergic to vitamin D_3 soft capsules;

6. Use of sexual hormone, mifepristone, GnRHa, or other medication which is likely to interfere with UFs in the past 3 months;

7. Pregnancy, lactation, postmenopause, or planned pregnancy within two years;

8. Suspected or identified as other tumors of genital tract;

9. History of osteoporosis or vitamin D deficiency taking vitamin D supplements within previous one month;

10. History of autoimmune diseases, infectious diseases (tuberculosis, AIDS), autoimmune diseases, digestive system diseases (malabsorption, crohn disease, and dysentery);

11. ALT or AST more than 3 times of the normal upper limit,TBIL more than 2 times of the normal upper limit;

12. Creatinine levels ≥1.4 mg/dl (123 μmol/l) or creatinine clearance ≤50 ml/min;

13. History of malignant tumors;

14. Some cases those uteruses are difficult to scan or the amount of UFs is more than 4.

15. Simultaneous participation in another clinical study with investigational medicinal product(s).

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). The volume of the largest uterine leiomyoma (in cm^3) was calculated with the following formula:

$$volume = \frac{4\pi}{3} \times \frac{a}{2} \times \frac{b}{2} \times \frac{c}{2} = \frac{\pi \cdot a \cdot b \cdot c}{6}$$

Secondary outcomes: percentage of subjects undergoing other medical or surgical treatment, hypercalcemia, urinary calculus, abnormal liver and renal function. Transvaginal ultrasound examinations will be performed by a well-experienced gynecologist. If possible, the same examiner should conduct all examinations for each subject throughout the study and the same ultrasound machine should be used 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. Patients with poor compliance;

4. Lost to follow-up;

5. Pregnancy;

6. Other medical or surgical treatments for UFs;

7. Receive other medical treatments which may affect the level of serum 25-hydroxyvitamin D_3 or other surgical treatments;

8. The level of serum calcium >3.5 mmol/L or serum 25-hydroxyvitamin D_3 >150 ng/ml.

Safety assessments

Safety of vitamin D administrated in patients with UFs will be assessed by the same methods as part I.

Treatment compliance assessment

All research medications (Vitamin D3 soft capsules) should be recorded. Standard with good compliance is defined as: 80%≤ Actual oral dose/dose * 100% ≤120%. Criteria for poor compliance is as follows: Actual oral dose/dose * 100% ≤80% or

Actual oral dose/dose * 100% ⩾ 120%. Each follow-up should be based on the number of returned study drugs to determine the drug compliance. Subjects should return all unused research drugs and empty packages of used drugs every follow-up.

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 Random Number Table. The normal distribution of continuous variables is 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-Wallistest are used, respectively, to compare means of 2 and 3 or more groups of variables that are not normally distributed. The frequencies of categorical variables are compared using Pearson $\chi 2$ or Fisher's exact test, when appropriate. A value of $P < 0.05$ is considered statistical significance.

Ethics and dissemination

The study has been approved by the ethics committee of the Second Affiliated Hospital of Wenzhou Medical University (No. LCKY2018-35) and registered in the United States National Institutes of Health Clinical Trials Registry: NCT03586947 and NCT03584529. The procedure will be performed following the principles described in the declaration of helsinki. We will publish the results of this study in peer-reviewed journals and related websites.

No Patient and Public Involvement

There were no funds or time allocated for patient and public involvement so we were unable to involve patients. We have invited patients to help us develop our dissemination strategy.

Discussion

There is an increasing awareness that vitamin D deficiency is associated with many health outcomes. Up to now, the roles of vitamin D in calcium homeostasis and

bone health have been well characterized.[29] In the last decade, it has been recognized that vitamin D also prevented cardiovascular diseases, infections, adverse pregnancy outcomes and tumors.[30] [31] Nevertheless, the cut-off thresholds for vitamin D deficiency and optimal levels are still controversial. The guideline of The Endocrine Society suggests vitamin D insufficiency is defined as a serum 25-hydroxyvitamin D_3 of 21–29 ng/ml, and vitamin D deficiency as a serum 25-hydroxyvitamin D_3 below 20 ng/ml.[32] The guideline also recommends that patients with vitamin D deficiency should be treated with 50,000 IU of vitamin D once a week for 8 weeks to achieve a blood level of serum 25-hydroxyvitamin D_3 above 30 ng/ml, followed by maintenance therapy of 1500–2000 IU/day. Patients with vitamin D insufficiency require at least 600 IU/day of vitamin D for the prevention of vitamin D deficiency.[32] In fact, the level of serum 25-hydroxyvitamin D_3 varies from race to race. It is reported that caucasian females have higher serum 25-hydroxyvitamin D_3 concentrations than the others.[33] [34] According to the guideline of Institute of Medicine, however, vitamin D deficiency is defined as a serum 25-hydroxyvitamin D_3 below 12 ng/ml, and vitamin D insufficiency as a serum 25-hydroxyvitamin D_3 of 12-20 ng/ml.[35] Specialists from Osteoporosis Committee of China Gerontological Society recommend that patients with high risks (history of osteoporosis, inadequate sun exposure, use of glucocorticoid, etc.) whose blood levels of serum 25-hydroxyvitamin D_3 are between 12 and 20 ng/ml should receive at least 600 IU/day vitamin D supplementation.[36] Patients with blood levels of serum 25-hydroxyvitamin D_3 exceed 20 ng/ml could obtain an adequate amount of vitamin D from dietary sources and sun exposure.[36] It is reported that the single-nucleotide polymorphisms in vitamin D receptor genes modified the efficacy of vitamin D_3 supplementation to increase circulating serum 25-hydroxyvitamin D_3 levels.[37] To determine the potential relationship between them, the vitamin D receptor genotype of all patients will be tested. Females over the age of 35 years are more likely to suffer from UFs. Furthermore, post menopause and pregnancy might affect the development and progression of UFs.[1] So the females aged 35-50 years are chosen in this trial. We will exclude patients who are pregnant, lactant, postmenopausal, or planned pregnancy within two years. In this study,

patients with vitamin D deficiency or insufficiency will receive adequate vitamin D supplementation. Dietary vitamin D intake and other supplements of vitamin D will be limited.

In conclusion, this is the first study protocol of an open-label, randomised controlled trial to evaluate the efficacy and safety of vitamin D supplementation in preventing and inhibiting the UFs. However, our study should be interpreted within the context of two limitations. First, the trial is not a double-blind, placebo-controlled trial. Furthermore, another limitation is that the trial is implemented in only one hospital. Notwithstanding these limitations, the results from this study will provide new evidences about vitamin D preparations in UFs from a well-designed trial. Once our hypothesis is confirmed, this study will provide a more effective, safe, and low-cost therapy in the prevention and treatment of UFs.

Author Contributions

X.Q.Z. is the principal investigator of this study and refined the protocol. B.S. and Y.Z.S. wrote the manuscript and contributed to the design of the study. B.S. will recruit the patients and conduct the trial. Y.Z.S., X.Q.Z. and Y.L. will supervise the trial. C.C.J., the medical statistician for the study, will contribute to the statistical design and analysis of data. All authors have revised the protocol critically for important intellectual content and approved the final manuscript.

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Competing interests statement

All authors declare that they have no conflict of interest.

Word Count: 3900

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Figure legends

Figure 1: Flow chart showing the steps of part Ⅰ in participant recruitment, treatment and analysis.

Figure 2: Flow chart showing the steps of part Ⅱ in participant recruitment, treatment and analysis.

Table 1: The doses of vitamin D supplementation in different clinical trials published since 2010.

Group	Country	Population	Age (years)	Oral vitamin D	Reference
Endocrine Society	USA	General population	>19	Risk of vitamin D deficiency: 1500-2000 IU/day; Vitamin D deficiency: 50,000 IU/wk for 8 wk followed by maintenance therapy of 1500-2000 IU/day	[32]
Boer et al.	USA	Participants with type 2 diabetes	Men aged ≥50 and women aged ≥55	Vitamin D_3 (cholecalciferol 2000 IU/day) and matching inert placebo	[38]
Burt et al.	Canada	Healthy adults without osteoporosis	55-70	Vitamin D_3 at 400 IU/day (n=109), or 4000 IU/day (n=100), or 10,000 IU/day (n=102) for 3 years	[39]
Urashima et al.	Japan	Patients with digestive tract cancers ovarall	30-90	Vitamin D 2000 IU/day and matching placebo	[40]
Scragg et al.	New Zealand	General population	50-84	Vitamin D at initial bolus dose of 200,000 IU, followed by maintenance dose of 100,000 IU/month	[41]
Aglipay et al.	Canada	Children	1-5	Vitamin D at doses of 2000 IU/day (n=349) and 400 IU/day (n=354)	[42]
Zittermann et al.	Germany	Heart failure patients	18-79	Patient with serum 25-hydroxyvitamin D_3 level <75 nmol/l were randomized to receive 4000 IU/day vitamin D or matching placebo for 3 years	[43]
Lappe et al.	USA	Postmenopausal women	≥55	Treatment group: 2000 IU/d vitamin D_3 and	[44]

				1500 mg/d calcium; The placebo group: identical placebos	
Arora et al.	USA	Low vitamin D status (25-hydroxyvitamin D levels ≤25 ng/mL) patients with SBP of 120-159 mmHg	18-50	High-dose group: 4000 IU/day for 6 months; Low-dose group: 400 IU/day for 6 months	45
Rusinska et al.	Poland	Healthy adults	19-65	800-2000 IU/day	46

Abbreviations: SBP, systolic blood pressure.

Table 2: Flow chart of the study showing timing collection of different variables.

Follow-up (times)	1	2	3	4	5	6	7	8	9
Time points (Months)	0	3	6	9	12	15	18	21	24
Informed consent	X								
Medical history	X								
Physical examination	X	X	X	X	X	X	X	X	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
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 System ultrasound	X				X				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								

Table 3: Flow chart of the study showing timing collection of different variables.

Follow-up	1	2	3	4	5	6	7	8	9
Months	0	3	6	9	12	15	18	21	24
Informed consent	X								
Medical history	X								
Physical examination	X	X	X	X	X	X	X	X	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 System ultrasound	X				X				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								

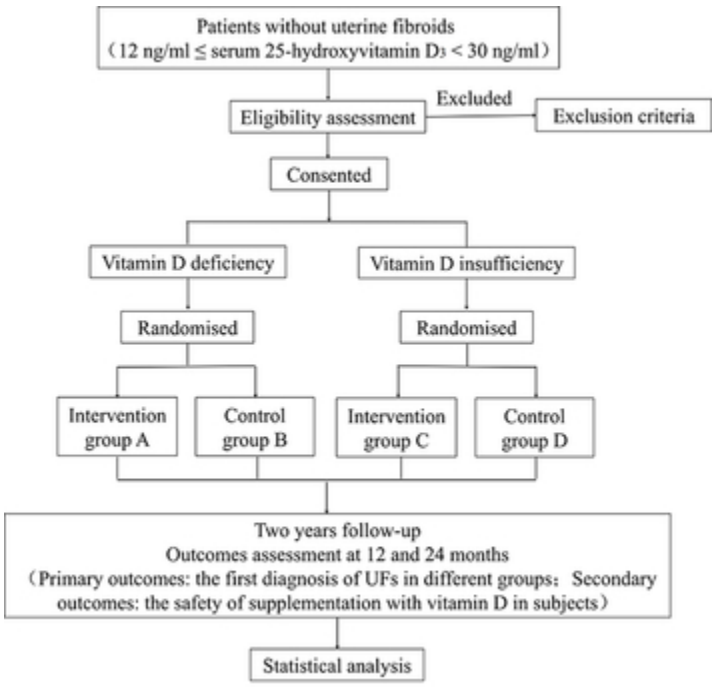

Figure 1: Flow chart showing the steps of part Ⅰ in participant recruitment, treatment and analysis.

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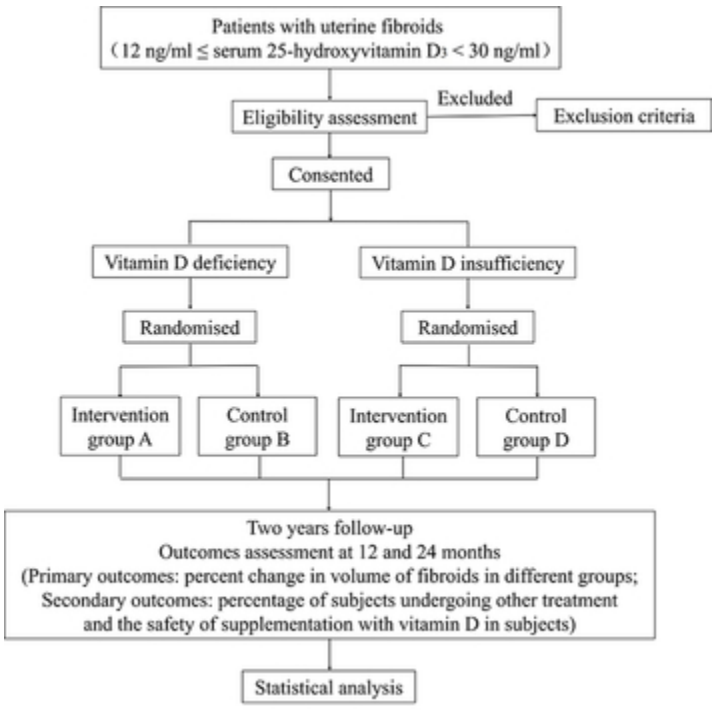

Figure 2: Flow chart showing the steps of part Ⅱ in participant recruitment, treatment and analysis.

29x29mm (300 x 300 DPI)

Reporting checklist for protocol of a clinical trial.

Based on the SPIRIT guidelines.

Instructions to authors

Complete this checklist by entering the page numbers from your manuscript where readers will find each of the items listed below.

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		Reporting Item	Page Number
Administrative information			
Title	#1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1

Trial registration	#2a	Trial identifier and registry name. If not yet registered, name of intended registry	2
Trial registration: data set	#2b	All items from the World Health Organization Trial Registration Data Set	n/a
Protocol version	#3	Date and version identifier	n/a
Funding	#4	Sources and types of financial, material, and other support	14
Roles and responsibilities: contributorship	#5a	Names, affiliations, and roles of protocol contributors	1
Roles and responsibilities: sponsor contact information	#5b	Name and contact information for the trial sponsor	14
Roles and responsibilities: sponsor and funder	#5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	14
Roles and responsibilities: committees	#5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	n/a

Introduction			
Background and rationale	#6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	4
Background and rationale: choice of comparators	#6b	Explanation for choice of comparators	5
Objectives	#7	Specific objectives or hypotheses	5
Trial design	#8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, non-inferiority, exploratory)	5
Methods: Participants, interventions, and outcomes			
Study setting	#9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	5
Eligibility criteria	#10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and	6, 7, 9, 10

		individuals who will perform the interventions (eg, surgeons, psychotherapists)	
Interventions: description	#11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	6, 9
Interventions: modifications	#11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving / worsening disease)	6, 9
Interventions: adherance	#11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return; laboratory tests)	6, 9
Interventions: concomitant care	#11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	6, 9
Outcomes	#12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	7, 10, 11
Participant timeline	#13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended	Figure 1 and 2

		(see Figure)	
Sample size	#14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	6, 9
Recruitment	#15	Strategies for achieving adequate participant enrolment to reach target sample size	6, 9
Methods: Assignment of interventions (for controlled trials)			
Allocation: sequence generation	#16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	12
Allocation concealment mechanism	#16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	12
Allocation: implementation	#16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	12

Blinding (masking)	#17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	n/a
Blinding (masking): emergency unblinding	#17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	n/a
Methods: Data collection, management, and analysis			
Data collection plan	#18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	19, 20
Data collection plan: retention	#18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	19, 20
Data management	#19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values).	19, 20

		Reference to where details of data management procedures can be found, if not in the protocol	
Statistics: outcomes	#20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	11, 12
Statistics: additional analyses	#20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	11, 12
Statistics: analysis population and missing data	#20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	11, 12
Methods: Monitoring			
Data monitoring: formal committee	#21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	n/a
Data monitoring: interim analysis	#21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	n/a
Harms	#22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial	n/a

		conduct	
Auditing	#23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	n/a
Ethics and dissemination			
Research ethics approval	#24	Plans for seeking research ethics committee / institutional review board (REC / IRB) approval	12
Protocol amendments	#25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC / IRBs, trial participants, trial registries, journals, regulators)	n/a
Consent or assent	#26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	6, 9
Consent or assent: ancillary studies	#26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	n/a
Confidentiality	#27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	19, 20
Declaration of	#28	Financial and other competing interests for principal	14

interests		investigators for the overall trial and each study site	
Data access	#29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	14
Ancillary and post trial care	#30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	n/a
Dissemination policy: trial results	#31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	14
Dissemination policy: authorship	#31b	Authorship eligibility guidelines and any intended use of professional writers	14
Dissemination policy: reproducible research	#31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	n/a
Appendices			
Informed consent materials	#32	Model consent form and other related documentation given to participants and authorised surrogates	n/a
Biological specimens	#33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	n/a

Notes:

- 10: 6, 7, 9, 10 The SPIRIT checklist is distributed under the terms of the Creative Commons Attribution License CC-BY-ND 3.0. This checklist was completed on 20. March 2020 using https://www.goodreports.org/, a tool made by the EQUATOR Network in collaboration with Penelope.ai