

Research Article

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Ginger (*Zingiber officinale*) and turmeric (*Curcuma longa* L.) supplementation effects on quality of life, body composition, bone mineral density and osteoporosis related biomarkers and micro-RNAs in women with postmenopausal osteoporosis: a study protocol for a randomized controlled clinical trial

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Abstract

Objectives: Phytomedicine is widely suggested for the prevention of chronic disease, but evidence for a favorable effect on bone health is lacking. The present study will investigate the *Zingiber officinale* (ZO) and *Curcuma longa* L. (CL) supplementation effects on quality of life, body composition, bone mineral density (BMD) and osteoporosis related biomarkers and micro-RNAs in women with postmenopausal osteoporosis (PMO).

Methods: This study protocol is designed as prospective triple-blind randomized controlled trial. One hundred and 20 patients with PMO will be enrolled in a 4 month, prospective, triple-blind, placebo-controlled trial and

randomly assigned to four groups: ZO (500 mg b.i.d.) + CL (500 mg b.i.d.) (ZO + CL); ZO (500 mg b.i.d.) + placebo CL (b.i.d.) (ZO + P); placebo ZO (b.i.d.) + CL (500 mg b.i.d.) (CL + P); and placebo ZO (b.i.d.) + placebo CL (b.i.d.) (P + P). Quality of life, body composition and BMD will be defined as the primary endpoints and osteoporosis related serum biomarkers and circulating micro-RNAs will be defined as the secondary endpoints. The ANCOVA statistical method will be used to investigate the effect of the interventional variables on the response variable.

Conclusion: To our knowledge, this trial is the first clinical study exploring the effect of Ginger and turmeric on quality of life, body composition, BMD and osteoporosis related biomarkers and micro-RNAs in women with PMO. The findings of this trial could be the basis for the development of harmless and inexpensive preventive and therapeutic approaches for PMO.

Keywords: biomarker; erginger; micro-RNA; postmenopausal osteoporosis; turmeric.

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Trial registration

Iranian Clinical Trial Registry, Identifier: IRCT20161022030424N3, Registered on 29 April 2018.

Trial status

The study has been planned in 2017 and registered in Iranian Clinical Trial Registry, IRCT20161022030424N3, on 29

April 2018. Participant recruitment began on August 1, 2018, and 105 participants have been recruited until now. Recruitment will be completed in March 2020.

Background

Osteoporosis (OP) is the most common metabolic bone disease and characterized with reduced bone mass, which may result in fragile and brittle bones [1]. Studies have confirmed the increasing trend of the occurrence and prevalence of OP and its effect on life, especially in women [2]. High incidence and disabling complications make OP one of the most important health issues in Iran, which requires attention and planning for screening, treatment and prevention.

Generally, in addition to conventional therapies, hormone therapy is used for the prevention and treatment of OP after menopause, but it can lead to serious side effects [3, 4]. Also, bisphosphonates are increasingly used to manage postmenopausal OP (PMO), although these drugs have been linked to esophageal cancer and osteonecrosis of the jaw [5, 6]. Therefore, there is an urgent need for more extensive research on the discovery and development of healthy foods containing natural compounds that can effectively compensate for estrogen deficiency [7].

The use of ginger as a traditional treatment in many countries is old enough. Ginger extract is used to manage various conditions such as inflammation, infection, constipation, hyperlipidemia, blood pressure and metabolic syndrome [8–13]. According to Fan JZ et al. [14] 6-gingerol treatment did not have significant effects on mineralization, but led to the differentiation of osteoblast-like cells and increased transection of osteogenic markers, increased alkaline phosphatase activity, and enhanced mineralization of nodule production.

Curcumin, as a major active component of Turmeric (*Curcuma longa*), is potentially anti-inflammatory [15] and has anti-arthritis properties [16]. According to Tsuji-Naito K et al. [17], turmeric inhibited the formation of osteoclast-like cells in concentrations of 12.5–50 $\mu\text{g}/\text{mL}$, which could be a new approach for treating patients with OP. Recently, Khanizadeh et al. [18] evaluated the effects of combination therapy of curcumin (110 mg/day) and alendronate (5 mg/day) for 12 months in comparison with alendronate and control (calcium supplement) on bone mineral density (BMD) and bone turnover markers in 60 women with PMO. Combination therapy significantly decreased bone-specific alkaline phosphatase and C-terminal cross-linking telopeptide of type I collagen concentrations and increased osteocalcin levels and BMD indexes (in four areas) at the end of trial in comparison with alendronate and control

groups. However, data on the impact of Ginger and turmeric supplementation separately and together on clinical outcome in women in PMO are scarce.

Given the cost-effectiveness of using the herbal compounds compared to the drug products, as well as the lack of sufficient clinical studies on human cases with OP, we decided to examine the effects of the use of Ginger (*Zingiber officinale*) and turmeric (*Curcuma longa* L.) supplements alone and in combination in postmenopausal women with OP. Based on our knowledge and the search made on reputable scientific sites, a clinical trial that examines the effects of turmeric and ginger supplement alone and in combination in PMO has not been performed so far.

Methods

Study design

The present study is prospective, randomized, triple-blind, placebo-controlled parallel group clinical trial to evaluate whether Ginger (*Zingiber officinale*) and turmeric (*Curcuma longa* L.) supplementation for 4 months can improve quality of life (QOL), body composition, BMD and osteoporosis related biomarkers and micro-RNAs in women with PMO, in which the researcher, the participants and the analyst should be blind to the group assignment and received intervention and treatment. Sampling started from August 2018 (Figure 1).

Participants

Sampling

The participants from the Centers for Disease Control (Physical Medicine and Rehabilitation Outpatient Clinics of Tabriz University of Medical Sciences), who are diagnosed

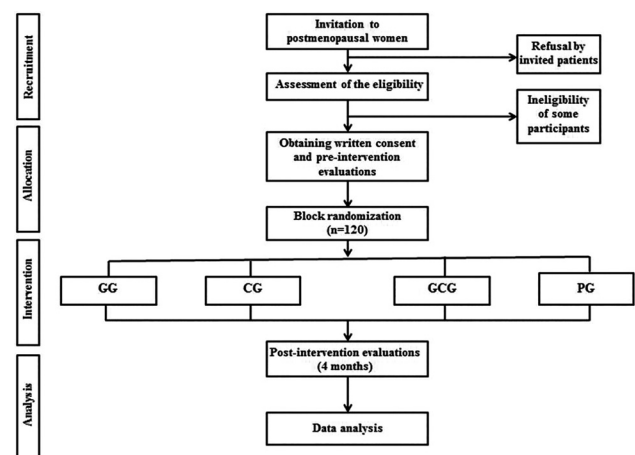


Figure 1: Participant flow diagram.

with OP, will be contacted to provide information about the project and its methods and objectives. OP is established when the BMD value is 2.5 or more standard deviations below the mean of a young reference population (T-score). They will be presented and then invited to participate in the initial assessment.

After selecting and obtaining informed consent by a trained technician of physical medicine and rehabilitation research center, the patients will be randomly assigned to receive 1) Ginger supplement 2) Turmeric supplement 3) Ginger-Turmeric supplement and 4) Placebo for 4 months. Other evaluations planned for the pre-intervention period will be conducted for participants who meet the criteria for inclusion and exclusion criteria.

Inclusion criteria

Natural menopause, primary OP, age ≥ 45 years, menstrual cessation for 12 consecutive months, low BMD (T-score < -2.5), no history of fracture, and verbal communication ability to answer questions.

Exclusion criteria

Use of oral contraceptives or corticosteroids during the study, kidney failure and diseases, metastatic bone diseases, coagulation disorders, unwillingness to continue the study, taking medications affecting bone metabolism other than taking calcium-D supplements with the same dose prescribed to the whole participants, mental disease and malignancies.

Intervention group 1

Vomigan tablets made by Dineh Iran Pharmaceutical Company containing 470 ± 30 mg of *Zingiber officinale* Rhizome powder two daily, plus Curcuma Placebo (flavored maltodextrin powder) Tablet, made by Dineh Pharmaceutical Company, which is similar to the curcuma tablet in terms of shape, size, taste, smell and other apparent qualities and does not contain powder and extract of turmeric, two daily with food in addition to routine treatment for 4 months.

Intervention group 2

Curcuma tablet made by Iran Dineh Pharmaceutical Company contains 450 mg powder of rhizome turmeric and

50 mg of turmeric extract two daily, plus Vomigan placebo (flavored maltodextrin powder) tablets, made by Dineh pharmaceutical company, which in terms of shape, size, taste, odor and other apparent characteristics are quite similar to Vomigan tablets and is free of zinger powder, two daily with food in addition to routine treatment for four months.

Intervention group 3

Vomigan tablets made by Dineh Iran Pharmaceutical Company containing 470 ± 30 mg of *Zingiber officinale* Rhizome powder two per day with food, plus curcuma tablets of Dineh Iran Pharmaceutical Company containing 450 mg of powdered rhizome turmeric and 50 mg of Turmeric extract two daily with food in addition to routine treatment for 4 months.

Control group

Curcuma placebo tablets (flavored maltodextrin powder) which in terms of shape, size, taste, smell and other exfoliation characteristics are quite similar to the curcuma tablet, are free of powdered and turmeric extract, two daily with food plus Vomigan placebo tablets (flavored maltodextrin powder), which in terms of shape, size, taste, smell is quite similar to Vomigan and does not contain ginger powder, two daily with food in addition to routine treatment for 4 months.

Participants will be requested to restrict their intake of ginger and turmeric from diet throughout the study period.

Outcomes

Primary outcomes

Determining and comparing differences in score changes in QOL, body composition (BC) and bone mineral density (BMD) in the postmenopausal women with OP who received ginger, turmeric, turmeric-ginger supplements, and placebo.

Secondary outcomes

Determining and comparing differences in serum levels of OP associated biomarkers [Osteocalcin, Procollagen type 1 amino-terminal propeptide (P1NP) and Carboxy-Terminal

cross-linked telopeptide of type 1 collagen (CTX)], and the circulating levels of OP-related micro-RNAs (miR422a, miR-133a, miR-21 and miR-503) in postmenopausal women with OP who received ginger, turmeric, turmeric-ginger supplements, and placebo.

Sample size

Using G-POWER software and taking into account the lumbar spine mL = 0.92 (Mean lumbar vertebral BMD [19] and 20% increase by default ($m2 = 0.104$), $sd1 = sd2 = 0.178$, two-sided $\alpha = 0.05$ and power = 95%, sample size for each group was calculated as 26 participants. Considering the 10% loss to follow up, the final sample size for each group will be 30 and a total of 120 for whole trial.

Randomization and blindness

To randomize the allocation of surveyed study groups, Random Allocation Software will be identified through blocks of four and 8 with an allocation ratio of one to one by a trained statistician. To conceal the allocation, the same packages will be used in the opaque package to be numbered sequentially. Therefore, none of the participants, surveyors, and analysts of the type of received intervention will be notified. Envelopes will be numbered from 1 to 120. The first envelope will be given to the first person who will be included in the study and it will be continued to complete the sampling.

Adverse outcomes

All participants will be educated to report any health difficulty at any time throughout the study. If any contributor stops with the procedure, follow-up data will however be gathered and the adverse events will be stated in the publication of the results.

Participant timeline

The timetable of visits and measurements is identified in Figure 2 consistent with SPIRIT guidelines.

Study product compliance

At the end of every two weeks, contributors will be asked to restore the envelope of supplements to control the amount of

Time point	Study Period											
	Enrolment	Allocation	Post-allocation									
	-1 week	0	0	Week 2	Week 4	Week 6	Week 8	Week 10	Week 12	Week 14	Week 16	Week 17
Enrolment:												
Eligibility screen	x											
Informed consent	x											
Medical history	x											
Allocation		x										
Interventions:												
20 + CL												
20 + P												
CL + P												
P + P												
Assessments:												
Anthropometric measurement	x											
Food records			x				x					x
Physical activity			x				x					x
Blood pressure												
Quality of Life			x									x
Bone mineral density			x									x
Body composition			x									x
Blood test			x									x
Adverse events monitoring												

Figure 2: SPIRIT schedule of enrolment, interventions and assessments.

the residual tablets. Patients will be considered as non-compliant if they have used less than 80% of the tablets on every case.

Physical activities

Physical activity of the participants will be assessed by the short-form international physical activity questionnaire [20].

Quality of life

The Menopause Specific Quality of Life Questionnaire will be used to address QOL at baseline, after 2 month and 4 months of study, which measures QOL in the context of vasomotor, psychosocial, physical and sexual activities [21].

Body composition

Measurement of anthropometric indicators will be performed in fasting mode, with light clothing and no shoes (basic and follow up). The Zeus 9.9 BC analyzer system will be used to provide accurate estimates of body weight, body fat percentage, body fat mass, body mass index, fat free mass, and muscle mass estimates and percentages by bioelectric impedance at baseline, 2 month and 4 months of study.

Bone mineral density (BMD)

Bone densitometry using dual X-ray absorptiometry will be performed at the lumbar spine (L1-L4) and the left femoral

neck in all participants at baseline and also after intervention as absolute value (g/cm²).

Blood sampling and preparation

Fasting venous blood samples (two samples from each subject) will be taken by a trained expert in two steps (baseline and after intervention). Samples will be taken during a time of day to minimize changes in circadian rhythm. Serum specimens will be extracted from one of two specimens within 1 h (after centrifugation) and stored at -80 °C until the examinations.

The second blood sample will be prepared for RNA analysis. The entire RNA will be extracted immediately and within 2 h of blood collection according to the manufacturer's protocol. Extracting RNA samples will be stored at -80 °C. The circulating levels of OP-related micro-RNAs (miR422a, miR-133a, miR-21 and miR-503) will be evaluated and measured.

In synthesizing cDNA from the extracted RNA, QuantiTect Rev. Transcription kit will be used and the cDNA version will be built according to the executive instruction of manufacturer.

Measuring food intake

Measurement of dietary intake will be based on the 24 h food intake questionnaire. For each individual three times (at the beginning of the study, after 2 months of intervention and at the end of the intervention), and each time three 24 h questionnaires, including two normal days and one day off will be completed [22].

At each stage, the average of three questionnaires will be considered and analyzed. Food units will be turned out to be gram per day using the "Home Scales Guide." Also, every food and drink will be encoded and entered to Nutritionist IV nutrition program adapted for Iranian foods in order to assess the amount of energy and nutrients received.

Statistical analysis

Data analysis will be conducted by SPSS version 17. Normal distribution of data will be evaluated using the Kolmogorov-Smirnov test. Regarding the correlation between the studied variables, the Mixed ANOVA statistical method will be used to compare the traits and basic measurements of the biochemical variables of patients between the four

groups and at different times. Also, the ANCOVA statistical method will be used to investigate the effect of the interventional variables on the response variable. In this study, P value less than 0.05 will be considered significant.

Ethical considerations

In order to observe ethical considerations, the present study, with the informed consent from the research units, will be followed by a description of the goals and method of work. Patients will have the right to refuse to participate in a study or to withdraw at any time without reason.

At all stages of the research, researchers will be committed to the ethics of the Ministry of Health's Medical Ethics Statement, and no costs will be charged to the participants. This study has been approved by the Ethics Committee of the Research Vice-Chancellor of Tabriz University of Medical Sciences (IR.TBZMED.REC.1396.720) and registered on the website of the Iranian Clinical Trial Registry (IRCT20161022030424N3).

Discussion

To the best of our awareness, this is the first randomized clinical trial that will evaluate the effect of Ginger (*Zingiber officinale*) and turmeric (*Curcuma longa* L.) supplementation on QOL, BC and OP related biomarkers and micro-RNAs in women with PMO.

Food intake and other lifestyle factors are environmental variables that have been changed dramatically since the Industrial Revolution, and these alterations have been linked to many chronic diseases such as OP [23]. As age-related changes and sex hormones lead to inflammation and oxidative conditions contributing to the pathogenesis of OP, other dietary components and phytochemicals that have anti-oxidative effects could have protective effects on bone loss.

Although calcium and vitamin D are the necessary nutrients for bone development, human diet contains a very wide range of natural bioactive molecules that have important beneficial effects on bone health [24]. The question that remains unanswered is the importance of plant compounds in the prevention and treatment of bone disorders.

Since both ginger and turmeric have shown promising beneficial effects on bone health in preliminary studies, we hypothesized that they may be beneficial in human as a novel therapeutic option in PMO management, which reduces the socio-economic burden of OP.

The study also has some limitations that must be addressed in future studies. Twenty four- hour food records are doubtful to be accurate measurements of usual dietary intake. The likelihood of measurement and laboratory error exists. Despite these limitations, this is the first randomized, triple-blind, placebo-controlled clinical trial exploring the effects of Ginger (*Zingiber officinale*) and turmeric (*Curcuma longa* L.) supplementation on QOL, BC and osteoporosis related biomarkers and micro-RNAs in women with PMO.

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Author contributions: All authors have accepted responsibility for the entire content of this manuscript and approved its submission.

Competing interests: Authors state no conflict of interest.

Informed consent: Informed consent was obtained from all individuals included in this study.

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