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Assessment of the effects of pine bark extract supplementation on bone turnover and the possible mechanism through OPG/RANKL signaling in postmenopausal osteopenic women: A clinical trial protocol

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A B S T R A C T

Objective: Despite the high prevalence of osteopenia in post-menopausal women, its current treatments are controversial. The present study was designed to investigate the effect of pine bark extract on the bone turnover process and its possible mechanism through OPG/RANKL signaling in postmenopausal osteopenic women.
Methods: This randomized double-blind clinical trial will be conducted on postmenopausal osteopenic women in Shariati Hospital of Tehran University of Medical Sciences, Tehran, Iran. A total of 44 postmenopausal women with osteopenia will be randomly assigned to two study groups ─ one (n = 22) receiving a PBE supplement (250 mg/d) and one (n = 22) a placebo. Demographic characteristics and the past medical history (recorded measurements of bone turn-over markers, namely, PINP, BAP, CTx and OPG/RANKL) will be recorded before and after the intervention.
Ethics and dissemination: The research was performed after obtaining approval from the Ethics Committee of Tehran University of Medical Sciences (IR.TUMS.VCR.REC.1395.1408), in line with the Helsinki Declaration. Prior to the commencement of the study, a written informed consent form was signed and dated by participants and investigators. The results will be released at the right time.
Trial registration number: IRCT2015071123153N1
Discussion: This study will investigate the effect of pine bark extract supplementation on bone turnover in postmenopausal osteopenic women. The results may help practitioners to choose the better palliative treatment for osteopenic women.

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Introduction

Osteopenia, a morbid condition of reduced bone mass, is a serious risk factor for the development of osteoporosis characterized by a bone mineral density (BMD) with a T-score between -1.0 and -2.5. It does not always progress to osteoporosis [1]. In Iran, local BMD studies have demonstrated that two out of five postmenopausal women suffer from either osteopenia or osteoporosis, and one has a normal BMD [2]. It is estimated that currently around 34% of the total population are afflicted with osteopenia, and according to the Endocrinology and Metabolism Research Center (EMRC), Tehran, Iran the prevalence of osteopenia among Iranian women aged 50 years or older in one region is 59.9%. However, among women younger than 50 years old about 33.0% have a reduced bone mass [3, 4].

Osteopenia leads to fractures and could result in serious adverse health outcomes. It has been reported in the literature that low bone mass is a main risk factor for fractures, although it is largely preventable [1]. Due to the fact that it is responsible for morbidity and mortality in elderly women, osteoporosis prevention has received much attention. Since osteoporosis is an important factor in morbidity and mortality among elderly women, its prevention is particularly important in this age group [5]. The best method to prevent osteoporosis is diagnosing osteopenia and curing it before it progresses into osteoporosis [6]. Moreover, it appears that osteoporosis can be prevented with the administration of suitable drugs at this stage of disease, although specific treatment indications for osteopenia are yet to be defined [7].

Nutritional approaches can play an important role as part of primary prevention strategies, decreasing the risk of loss of bone density. In addition to a suitable diet and other lifestyle factors such as physical activity, there is some evidence showing that phytochemicals, especially polyphenols, may protect bone health. Previous studies have demonstrated that phytochemicals and polyphenols can prevent osteopenia through their anti-oxidative, anti-inflammatory and modulatory properties in osteoblastogenesis, osteoclastogenesis, and osteoimmunology processes [7, 8]. Among the polyphenol-containing plant products reported to have a bone-protective property, pine bark extract (PBE) has been shown to play a special role in preventing and reversing bone loss in osteoporotic animal models [9, 12].

Pine bark extract contains polyphenols such as proanthocyanidins (70-50%), catechins, epicatechins, bioflavonoids and taxifolin, as well as tartaric acid, organic acids and other bioactive substances [13-17]. Some of the properties of pine bark extract are its anti-oxidant, anti-inflammatory and α-glucosidase inhibitory activities, as well as stimulation of the synthesis of hyaluronic acid, collagen, and nitric oxide [17, 18]. Recently, it has also been revealed that catechins may reduce bone loss resulting from estrogen deficiency in postmenopausal women [19-21]. Furthermore, a recent study recorded an inhibitory effect of cyaniding on the receptor activator of nuclear factor Kappa-B ligand (RANKL) [22]. RANKL and osteoprotegerin (OPG) are cytokines that may modulate bone remodeling via the receptor activator of the NF-κB pathway. The synthesis of osteoclasts from precursors is regulated by the RANKL/RANK signaling system. Also, this signaling system is responsible for the activation and survival of osteoclasts in normal bone remodeling and in different pathologic conditions, including osteoporosis. Bone resorption is prevented by OPG through binding to RANKL and thereby preventing its binding to RANK. Bone mass and stability are determined on the basis of the relative concentrations of RANKL and OPG in the bone [23].

Several studies have been conducted to assess the effect of pine bark extract on bone density in ovariectomized rats (OVXs) [9-12] and its possible impact on the inhibition of RANKL. The present study was designed to investigate the effect of pine bark extract on the bone turnover process and its possible mechanism through OPG/RANKL signaling in postmenopausal osteopenic women.

Subjects and methods

Study design

This randomized double-blind clinical trial will be conducted in Shariati Hospital of Tehran University of Medical Sciences, Tehran, Iran. Two groups of eligible postmenopausal osteopenic women were assigned, for 3 months, to either a 250mg/day of PBE supplement (n =22; intervention group) or a placebo (n= 22; control group). The intervention will be carried out after obtaining an informed consent from the participants. Data will be collected, initially and at the end of the period, using questionnaires for demographic and anthropometric characteristics, supplement intake and medications, and a 24-

hour dietary recall for food intake. Weights and heights of the participants will be measured using a digital scale and measuring tape, respectively, followed by the following tests: bone formation and resorption and calcium homeostasis markers. The supplements will be distributed monthly. The participants will be asked to record the number of supplements they missed (according to the study protocol) and return any unused supplements for compliance monitoring purposes. In the second month, the daily physical activity level of the patients will be recorded using a short IPAQ questionnaire.

Setting

The study will take place at Shariati Hospital, Tehran University of Medical Sciences, Tehran, Iran. The project begins after obtaining approval from the Ethics Committee of Tehran University of Medical Sciences (IR.TUMS.VCR.REC.1395.1408) in line with the Helsinki Declaration and registering the experimental procedure at the Iranian Registry of Clinical Trials (IRCT2015071123153N1), according to the International Committee of Medical Journal Editors. The aim of the project will be explained to the participants, and then they signed a written informed consent.

Participants

The participants will be recruited from the women referring to the Center for Osteoporosis in the Shariati Hospital. After initial screening, participants will be entered in the intervention groups based on the inclusion/exclusion criteria. (Figure 1). The participants abstained from taking any new dietary supplements (including calcium and Vitamin D), maintained their body weight (BW) and lifestyle (such as dietary patterns, physical exercises, etc), during the study. At each visit, information about any possible adverse events will be obtained and compliance will be estimated based on the number of tablets returned.

Screening

Nurses as research coordinators will screen potential applicants over the phone by obtaining information about their medical history, menopause history, chronic diseases, accessibility for the study, most recent BMD measurement results, as well as any bone metabolic diseases. Potential subjects who are interested in participating in the study and qualify for further screening will be identified by this pre-screening method. Eligible subjects will attend an informed consent meeting. Those who sign the informed consent form would complete the demographic, medical and dietary questionnaires.

Inclusion and exclusion criteria

A. Inclusion criteria

1. Age between 50-65 years;
2. Suffering from osteopenia, defined as a BMD between -1 to –2.5 SD (spine and/or hip);
3. Having a healthy normal functioning thyroid, liver, and kidney.

B. Exclusion criteria

1. Unwillingness to accept randomization;
2. Morbid Obesity (BMI ≥ 40);
3. Report of fractures during the study;
4. Unwillingness to continue participating in the project;
5. Any visible side effects due to the supplements;
6. A history of the following diseases: bone disease (Paget's disease, osteomalacia, etc.); chronic diseases, including cancer, diabetes, kidney failure, liver disease, systemic inflammatory disease, degenerative joint diseases or rheumatological disorders;
GI disease, including celiac disease, chronic diarrhea, ulcerative colitis, Crohn's disease or gastric or duodenal ulcer or untreated GI bleeding;
7. A history of surgery: ovariectomy, gastrectomy, intestinal bypass or organ transplant;
8. A history of diseases or treatments that might influence bone turnover: COPD, hyperparathyroidism, thalassemia or hemiplegia;
9. A history of taking medications that might affect bone metabolism;
10. A history of regular consumption of tobacco
or any of the following drugs for at least six months during the previous two years: diuretics, such as Lasix and thiazides; anticonvulsants (phenytoin, phenobarbital); heparin; lithium; corticosteroids; cyclosporine; NSAID; HRT; or bisphosphonates;;
11. Motor disabilities; skeletal disorders; untreated psychiatric illnesses such as psychosis, Alzheimer's, Parkinson's, etc.

Sample size

The minimum sample size was calculated based on data from the studies of Shen et al. [24].

Shen et al. reported the standard deviations of BAP of the intervention and placebo groups as 1.5 U/L and 1.6 U/L, respectively. The mean difference of BAP was reported to be 1/8 U/L. With a 95% confidence interval, and 90% power of study. The sample size of the experimental group was calculated to be 17. Assuming a dropout of 30%, the final size was increased to 22 patients; hence a total of 44 subjects entered the study.

Randomization

All participants eligible for the study based on the stratified random sampling will be placed in one of the 6 treatment units and will receive supplements for 3 months. To assign the participants to experimental groups, a stratified randomization strategy will be used based on age (50-55, 55-60 and 60-65 years old) and a history of vitamin D consumption, so as to minimize any bias.

Intervention

The placebo (50 mg medicinal starch) and PBE (50 mg PBE consisting of 67% procyanidins and other oligomers including monomeric and dimeric phenolic acids) capsules (oligopin®) were purchased from Aramis Pharmed Co., representative of France DTR (Purextract) Co., (IRC No.: 1228239427). The participants were instructed to take daily 5 capsules ─ 2 at breakfast, 2 at lunch and 1 at dinner.

General Assessment

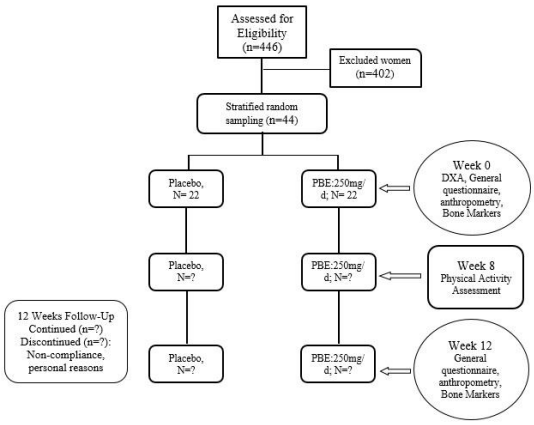
Important dietary changes recorded during the intervention period will be determined by a dietary assessment. A 24h-dietary recall questionnaire will be completed at the beginning and end of the study to ascertain the usual dietary intake (energy, macro- and micronutrients). In addition, an IPAQ short form questionnaire will be completed at the end of the 8-week treatment to record any variation from the usual activities. Also, during the intervention period supplements and medications (types and reasons for taking) taken by the participants will be recorded.

Blinding

All personnel involved in the study, as well as the investigators, will be blinded to the study.

Determination of compliance

To determine compliance, the number of capsules not taken by the participants will be recorded at each visit. Also, information about



any adverse events will be obtained at each visit and by self-report phone calls. In addition, adverse events observed will be recorded throughout the study.

Blood sample collection

Fasting blood samples will be collected from a superficial arm vein before and after the intervention, centrifuged at 2000 rpm for 10 minutes and stored at -80°C prior to analyses.

Measurements

Five ml of blood will be taken from a vein in the arm and centrifuged at 2000g rpm for 10 minutes, kept at -270C^{o} and, then, will analyze for bone biomarkers of bone formation (PINP), bone resorption, CTx, BAP, OPG and RANKL, using the ELISA method PINP, CTx, BAP, OPG.

Statistical Analysis

Data analysis will be done using the SPSS software, the normality of data distributions being checked by the Kolmogorov-Smirnov test. Comparisons of the baseline characteristics between the PBE and placebo groups will be performed using the Student's two-tailed t-test. Independent t-test and Mann-Whitney test for quantitative comparison of variables between the PBE and control groups will be used for normal and non-normal data distributions, respectively. To compare the mean values before and after the test, the paired t-test or the Wilcoxon test will be used. The analysis of covariance for the quantitative variables will be done with adjusting for confounders. A multivariate ANCOVA will be used to determine the effects of age, BMI, BMD and other variables (years of menopause etc).

Primary Results

Table 1 shows the demographic characteristics of the 44 participants who completed the study.

Table 1. Demographic characteristics of the participants (Means)

Group	Intervention 1	Intervention 2
Number	22	22
Age (yr)	57.52	56.71
Menopause Age (yr)	49.00	49.43
Weight (Kg)	69.68	70.67
Height (m)	156.9	158.4

Discussion

Based on previous findings, pine bark extract can have desirable effects on bone metabolism. Anthocyanidins (genistein) and catechins (monomeric flavonols) are known to have many positive effects on bone density in osteoporosis patients. Pine bark extract contains polyphenolic compounds and proanthocyanidins (polymeric flavonols). Previous studies have reported desirable properties for PBE as regards bone turnover in animal models. Based on the results of previous studies, it was hypothesized that PBE would have positive effects on osteogenic bone turnover. In case the beneficial effects of PBE supplementation in decreased bone turnover are confirmed, a new window will be opened for research to find its possible additional effects on osteoporosis, which will have useful clinical applications.

Limitations

One limitation was that some patients refused to take supplements. Two other limitations were the self-reporting of supplement consumption for compliance estimation and lack of cooperation of some of the participants to complete the intervention.

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Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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