Effect of Zuogui pill and Yougui pill on osteoporosis: a randomized controlled trial

Li Wenxiong, Zhang Kuaiqiang, Liu Zhu, Liu Li, Cheng Yan, Yin Jichao, Sun Yindi, Yang Feng

Abstract

OBJECTIVE: To evaluate the effect and safety of Zuogui pill and Yougui pill, classic Yin and Yang tonic formula (CYYTF), in the treatment of osteoporosis and the underlying mechanism.

METHODS: Participants aged 55 to 75 with osteoporosis and Kidney deficiency in Traditional Chinese Medicine (TCM) will be included and randomly allocated into two groups: treatment group and control group. Participants in the treatment group were treated with Zuogui pill or Yougui pill TCM formula granule, while the control group received placebo. Primary outcomes are the lumbar spine on bone mineral density (BMD) (L1-4) and femoral BMD. Secondary outcomes include pain intensity, health-related quality of life (HRQoL), bone turnover markers and safety.

RESULTS: Totally 200 patients were enrolled from December 2014 to April 2016 from four hospitals. There were no statistically significant differences between the two groups at baseline (P > 0.05) and it was good to comparability. Statistically significant differences between the two groups were observed for the lumbar BMD (L1-4), pain VAS scores and HRQoL at six months and twelve months and femoral BMD at twelve months (P < 0.05), but no significant differences for femoral BMD and bone turnover markers at six months (P > 0.05). Moreover, significant difference was observed at different time before and after treatment in terms of lumbar spine (L1-4) BMD, femoral BMD, pain VAS scores and health-related quality of life, and there was an crossover effect between the time and groups before and after treatment. In additional, in the treatment group, 8 patients lost to follow-up and 3 patients had adverse events (AEs) and in the control group, 10 patients lost to follow-up and 2 patients had AEs. No remarkable differences were observed between the two groups with regard to AEs, lost rate and safety (P > 0.05).

CONCLUSION: Zuogui pill or Yougui pill could improve BMD, ease pain, relieve Kidney deficiency and have a better effect than placebo.
syndrome, improve the quality of life osteoporosis patients, inhibit bone conversion and regulate the coupling balance of bone formation and bone resorption, but long-term efficacy should be confirmed by a longer term follow-up and larger of samples clinical randomized controlled trials.

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Keywords: Bone resorption; Osteoporosis; Zuo Gui Wan; You Gui Wan; Treatment outcome; Quality of life

INTRODUCTION

The internationally recognized description of osteoporosis is: a systemic skeletal disease characterized by low bone mass, microarchitectural deterioration of bone tissue and decrease of bone strength with a consequent increase in bone fragility and susceptibility to fracture.\(^1\)\(^-\)\(^3\) This description captures the notion that osteoporosis is an important component of fracture risk. Many of osteoporotic fractures are associated with significant morbidity and mortality not only in developing countries, but also in developed countries.\(^4\) The impact of osteoporosis is projected to increase exponentially due to the aging of the population.\(^1\) It has been estimated that osteoporosis contributes to 90% of hip and spine fractures in women 65 to 84 years of age.\(^5\)\(^,\)\(^6\) However, this disease continues to be under-diagnosed, and its management with a variety of treatments, including adequacy of calcium and vitamin D, exercise to improve balance and prevent falls, and pharmacological therapy as indication to lower fracture risk, remains suboptimal.\(^7\) Furthermore, the short- and long-term consequences (adverse effects) of these therapies continue to be discovered. The adverse outcomes include cancer and neurological, psychiatric, cardiac, musculoskeletal, respiratory, gastrointestinal, gynecologic, dermatologic, immunologic and metabolic events. Therefore, there has been an interest in developing approaches to prevent osteoporosis. Since long-term drug therapy is an expensive option with uncertain consequences and side effects, natural herbal therapy offers a possible alternative. "Kidney dominates bone" is one of the most important theories in Traditional Chinese Medicine (TCM). The physiopathological changes of bone depend on Kidney essence, and the conditions of bone reflect the status of Kidney essence. According to this theory, Kidney-tonifying herbs are used to treat bone related diseases including osteoporosis for thousands of years with definite treatment effects.\(^8\) And Kidney Yin- or Yang-tonifying herbs are always used together for osteoporosis treatment in accordance with the TCM theory "treating Yang for reinforcing Yin and treating Yang for reinforcing Yin" and its representative prescriptions are Zuogui pill and Yougui pill, classic Yin and Yang tonic formula (CYYTF). Moreover, several putative mechanisms for this have been proposed, including stimulation of osteoblast proliferation and differentiation, osteogenesis, and inhibition of bone resorption, as an estrogen-like function. For this reason, Chinese guidelines for the treatment of osteoporosis include natural herbal therapy. Meanwhile, there is currently little randomized placebo-controlled trial to verify its efficacy in treating bone mass loss. This trial was to examine effects of Zuogui pill and Yougui pill on osteoporosis by improving bone mineral density (BMD) and health-related quality of life (HRQoL).

METHODS AND DESIGN

Study design
This study was a multicenter randomized double-blind placebo-controlled trial. The first subject was screened in December 2014 and the last subject observation occurred in April 2016. Subjects were recruited at four hospitals all located in China: (a) Affiliated Hospital of Shaanxi University of Traditional Chinese Medicine; (b) Shaanxi Province Hospital of TCM; (c) Xi’an Hospital of TCM; (d) Xi’an Honghui Hospital. The study was performed in accordance with good clinical practices and ethical principles from the Declaration of Helsinki, and has been approved by the Ethics Board of Shaanxi University of TCM (No. SZFYIEC-PJ-2014-15). Each participating center also obtained a local Institutional Review Board Approval. All subjects gave written and informed consent to participate.

Diagnostic criteria for osteoporosis
Diagnostic criteria referred to Clinic Manual of the Diagnosis of Osteoporosis in Chinese Population (2010 edition) compiled by the Osteoporosis Committee of China Gerontological Society (OCCGS)\(^9\), used diagnostic method of standard deviation of BMD. Normal: a value for BMD that is higher than 1 SD below the young adult female reference mean (T-score greater than or equal to -1 SD).

Low bone mass (osteopenia): a value for BMD more than 1 SD below the young female adult mean, but less than 2.0 SD below this value (T-score < -1 and > -2.0 SD).

Osteoporosis: a value for BMD 2.0 SD or more below the young female adult mean (T-score less than or equal to -2.0 SD).

Severe osteoporosis (established osteoporosis): a value for BMD 2.0 SD or more below the young female adult mean in the presence of 1 or more fragility fractures.

Diagnostic criteria for kidney deficiency patterns
The National Standard of the People’s Republic of China (GB/T16751. 2-1997), Clinic Terminology of Tra-
ditional Chinese Medicine Diagnosis and Treatment-patterns and Guidance Principle of Clinical Study on New Drug of Traditional Chinese Medicine were consulted to define the diagnostic criteria for kidney deficiency patterns. The symptoms mainly include: low back pain, soreness and weakness of the lumbar regions and knees, dizziness, fatigue, spontaneous sweating, a hot or cold sensation in the palms, soles and chest, dysphoria, insomnia. Pulse is weak, and the tongue is red and covered without fur or bulky, moist, and covered with white fur.

**Inclusion criteria**

Included were the subjects in conformity with the diagnostic criteria of osteoporosis, aged 55 to 75 years old and whose symptom pattern was identified as kidney deficiency. And they did not receive any other treatments. Each participant signed the written informed consent before undergoing any examination or study procedure.

**Exclusion criteria**

Subjects were excluded if they had any disorders such as primary hyperparathyroidism, Cushing’s syndrome, premature menopause due to hypothalamic, pituitary, or gonadal insufficiency, poorly controlled diabetes mellitus (HbA1c > 8.0%) or other causes of secondary osteoporosis. The trial excluded individuals who have taken bisphosphonates, glucocorticoids, calcitomin, vitamin K, active vitamin D compounds or hormone replacement therapy within the previous 2 months. In addition, serum calcium levels above 2.7 mmol/L or below 2.0 mmol/L, serum creatinine levels above 1.13 mmol/L or clinically significant hepatic disorders were also exclusion. Furthermore, subjects that had malignancies, physical or mental disabilities were excluded, as well as lactating or pregnant patients.

**Treatments**

Treatments allocation occurred when the study participants met the inclusion criteria and signed the informed consent form. Eligible patients were randomly assigned to treatment group and control group. Subjects in the treatment group were treated with either Zuogui pill or Yougui pill according to symptom pattern identified as kidney-Yang deficiency or kidney-Yin deficiency, 18 g each time, 2 times a day. Zuogui pill was made from: prepared Dihuang (Radix Rehmanniae) 24 g, Shanyao (Rhizoma Dioscoreae Oppositae) 12 g, Gouqizi (Fructus Lycii) 12 g, Shanzhuyu (Fructus Macrocarpi) 12 g, Niuxi (Radix Achyranthis Bidentatae) 9 g, Tusizi (Semen Cuscutae) 12 g, Lujiaojiao (Colla Cornus Cervi) 12 g. The composition of Yougui pill: prepared Dihuang (Radix Rehmanniae) 24 g, prepared Fuzi (Radix Aconiti Lateralis Pretata) 6 g, Gouqizi (Fructus Lycii) 12 g, Shanyao (Rhizoma Dioscoreae Oppositae) 12 g, Shanzhuyu (Fructus Macrocarpi) 12 g, Tusizi (Semen Cuscutae) 12 g, Lujiaojiao (Colla Cornus Cervi) 12 g, Gouqizi (Fructus Lycii) 12 g, Duzhong (Cortex Eucommiae) 12 g, Danggui (Radix Angelicae Sinensis) 9 g.

Subjects in the control group took orally placebo, 18 g each time, 2 times a day. The placebo and TCM formula granule were produced by JiangYin Tianjiang Pharmaceutical Co., Ltd., in China and have the same appearance, size, color, form, weight, taste and smell. All drugs were administered orally for 6 months. Patients visited the doctor at 6 and 12 months follow-up. In this trial, the treating physicians, subjects, investigators and statisticians were blinded to treatment assignment.

**Outcome measurement**

The primary outcome are the lumbar spine BMD (L1-4) and femoral BMD pretherapy and post-treatment. Secondary outcomes include pain intensity, health-related quality of life (HRQoL), bone turnover markers and safety.

**BMD assessments**

Lumbar spine BMD (L1-4) and femoral BMD were measured at baseline, 6 months and 12 months after the treatment using the dual energy X-ray absorptiometry (DXA) densitometer obtained from Hologic, Inc. USA. The accuracy of the measurements recorded by the DXA instruments was evaluated by the use of serial measurements of a local spine “phantom”. The variability of DXA measurements across the different participant centers were assessed via utilization of the same spine “phantom” technique. Using this technique, the long-term coefficient of variation of each instrument in the study was estimated as less than 1%. These “phantom” measurements were used to adjust for any “drift” in measurements of BMD during the study.

**Pain intensity assessments**

The visual analogue scale (VAS) measures amount of pain, which is a pain score ranging from 0 (no pain) to 100 (worst pain ever). Operationally, the VAS score is usually a horizontal line, 100 mm in length, anchored by word descriptors at each end. The patients marked on the line the point that they felt representing their perception of their current pain. The VAS score was then determined by measuring in millimeters from the left hand end of the line to the point that the patient marks. The VAS score was measured at baseline, 6 months and 12 months after the treatments.

**Health-related quality of life**

The ECOS-16 questionnaire is developed with the aim of measuring HRQoL in postmenopausal women with osteoporosis. It is based on the combination of two disease-specific questionnaires for women with osteoporosis: the Osteoporosis Quality of Life Questionnaire (OQLQ). The Quality of Life Questionnaire of the European Foundation for Osteoporosis (QUAL-EFFO). The 16 items in the new questionnaire are di-
vided qualitatively into four dimensions. The nature of the four dimensions also suggests that they can be further combined to produce two summary scores that would include Physical Function and Pain in one Physical score and another that would include Fear of Illness and Psychosocial Function in a Mental score. These two summary scores could, in turn, be combined to provide an overall score for the questionnaire. However, although the 16 items can be classified qualitatively into four dimensions, this is a unidimensional questionnaire, according to quantitative analysis. The score of each item ranges from 1 to 5. The ECOS-16 questionnaire generates a single summary score obtained from the arithmetic mean of the answered items, so the total score ranges from 1 (best HRQoL) to 5 (worst HRQoL). This questionnaire was completed at baseline, 6 months and 12 months after the treatments.

**Bone turnover markers assessments**
Serum samples were collected at baseline and 6 months after the treatments for measurement of bone turnover markers, including serum osteocalcin (BGP), serum Procollagen type I N-terminal propeptide (PINP), serum procollagen I carboxy-terminal propeptide (PICP), carboxy-terminal telopeptide of type I collagen (CTX), type I collagen N-telopeptide (NTX) and tartrate resistant acid phosphatase (TRACP).

**Adverse events assessments**
All subjects were questioned about adverse events (AEs) during treatments at each visit point, and all AEs reports were analyzed regardless of the investigators’ assessments of causality. The Medical Dictionary for Regulatory Activities (Med DRA, Version 17.1) was used to categorize reported AEs.

**Sample and randomization**
In this clinical trial, treatment group and control group were administered in the ratio of 1:1 based on the number of cases and calculate the required sample size based on the following calculation: \( n = 2\sigma^2 \times \frac{1}{\alpha (\bar{\beta})^2} \). The estimated sample size for each group was 84 to have at least a 90% power \( \beta = 0.1 \) and to rule out a two-sided type I error of 5% \( \alpha = 0.05 \). Assuming an withdrawal rate of 20%, the appropriate sample size for recruitment was 100 cases in each group (a total of 200 cases).

Randomization of subjects were occurred centrally using a random number generator and were stratified by syndrome differentiation of TCM. All subjects were registered into the database by the teletherapist and then randomly generated the participants’ group assignment numbers (placebo versus Chinese medical herb) by the site-specific randomization program if the participants had been ready to be randomized. This method was used at all four study locations. The information was concealed from the researchers by a senior data manager who was not involved in the study and remained confidential to the study sites, in concordance with the CONSORT guidelines.

**Statistical analysis**
The data were collected and analysed according to the intention-to-treat (ITT) principle. The primary analysis comparing the efficacy of the two group used the full analysis set (FAS) and the per protocol set (PPS). In the FAS analysis, the population included the total subjects who received at least one dose of study drug and an interview record during the treatment period. In the PPS analysis, patients with important protocol deviations were excluded from the analyses. A list of protocol violators was issued before unblinding the database. No missing data were imputed. Safety was evaluated in patients who received at least one dose of study drug during the treatment period, used safety set (SS).

Analysis of variance was performed to test the differences between the treatment and the control groups in terms of BMD, pain VAS scores and health-related quality of life. The Bone turnover markers was analysed by means of paired-sample \( t \) test. Between-group comparisons at different time-points data and baseline were performed using the independent-sample \( t \) test. Data were analysed using SPSS 22.0 (IBM Corp. Released 2013. IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY, USA). \( P < 0.05 \) was the statistical significant level.

**RESULTS**

**Subjects**
The program enrolled 320 patients initially, but 120 subjects were excluded, including 68 participates not conforming with the inclusion criteria, 23 patients refusing to participate and 29 participates receive other therapy and so only 200 patients for the actual enrollment. Of the 100 patients in the treatment group, the PPS analysis included 92 patients, and the FAS analysis included 100 patients. Of the 100 patients in the control group, the PPS analysis included 90 patients, and the FAS analysis included 100 patients. A total of 18 patients did not enter the PPS (8 patients from treatment group and 12 patients from control group). The main reasons were AEs, lost contact, patients choosing to quit the study or having taken bisphosphonates and calcitonin during the study period. The two groups were not significantly different in the numbers of cases that were disqualified for PPS (\( P > 0.05 \), Figure 1).

Table 1 shows the distribution of demographic characteristics, physical measurements, primary outcome and secondary outcomes of the osteoporosis patients at baseline. There were no statistically significant differences between the two groups (\( P > 0.05 \)).
Change of lumbar spine (L1-4) and femoral BMD

Significant difference was observed at different time before and after the treatments regarding the lumbar spine (L1-4) BMD ($F = 18.467, P < 0.05$), and as well as in the treatment group ($F = 1.994, P > 0.05$), but not in the control group ($F = 1.994, P > 0.05$). BMD in the treatment group was the lowest at baseline, and reached the peak at twelve months, which showed a rising trend. From the different level of time, there was no significant difference between the two groups at baseline ($t = 1.603, P > 0.05$), but significantly different between the two groups at six months ($t = 2.126$, $P < 0.05$).
was the highest at baseline, and the lowest at twelve months, showing a downward trend, but the pain VAS scores was lower than that in the control group. From the different level of time, there was no significant difference between the two groups at baseline ($t = 0.611$, $P > 0.05$), but significantly different between the two groups at six months ($t = -2.378$, $P < 0.05$) and twelve months ($t = -4.426$, $P < 0.05$). Moreover, There was an crossover effect ($F = 28.348$, $P < 0.05$) between the time and groups before and after treatments (Table 4).

### Pain VAS scores

Significant difference was observed at different time before and after treatments regarding the pain VAS scores ($F = 260.505$, $P < 0.05$), and as well as in the treatment group ($F = 172.215$, $P < 0.05$) and control group ($F = 88.888$, $P < 0.05$). BMD in the treatment group was the highest at baseline, and the lowest at twelve months, showing a downward trend, but the pain VAS scores was lower than that in the control group. From the different level of time, there was no significant difference between the two groups at baseline ($t = 0.611$, $P > 0.05$), but significantly different between the two groups at six months ($t = -2.378$, $P < 0.05$) and twelve months ($t = -4.426$, $P < 0.05$). Moreover, There was an crossover effect ($F = 28.348$, $P < 0.05$) between the time and groups before and after treatments (Table 4).

### Health-related quality of life

Significant difference was observed at different time before and after treatments regarding the health-related quality of life ($F = 187.07$, $P < 0.05$), and as well as in the treatment group ($F = 113.935$, $P < 0.05$) and control group ($F = 78.442$, $P < 0.05$). BMD in the two groups were the highest at baseline, and the lowest at twelve months shows a downward trend, but the level of the health-related quality of life was lower than that in the control group. From the different level of time, there was no significant difference between the two groups at baseline ($t = -0.55$, $P > 0.05$), but significantly different between the two groups at six months.

#### Table 2 Comparison of lumbar spine (L1-4) BMD between two groups ($ \bar{x} \pm s $)

<table>
<thead>
<tr>
<th>Group</th>
<th>Baseline</th>
<th>Six months</th>
<th>Twelve months</th>
<th>$F$ value</th>
<th>$P$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>0.77±0.12</td>
<td>0.77±0.13</td>
<td>0.79±0.12</td>
<td>20.182</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Control</td>
<td>0.74±0.11</td>
<td>0.74±0.11</td>
<td>0.74±0.11</td>
<td>1.994</td>
<td>0.158</td>
</tr>
<tr>
<td>$t$ value</td>
<td>1.603</td>
<td>2.126</td>
<td>2.728</td>
<td>18.467</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>$P$ value</td>
<td>0.111</td>
<td>0.035</td>
<td>0.007</td>
<td>10.771</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Notes: BMD: bone mineral density; $t$ statistic and $P$ value of independent-sample $t$ test; $F$ statistic and $P$ value of main effect of repeated measures analysis of variance (ANOVA) in treatment or control group. $F$ statistic and $P$ value of main effect of repeated measures ANOVA between two groups. $F$ statistic and $P$ value of crossover effect of repeated measures ANOVA between two groups.

#### Table 3 Comparison of femoral BMD between two groups ($ \bar{x} \pm s $)

<table>
<thead>
<tr>
<th>Group</th>
<th>Baseline</th>
<th>Six months</th>
<th>Twelve months</th>
<th>$F$ value</th>
<th>$P$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>0.80±0.10</td>
<td>0.81±0.10</td>
<td>0.81±0.10</td>
<td>13.977</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Control</td>
<td>0.78±0.10</td>
<td>0.78±0.10</td>
<td>0.78±0.10</td>
<td>0.021</td>
<td>0.939</td>
</tr>
<tr>
<td>$t$ value</td>
<td>1.677</td>
<td>1.723</td>
<td>2.331</td>
<td>4.058</td>
<td>0.030</td>
</tr>
<tr>
<td>$P$ value</td>
<td>0.095</td>
<td>0.086</td>
<td>0.021</td>
<td>4.185</td>
<td>0.028</td>
</tr>
</tbody>
</table>

Notes: BMD: bone mineral density; $t$ statistic and $P$ value of independent-sample $t$ test; $F$ statistic and $P$ value of main effect of repeated measures analysis of variance (ANOVA) in treatment or control group; $F$ statistic and $P$ value of main effect of repeated measures ANOVA between two groups; $F$ statistic and $P$ value of crossover effect of repeated measures ANOVA between two groups.

#### Table 4 Comparison of pain VAS scores between two groups ($ \bar{x} \pm s $)

<table>
<thead>
<tr>
<th>Group</th>
<th>Baseline</th>
<th>Six months</th>
<th>Twelve months</th>
<th>$F$ value</th>
<th>$P$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>50±24</td>
<td>29±16</td>
<td>22±14</td>
<td>172.215</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Control</td>
<td>48±24</td>
<td>37±25</td>
<td>34±24</td>
<td>88.888</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>$t$ value</td>
<td>0.611</td>
<td>-2.378</td>
<td>-4.426</td>
<td>260.505</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>$P$ value</td>
<td>0.542</td>
<td>0.019</td>
<td>&lt;0.001</td>
<td>28.348</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Notes: VAS: visual analogue scale; $t$ statistic and $P$ value of independent-sample $t$ test; $F$ statistic and $P$ value of main effect of repeated measures analysis of variance (ANOVA) in treatment or control group; $F$ statistic and $P$ value of main effect of repeated measures ANOVA between two groups; $F$ statistic and $P$ value of crossover effect of repeated measures ANOVA between two groups.
(t = −4.238, P < 0.05) and twelve months (t = −5.824, 
P < 0.05). Moreover, there was an crossover effect (F = 22.863, 
P < 0.05) between the time and groups before and after treatments (Table 5).

**Bone formation markers**

No significant difference of bone formation markers between two groups at the different level of time before and after treatments (P > 0.05), but the level of treatment group’s BGP and PICP of the two groups was statistically significant improvement comparing with the baseline (P < 0.05) (Tables 6, 7).

**Bone resorption markers**

No significant difference of bone resorption markers between two groups at the different level of time before and after treatments (P > 0.05), but the level of NTX of two groups was statistically significant improvement comparing with the baseline (P < 0.05) (Tables 8, 9).

**Safety assessments**

It was reported, in the treatment group, 3 patients complained of mild diarrhea after medication, 2 patients spontaneously relieved and completed the trial, 1 patients relieved after withdrawal of the drug, but unceasingly refused to complete the experiment. In the control group, 2 patients complained of mild diarrhea after medication and alleviated spontaneously 2 d later and completed the trial. There were no serious AEs in the two groups. No remarkable differences were observed between the two groups with regard to AEs (P = 1.000).

**Loss to follow-up**

In the treatment group, 8 patients lost to follow-up. Among them, 2 patients moved to other places, 4 patients lost to contact, 1 patient received surgical treatment because of tibia and fibula bone fracture and 1 patient refused to complete the trial due to diarrhea. In the control group, 10 patients lost to follow-up. Among them, 5 patients lost to contact, 1 patient received surgical treatment because of fracture of neck of femur, 2 patients terminated the test due to complication of systemic lupus erythematosus and breast cancer needing to take drugs which was prohibited in the trail, 2 patients terminated the test as a result of the use of calcitonin and bisphosphonates.

### Table 5 Health-related quality of life ECOS-16 questionnaire (x ± s)

<table>
<thead>
<tr>
<th>Group</th>
<th>Baseline</th>
<th>Six months</th>
<th>Twelve months</th>
<th>F value</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>37±12</td>
<td>27±7</td>
<td>23±7</td>
<td>113.935</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Control</td>
<td>37±12</td>
<td>32±10</td>
<td>31±11</td>
<td>78.442</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>t value</td>
<td>−0.550 a</td>
<td>−4.238 b</td>
<td>−5.824 b</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P value</td>
<td>0.956 c</td>
<td>&lt;0.001 d</td>
<td>&lt;0.001 d</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Notes: 't' statistic and P value of independent-sample t test; 'F' statistic and P value of main effect of repeated measures analysis of variance (ANOVA) in treatment or control group; F statistic and P value of main effect of repeated measures ANOVA between two groups; 'F' statistic and P value of crossover effect of repeated measures ANOVA between two groups.

### Table 6 Change of bone formation markers (x ± s)

<table>
<thead>
<tr>
<th>Item</th>
<th>BGP</th>
<th>PINP</th>
<th>PICP</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Treatment group</td>
<td>Control group</td>
<td>Treatment group</td>
</tr>
<tr>
<td>Baseline</td>
<td>15.4±6.9</td>
<td>16.2±7.0</td>
<td>44.9±21.5</td>
</tr>
<tr>
<td>Six months</td>
<td>16.4±6.7</td>
<td>15.8±6.8</td>
<td>46.2±18.5</td>
</tr>
<tr>
<td>t value</td>
<td>−2.337</td>
<td>0.900</td>
<td>−0.994</td>
</tr>
<tr>
<td>P value</td>
<td>0.021</td>
<td>0.370</td>
<td>0.322</td>
</tr>
</tbody>
</table>

Notes: paired-sample t test. BGP: osteocalcin; PINP: procollagen type I N-terminal propeptide; PICP: procollagen I carboxyterminal propeptide. Compared with the baseline period, 'P' < 0.05.

### Table 7 Change of bone formation markers (x ± s)

<table>
<thead>
<tr>
<th>Group</th>
<th>BGP</th>
<th>PINP</th>
<th>PICP</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>Six months</td>
<td>Baseline</td>
</tr>
<tr>
<td>Treatment</td>
<td>15.4±6.9</td>
<td>16.4±6.7</td>
<td>44.9±21.5</td>
</tr>
<tr>
<td>Control</td>
<td>16.2±7.0</td>
<td>15.8±6.8</td>
<td>47.5±25.1</td>
</tr>
<tr>
<td>t value</td>
<td>−0.805</td>
<td>0.580</td>
<td>−0.786</td>
</tr>
<tr>
<td>P value</td>
<td>0.422</td>
<td>0.562</td>
<td>0.433</td>
</tr>
</tbody>
</table>

Notes: independent-sample t test. BGP: osteocalcin; PINP: procollagen type I N-terminal propeptide; PICP: procollagen I carboxyterminal propeptide. Compared with the control group, 'P' > 0.05.
Corresponding individualized treatment to accommodate TCM principle, that is, replicating TCM takes a holistic approach in treating the individual treatment protocol defined for each disease entity, adopted by Western Medicine where there is a standard treatment, that is, in accordance with evidence-based medicine, which are accepted in the evaluation of mainstream medicine. It is essential for TCM to demonstrate its efficacy to be rationally used for patients whom may benefit from it. It is essential for TCM to demonstrate its efficacy and safety by high-level evidence using methods which are accepted in the evaluation of mainstream medicine, that is, in accordance with evidence-based medicine. However, in contrast to the disease-targeted approach adopted by Western Medicine where there is a standard treatment protocol defined for each disease entity, TCM takes a holistic approach in treating the individual with customized treatment based on the concept of "Syndrome Differentiation". This difference in approach, however, is not entirely reconcilable. In order to accommodate TCM principle, that is, replicating the diagnosis using "Syndrome Differentiation" and giving the corresponding individualized treatment within the rigorous framework of EBM, a systematic process for assessing symptoms and signs, identification and quantification of TCM syndrome for a sample group of study subjects with the same Western medical diagnosis can be applied before and after treatment. The trial based on the concepts of "Syndrome Differentiation" and RCTs, under the guidance of the TCM theory "Kidney dominate bone", CYYTF were selected as the intervention depending on the different types of Kidney deficiency syndrome, placebo control, to treat osteoporosis patients at four hospitals, and the data during the trial were concealed from the researchers by a senior data manager who was not involved in the study and remained confidential to the study sites, in concordance with the CONSORT guidelines.

Osteoporosis is a chronic skeletal disorder characterized by the decrease of the BMD, bone quality and strength leading to increased fracture risk, even result in fragility fractures, the most devastating clinical consequence of osteoporosis. Hence, decreasing the rate of fracture becomes the main goal in the evaluation of curative effect of osteoporosis. Whereas, due to the trial needing a large of samples, treatment and follow-up for a long time, it is difficult to study. Epidemiological survey shows low BMD is the major risk factors for osteoporotic fracture, and most of the literatures select BMD as the main outcome measures to evaluate the efficacy. Therefore, this trial chose lumbar spine BMD (L1-4) and femoral BMD as the primary outcomes. Results indicated that CYYTF could improve the BMD of osteoporosis patients and reached the peak at twelve months, which showed a rising trend. But without comparison with the existing treatments for osteoporosis, it needs more positive control trial of RCTs to verify its efficacy.

### Table 8 Change of bone resorption markers (±s)

<table>
<thead>
<tr>
<th>Group</th>
<th>CTX Treatment</th>
<th>CTX Control</th>
<th>NTX Treatment</th>
<th>NTX Control</th>
<th>TRACP Treatment</th>
<th>TRACP Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>0.30±0.18</td>
<td>0.32±0.20</td>
<td>337.09±149.78</td>
<td>338.52±162.29</td>
<td>10.51±7.92</td>
<td>10.34±9.55</td>
</tr>
<tr>
<td>Six months</td>
<td>0.31±0.21</td>
<td>0.34±0.20</td>
<td>316.03±137.43</td>
<td>355.63±154.67</td>
<td>10.18±7.39</td>
<td>10.54±9.39</td>
</tr>
<tr>
<td>t value</td>
<td>-1.108</td>
<td>-1.055</td>
<td>2.734</td>
<td>-3.141</td>
<td>0.968</td>
<td>-0.633</td>
</tr>
<tr>
<td>P value</td>
<td>0.271</td>
<td>0.317</td>
<td>0.007</td>
<td>0.002</td>
<td>0.335</td>
<td>0.528</td>
</tr>
</tbody>
</table>

Notes: paired-sample t test. CTX: carboxy-terminal telopeptide of type-I collagen; NTX: type I collagen N-telopeptide; TRACP: tartrate resistant acid phosphatase. Compared with the baseline period, *P < 0.05.

### Table 9 Change of bone resorption markers (±s)

<table>
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<tr>
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<td>10.34±9.55</td>
<td>10.54±9.39</td>
</tr>
<tr>
<td>t value</td>
<td>-0.781</td>
<td>-0.764</td>
<td>-0.065</td>
<td>-1.914</td>
<td>0.134</td>
<td>-0.307</td>
</tr>
<tr>
<td>P value</td>
<td>0.435</td>
<td>0.480</td>
<td>0.948</td>
<td>0.057</td>
<td>0.894</td>
<td>0.759</td>
</tr>
</tbody>
</table>

Notes: independent-sample t test. CTX: carboxy-terminal telopeptide of type-I collagen; NTX: type I collagen N-telopeptide; TRACP: tartrate resistant acid phosphatase. Compared with the control group, *P > 0.05.

### DISCUSSION

Despite the growing prevalence of TCM usage and the worldwide interest in its therapeutic benefits, the fundamental issue hindering its acceptance by the Western Medicine community and integration into mainstream health-care is still the lack of robust evidence from the evidence-based medicine (EBM) perspectives. EBM is defined as "the conscientious, explicit, and judicious use of current best evidence in making decisions about the care of individual patients." The practice of EBM integrating individual clinical expertise with the best available external clinical evidence from systematic research. In the EBM system, different types of evidences are prioritized into a set of hierarchical levels. Randomized controlled trials (RCTs) or systematic reviews of RCTs are the golden standard for the highest level of evidence, followed by other types of evidences such as cohort studies, case-control studies, case series, case reports, animal/in vitro studies, and expert opinion. In order to be rationally used for patients who may benefit from it, it is essential for TCM to demonstrate its efficacy and safety by high-level evidence using methods which are accepted in the evaluation of mainstream medicine, that is, in accordance with evidence-based medicine. However, in contrast to the disease-targeted approach adopted by Western Medicine where there is a standard treatment protocol defined for each disease entity, TCM takes a holistic approach in treating the individual with customized treatment based on the concept of "Syndrome Differentiation". This difference in approach, however, is not entirely reconcilable. In order to accommodate TCM principle, that is, replicating the diagnosis using "Syndrome Differentiation" and giving the corresponding individualized treatment within the rigorous framework of EBM, a systematic process for assessing symptoms and signs, identification and quantification of TCM syndrome for a sample group of study subjects with the same Western medical diagnosis can be applied before and after treatment. The trial based on the concepts of "Syndrome Differentiation" and RCTs, under the guidance of the TCM theory "Kidney dominate bone", CYYTF were selected as the intervention depending on the different types of Kidney deficiency syndrome, placebo control, to treat osteoporosis patients at four hospitals, and the data during the trial were concealed from the researchers by a senior data manager who was not involved in the study and remained confidential to the study sites, in concordance with the CONSORT guidelines.

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Study shows that osteoporosis clinically mainly manifested as low back pain. Large population studies have reported a link between low back pain and osteoporosis, especially in the elderly. Although the perception and response to pain change with reasons that remain unknown, most people believe that those may result from skeletal deformities, joint imbalance, and tension in muscular structures. Therefore, relieving patients' pain are very important for treating osteoporosis. The study confirmed that CYYTF could relieve pain of osteoporosis patients. However, the mechanism of CYYTF for relieving pain remained to be further studied.

Osteoporosis is highly disabling, seriously affecting the elderly’s physical and mental health and quality of life. So rehabilitation of osteoporosis can not only focus on improvement in patients’ physiological function, but also focus on assessment and intervention of patients' psychological and social function and quality of life. This trial was conducted to evaluate HRQoL of osteoporosis patients using ECOS-16 questionnaire. Its items were obtained from the Spanish versions of the OQLQ and QUALEFFO questionnaires, and were then reduced by using the Rasch analysis to obtain a total of 16 items. 12 items from the QUALEFFO and 4 items from the OQLQ, including subjective emotions, self-care ability, pain experience, fear of falling, and other aspects of the assessment. Compared with the previous special volume table (e.g. OQLQ, QUALEFFO-41) and universal volume table (e.g. SF-36), it is more simple and easier to operate. The results show that CYYTF could significantly improve HRQoL of osteoporosis patients, including physiological, psychological and social function.

Osteoporosis is characterized by reduced bone mass and strength leading to increased risk of fractures. Pharmacological interventions aim to decrease the risk of fractures and associated clinical consequences by correcting the imbalance between bone resorption markers and bone formation markers that constitutes the pathophysiological basis of the disease. Bone turnover markers could indirectly show the state between bone resorption and bone formation, which are widely used in prediction of fracture risk, monitoring the response of drug therapy and diagnosis of metabolic osteopathy. The combination of bone turnover markers and BMD can be more comprehensive to evaluate the state between bone resorption and bone formation.

BGP is the specific markers of bone formation when the uncoupling of bone formation and bone resorption, the level of serum PICP and PINP is a specific markers reflecting the osteogenic activity of bone formation and reflecting the rate of type I collagen synthesis. However, Serum TRACP levels were considered as biochemical markers of bone resorption, mainly reflecting the osteoclast activity and bone resorption status. CTX and NTX are extracellular collagen degradation products and indicators of bone resorption and bone metastasis. The trail implied that CYYTF could increase the level of BGP, PINP and PICP but reduce the level of TRACP, CTX and NTX to some extent. In additional, it suggested that CYYTF may inhibit bone conversion and regulate the coupling balance of bone formation and bone resorption through different pathways.

In conclusion, the short-term curative effect of the CYYTF in the treatment of primary osteoporosis is affirmative, which can improve BMD, ease pain, relieve Kidney deficiency syndrome, improve the quality of life osteoporosis patients, inhibit bone conversion and regulate the coupling balance of bone formation and bone resorption. However, the long-term efficacy should be confirmed by a longer term follow-up and larger samples clinical randomized controlled trials.

REFERENCES


