Effectiveness of ZiYin Xiehuo granules and Zishen Qinggan granules on partial precocious puberty in girls: a multicenter, randomized, single-blind, controlled trial

Sun Wen, Han Xinghui, Wang Yonghong, Yu Jian, Yan Weili, Zhao Jun, Chen Weibin, Xue Zheng

Abstract

**OBJECTIVE:** To evaluate the effect of ZiYin Xiehuo granules (ZYXH) and Zishen Qinggan granules (ZSQG) on partial precocious puberty (PPP).

**METHODS:** This was a multicenter, randomized, single-blind, positive-controlled trial. A total of 143 patients were assigned to either the ZYXH group or the ZSQG group using a random number table. The ZYXH group received ZYXH three times daily for 6 months and the ZSQG group received ZSQG three times daily for 6 months. Mammary nucleus diameter; the results of uterus, ovarian, and maximum follicle measures; and Chinese medicine symptom pattern scores were compared at baseline and after 3 months and 6 months of treatment.

**RESULTS:** After 3 months’ treatment, there were no significant differences between the two groups in mammary nucleus index changes (left: 3.4 ± 3.1 vs 3.5 ± 3.1, P = 0.79; right: 3.0 ± 2.9 vs 3.6 ± 3.0, P = 0.719). The uterine volume in the ZYXH group was smaller than that in the ZSQG group (2.1 ± 1.6 vs 2.6 ± 2.2, P = 0.006). There were no significant between-group differences in ovarian volume and maximum follicular diameter on either side (ovarian volume: left 1.2 ± 0.7 vs 1.3 ± 0.6, P = 0.8; right 1.2 ± 0.7 vs 1.4 ± 1.1, P = 0.984; maximum follicular diameter: left 3.9 ± 1.7 vs 3.5 ± 2.2, P = 0.158; right 3.5 ± 1.7 vs 3.9 ± 2.1, P = 0.314).

**CONCLUSION:** ZYXH granules and ZSQG granules both affected the size of the mammary nucleus in girls with PPP, and improved Chinese medicine symptom patterns. ZYXH granules showed slight advantages over ZSQG granules in terms of the decrease in the size of the uterus, ovaries, and ovarian follicles.
INTRODUCTION

Precocious puberty has become one of the most common pediatric endocrine diseases and can be caused by premature activation of the hypothalamic-pituitary-gonadal axis. If left untreated, patients can develop symptoms such as early appearance of sexual characteristics, advanced bone age, and early fusion of the epiphysis, all of which influence the patient’s expected adult height. Patients may also have psychological and behavioral problems and a higher likelihood of endocrine disorders in adulthood. Partial precocious puberty (PPP) is a special type of idiopathic precocious puberty. Traditional Chinese medicine (TCM) is now commonly used by pediatricians nationwide, and there have been some reports on the use of TCM to treat precocious puberty in China. However, few evidence-based medical studies have been conducted. A previous study has shown that kidney deficiency and a hyperactive pattern, as well as Yin deficiency and a fire excess pattern in the liver and kidney, are the most common TCM symptom patterns in precocious puberty. ZiYin Xiehuo granules (ZYXH) were designed based on this theory, which in turn was derived from the ancient TCM prescription Zhi Bai Di Huang pill. This prescription has been used for many years and has a clear efficacy in improving the symptoms of children with precocious puberty. Zishen Qinggan granules (ZSQG) were used as a positive control drug. Therefore, we hypothesized that ZYXH would have superior clinical effectiveness to ZSQG.

METHODS

Diagnostic, inclusion, and exclusion criteria

PPP was diagnosed according to the guidelines established by the Endocrine Professional Group of the Chinese Association of Pediatrics and the China Association of Traditional Chinese Medicine. The inclusion criteria were as follows: (a) onset of puberty in girls aged ≤8 years, diagnosed based on early breast development; (b) mammary nucleus in Tanner stage II or III, and with a diameter < 3 cm; (c) pelvic ultrasound showing that the uterine and ovarian volume, and the follicle diameter, were within normal range; (d) bone age equal to chronological age ± 0.5 years; (e) maximum increment of follicle-stimulating hormone (FSH) as the main inspired peak following a luteinizng hormone-releasing hormone test, a luteinizing hormone (LH) peak < 3 IU/L, and an LH/FSH ratio < 0.6; (f) no previous treatment with gonadotropin-releasing hormone-agonist (GnRHa). Patients were included and data were collected only after informed consent was obtained. Patients were excluded if (a) it could not be guaranteed that they would take the drug according to doctors’ instructions (e.g., if they were boarding school students), or (b) they did not understand how to take tests, refused to participate, or had a history of mental illness.

Study protocol and patients

This is a multicenter, randomized, single-blind, positive controlled experimental research. We generates a random number table by Excel (Microsoft Corporation, Redmond, Washington, USA), each hospital alone assigns groups by random number table. This is a single-blind trial, which means only researchers know the group information. This trial was registered at http://ClinicalTrials.gov (No. NCT02650141). Based on a previous study, the target sample size was 142 participants. Following the diagnosis and treatment guidelines for common diseases in pediatric TCM, published by the China Association of Chinese Medicine, the ZSQG group was the control group. Because the subjects had PPP, GnRHa was not chosen as the positive control drug. Patients were assigned to either the ZYXH group or the ZSQG group using a random number table generated in Excel. The subjects were blind to their own grouping and medication. The study protocol was approved by the Ethics Committee of the Children’s Hospital of Fudan University (No. [2013]096). The patients were enrolled between November 2013 and May 2015 at the TCM Department of the Children’s Hospital of Fudan University, the Pediatrics Department of Shuguang Hospital (which is affiliated with Shanghai University of TCM), the Pediatrics Department of Shanghai Hospital of TCM, and the YueYang Hospital (which is affiliated to Shanghai University of TCM). The patients took a dosage three times daily for two courses, with each course lasting for 3 months. The ZYXH granules were composed of Dihuang (Radix Rehmanniae) 5 g, Xuanshen (Radix Scrophulariae) 3 g, Zexie (Rhizoma Alismatis) 3 g, Zhihu (Rhizoma Anemarrhenae) 3 g, Huangbai (Cortex Phellodendri Amurensis) 3 g, stir-frying with liquid adjuvant Guijia (Carapax et Plastrum Testudinis) 3 g, Maiya (Fructus Hordei Germinati) 2 g, and stir-frying with liquid adjuvant Gancao (Radix Glycyrrhizae) 2 g in a bag. The ZSQG granules were composed of Dihuang (Radix Rehmanniae) 5 g, Baishao (Radix Paeoniae Alba) 3 g, Huangqin (Radix Scutellariae Baicalensis) 3 g, Fuling (Poria) 4 g, Mudanpi (Cortex Moutan Radicis) 3 g, Mudanpi (Cortex Moutan Radicis) 6 g, Shanzha (Fructus Crataegi Pinnatifideae) 3 g, Zhebeimu (Bulbus Fritillariae Thunbergii) 3 g, and stir-frying with liquid adjuvant Gancao (Radix Glycyrrhizae) 2 g in a bag. The granules used in this study were provided by Nanning Pelli Pharmaceutical Co., Nanning, Guangxi, China (Lot No. 1501453).

Outcome measurement

Data were collected at three time points: before taking the medicine, at the end of the third month, and at the end of the sixth month. A baseline survey was carried out that recorded general demographic information,
anthropometric measurements, mammary nucleus diameter, Tanner staging, bone age, uterine appendage ultrasound (long diameter, width, and thickness of the uterus and ovaries, as well as maximum follicular diameter), measurement of serum sex hormone levels (FSH, LH, prolactin [PRL], and estrogen [E2]), and TCM symptom pattern scores. At the end of the third month and sixth month, the following data were collected: mammary nucleus diameter; mammary nucleus Tanner staging; uterine ultrasound, ovarian, and follicular measurements; and TCM symptom pattern scores.

The primary outcome measures were the mammary nucleus index, uterine volume, ovarian volume, and changes in follicle size after the third month. The mammary nucleus index was calculated by measuring the mammary nucleus diameter through the breast midline and squaring the result. Uterus volume, ovarian volume, and maximum follicle diameter were measured using ultrasound testing.

Secondary variables included the change in mammary nucleus index, uterine volume, ovarian volume, and changes in follicle size after the sixth month, as well as the change in TCM symptom pattern scores after the third and sixth months. Following the diagnosis and treatment guidelines for common diseases in pediatric TCM, published by the China Association of Chinese Medicine (precocious puberty: ZYYXH/T2010-2012),

as well as clinical experience, we screened for 12 common symptom patterns of precocious puberty to calculate TCM symptom pattern scores (heat intolerance; thirst; fire pattern on the face; dysphoria with feverish sensation in the chest, palms, and soles; preference for greasy and sweet food; irritability; swelling and pain in the eyes; breast bulging pain; constipation; night sweats; halitosis; and red tongue). For each symptom found, a score of 2 points was given; 2 points were given for a specific condition, 1 point was given if the condition had improved after treatment, and no points were assigned if there were no signs of the specific condition. TCM symptom pattern scores were then calculated from the sum of the scores of each patient.

Safety assessment
All subjects were questioned about adverse events during the treatment. All adverse events reported by the patients were analyzed. The safety assessment was based on liver and kidney function examinations.

Statistical analysis
Microsoft Excel was used to establish a database, and SPSS 14.0 (SPSS for Windows, Version 16.0. Chicago, SPSS Inc., Chicago, IL, USA) was used to analyze the data. Statistical analysis was performed using an intention-to-treat analysis strategy. Measured data were expressed in terms of mean ± standard deviation (x ± s), and normality and homogeneity of variance were calculated. If the two samples were normally distributed and showed homogeneity of variance, the independent samples approximate t-test was used. If the distribution was not normal, the Mann-Whitney U-test was used. In the case of counted data, the \( \chi^2 \) test was used. A \( P \)-value < 0.05 was considered statistically significant.

RESULTS

Baseline clinical characteristics
A total of 156 patients were screened; of these, 143 signed informed consent forms: 25 from the Children’s Hospital of Fudan University, 45 from Shuguang Hospital (affiliated with Shanghai University of TCM), 53 from Shanghai Hospital of TCM, and 20 from YueYang Hospital (affiliated with Shanghai University of TCM) (Figure 1). All patients were girls. Patients were randomly divided into two groups: 71 were assigned to the ZSYXH group and 72 to the ZSQG group. During the study, three patients disliked the taste of the Chinese medicine and five patients were unable to follow up because they were non-locals (Figure 1). Therefore, the dropout rate was 5.6%.

The baseline characteristics of the patients in both groups are shown in Tables 1 and 2. There were no significant differences between the groups in age, height, weight, mammary nucleus index, Tanner staging, bone age, ovary and uterus volume, maximum follicular diameter, serum sexual hormone (FSH, LH, PRL, and E2) levels, and TCM symptom scores.

Comparison of mammary nucleus index
At baseline, 3-month, and 6-month assessments, there were no significant differences in mammary nucleus index changes between the two groups (left side: \( P = 0.224, 0.790, \) and 0.310, respectively; right side: \( P = 0.345, 0.719, \) and 0.579, respectively; Table 2). Compared with baseline in the ZYXH group, the differences were statistically significant (left side: \( P = 0.004 \) and 0.001, respectively; right side: \( P = 0.006 \) and 0.002, respectively; Table 2). Compared with baseline in the ZSQG group, the differences were also statistically significant (left side: \( P = 0.001 \) and < 0.001, respectively; right side: \( P = 0.004 \) and < 0.001, respectively; Table 2).

Comparison of uterine volume, ovarian volume, and maximum follicle diameter
At baseline, 3-month, and 6-month assessment, there were significant differences in uterine volume between the two groups (\( P = 0.435, 0.006, \) and 0.001, respectively; Table 3). Compared with baseline in the ZYXH group, the differences in uterine volume were statistically significant (\( P = 0.009 \) and < 0.001, respectively; Table 3). Compared with baseline in the ZSQG group, the differences in uterine volume were not statistically significant (\( P = 0.542 \) and 0.239, respectively; Table 3).
At baseline, 3-month, and 6-month assessment, the differences in ovarian volume between the two groups were not statistically significant (left side: $P = 0.566$, 0.809, and 0.407, respectively; right side: $P = 0.136$, 0.984, and 0.415, respectively; Table 3). Compared with baseline in the ZYXH group, the differences in the left ovary volume were not statistically significant ($P = 0.240$ and 0.112, respectively; Table 3), whereas those in the right ovary volume were statistically significant, especially at 3-month assessment ($P = 0.006$ and 0.024, respectively; Table 3). Compared with baseline in the ZSQG group, the differences in ovarian volume were not statistically significant on either side (left side: $P = 0.211$ and 0.462, respectively; right side: $P = 0.376$ and 0.541, respectively; Table 3).

At baseline and 3-month assessment, the difference in maximum follicular diameter between the two groups was not statistically significant on either side (left side: $P = 0.279$ and 0.158, respectively; right side: $P = 0.609$ and 0.314, respectively; Table 3). At 6-month assessment, the difference in left maximum follicular diameter was statistically significant ($P = 0.043$, Table 3), whereas the difference in right maximum follicular diameter was not statistically significant ($P = 0.830$, Table 3). Compared with baseline in the ZYXH group, the differences in left maximum follicular diameter were not statistically significant ($P = 0.366$ and 0.309, respectively; Table 3), whereas the differences in right maximum follicular diameter were statistically significant ($P = 0.030$ and 0.040; Table 3). Compared with

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**Table 1 Baseline characteristics of patients ($n \pm s$)**

<table>
<thead>
<tr>
<th>Index</th>
<th>ZYXH ($n = 71$)</th>
<th>ZSQG ($n = 72$)</th>
<th>$P$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>7.79±1.10</td>
<td>7.66±1.12</td>
<td>0.487</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>129.26±8.19</td>
<td>130.89±8.44</td>
<td>0.257</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>27.71±5.49</td>
<td>29.6±7.18</td>
<td>0.089</td>
</tr>
<tr>
<td>Breast Tanner staging (II/III)</td>
<td>51/20</td>
<td>56/16</td>
<td>0.413</td>
</tr>
<tr>
<td>FSH (mIU/mL)$^*$</td>
<td>2.17±0.24</td>
<td>2.39±0.24</td>
<td>0.552</td>
</tr>
<tr>
<td>LH (mIU/mL)$^*$</td>
<td>2.38±0.60</td>
<td>2.61±0.63</td>
<td>0.105</td>
</tr>
<tr>
<td>PRL (ng/mL)$^*$</td>
<td>3.26±1.01</td>
<td>3.36±1.19</td>
<td>0.715</td>
</tr>
<tr>
<td>E$_2$ (pg/mL)$^*$</td>
<td>2.12±0.33</td>
<td>2.13±0.48</td>
<td>0.907</td>
</tr>
<tr>
<td>Bone age (years)</td>
<td>8.62±1.33</td>
<td>8.36±1.19</td>
<td>0.245</td>
</tr>
</tbody>
</table>

Notes: $^*$Raw data multiplied by 100 and then transformed using logarithmic transformation. ZYXH: ZiYin Xiehuo granules; ZSQG: Zishen Qinggan granules.
baseline in the ZSQG group, the differences in maximum follicular diameter were not statistically significant on either side (left side: \( P = 0.262 \) and 0.120, respectively; right side: \( P = 0.376 \) and 0.805, respectively; Table 3).

### Comparison of TCM symptom pattern scores

At baseline, 3-month, and 6-month assessment, there were no statistically significant between-group differences in TCM symptom pattern scores (11.6 \( \pm \) 3.6 vs 11.6 \( \pm \) 3.6, \( P = 0.899 \); 5.2 \( \pm \) 2.3 vs 5.6 \( \pm \) 2.2, \( P = 0.954 \); 3.1 \( \pm \) 2.5 vs 3.12 \( \pm \) 2.0, \( P = 0.142 \); respectively). Compared with baseline in the ZYXH and ZSQG groups, the differences were statistically significant (ZYXH group: \( P < 0.001 \) in both cases; ZSQG group: \( P < 0.001 \) in both cases).

The ancient TCM classical texts contain no information regarding the name, etiology, or pathogenesis of the congenital foundation and is in charge of reproduction and growth. Since the early 1980s, other researchers in our department have discovered that girls with precocious puberty present with the TCM symptom pattern of “kidney deficiency and exuberance of liver fire,” or “\( Yín \) deficiency and fire effulgent is rarely seen."

### Table 2: Between-group comparison of mammary nucleus index (\( x \pm \delta \))

<table>
<thead>
<tr>
<th>Group</th>
<th>( n )</th>
<th>Time</th>
<th>LMNI (cm(^2))</th>
<th>RMNI (cm(^2))</th>
</tr>
</thead>
<tbody>
<tr>
<td>ZYXH</td>
<td>71</td>
<td>Baseline</td>
<td>4.1 ( \pm ) 2.9</td>
<td>3.6 ( \pm ) 2.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3-month</td>
<td>3.4 ( \pm ) 3.1 ( ^{1} )</td>
<td>3.0 ( \pm ) 2.9 ( ^{1} )</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6-month</td>
<td>3.2 ( \pm ) 3.1 ( ^{1} )</td>
<td>2.9 ( \pm ) 3.0 ( ^{1} )</td>
</tr>
<tr>
<td>ZSQG</td>
<td>72</td>
<td>Baseline</td>
<td>4.0 ( \pm ) 3.3</td>
<td>4.0 ( \pm ) 3.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3-month</td>
<td>3.5 ( \pm ) 3.1 ( ^{1} )</td>
<td>3.6 ( \pm ) 3.0 ( ^{1} )</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6-month</td>
<td>2.9 ( \pm ) 2.7 ( ^{1} )</td>
<td>3.0 ( \pm ) 2.8 ( ^{1} )</td>
</tr>
</tbody>
</table>

Notes: LMNI: left mammary nucleus index; RMNI: right mammary nucleus index; ZYXH: treated with ZiYin Xiehuo granules, three times daily for two courses and each course lasting for 3 months; ZSQG: treated with Zishen Qinggan granules, three times daily for two courses and each course lasting for 3 months. \( \delta \leq 0.05 \), \( \delta \leq 0.01 \), compared with baseline in the same group; \( \delta \leq 0.05 \), \( \delta \leq 0.01 \), compared between the two groups at 3-month and 6-month.

### Table 3: Between-group comparison of uterus volumes, ovary volumes, and maximum follicle diameter (\( x \pm \delta \))

<table>
<thead>
<tr>
<th>Group</th>
<th>( n )</th>
<th>Time</th>
<th>UV (cm(^3))</th>
<th>LOV (cm(^3))</th>
<th>ROV (cm(^3))</th>
<th>LMFD (mm)</th>
<th>RMFD (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ZYXH</td>
<td>71</td>
<td>Baseline</td>
<td>2.4 ( \pm ) 1.9</td>
<td>1.3 ( \pm ) 0.8</td>
<td>1.6 ( \pm ) 1.3</td>
<td>3.6 ( \pm ) 1.8</td>
<td>4.2 ( \pm ) 2.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3-month</td>
<td>2.1 ( \pm ) 1.6 ( ^{1} )</td>
<td>1.2 ( \pm ) 0.7</td>
<td>1.2 ( \pm ) 0.7 ( ^{1} )</td>
<td>3.9 ( \pm ) 1.7</td>
<td>3.6 ( \pm ) 1.7 ( ^{1} )</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6-month</td>
<td>1.9 ( \pm ) 1.2 ( ^{1} )</td>
<td>1.2 ( \pm ) 0.7</td>
<td>1.3 ( \pm ) 0.8 ( ^{1} )</td>
<td>3.7 ( \pm ) 1.9</td>
<td>3.6 ( \pm ) 2.5 ( ^{1} )</td>
</tr>
<tr>
<td>ZSQG</td>
<td>72</td>
<td>Baseline</td>
<td>2.4 ( \pm ) 2.1</td>
<td>1.4 ( \pm ) 1.6</td>
<td>1.4 ( \pm ) 1.2</td>
<td>3.8 ( \pm ) 2.0</td>
<td>4.0 ( \pm ) 2.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3-month</td>
<td>2.6 ( \pm ) 2.2 ( ^{1} )</td>
<td>1.3 ( \pm ) 0.6</td>
<td>1.4 ( \pm ) 1.1</td>
<td>3.5 ( \pm ) 2.2</td>
<td>3.9 ( \pm ) 2.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6-month</td>
<td>2.4 ( \pm ) 1.8 ( ^{1} )</td>
<td>1.4 ( \pm ) 0.7</td>
<td>1.4 ( \pm ) 1.0</td>
<td>3.7 ( \pm ) 2.4 ( ^{1} )</td>
<td>4.0 ( \pm ) 2.2</td>
</tr>
</tbody>
</table>

Notes: UV: uterine volume; LOV: left ovarian volume; ROV: right ovarian volume; LMFD: left maximum follicle diameter; RMFD: right maximum follicle diameter; ZYXH: treated with ZiYin Xiehuo granules, three times daily for two courses and each course lasting for 3 months; ZSQG: treated with Zishen Qinggan granules, three times daily for two courses and each course lasting for 3 months. \( \delta \leq 0.05 \), \( \delta \leq 0.01 \), compared with baseline in the same group; \( \delta \leq 0.05 \), \( \delta \leq 0.01 \), compared between the two groups at 3-month and 6-month.
third of PPP cases develop into CPP, TCM may effectively slow the progression to CPP. The present findings showed that both ZYXH granules and ZSQG granules reduced the early development of secondary sexual characteristics and improved TCM symptom pattern scores, providing preliminary evidence that TCM therapy is beneficial for PPP. Further studies on TCM treatments for PPP are needed and should be based on TCM symptom pattern identification and personalized treatment.

For the rapidly progressive (severe) type of CPP, GnRHa treatment may be a better choice in clinical practice; however, long-term use of GnRHa has adverse effects on gonadal function and bone mineral content.\(^1\) Doctors often use TCM therapies for special types of CPP, like simple early breast development, as well as some types that manifest in their early stages as non-rapidly progressing CPP. At the early stages of precocious puberty, TCM treatment can slow down or even reverse the development of rapidly progressive precocious puberty, delay the appearance of advanced bone age and impairment of growth potential, and reduce future development of GnRHa. Further studies are needed to clarify the role of TCM in the treatment of PPP.

REFERENCES