Comparison of the use of letrozole and clomiphene citrate in regularly ovulating women undergoing intrauterine insemination

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ABSTRACT

Objective: To compare the effects of letrozole 5mg and clomiphene citrate (CC) 100mg on total follicle number, endometrial thickness, hormone levels, pregnancy, miscarriage and OHSS rate in women undergoing superovulation and I.U.I.

Material and methods: This study was done as a prospective randomized trial in Research and Clinical Center for Infertility (Shahid Sadoughi University) and Mother Hospital, Yazd, Iran. In this study, 115 patients with unexplained and mild male factor infertility were studied. Patients were randomized, using a computer-generated random table into two groups which were treated with 5 mg of letrozole daily (60 patients, 60 cycles) or 100mg of CC daily (55 patients, 55 cycles) from day 3-7 followed by FSH on day 8 of the cycle. Then data were analyzed using student's T Test and chi-square.

Results: The mean age and duration of infertility in both groups were similar. There was a significant difference in the total numbers of follicles during stimulation between two groups (5.45± 4.3 in CC group vs. 3.28± 2.5 in letrozole group) (p-value= 0.01). No significant difference was found in the endometrial thickness between two groups (letrozole group= 6.9±2.5, CC group= 7.6±1.8). The mean level of LH (IU/L) and FSH (IU/L) in both groups were similar. (P-value of hormone levels between two groups were respectively: 0.33 and 0.47), but there was a significant difference in mean estrogen (pg/ml) levels between the groups (220.28±150.5 in letrozole group vs. 867.34±240.6 in CC group) (p-value=0.018). The mean number of used gonadotropin ampoule was the same in both groups. Chemical pregnancy rate per cycle was 8.3% in the letrozole group and 5.5% in the CC group. Clinical pregnancy rate per cycle was 6.6%in the letrozole group and 1.8% in the CC group (p-value= 0.6).Two out of the three pregnancies in the CC group and one out of the five pregnancies in the letrozole group resulted in a miscarriage. One twin pregnancy occurred in the letrozole group and none in the CC group. Ovarian hyperstimulation syndrome (OHSS) has not occurred in any group.

Conclusions: In spite of comparable pregnancy rate in clomiphene citrate and letrozole group, C.C. in these patients could be prescribed because of its lower cost.

Keywords: letrozole, clomiphene citrate, superovulation, I.U.I
pregnancy rate and high miscarriage rate. Successful implantation requires a receptive endometrium, with synchronous development of glands and stroma (5, 6).

In one study, CC was found to have a deleterious effect on the endometrium, as demonstrated by a reduction in glandular density and an increase in the number of vacuolated cells (7). In addition, Gonen et al (1990) demonstrated a reduction in endometrial thickness, below the level thought to be needed to sustain implantation, in up to 30% of women receiving CC for ovulation induction or for unexplained infertility (8). Recently, it was suggested that letrozole, a specific reversible, nonsteroidal aromatase inhibitor that suppresses estrogen biosynthesis (9), could successfully replace CC in superovulation treatment of patients with unexplained infertility or polycystic ovarian syndrome in addition to the poor responder patients (10).

The new third generation aromatase inhibitors agents commercially available include two nonsteroidal preparations, anastrozole and letrozole and a steroidal agent, exemestane (11, 12). Letrozole has a short half-life (around 2 days) and it clears rapidly from the body (13). This drug is a potent and highly specific nonsteroidal aromatase inhibitor that initially was approved for use in postmenopausal women with breast cancer to suppress estrogen production (14, 15).

Letrozole inhibits the aromatase enzyme by competitively binding to the heme of the cytochrome p450 subunit of the enzyme, resulting in a blockade of androgens conversion into estrogens with subsequent increase in intraovarian androgens (16). Letrozole can be administered early in the follicular phase to induce ovulation by releasing the hypotalamus or pituitary from estrogen negative feedback on GnRH and gonadotropin secretion, leading to an increase in gonadotropin production which would stimulate ovarian follicular development (13) but unlike clomiphene citrate, it doesn’t lead to estrogen receptor depletion (17). By using letrozole, intraovarian androgen levels increase and this may synergize with central effects of decreased estrogen to enhance ovarian response to gonadotropin stimulation (1). In specifically defined subgroup of patients according to the Mitwally et al (2003) study, the combined use of aromatase inhibitors with gonadotropin injection was associated with improved ovarian response (18). The purpose of the present study was to compare letrozole effect concerning pregnancy rate, in regularly menstruating women undergoing IUI with clomiphene citrate.

**MATERIALS AND METHODS**

The present study was performed at Yazd Research and Clinical Center for Infertility and Mother Hospital between October 2004 and September 2005. Informed consent was obtained from all the patients with unexplained and mild male factor infertility. Inclusion criteria were age younger than 40 years, duration of infertility more than 1 year, patent fallopian tubes on hysterosalpingogram or laparoscopy and the presence of at least 10 million rapidly motile sperm/ml (17). Patients were randomized by using a computer-generated random table, into two groups which were treated with 5 mg of letrozole daily (60 patients, 60 cycles) or 100mg of CC daily (55 patients, 55 cycles). Patients received either letrozole (Femara; Novartis pharmaceuticals, Dorval, Quebec, Canada) 5mg/day or clomiphene citrate 100mg/day from day 3-7 of their menstrual cycle plus FSH (Gonal-F, Serono, Switzerland) starting on day 8. The starting dose of gonadotrophin in both groups was150IU/day. The decision of combining FSH to letrozole or CC was based on previous study by authors (19). Transvaginal ultrasonography scans were performed on day 12 of the menstrual cycle and then daily until the mean diameter of the largest follicle reached 18 mm. One physician did all ultrasound examination and she was blinded to the group status. At ultrasonographic scan, the internal diameter of each visible follicle was measured in two dimensions and the average diameter was calculated. In addition, the endometrial thickness (mm) was measured at the greatest diameter perpendicular to the midsagittal plane in the fundal region, including both layers of the endometrial cavity. The image was oriented so that the endometrial canal and the cervical canal were visualized in the same plane to ensure measurement.
Table 1. Characteristics of patients undergoing superovulation with letrozole or clomiphene citrate (CC)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Letrozole</th>
<th>CC</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of subjects</td>
<td>60</td>
<td>55</td>
<td></td>
</tr>
<tr>
<td>Mean (±SD) age of women, years</td>
<td>28.7±2.9</td>
<td>25.7±3.8</td>
<td>NS*</td>
</tr>
<tr>
<td>Mean (±SD) age of men, years</td>
<td>33.8±4.3</td>
<td>32.6±4.01</td>
<td>NS</td>
</tr>
<tr>
<td>Mean (±SD) duration of infertility, years</td>
<td>6.2±2.4</td>
<td>5.4±2.5</td>
<td>NS</td>
</tr>
<tr>
<td>Causes of infertility</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male factor (%)</td>
<td>21.8</td>
<td>34.8</td>
<td>NS</td>
</tr>
<tr>
<td>Unexplained (%)</td>
<td>78.2</td>
<td>65.2</td>
<td>NS</td>
</tr>
</tbody>
</table>

*NS= Not Significant

through the center of the endometrium (8). When the mean diameter of at least one follicle had reached 18mm, 10,000IU of HCG (Profasi; Serono) was administered intramuscularly. Serum FSH, LH and E2 levels were measured by the same lab technician in all patients on day of HCG administration by Elisa technical. Moreover, Semen analysis was prepared by swim down procedure.

Intra uterine insemination (I.U.I) was performed 34 – 36 hours after administration of HCG. Serum B hCG was measured two weeks after HCG administration. Chemical pregnancy rate was calculated based on a positive result of serum BhCG (greater than 25mIU/ml) and clinical pregnancy rate was assessed by detection of fetal heart beat on sonography. In addition, we evaluated the total number of follicles, the number of gonadotrophin ampules, and miscarriage rate. For statistical analysis, the data were entered into statistical package for social sciences (SPSS), PC version13. The appropriate statistical tests including student's T Test and chi-square were used to compare the results, which are expressed as mean and standard deviation. The differences were considered to be statistically significant if p value was<0.05.

RESULTS

All patients were included in the analysis. Our results showed that the causes of infertility, mean age and duration of infertility in both groups were similar (Table 1). The total numbers of follicles in the letrozole group were significantly lower than those in the clomiphene citrate group (Table 2).

There was no significant difference in the endometrial thickness between the two groups (6.9±2.5 mm in the letrozole group, 7.6±1.8mm in the CC group) (Table 2). The mean number of gonadotrophin ampules was the same in both groups. (p-value=0.19) (Table 2).

Hormones were measured on day of hCG administration. The mean levels of LH (IU/L) and FSH (IU/L) in both groups were similar. (p-value of hormone levels between two groups were respectively: 0.33, .0.47) but there was a significant difference in mean E2 (pg/ml) levels between two groups (220.28±150.5 in letrozole group vs. 867.34±240.6 in CC group) (p-value=0.018) (Table 2). Chemical pregnancy rate per cycle was 8.3% in the letrozole group and 5.5% in the CC group. Clinical pregnancy rate per cycle was 6.6% in the letrozole group and 1.8% in the CC group (p-value=0.6). Therefore, no difference in the pregnancy rate between the two groups was noted (Table 3).

Table 2. Superovulation with letrozole or with clomiphene citrate (CC).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Letrozole</th>
<th>CC</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of subjects</td>
<td>60</td>
<td>55</td>
<td></td>
</tr>
<tr>
<td>Mean(±SD) no. of total follicles</td>
<td>3.28±2.5</td>
<td>5.45±4.3</td>
<td>p= 0.01</td>
</tr>
<tr>
<td>Mean(±SD) Endometrial thickness(mm)</td>
<td>6.9±2.5</td>
<td>7.6±1.8</td>
<td>NS</td>
</tr>
<tr>
<td>Mean(±SD) of L.H(IU/L)</td>
<td>8.17±4.9</td>
<td>8.24±7.8</td>
<td>NS</td>
</tr>
<tr>
<td>Mean(±SD) of F.S.H (IU/L)</td>
<td>6.5±3.3</td>
<td>7.3±4.2</td>
<td>NS</td>
</tr>
<tr>
<td>Mean(±SD) of E2 (pg/ml)</td>
<td>220.28±150.5</td>
<td>867.34±240.6</td>
<td>p=0.018</td>
</tr>
<tr>
<td>Mean(±SD) no. of Gonadotrophin Ampoule</td>
<td>6.8±2.4</td>
<td>7.4±3.4</td>
<td>NS</td>
</tr>
</tbody>
</table>
Table 3. Outcome of letrozole or clomiphene citrate in women undergoing I.U.I.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Letrozole</th>
<th>CC</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of subjects</td>
<td>60</td>
<td>55</td>
<td></td>
</tr>
<tr>
<td>Chemical pregnancy</td>
<td>5 (8.3%)</td>
<td>3 (5.5%)</td>
<td>NS</td>
</tr>
<tr>
<td>Clinical pregnancy</td>
<td>4 (6.6%)</td>
<td>1 (1.8%)</td>
<td>NS</td>
</tr>
<tr>
<td>Miscarriage</td>
<td>1</td>
<td>2</td>
<td>NS</td>
</tr>
</tbody>
</table>

One out of the five pregnancies in the letrozole group resulted in a miscarriage and two out of the three pregnancies in the CC group resulted in a miscarriage (Table 3). One twin pregnancy occurred in the letrozole group and none in the CC group. OHSS has not occurred in any group.

**DISCUSSION**

Clomiphene citrate is the most commonly prescribed agent for ovulation induction. Unfortunately, despite high rates of ovulation, pregnancy rates per cycle remain relatively low (20).

Mitwally et al (2001) have shown that the use of CC may be complicated because of antiestrogenic effects on endometrial development. Therefore, a simple inexpensive and safe alternative to CC for use in normally ovulatory woman is required (20).

Fisher et al (2002) compared the effect of clomiphene citrate and letrozole on normal ovulatory women, and found that profiles of both LH and FSH were similar in natural and medicated cycles with letrozole and CC but E₂ level was more than two times higher in clomiphene-treated cycles (21). Despite significantly lower E₂ levels in letrozole-treated women, endometrial development was unaffected (21). In our study, CC resulted in a significant increase in E₂ levels while there was no difference in the endometrial thickness at midcycle between two groups. Al-Fozan et al (2004) compared the effect of CC and letrozole in women undergoing superovulation, and found that there was no difference in the pregnancy rates or in the endometrial thickness between the letrozole and the CC group. Of interest, the miscarriage rate was higher in the CC group (22). Perhaps, this is due to the different mechanism of action between letrozole and CC (22). In our study, there was no difference in pregnancy rates or in the endometrial thickness between the groups. Mitwally et al (2005), studied the effect of an aromatase inhibitor for ovarian stimulation on pregnancy outcome, their results indicated that CC treatment is consistently associated with development of more ovarian follicles than with aromatase inhibitor and the lowest multiple gestation rate is associated with letrozole treatment (23). In our study, there was a significant difference in the total numbers of follicles between two groups. The numbers of follicles were more in the CC group than those in the letrozole group, but multiple gestation rate was higher with letrozole treatment. More studies are needed to confirm that letrozole is associated with higher multiple gestation rate.

Our results showed significantly lower estradiol concentrations in the letrozole group than in the CC group and more follicles were observed in cycles stimulated with 100mg CC from day 3 to 7 of the cycle than letrozole group. Fatemi et al (2003) compared endocrinological environment of cycles stimulated with letrozole compared to CC (24). Sohrabvand et al (2006) compared the efficacy of combined metformin-letrozole with metformin-clomiphene citrate in clomiphene resistant infertile women with polycystic ovarian disease. They showed that the mean concentration of total E₂ were significantly higher in CC without a difference in mean number of follicle and ovulation rate. In addition, endometrial thickness was significantly higher in letrozole group than CC group. Full term pregnancies were higher in letrozole group (25). The estrogen level in women on aromatase inhibitors was found to be 2-3 times lower as well than that reported in CC cycles, but despite this, the endometrial thickness was greater in the aromatase inhibitor cycles (2). In our study, the estrogen level was greater in the CC group with a significant difference in the mean number of follicle than letrozole group, but there was no difference in the endometrial thickness or
pregnancy rate between two groups. Letrozole, at doses of 1-5 mg/day, inhibits of the 97%-99% aromatase activity (11). In all studies reported so far, the aromatase inhibitor letrozole was administered as a 5-day regimen, usually from day 3 to 7 of the menstrual cycle, at a dose of 2.5-7.5mg/day (26). Even in one study (12), the new approach of a single-dose regimen of an aromatase inhibitor for ovarian stimulation seems to be as effective as the previously reported 5-day regimen. Al-Fadhli et al (2006) showed that 5mg daily for 5 days is a preferable letrozole dose for superovulation (27).

In the present study, letrozole was administered at a dose of 5mg/day from day 3 to 7 of the menstrual cycle. It was shown that CC is associated with increased risk of ovarian hyperstimulation syndrome and high multiple pregnancies (1). In the present study, O.H.S.S has not occurred in any group. Mitwally et al (2004) proposed that aromatase inhibitors would replace CC in the future as the new primary treatment for ovulation induction in P.C.O patients (11). Letrozole can be used for ovulation induction or ovarian stimulation with higher pregnancy rates compared with CC (20), but in our study the pregnancy rate between two groups was the same.

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