

# EVALUATION OF LETROZOLE THERAPEUTIC EFFECT IN INFERTILE WOMEN

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**Abstract-** Combination therapy with clomiphene citrate plus gonadotropin (clomiphene citrate plus HMG) in induction ovulation reduce the gonadotropins dose but antiestrogenic effect of c.c. can decrease pregnancy rate. In a randomized clinical trial we compared 3 treatment protocol for induction ovulation and intra uterine insemination (I/o plus IUI) in women with infertility history more than one year, normal hysterosalpingogram (HSG), normal spermiogram and follicular stimulating hormone (FSH) < 10 IU/ml. 52 women were taken clomiphene citrate plus HMG, 52 women were received letrozole plus HMG and 28 women were taken HMG alone. Gonadotropin dose for induction ovulation in clomiphene citrate plus HMG group [mean 4.9 (1.5)] and letrozole plus HMG group significantly was lower than HMG group [mean 11 (4.5)]( $P < 0.05$ ). Although size and number of mature follicule in 3 group were equal. In clomiphene citrate plus HMG group endometrial thickness was lower but level of estradiol obviously was higher than others ( $P < 0.05$ ). Pregnancy rate in letrozole plus HMG group was 28%, in clomiphene citrate plus HMG group was 23% and in HMG group was 25%. In conclusion aromatase inhibitors like letrozole same as clomiphene can reduce gonadotropin dose in induction ovulation without anti estrogenic effect.

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**Key words:** Aromatase inhibitors, Letrozole, Clomiphene citrate, Human menopausal gonadotropin

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## INTRODUCTION

In recent years increasing infertility rate, drug's side effects, expensive treatments and low rate of success induced investigators to search for more effective treatments. Induction ovulation plus intra uterine

insemination is one of the ART methods in treatment of infertile couples. Gonadotropins are effective but high cost drugs that were used for I/O from many years ago. Anti estrogenic dugs such as clomiphene citrate are first line alternative drugs that were used from 1962 for anovulatory infertile women (1-3).

In central nervous system clomiphene causes decrement estrogen receptor, as an anti-estrogen agent, the result of its action is increasing secretion of FSH and LH and at the end we can observe hyper stimulation of ovary and ovulation. Combination of gonadotropin plus clomiphene citrate in a treatment

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cycle, in addition to improve response of patient to clomiphene citrate, decreases HMG dose and finally causes the reduction of treatment price (1, 4, 5). Anyway local antiestrogenic effect of clomiphene citrate can decrease pregnancy rate. Aromatase is a cytochrome P-450 hemoprotein- containing complex that catalyze the rate limiting step in the production of estrogens, *i.e.* the conversion of androstenedione and testosterone. From many years ago aromatase inhibitors like letrozole and anastrozol had been used in post menopausal breast cancer to suppress estrogen production (1, 3). Aromatase inhibitors have a relatively short half life (48 h) and can mimic clomiphene function without estrogen receptor depletion therefore in recent years this drugs administer for induction ovulation (2). Their mechanism is release of the hypothalamic-pituitary axis from estrogenic negative feedback, thereby increasing gonadotropin secretion and resulting in stimulation of ovarian follicles (6, 7). In addition it has a local effect on ovary and by androgen accumulation increases follicle sensitivity to FSH (4, 6). In comparison with clomiphene citrate in spite of decreasing estrogen level it can improve endometrial thickness (2, 3).

In recent years many researchers evaluate letrozole efficacy in induction ovulation (3, 8). In this study our object was to compare letrozole efficacy with clomiphene citrate and gonadotropins and to evaluate its effect in decreasing required gonadotropin dose.

## MATERIALS AND METHODS

This research was done in infertility ward of Mirza Hospital, Tehran University of Medical Sciences, in Tehran. The patients consist of infertile women referred to infertility unit from Jan. 2005 till Jan. 2006. In this clinical trial study samples were consist of 150 patients with infertility history more than one year, normal HSG or laparoscopy and normal

spermiogram. In hormonal evaluation all of them have FSH < 10 IU/ml in day two or three of cycle. They were less than 40. At first visit the necessary knowledge about treatment methods were explained to them. After their agreement, we divided them according to simple randomization method in to three groups.

Due to different reasons such as use of different brand of HMG amp (we use only one brand in all cases), unresponsiveness of ovary and disruption of treatment, some of the cases (basically in HMG group) were excluded from the study. At last 132 patients completed treatment cycles. The first group consisted of 52 persons received cc 100 mg/day from day 3 till 7 of cycle plus HMG (Menogon-Ferring-Germany) 150 IU/day from day 5 of cycle. The second group consisted of 52 patients were under treatment by letrozole (Novartis-Switzerland) 5 mg/day from day 3 till 7 plus HMG from day 5. In third group 28 patients completed treatment cycle. To those patients HMG 150 IU/day was given from day 3 cycle. Clinical evaluations and sonography were done at infertility unit. Transvaginal sonographic evaluations were done at the first of cycle, before beginning treatment, and then at the day 9 or 10 of cycle and after that if necessary. When the size of mature follicle reached  $\geq 18$  mm 5000 IU HCG was injected. 36-38 h after that IUI was done. The level of Progesterone was checked at the day 21-23 cycle. Two weeks after IUI if the patient had retard of mense BHCG was controlled.

If menstruation happened sonography was done to evaluate ovarian cyst formation (ovarian cyst with diameter > 3 cm) Main outcome measures were include: number of follicles, estradiol level (in the day of HCG injection), Progesterone level (on the days 21-23), pregnancy rate and cyst formation. The study group did not differ demographically or in age, duration of infertility, cause of infertility and FSH level. Primary characteristics of 3 groups demonstrated in Table 1.

**Table 1.** Patients' demographic and baseline characteristics in three study groups\*

Characteristic	CC + HMG (n=52)	Letrozole + HMG (n=52)	HMG (N=28)	P value
Age	27.3 (3.3) (21-32)	27 (4) (19-37)	29 (4) (22-39)	NS
FSH	5.8 (1.5) (3-9)	5.8 (1.6) (3-9)	5.5 (1.7) (2.8-9)	NS
Duration of Infertility	4.3 (2.7) (1-13)	4.3 (2) (1-10)	3.9 (2.3) (1-10)	NS

\*Data are given as mean (SD), range.

**Table 2.** Features of all treatment cycles: clomiphene citrate plus HMG group versus HMG group\*

	Clomiphene citrate plus HMG (n=52)	Letrozole plus HMG (n= 52)	HMG (n=28)	P value
Number of follicle $\geq$ 15 mm	1.23 (1.32) (0-6)	0.7 (0.8) (0-4)	0.7 (0.7) (0-3)	NS
Num. of follicle $\geq$ 18 mm	1.8 (1.1) (0-5)	1.4 (0.6) (1-3)	1.5 (1.2) (1-5)	NS
Endometrial thickness	6.4 (0.8) (5-9)	7 (1.1) (5-9)	7.3 (1.1) (6-10)	< 0.05†
Num of consumed ampoules	4.9 (1.5) (3-10)	4.8 (1.6) (3-12)	11 (4.5) (6-24)	< 0.05‡
Estradiol level	619.9 (451.3) (58-2018)	209.7 (195.1) (11-1039)	379.7 (487.7) (139-2138)	< 0.05¶
Progesteron (day 21-23)	22.8 (13.5) (0.5-75)	20.7 (11.8) (0.4-60)	17.3 (21.2) (2-100)	NS
Pregnancy rate	23% (n=12)	28% (n= 15)	25% (n= 7)	NS
Cyst formation	17.3% (n=9)	9.6 (n= 5)	8% (n= 2)	NS

Abbreviations: HMG, human gonadotropins; NS, not significant.

\* Data are given as mean (STD) (range).

† Significant difference between “clomiphene citrate plus HMG and letrozole plus HMG” and between “clomiphene citrate plus HMG and HMG”.

‡ Significant difference between “clomiphene citrate plus HMG and HMG” and between “letrozole plus HMG and HMG”.

¶ Significant difference between “clomiphene citrate plus HMG and letrozole plus HMG” and between “clomiphene citrate plus HMG and HMG”.

## Statistical analysis

Quantitative data presented by means (SD) and range and qualitative data demonstrated by frequency and percent. The statistical analysis was undertaken using parametric and non parametric tests including Fisher exact test, ANOVA and Bonferroni test. All of these analyses were done in the SAS system version 9 software. A  $P < 0.05$  was considered significant.

## RESULTS

A total of 132 patients were enrolled in this study. Number of follicles  $\geq$  15 and 18 mm and mean of Progesterone in the day 21-23 were not statistically different between 3 groups (Table 2). Endometrial thickness between group clomiphene citrate plus HMG and group letrozole plus HMG was significantly different ( $P < 0.05$ ) (Table 2). This difference was observed between group clomiphene citrate plus HMG and group HMG this thickness was thinner in group clomiphene citrate plus HMG (Table 2). Thickness of endometrium was statistically equal between group HMG and letrozole plus HMG (Table 2). Number of consumed ampoules were equal between group HMG plus clomiphene citrate and group letrozole plus HMG but were higher in group HMG (Table 2). Level of blood estradiol in the day of HCG injection was not

statistically different between group HMG and HMG plus letrozole and also between group HMG and clomiphene citrate plus HMG but demonstrated statistically difference between group HMG plus letrozole and clomiphene citrate plus HMG (was obviously lower in group letrozole plus HMG) (Table 2). Pregnancy rate was 28% in letrozole plus HMG 23% in clomiphene citrate plus HMG and 25% in HMG Group and they did not show any statistically difference.

Cyst formation is one of the common side effect of infertility treatments and in this study its prevalence was 17.3 in group clomiphene citrate plus HMG, 9.8% in letrozole plus HMG and 8% in HMG group that were not statistically different.

We had one case of twine pregnancy in letrozole plus HMG group and one case of ectopic pregnancy in clomiphene citrate plus HMG group. We observed primary sign of hyperstimulation (more than 6 follicle  $>$  15 mm) in one patient in HMG group and in 3 patients in clomiphene citrate plus HMG group and for prophylaxis we used GnRH analog injection for triggering of ovulation (not HCG) and we did not have any case of ovarian hyperstimulation.

## DISCUSSION

In this study co-treatment with the letrozole plus HMG such as co-treatment with clomiphene citrate plus HMG significantly reduced the required

gonadotropin dose. In addition it did not has undesirable antiestrogenic effect such as decreasing endometrial thickness. Letrozole effect in reduction of gonadotropin dose may be due to central and peripheral mechanism of action (3, 6). Suppression of estrogen production in CNS release the negative feedback on the hypothalamus that resulting in endogenous gonadotropin secretion leading to enhancement of ovarian follicular development. Peripherally inhibition of estrogen production may lead to accumulation of androgens. Androgens through implication of the FSH receptor gene expression can increase follicular sensitivity to FSH (2)

Co-treatment with clomiphene citrate plus gonadotropin can reduce cost of treatment cycle but its antiestrogenic effect can have negative effects on endometrium, cervical mucus, transtubal transport, oocyte and embryo quality. On the other hand accumulation of clomiphene citrate because of long half life can amplified its negative effects (1, 3). Aromatase inhibitors in addition to absence of any antiestrogenic effect because of short half life (~ 45 h) supposed that have no deleterious effects on oocyte maturation or early development of embryo (2).

Measuring of the endometrial thickness by transvaginal ultrasonography is a relatively simple, non-invasive and clinically applicable method to monitor the antiestrogenic effect on the endometrium. In this study in group clomiphene citrate plus HMG blood estradiol was higher and endometrial thickness is lower than others.

In some researches modification of treatment protocol or administration of ethinyl estradiol in follicular phase or addition of other antiestrogenic drugs had no significant benefit (1, 9, 10).

In some studies such as Al-Fozan in 2003 (9), Barroso in 2004 (11), Jee in 2006 (12) and Al-Fadhli in 2006 (13) endometrial thickness in letrozole users was equal with other groups but like some studies including Mitwally in 2003 (3) and Atay in 2006 (14) endometrial thickness was higher in our study. Level of blood estradiol in the day of HCG injection in other research (3, 6, 7, 12, 15) like our study was lower than clomiphene users. Number of follicles in most of studies (3, 4, 8, 9, 11) like our research did

not show any statistical difference, but in a study in 2002 by Healy (8) in letrozole users they had more follicles.

Goswami (6), Mitwally (3-5) and Healy (8) in their studies (like us) found that gonadotropin consumption in co-treatment with letrozole plus HMG was lower than HMG alone but pregnancy rate in both group was equal (6, 8, 11, 12) or higher in letrozole group (3, 4).

In summary, the results of this study present that concomitant use of letrozole plus HMG during induction ovulation results in reduction of required gonadotropin dose in addition to lower estradiol level, higher endometrial thickness and desirable pregnancy rate. We suppose further randomized controlled trials with larger samples help us to do a better judgment.

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### **Conflict of interests**

The authors declare that they have no competing interests.

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