Zainab Hussein et al /J. Pharm. Sci. & Res. Vol. 9(11), 2017, 2291-2295

ISSN:0975-1459
Journal of Pharmaceutical Sciences and Research
www.jpsr.pharmainfo.in

Comparison of the Effect of Clomiphene citrate and Letrozole on the Endometrial Parameters of PCOS Women

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Abstract:

Background: Receptive endometrium is fundamental for the successful implantation of an embryo. It is adequately primed for implantation; its growth is regulated by steroid hormones, various growth factors and cytokines. Clomiphene citrate (CC) is an orally active nonsteroidal drug with mixed estrogen agonist/ antagonist properties. While Letrozole, a selective reversible third-generation aromatase inhibitor, has the potential to be used for ovulation induction.
Objective: to decide which induce better endometrial receptivity either clomiphene citrate or Letrozole in PCOS patient.
Subject, Materials and Method: In prospective study, 120 infertile women with PCOS, undergo basic hormonal profile in cycle day 2, pelvic ultrasound to confirm diagnosis and to rule out presence of cyst, few number of patients get resistance to CC but the resistance to treatment was higher in clomiphene citrate group compared to letrozole group (36.76% *vs* 21.15%) 120 patients enrolled in the study and they received standard dose of either clomiphene citrate or letrozole as induction protocol so only 80 patients apart from those enrolled in the study of endometrium. On cycle day 12 or 13 another ultrasound done to examine growth of follicle, endometrial thickness, pattern and echogenicity, Human chorionic gonadotropin (hCG) was injected subcutaneously when at least one mature follicle 18 mm diameter was detected and asking couple to have intercourse 34-36 hour, follow up the patient to do pregnancy test after two weeks.
Results: Clomiphene citrate and letrezole achieved follicle maturation with a mean ±SD of 12.13±0.52 and 11.9±0.5 days respectively; the mean number of dominant follicle in clomiphene citrate was significantly higher than letrozole (2.35±0.92 *vs* 1.83±0.38). letrozole group had a significantly greater endometrial thickness, than clomiphene citrate (0.85±0.07 *vs* 0.77±0.09) and significant triple hypoechogenic pattern in letrozole compared to clomiphene citrate. The pregnancy rate achieved in letrozole group (30%) was greater than clomiphene citrate group (17.5%) although non-significant difference.
Conclusion: Letrozole induce better endometrial receptivity parameters than Clomiphene citrate.

Key words: polycystic ovarian syndrome, clomiphene citrate, Letrozole, endometrial parameters.

INTRODUCTION

PCOS is commonest causes of anovulation and amenorrhea. It is classically characterized by infertility, oligo and amenorrhea, hirsutism, acne or seborrhea, and obesity. The Rotterdam consensus defined the polycystic ovary as having 12 or more follicles, measuring between 2 and 9 mm follicle number per ovary, and/or an ovarian volume (OV) >10 cm^3.[(1)] Clomiphene citrate (CC) considered as first line of treatment, it is an isomeric mixture of ~38% zuclomiphene and 62% enclomiphene. Zuclomiphene is a mild estrogenic activity, it is estrogenic agonist propertied occur when endogenenous estrogen level are extremely low, as well as antiestrogenic, while enclomiphene is entirely antiestrogenic. CC is a safe and effective oral agent with good ovulatory rates; it is associated with several drawbacks, like adverse antiestrogenic effects systemically and on the endometrium and cervical mucus, low conception rates despite good ovulatory rates. However, since over 75% of ovulations occur within a dose range of 100–150 mg/day for 5 days; CC resistance was defined as the absence of ovulation after using this dose [(2)].

Letrozole is a selective reversible third-generation aromatase inhibitor, has the potential role to be used for ovulation induction with demonstrable endometrial sparing effect. It induces the ovulation by inhibition of the conversion of androgens to estrogen and creating an estrogen-deficient environment; mimic the central reduction of negative feedback through which CC works [(3)]. Some studies revealed that letrozole is superior or at least equal to CC in ovulation and pregnancy rates in PCOS women with anovulatory cycle PCOS and inadequate clomiphene response. Endometrial thickness is known as the maximal distance between the echogenic interfaces of the myometrium and the endometrium, measured in the plane through the central longitudinal axis of the uterus. There is correlation between endometrial thickness and the likelihood of conception, in the context of assisted conception. However, a very thin endometrium (below 7 mm) seems to be accepted as a reliable sign of suboptimal implantation potential.[(4)] Endometrial pattern which is the relative echogenicity of the endometrium and the adjacent myometrium as demonstrated on a longitudinal ultrasound scan. A researcher recognizes that multilayered pattern to be more predictive of implantation than any other parameter measured [(5)].

SUBJECTS, MATERIALS AND METHODS

A prospective study conducted in Higher Institute of Infertility diagnosis and Assisted Reproductive Techniques /AL-Nahrain University (Baghdad/Iraq) during the period from (July 2016 to July 2017).The study was

approved by the Local medical ethical committee of college of Medicine, AL-Nahrain University, and written consent was obtained from patients or their surrogates.120 PCOS women-part of them show resistance to induction and other got cyst those not enrolled in current endometrial study and 80 patients who response to induction were enrolled in this study and classified randomly in to two group .The diagnoses of PCOS is basis on history taking and physical examination, and fulfilling at least 2 of the following three criteria of Rotterdam European Society of Human Reproduction and Embryology / American Society for Reproductive Medicine (ESHRE/ASRM) sponsored PCOS consensus workshop group [(1)]: Polycystic ovaries on ultrasound.(defined by increase number of small antral-follicles more or equal to 12 follicles that were less than 8 mm diameter) or an increase in ovarian volume more than 10 cm3 in one ovary or both, chronic anovulation or oligovulation and clinical (depend on basis of hirsutism) or biological signs of hyperandrogenism (elevated testosterone level).

The PCOS women included in this study with age range 18-40 years, more than 2 years of infertility, the FSH level less than 10 mIU/ml and have bilateral patent tubes confirmed by hysterosalpingography, in addition their partners have normal seminal fluid analysis according to WHO criteria [(6)]. All patients should have no history of ovarian drilling, any uterine pathology or endocrinopathies and not taking any other medications.

All patients undergo assessment by taking full history and examination, hormonal assay (FSH, LH, prolactin, E2 and testosterone) at day two of cycle. Measurement of body mass index (BMI). Base line ultrasound to confirm polycystic ovaries on day 2 or 3 of cycle and to exclude ovarian cyst and other ovarian.

Fifty two PCOS Patients were given letrozole (Femara®, Novartis, Basel, Switzerland) orally 2.5 mg twice daily 12 hours apart from day 3 for 5 days of menstrual cycle. While sixty eight PCOS patients were given Clomiphene citrate (Clomid®, Sanofi Aventis, France) 50mg twice daily in two doses 12 hour apart from Day3 of menses for 5 days long.

Transvaginal ultrasound was done from day 10 of the menstrual cycle and every other day till we reach the optimal size of follicle where at least one follicle with mean diameter more than 18mm. A recombinant HCG (1-2) ampules (Ovitrelle®; Merck serono) was administrated subcutaneously to trigger ovulation and timed intercourse (24-36hour after hCG injection) was advised. All patients had a blood-based pregnancy test at 2 weeks after HCG injection. Followed-up by an ultrasound which could document the viability of pregnancy (fetal heart +ve).

Statistical analysis:

Most of the data was continuous so presented as mean ± standard deviation, and comparison between means of study groups done by using unpaired student t-test. Only non-continuous data presented as frequency and comparison between frequencies of study groups done by using Fisher exact test. Pearson correlation was done between different variables of each group. P value less than 0.5 was considered as significant. Microsoft excels 2016 and SPSS (statistical package for social sciences) version 23 was used as software do the statistics.

RESULTS

The demographic and hormonal parameter of the PCOS patients in both CC and letrozole groups). No significant difference between two groups in all parameter as shown in table (1)

Regarding stimulation parameters, table (2), letrozole group has significantly lower no. of developing follicle and lower estradiol level at day of HCG, but on the other hand has significantly larger size of follicles, also significant shorter duration of stimulation. In addition letrozole group has thicker endometrium if compared to CC group. Regarding pregnancy rate, letrazole group have higher although not significant pregnancy rate where 12 patients from 40 became pregnant (30%), if compared with those using CC in which 7 patients get pregnancy out of 40 (17.5%).

Table (1): Comparison of demographic and hormonal parameters between Clomiphene citrate and letrozole groups by unpaired t-test

Parameter		CC N=40 Mean ± SD	Letrozole N=40 Mean ± SD	P value
Age (yr)		29.58±5.81	28.45±5.95	0.395
BMI (kg/m^2)		24.87±2.85	25.29±2.76	0.505
Duration of infertility (yr)		3.5±1.88	3.53±1.87	0.953
*Type of Infertility	Primary	19	22	0.655
	Secondary	21	18	
S. FSH (mIU/ml)		7.33±2.15	7.38±1.71	0.914
S. LH (mIU/ml)		5.81±3.34	5.69±2.24	0.853
S. E2 (pg/ ml)		42.11±19.45	45.37±19.15	0.458
S. Prolactin (ng/ml)		14.31±7.16	14.97±5.93	0.653
S. Testosterone (ng/ml)		0.21±0.2	0.19±0.2	0.746

FSH= Follicle stimulating hormone, LH= Luteinizing hormone, E2= Estradiol , CC= clomiphene citrate, BMI= Body mass index, * Data expressed as frequency & percentage

Table (2): Stimulation protocols results parameters by unpaired t-test

Parameters	CC N=40 Mean±SD	Letrozole N=40 Mean±SD	P value
No. of follicles	2.35±0.92	1.83±0.38	**0.001**
Size of follicles	17.66±1.46	18.82±1.3	**< 0.001**
Duration of stimulation (days)	12.13±0.52	11.9±0.5	0.05
E2 level at day of hCG (pg/ml)	271.65±142.24	220.8±60.16	**0.041**
Average endometrial thickness (mm)	0.77±0.09	0.85±0.07	**< 0.001**
Pregnancy outcome No. (%)	12 (30%)	7 (17.5%)	0.293

CC= clomiphene citrate

Table (3): Comparison of endometrial pattern and echogenicity between of Clomiphene citrate group and Letrozole group

Endometrium	Parameter	CC N=40	Letrozole N=40	P value
Pattern	NTET	23	12	**0.024**
	TET	17	28	
Echogenicity	Hypoechogenic	10	30	**< 0.001**
	Hyperechogenic	30	10	

CC= clomiphene citrate, NTET: Non Triple Endometrium, TET: Triple Endometrium

Table (4): Comparison of pregnancy test between categorical parameters in Clomiphene citrate and letrozole groups by unpaired t-test

Parameter		CC N=40		Letrozole N=40	
		Pregnant	Non pregnant	Pregnant	Non pregnant
Endometrial Echogenicity	Hyper-echogenic	1	29	0	10
	Hypo-echogenic	6	4	12	18
	P value	**< 0.001**		**0.019**	
Endometrial pattern	NTET	1	24	2	10
	TET	6	11	10	18
	P value	**0.012**		0.285	

NTET= Non-triple endometrial thickness, TET= Triple endometrial thickness, CC= clomiphene citrate

The endometrial pattern of PCOS patient using letrozole showed significant more frequent triple endometrial pattern than in CC group, as shown in table (3). Furthermore PCOS patients using letrozole have highly significant more hypoechogenic endometrium than those use CC.

Table (4) showed that hypoechogenic endometrium was highly significant associated with pregnancy in both CC and letrozole groups. The same as the triple endometrium pattern was significantly higher in pregnant than non-pregnant in CC group, while it was also higher in letrozole group but without significance

The endometrial thickness shows on significant difference between pregnant and non-pregnant patients of both CC and letrozole groups. Table (5)

DISCUSSION

The data of this study showed that the mean no. of follicle which is observed by transvaginal ultrasound was significantly higher in clomiphene citrate compared to letrozole groups as demonstrated in table (2). This is agreed by other others, there were more follicles ≥16 mm in clomiphene group.[7,8] Another study show there was no significant difference between the total number of developing follicles in the letrozole and in the clomiphene citrate groups. [9] In the ovary, aromatase inhibitors increase follicular sensitivity to FSH by the accumulation of intraovarian androgens. As the dominant follicle grows and estrogen levels rise, normal negative feedback occurs centrally, resulting in suppression of FSH secretion and atresia of the smaller growing follicles resulting in single dominant follicle and mono-ovulation [10-12]. Other study explains that, the hypoestrogenic state created by letrozole not last late in the follicular phase of the menstrual cycle due to its short half-life, create a higher likelihood of mono-follicular growth.[13]

Current study showed that average size of dominant follicle was larger in letrozole group than that in clomiphene citrate with highly significant difference. As explained earlier the mechanism of Letrozole action, the selected follicle receive FSH stimulation allowing greater growth of follicle also it increase intrafollicular androgen in ovary which upregulate and sensitize FSH receptors in ovary.[11,12]

Also a higher level of estradiol on the day of HCG injection as shown in table (2) was noticed in Clomiphene Citrate group than that in letrozole group, the E2 elevation may have a detrimental effect on receptivity of endometrial. Some study show that excessive estrogen administered post ovulation can prevent implantation of blastocyst, there is little understanding of how varying levels of estrogen in serum and progesterone within the wide normal range, may influence receptivity [14]. This explains some of the adverse effects of Clomiphene on the outcome of infertility

treatment, although reducing estrogen synthesis by aromatase inhibitor may ameliorate such deleterious effects [15].

In addition, clomiphene citrate affects the endometrial development, causing implantation failure and thinning of the endometrium, this we clarified in our study in table (2). The mean thickness of endometrium in this study was higher significantly in a letrozole than that of clomiphene groups, these findings were consistent with other studies reporting that most patients taking letrozole had a thicker endometrium. Endometrial thickness (ET) measurement is a predictor for a successful implantation following ovulation induction, with many studies reporting more success with a thickness of 9 - 10 mm.[10,18] , there is no negative effect on endometrial thickness. This is due to the relatively short half-life of Letrozole, which allows complete recovery of endometrium before implantation.[18]. Compared to clomiphene citrate, letrozole is cleared from the circulation more rapidly due to a shorter half-life; and it does not deplete estrogen receptors and is devoid of any antiestrogenic peripheral actions; therefore, it has no adverse effect upon the receptivity of endometrium and the cervical mucus quality [18].

The endometrial echogenicity and pattern were significantly different between pregnant and non-pregnant in both clomiphene and letrozole groups, table (4), on the other hand as mentioned earlier letrozole has significant more hypoechogenic and triple endometrium pattern if compared to clomiphene as shown in table (3) ,this is agreed with other authors who stated that the triple layer of endometrial pattern was the most suitable for conception [19,20]. Contrary to other research results, Ng *et al.*, reported, no relationship between the endometrial thickness, morphology and pregnancy outcomes [21]. However, this reduction of the endometrial thickness did adversely affect pregnancy or ongoing pregnancy rates. The statistical data of the current study showed in table (4) that PCOS patient in letrozole group has significantly higher hypoechogenic endometrium if compared with clomiphene group that is agreed with Wei Liu *et al*[22] The endometrial thickness of 10–12.9 mm with a trilaminar appearance produced a highest clinical pregnancy rate (56.5%) compared to the least clinical pregnancy rate was recorded in the echogenic pattern group (6.5%).[23] This is in agreement with Check *et al*[15], who found that no pregnancies occurred in those patients with a homogenous hyperechoic endometrium [15]. Another study disagree with our result demonstrated that the homogeneous hyperechogenic endometrial texture on the day of ET was a predictive of implantation.[24]

In the current study, the trilaminar endometrial pattern was significantly higher in the letrozole group compared to the clomiphene citrate group, as clarified in table (3). This result agreed with the study of Jang and Jee which got significant difference between Clomiphene Citrate and letrozole group, which was more frequent in letrozole.[16]

Receptive endometrium is essential for the implantation of an embryo. Endometrial thickness, trilaminar pattern are the essential detriments of endometrial receptivity [25,26]. The pregnancy rate was lower in Clomiphene Citrate group than letrozole group with pregnancy rate 17,5% for Clomiphene Citrate and 30% in letrozole group, this is agreed by [17][27-30]. A likely explanation for the mechanism underling the lower rate of pregnancy in Clomiphene Citrate group was Clomiphene citrate is an anti-estrogen with a 60% - 80% ovulation rate and a 10% - 20% pregnancy rate. This is due to the anti-estrogen effect of Clomiphene citrate, resulting in long-lasting estrogen receptor (ER) depletion, also it accumulates in the body due to its long half-life (2 weeks), causing adverse effects on the quality and quantity of cervical mucus, these undesirable effects of Clomiphene citrate on the endometrium may explain the relatively poor pregnancy rate associated with Clomiphene citrate despite the high rate of ovulation [31].

CONCLUSION:

Letrozole induce better endometrial receptivity parameters than Clomiphene citrate.

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