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Efficacy of combined letrozole-metformin in comparison with letrozole only in clomiphene resistant infertile women with polycystic ovarian syndrome: a randomized controlled trial

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***Abstract*---**The aim of the work is to compare and determine the efficacy of combined metformin-letrozole administration in comparison with letrozole only in ovulation induction and the reproductive outcome in clomiphene – resistant infertile women with PCOS.The sample was 80 Women with PCOS and clomiphene citrate resistance. This study sample was allocated into two groups group (1) that received letrozole plus metformin, and group (2) that received letrozole only. All participants were followed up for 6 cycles and during this period monitoring through transvaginal ultrasonography was done every other day from day 12 of the cycle to assess follicular growth,number of follicles and endometrial thickness until at least one follicle reach 18 mm or more or reaching day 18.HCG was administered to those in whom at least one ovarian follicle reached 18 mm or more in size. The study revealed that Ovulation was 43.68 % in group (1) and 31.69 % in group (2) but without significant differences between both groups. pregnancy occurred in 8 women in group (1) and in 5 women in group (2) (20 % vs. 12.5 %) with no statistically significant differences between the two groups.

***Keywords*---**PCOS ; Polycystic ovary syndrome ; clomiphene resistant PCOS.

Introduction

Polycystic ovary syndrome "PCOS" is a complex endocrino-metabolic disorder characterized by hyperandrogenism,ovulatory dysfunction and polycystic ovarian morphologic features **(Waldman and Legro, 2019)**.It is the most common metabolic and reproductive endocrinopathy in reproductive aged women affecting 5%–8%,though with changes in the diagnostic criteria the prevalence has also nearly doubled.Among women with anovulation,PCOS is the cause in 55%–91% of cases **(Ndefo et al., 2013)**.Polycystic ovary syndrome (PCOS) accounts for the vast majority of anovulatory symptoms and hyperandrogenism in women **(Rosenfield, and Ehrmann**, 2016).It has life-long implications,with increased risk for infertility,metabolic syndrome and type 2 diabetes mellitus,and possibly for cardiovascular disease and endometrial carcinoma **(Hart, and Doherty, 2015)**.

Clomiphene citrate is the most commonly used Selective Estrogen Receptor Modulators (SERM) in ovulation induction in women with PCOS **(Palomba S,2015)**.It acts as estrogen receptor antagonist in the hypothalamus and stimulates gonadotrophin-releasing hormone (GnRH) and subsequent follicle-stimulating hormone (FSH) secretion especially relative to the excess of luteinizing hormone (LH) secretion that characterizes women with PCOS and restores follicular development **(Legro RS et al.,2014)**.Although ovulation rates are about 70-80%,the actual pregnancy rates are significantly lower at around 30-40%. Clomiphene citrate has peripheral antiestrogenic action at the level of the endometrium and cervical mucus which is partly explaining the discrepancy in ovulation rate and pregnancy rate **(Homburg, 2005)**.

Clomiphene resistance together with side effects as cyst formation and multifollicular development with antiestrogenic side effects persist the desire for some effective alternatives (**Homburg, 2005)**.

Alternative treatments for Clomiphene citrate failures involve parenteral gonadotropin injections that are more complicated and uncomfortable to administer,are very expensive and associated with more frequent and more serious complications.Owing to all these potential problems **(Casper and Mitwally, 2011)**,an oral substitute for Clomiphene citrate in the form of aromatase inhibitors,specifically letrozole began to be used **(Kar, 2013)**.Unlike clomiphene citrate, letrozole is devoid of any antiestrogenic peripheral action and elicits a monofollicular response and dose not adversely affect the endometrium or the cervical mucus **(Holzer et al. 2006)**.It also cleared from the circulation more rapidly due to a short half-life as compared to clomiphene citrate **(Young and Fritz, 1999)**.

Metformin was introduced in 1957 as an oral glucose-lowering agent to treat non-insulin dependent diabetes mellitus.It becomes widespread in clinical practice for the treatment of PCOS-associated symptoms such as

infertility,hirsutism and acne,and cycle regulation **(Johnson, 2014)**.In women with PCOS, insulin sensitizers (metformin) promote ovulation and lower androgen levels by about 20 %,but there is little evidence of a clinically significant improvement in hirsutism with the use of these agents **(Naka et al., 2011)**.Metformin has an additive effect in achieving ovulation and pregnancy when combined with drugs to induce ovulation **(Dasari and Pranahita, 2009)**.

Method

2. Materials and Methods

2.1. Patients Selection

The study was a randomized controlled trial (clinical trial.gov identifier:NCT03135301) in which 80 Women with PCOS and clomiphene citrate resistance were chosen from 111 PCOS women attending the outpatient infertility clinic of Sohag General Hospital and identified as eligible participants.PCOS was diagnosed on the basis of the revised Rotterdam 2003 criteria **(Rotterdam ESHRE/ASRM, 2004)**.The presence of 2 out of 3 criteria (oligo and/or anovulation, clinical and/or biochemical signs of hyperandrogenism and polycystic ovary) were recommended as diagnostic of PCOS.Clomiphene citrate resistance is defined as failure to achieve adequate follicular maturation after 3 consecutive induction cycles with Clomiphene citrate at 150 mg/day for 5 days **(Hughes E. et al 2009)**.

Inclusion Criteria includes patients aged 18-38 years who had failed to ovulate or become pregnant after 3 courses of 150 mg of clomiphene citrate (considered as clomiphene resistant),whereas the above mentioned investigation were normal.Exclusion Criteria includes women with other causes of infertility as male factor,tubal factor,those with endocrine disorders as thyroid dysfunction and hyperprolactinemia,women who received hormonal treatment or ovulation induction drugs in the preceding 3 months before the study,women with history of liver,kidney or cardiovascular disease.

2.2. Intervention Procedure

A series of blind envelopes numbered from 1 to 80 were prepared.Each patient was invited to pull out an envelope thus patients were assigned randomly into group (1) that received letrozole plus metformin, and group (2) that received letrozole only.In patients with amenorrhoea,withdrawal bleeding was achieved using Norethisterone Acetate 5 mg tablets (Steronate,Hi pharm,Egypt) 1 tablet twice daily for 5 days before stimulation.In both groups, 5 mg (2 tablets) of letrozole oral tablets (Femara,Novartis pharma services,Switzerland) were administered only for 5 days from day 3 of menses each month of spontaneous or induced bleeding.In group (1),metformin was started (Cidophage,Chemical industries Development,Egypt) from the first day with a dose of 500 mg (1 tablet before lunch) daily for 1 week to avoid nausea and vomiting then the dosage increased to 1500 mg (1 tablets 3 times before meal) daily and continued.

2.3. Ethical Approval

Before beginning the study, A written informed consent was obtained from all women participating in the study. The study was approved by the Scientific Ethical Committee of Faculty of Medicine, Assiut University.

2.4. Records

The following routine records were obtained for each patient before treatment including age,type of infertility(1ry or 2ry),duration of infertility and menstrual history,complete physical examination including BMI,basal hormonal assays were performed on day 2 or 3 of the menstrual cycle in women with oligomenorrhea and at random in women with amenorrhea and included measurements of the serum concentrations of luteinizing hormone (LH), follicle-stimulating hormone (FSH),testosterone,TSH and prolactin level.hystrosalpingography was performed at the the start of the study to exclude tubal factor infertility and uterine anomalies and husband's semen analysis.

2.5. Follow up

All participants were followed up for 6 cycles and during this period all patients were monitored through transvaginal ultrasonography every other day from day 12 of the cycle by single sonographer to assess follicular growth,number of follicles and endometrial thickness until at least one follicle reach 18 mm or more or reaching day 18.Total of 10.000 IU of HCG (Choriomon,IBSA, Switzerland) was administered to those in whom at least one ovarian follicle reached 18 mm or more in size.The woman was advised to have intercourse every other day for one week starting 24-36 h after receiving HCG with luteal support using dydrogesterone 10 mg tablets (Duphaston,Abbott Biologicals BV,The Netherland) twice daily for 2 weeks or continued if pregnancy test is positive.In order to confirm ovulation,transvaginal ultrasonograghy was performed 3 days after HCG injection to confirm reduction in the size of the follicle by at least 1/3 of its size or appearance of corpus luteum with its festooned edges.Women who didn't reach mature follicle until day 18 of menstrual cycle received Norethisterone Acetate 5 mg tablets (Steronate,Hi pharm,Egypt)1 tablet twice daily for 10 days to withdraw menses.In patients with delayed menstruation who had ovulated,pregnancy was confirmed by measurement of β-HCG in blood followed by transvaginal ultrasound diagnosis of intrauterine gestational sac and subsequent positive fetal heart activity.The primary outcome was to measure the number of growing and mature follicles and ovulation rate.The secondary outcome measures the pregnancy rate.

3. Results and Discussion

Table (1) shows the baseline data of studied group in which there are no statistically significant differences between the two groups with regard to age (P-value = 0.392),BMI (P-value = 0.203),duration of marriage (P-value = 0.202),parity (P-value = 0.255), previous abortions (P-value = 0.185),type of infertility (P-value = 1.000) and duration of infertility (P-value = 0.123).

Table (1)
Baseline data of studied groups

	Group(I) letrozole-metformin (n= 40)		**Group(II) Letrozole only (n= 40)**		**P-value**
Age: (years)					
Mean ± SD	25.95 ± 5.05		27.00 ± 5.84		0.392
BMI:					
Mean ± SD	27.88 ± 3.99		29.00 ± 3.85		0.203
Duration of marriage: (years)					
Mean ± SD	4.72 ± 3.48		5.28 ± 3.19		
Median (Range)	3.0 (1.0-13.0)		4.5 (2.0-14.0)		0.202
Parity:					
Nullipara	27	67.5%	23	57.5%	
Para 1	5	12.5%	10	25.0%	0.255
Para 2	6	15.0%	7	17.5%	
Para 3	2	5.0%	0	0.0%	
Previous abortions:					
None	21	52.5%	24	60.0%	
Once	11	27.5%	10	25.0%	0.185
Twice	7	17.5%	2	5.0%	
3 times or more	1	2.5%	4	10.0%	
Type of infertility:					
Primary	17	42.5%	17	42.5%	1.000
Secondary	23	57.5%	23	57.5%	
Duration of infertility: (years)					
Mean ± SD	2.75 ± 1.41		3.17 ± 1.36		
Median (Range)	2.0 (1.0-7.0)		3.0 (2.0-7.0)		0.123

Regarding the number of follicles in both groups in which there is significant increase in the number of follicles in group (1) in day 12 of the 2nd cycle (P-value =0.033), day 14 of the 4th cycle (P-value =0.036) and day 14 of the 5th cycle (P-value =0.027).otherwise there are no statistically significant differences between the two groups.

With regard to follicular growth in each cycle, there is significant increase in follicular growth in group (2) than group (1) in day 12 of the 1st cycle (P-value = 0.031).On the other hands, there is significant increase in follicular growth in group (1) than group (2) in day 16 of the 2nd cycle (P-value =0.029),day 18 of the 5th cycle (P-value =0.007) and day 14 of the 6th cycle (P-value =0.036), otherwise

there are no statistically significant differences between the two groups regarding follicular growth.At the same time,endometrial thickness in both groups shows no statistically significant differences between group (1) and group (2).On the other hand,the Number of women who developed follicles 18 mm or more and HCG was administered is significantly more in group (1) in the 1st cycle only (with 37.5% in group 1 vs 12.5% in group 2).with no other significant difference between the two groups in the following cycles **(Table 2).**

Table(2)
HCG administration

HCG administration	**Group(I) letrozole-metformin (n= 40)**		**Group(II) Letrozole only (n= 40)**		**P-value**
	No.	**%**	**No.**	**%**	
1st cycle:	**n= 40**		**n= 40**		
Yes	15	37.5%	5	12.5%	0.010*
No	25	62.5%	35	87.5%	
2nd cycle:	**n= 36**		**n= 39**		
Yes	17	47.2%	11	28.2%	0.089
No	19	52.8%	28	71.8%	
3rd cycle:	**n= 35**		**n= 38**		
Yes	11	31.4%	11	28.9%	0.817
No	24	68.6%	27	71.1%	
4th cycle:	**n= 32**		**n= 36**		
Yes	16	50.0%	19	52.8%	0.819
No	16	50.0%	17	47.2%	
5th cycle:	**n= 32**		**n= 36**		
Yes	16	50.0%	13	36.1%	0.248
No	16	50.0%	23	63.9%	
6th cycle:	**n= 30**		**n= 35**		
Yes	15	50.0%	12	34.3%	0.200
No	15	50.0%	23	65.7%	

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Finally **table (3)** shows a total number of 430 cycles where studied in 80 patients in 6 months (206 cycles in 40 patients in group 1 and 224 cycles in 40 patients in group (2).Ovulation occurred in 90 cycles (43.68 %) in group (1) and 71 cycles (31.69 %) in group (2) where the number of ovulatory cycles is more in group (1) but without significant differences between both groups.Regarding pregnancy outcome,pregnancy occurred in 8 women in group (1) and in 5 women in group (2) (20 % vs. 12.5 %) with no statistically significant differences between the two groups.

Table(3)

	Group(I) letrozole-metformin (n= 40)		Group(II) letrozole only (n= 40)		P-value
No. of ovulatory cycles:	90 (43.68%)		71 (31.69)		
Mean ± SD	2.32 ± 1.75		1.78 ± 1.37		
Median (Range)	2.0 (0.0-6.0)		2.0 (0.0-4.0)		0.172
No. of follow-up cycles:	206		224		
Mean ± SD	5.22 ± 1.66		5.60 ± 1.17		0.246
Pregnancy outcome:					
Pregnant	8	20.0%	5	12.5%	0.363
Not pregnant	32	80.0%	35	87.5%	

PCOS is a common disorder, often complicated by chronic anovulatory infertility and hyperandrogenism with the clinical manifestation of oligomenorrhoea, hirsutism and acne **(Franks S et al.,1995)**.The use of metformin for the management of type 2 diabetes mellitus is evidence based.Furthermore, it may Theoretically have a beneficial effect on ovulation rates in infertile and severely insulin-resistant women **(Nawrocka and Starczewski, 2007).**

In the present study, metformin was responsible for fewer developing and mature follicles; however,no significant difference between both groups.Ovulation was achieved in 90/206 cycles (43.68 %) in letrozole-metformin group which was higher than that of letrozole only group,71/224 cycles (31.69 %) but with no statistically significant differences between both groups.Pregnancy occurred in 8 women in letrozole-metformin group and in 5 women in letrozol only group (20 % vs. 12.5 %) with no statistically significant differences between the two groups.

To our knowledge, no studies yet compared the effects of combined letrozole-metformin with letrozole only in clomiphene resistant PCOS women.In other trials reported by **Abu Hashim H.(2015)** in which 250 women were studied for three successive cycles comparing letrozole versus combined metformin and clomiphene citrate in clomiphene resistant PCOS women,In this study,ovulation occurred in 185/285 cycles (64.9%) in letrozole only group and 207/297 cycles (69.6%) in metformin-clomiphene citrate group without significant difference between both groups. In the same study,Pregnancy occurred in 42/285 cycles (14.7%) in letrozole group and 43/297 cycles in metformin- clomiphene citrate group (14.4%) and the difference was not statistically significant.

In the study performed by **Sohrabvand et al.(2006)**, comparing letrozole with clomiphene citrate in 60 clomiphene resistant infertile women with PCOS after initial 6-8 weeks of metformin treatment, there was no significant difference between both groups regarding the number of follicles reaching ≥ 18 mm in diameter (Mean ± SD 1.90 ± 0.29 vs 1.80 ± 0.39).in the same study, The rate of pregnancy per cycle was 7% (5 of 67 cycles) in the clomiphene group and 19% (10 of 53 cycles) in the letrozole group, which was not statistically significant (*P* = 0.06).

Another study performed by **Davar et al.(2004)** analyzing the effect of combined metformin-letrozole in comparison with combined metformin-clomiphene citrate in 20 clomiphene resistant PCOS patients undergoing IUI where there was no significant difference between both groups regarding pregnancy rate (8.3% in metformin–letrozole group as compared to 2% in metformin– clomiphene group; $p > 0.05$)

In the study carried out by **Abd Elgafor et al.(2013)** who compared the efficacy of combined metformin-letrozole with laparoscopic ovarian drilling in 146 clomiphene resistant PCOS women for 6 months,it was found that there was no significant difference in cycle regularity, ovulation, pregnancy rate and abortion rate between metformin plus letrozole group and LOD group.

In this study,there is a nonsignificant increase in endometrial thickness in letrozole only group.Similar results were reported by **Abu Hashim H.(2015)**.However **Sohrabvand et al.(2006)** showed that the mean endometrial thickness was significantly lower in the clomiphene citrate group (0.55 ± 0.28 cm versus 0.82 ± 0.13 cm) which is similar to the results achieved by **Davar et al.(2004)** and these results were explained by the negative effects of clomiphene citrate on the quality or quantity of the endometrial mucosa due to the anti-estrogenic effect of clomiphene citrate thus decreasing endometrial thickness by its long-term effect in decreasing the number of estrogen receptors **(Mitwally and Casper, 2001)**.

Conclusions

our findings suggest that combined letrozole-metformin and letrozole only are equally effective for induction of ovulation and achieving pregnancy in patients with clomiphene resistant PCOS.Metformin has no additional effect on ovulation or pregnancy rates. We recommednd that extended treatment of metformin is to be investigated to determine its efficacy when combined with letrozole and larger sample size is needed before making solid conclusion.

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