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GYNECOLOGIC ENDOCRINOLOGY AND REPRODUCTIVE MEDICINE

The effectiveness of inositol and metformin on infertile polycystic ovary syndrome women with resistant to letrozole

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Abstract

Purpose The purpose is a comparison of effectiveness of myo-inositol and metformin in infertile women with polycystic ovary syndrome (PCOS) treated with letrozole.

Methods This study is a randomized single-blind controlled clinical trial undertaken in 150 infertile PCOS women. For all patients, letrozole is prescribed at a dose of 7.5 mg per day from the third day of menstruation for 5 days. Patients who did not ovulate were included and divided into three pretreatment groups: group I(control group), 200 µg of folic acid (as a placebo); group II, 1500 mg of metformin daily plus 200 µg of folic acid, and group III, inositol 2 g plus 200 µg of folic acid received twice daily for 3 months. In the last cycle, 7.5 mg letrozole was prescribed for the induction of ovulation. Primary outcomes were ovary function and pregnancy.

Results The ovarian function was not significantly different in those groups, whereas the ovarian function of inositol + folic acid group in normal BMI found significantly higher than other BMI spectra. In addition, the ovarian function is significantly higher in the inositol + folic acid group by increasing the infertility duration. The incidence of pregnancy is lower in letrozole + folic acid + inositol group than the other groups; however, it is not significant.

Conclusion The addition of inositol and metformin to the treatment of infertile PCOS women with letrozole resistance improves the ovarian function; however, it is not significant. Of note, inositol was more effective than metformin in patients with normal BMI.

IRCT registration number IRCT2017070234845N1

Keywords Infertility · Inositol · Metformin · letrozole · PCOS

Introduction

Polycystic ovary syndrome (PCOS) is the most common endocrinopathy which affects 5–10% of women of reproductive age [1]. This syndrome is characterized by chronic anovulation, hyperandrogenemia, and the presence of polycystic ovary in ultrasound [2]. PCOS is the most common cause of infertility, and the clomiphene citrate is the first line in infertility treatment of PCOS patient [3]. About 20% of PCOS patients are resistant to clomiphene; therefore, the replacement of other drugs such as letrozole is required for their treatment.[4]. This drug is an aromatase inhibitor which controls the conversion of androgen to estrogen and increases ovarian androgens [5]. The administration of letrozole in the follicular phase removes the effect of negative estrogen feedback on the pituitary and hypothalamus, thus increasing the gonadotropins [5]. A 2.5 mg letrozole used daily by clomiphene-resistant women resulted in ovulation and increased pregnancy. It should be noted that some patients are also resistant to letrozole [6].

Moreover, insulin resistance is the most common feature in PCOS women; therefore, the use of insulin sensitizer such as metformin leads to weight loss, decreasing insulin resistance, which denotes the levels of androgen and restoration of normal menstrual cyclicity as well as ovulation [7];

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however, it has gastrointestinal side effects [8]. Recently, inositol introduced as a new insulin sensitizer in the treatment of subjects with PCOS. A few studies have reported the beneficial effects of inositol on insulin sensitivity and androgens [9]. In addition, it improved reproductive function in patients with PCOS by reducing the hyperinsulinemia state [10]. In contrast to metformin, no side effects have been reported following treatment with myo-inositol [11].

To the best of our knowledge, data on the comparison of inositol and metformin on ovarian function and incidence of pregnancy in PCOS patient with letrozole resistance are scarce. Therefore, the aim of this study was to compare inositol and metformin on ovarian function and incidence of pregnancy in these patients.

Material and methods

Design and data collection

This study was a single-blinded randomized clinical trial undertaken in infertility clinic of Hormozgan University of Medical Sciences, Hormozgan, Iran. A table of random numbers was used for randomization. The sample size was calculated; at least 45 people were estimated for each group.

After the approval of the ethics committee of Hormozgan University of Medical Sciences, Iran, this study was conducted in 2015–2016. Written consent was obtained from all participating women and the parents of individuals of younger age; all participants were aware that they could withdraw any time during the trial.

Recruitment of participants

Inclusion criteria were individuals of 15–38 years old with PCOS defined according to Rotterdam criteria as having at least two of the following three features: oligo and/or anovulation, hyperandrogenism (clinical and biochemical), and polycystic ovaries on ultrasound scan; inability to get pregnant despite having frequent, unprotected intercourse for at least a year; absence of tubal, anatomic and male factors; intact uterine cavity and normal level of thyroid hormones. The study excluded patients diagnosed with the other endocrine disorders like hyperprolactinemia as well as the patients who have no desire for cooperation.

Study design

For all patients, letrozole (Femara, Novartis Pharmaceuticals Canada, Inc., Dorval, Quebec) is prescribed at a dose of 2.5 mg per day from the third day of menstruation for 5 days. The patients were then evaluated during 12–16 days of menstrual cycles for the response of the drug, the size of the follicles reached in the ovary, and the thickness of the endometrium by vaginal ultrasonography using a 6–9 MHz convex-array transducer (Ultrasonix RP, Vancouver, BC, Canada). With the observation of at least one mature follicle (≥17 mm), 10,000 units of HCG were injected. Patients who did not ovulate after 2.5 mg letrozole received a 5 mg letrozole for next cycle, after which the patients who did not ovulate, the dose of letrozole increased to 7.5 mg in next cycle, and then, patients who did not ovulate were divided into three pretreatment groups: group I (control group) 200 µg of folic acid (as a placebo); group II 1500 mg of metformin(Apotex, Toronto, Canada) daily plus 200 micrograms of folic acid (Apotex, Toronto, Canada) and group III, 2000 mg myo-Inositol (amazing nutrition, Jersey, USA) plus 200 µg of folic acid twice daily for 3 months. In the last cycle of pretreatment, 7.5 mg letrozole was prescribed daily from the third day of menstruation for 5 days. The patients were then evaluated during 12–16 days of menstrual cycles for the response of the drug, the size of the follicles reached in the ovary, and the thickness of the endometrium by vaginal ultrasonography using a 6–9 MHz convex-array transducer (Ultrasonix RP, Vancouver, BC, Canada).With the observation of at least one mature follicle (≥17 mm), 10,000 units of HCG were injected. Primary outcomes were ovary function and incidence of pregnancy. The ovarian function was evaluated by the presence or absence of a mature follicle (≥17 mm seen by transvaginal ultrasound) during 12–16 menstrual cycles. The clinical pregnancies were identified by the presence of a gestational sac on ultrasonography 5 weeks after HCG injection.

Ethical considerations

The Ethics Committee of the Hormozgan University of Medical Sciences, Bandarabbas, Iran, approved the study.

Data analysis

Data were analyzed by descriptive statistics (standard deviation, mean, percent, and frequency), followed by Chi-square, ANOVA, and Kruskal–Wallis tests. The Scheffe test was used for post hoc analysis. Data were analyzed using statistical software (version 21) (SPSS Inc., Chicago, IL, USA). The significance level for all tests $P < 0.05$ was considered.

Result

Sample characteristics

We assessed 210 subjects for eligibility, out of which 24 were excluded, because they did not meet the inclusion criteria. 186 recruited patients randomly divided into

three groups (62 subjects in each group). Among recruited patients, 28 declined to participate for personal reason, 12 in control (letrozole + folic acid), 9 in group III (letrozole + myo-inositol + folic acid), and 7 in group II (letrozole + metformin + folic acid). The patients who continued the study were 158. Of these, 8 patients were dropped out of the study due to fear of adverse effects and drug intolerance. Finally, 50 subjects were placed in group I, 50 in group II and 50 in group III. The process of allocating participants during 2015–2016 is shown in Fig. 1.

Socio-demographic and clinical characteristic of the patients is presented in Table 1. There is no significant difference in appearance between study groups ($P > 0.05$) (Table 1). Our data have shown that there was no side effect in control and myo-inositol group, but the incidence of a side effect was 42% (21/50) in the metformin group.

Incidence of pregnancy

Table 2 shows a lower incidence of pregnancy in letrozole + folic acid + myo-inositol group than other groups; however, it is not significant ($p > 0.05$).

Ovarian function

Table 3 shows no significant difference between the three groups in terms of ovarian function ($p > 0.05$). Although the ovarian function is slightly lower in letrozole +folic acid + inositol than in metformin+ folic acid + letrozole groups.

Table 4 shows a significant difference in terms of ovarian function based on the duration of infertility between the three groups. Post hoc analysis showed a significant difference between letrozole + folic acid and myo-inositol + folic acid groups ($P = 0.001$).

It shows that improvement of ovarian function in the two mentioned groups is desirable by reducing the duration of infertility. In other words, in patients with longer duration of infertility, treatment with myo-inositol + folic acid is more effective in improving ovarian function.

There was also a significant difference between the three groups for ovarian function based on BMI (Table 4). Post hoc analysis showed that, in the normal BMI, ovarian function was significantly higher in myo-inositol + folic acid group than letrozole + folic acid group ($P = 0.002$), and it is significantly higher in letrozole + folic acid group than metformin +folic acid ($P = 0.002$), as well.

In addition, Table 4 shows that improvement of ovarian function in myo-inositol + folic acid group in normal BMI (18.5–24.9) is higher than other BMI spectra, and ovarian function decreased in this group by the increase of BMI. Despite overweight and obese patients, the ovarian function was significantly higher in metformin +folic acid group than other group.

Discussion

To our knowledge, for the first time, we compared the effect of inositol and metformin on ovarian function and incidence of pregnancy in infertile PCOS women resistant to letrozole. Our data show that metformin and myo-inositol have improved the ovarian function, but the difference was not statistically significant. Moreover, there was no side effect in control and myo-inositol group; however, 42% of patient affected by side effect of metformin. Our data were consistent to Ciotta and colleagues who suggested that myo-inositol may be useful in the treatment of PCOS patients undergoing ovulation induction, both for its insulin-sensitizing activity, and its role in oocyte maturation [12]. These results, like other trials, suggest that myo-inositol has a positive effect on mature oocytes development [13, 14]. The previous studies have shown that myo-inositol through inositol triphosphate regulates thyroid-stimulating hormone and follicle-stimulating hormone [15], and is responsible for glucose uptake [16], which, in turn, increases insulin sensitivity. The use of inositol directly influences steroidogenesis by reducing androgen production in theca cells. In fact, the administration of inositol has been demonstrated to increase the insulin action in PCOS patients, improving ovulatory function [10], and reducing serum testosterone concentration [17]. Moreover, Chiu and colleagues have reported that a higher concentration of inositol in the human follicular fluid leads to follicular maturation and provides a marker of good oocyte quality [18]. In addition, Zheng and colleagues conducted a systematic review and meta-analysis study which reported that inositol supplement improves oocyte and embryo quality resulting in the increase of the clinical pregnancy rate in infertile women undergoing ovulation induction for intracytoplasmic sperm injection (ICSI) and in vitro fertilization–embryo transfer (IVF-ET) [19].

Our data show that, in patients with longer duration of infertility, treatment with inositol + folic acid is more effective in the improvement of ovarian function. Furthermore, the current study has shown that the ovarian function of the inositol + folic acid group in normal BMI (18.5–24.9) is significantly higher than other BMI spectra and ovarian function decreased in this group by increasing BMI. Likely, it is related to insulin resistance associated with increasing BMI.

We found that myo-inositol administration for 12 weeks in letrozole-resistant PCOS subjects led to decreases in pregnancy rate in comparison to metformin and control groups, but their difference is not statistically significant. Our data were inconsistent with Raffone et al. compared the effectiveness of myo-inositol and metformin, and they

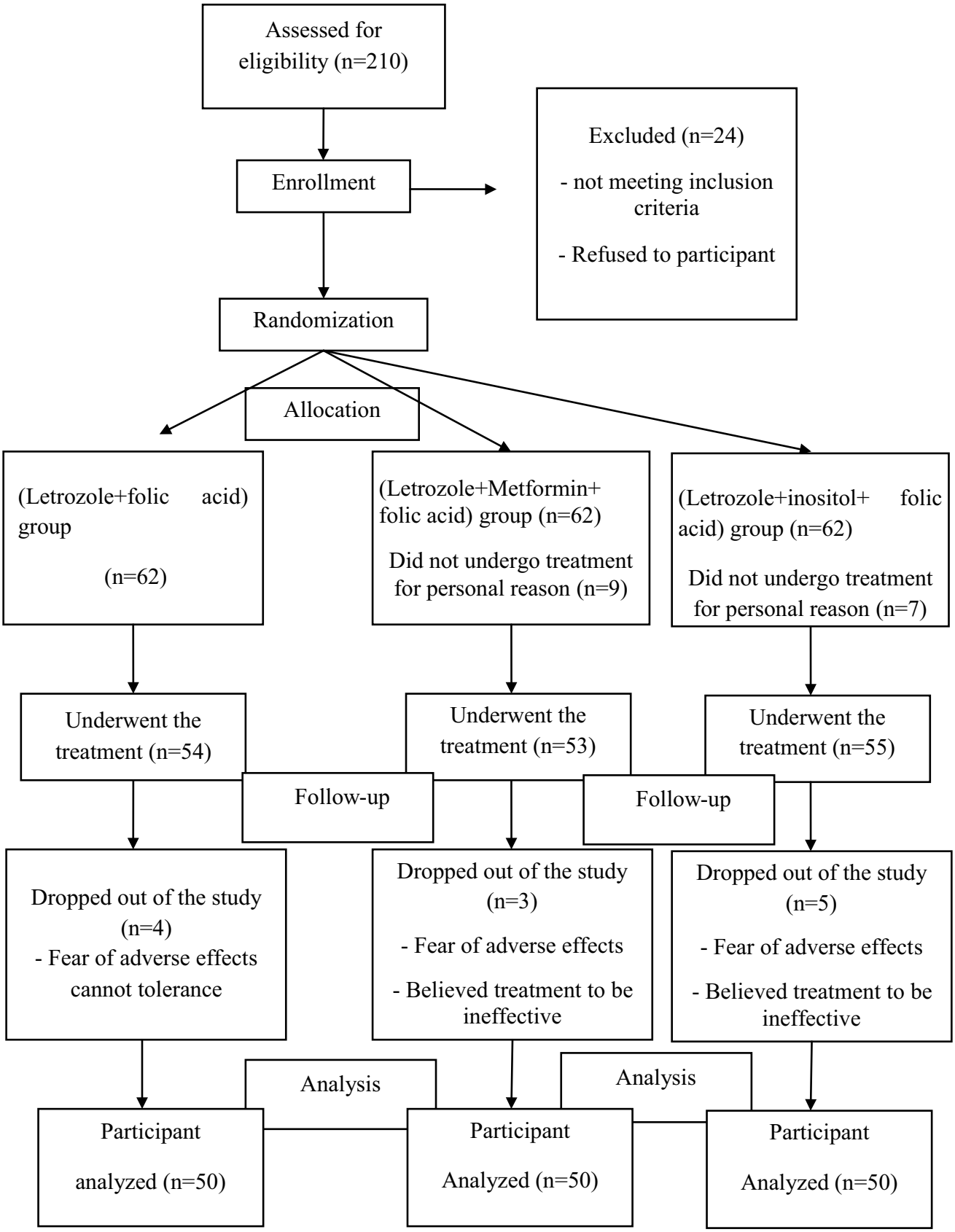

Fig. 1 The process of allocating participants during 2015–2016

Table 1 Clinical characteristics of the patient in study groups

Variable	Groups				
	Group I (letrozole + folic acid) ($n=50$)	Group II (letrozole + metformin + folic acid) ($n=50$)	Group III (letrozole + myo-inositol + folic acid)	Statistical test	*P* value
Age*	30.42 ± 2.58	31.06 ± 1.11	31.08 ± 3.31	$F = 0.75$	0.47
Infertility duration*	4.36 ± 1.005	5.10 ± 1.11	6.36 ± 2.25	$F = 0.61$	0.39
BMI*	27.38 ± 4.02	27.84 ± 3.68	29.79 ± 3.58	$F = 0.0.43$	0.54
FBS*	102.92 ± 18.55	113.01 ± 14.26	105.40 ± 16.92	$F = 0.96$	0.48
Type of infertility**					
Primary	27 (54)	26 (52)	26 (52)	$X2 = 0.05$	0.97
Secondary	23 (46)	24 (48)	24 (48)		
Triglyceride *	146.20 ± 16.35	157.12 ± 19.68	145.36 ± 16.28	$F = 0.39$	0.53
Clostrole *	176.14 ± 20.44	186.98 ± 19.95	181.54 ± 23.08	$F = 0.48$	0.16
Hirsutism**					
Yes	16 (32)	20 (40)	14 (28)	$X2 = 1.66$	0.43
No	32 (68)	30 (60)	36 (72)		
LH/FSH**					
Normal	5 (10)	3 (6)	4 (8)	$X2 = 0.54$	0.76
Abnormal	45 (90)	47 (94)	46 (92)		
Menstrual pattern **					
Amenorrhea	15 (30)	13 (26)	17 (34)	$X2 = 0.45$	0.29
Oligomenorrhea	24 (48)	27 (54)	29 (58)		
Normal	11 (22)	10 (20)	4 (8)		

The data show that all groups were comparable ($P \leq 0.05$)

BMI body mass index, *FBS* fasting blood sugar, *LH* luteinizing hormone, *FSH* follicle-stimulating hormone

* represents the level of significance was set at $P < 0.05$

Table 2 The comparison of the incidence of pregnancy in study groups

Variable	Groups				
	Group I (letrozole + folic acid) ($n=50$)	Group II (letrozole + metformin + folic acid) ($n=50$)	Group III (letrozole + myo-inositol + folic acid) ($n=50$)	Statistical test	*P* value
Pregnancy incidence*					
Yes	16 (32)	19 (38)	14 (28)	$X2 = 1.14$	0.56
No	34 (68)	31 (62)	36 (72)		

The data show that there is no significant difference between all groups ($P \leq 0.05$)

*n (%)

Table 3 The comparison of ovarian function in study groups

Variable	Groups				
	Group I (letrozole + folic acid) ($n=50$)	Group II (letrozole + metformin + folic acid) ($n=50$)	Group III (letrozole + inositol + folic acid) ($n=50$)	Statistical test	*P* value
Ovarian function*					
Abnormal	26 (52)	17 (34)	19 (38)	$X2 = 1.14$	0.56
Normal	24 (48)	33 (66)	31 (62)		

The data show that there is no significant difference between all groups ($P \leq 0.05$)

*n (%)

Table 4 The comparison of ovarian function in study groups based on age, infertility duration, and and BMI between the three groups

Variable	Groups			Statistical test	*P* value
	Group I (letrozole + folic acid) ($n=50$)	Group II (letrozole + metformin + folic acid) ($n=50$)	Group III (letrozole + myo-inositol + folic acid) ($n=50$)		
Age*					
24–28	7 (29.16)	7 (2.21)	8 (25.8)	Post hoc	0.67
29–33	13 (54.16)	17 (51.51)	10 (51.61)		
34–38	4 (16.66)	9 (27.27)	7 (22.58)		
Infertility duration*					
2–5 years	20 (83.33)	23 (69.69)	15 (48.38)	Post hoc	0.001
6–9 years	4 (16.66)	10 (30.30)	16 (51.61)		
BMI*					
18.5–24.9	10 (41.66)	3 (9.09)	18 (58.06)	Post hoc	0.002
25–29.9	10 (41.66)	19 (57.57)	9 (29.03)		
30–34.9	3 (12.5)	11 (33.33)	1 (3.22)		
35–39.9	1 (4.16)	–	3 (9.67)		

BMI ($P \leq 0.05$). There was a significant difference in terms of ovarian function based on the duration of infertility

*n (%)

found that 39/60 patients restored monthly ovulation in the myo-inositol group and 30/60 in metformin. Numbers of pregnancy/patients with restored ovulation were 18/39 (46.1%) in myo-inositol and 11/30 (36.6%) in the metformin group. In addition, in this study, there was no significant difference between treatments even if authors stated that myo-inositol treatment seems to be more effective than metformin [20]. Moreover, inconsistent with our study, Rezk et al. showed that the addition of metformin during ovulation induction with letrozole does not affect pregnancy outcome in infertile women with PCOS [21]. Therefore, we are suggesting that inositol administrated in combination with the other substance in future studies, because recent studies have found a major bioavailability of myo-inositol due to a higher absorption through the activity of alpha-lactalbumin [22].

In conclusion, our data show that the addition of inositol and metformin in the treatment of infertile PCOS women with letrozole resistance improves the ovarian function; however, there is no side effect in inositol group. Despite our data, we need further studies on larger RCT and with greater statistical power with the assessment of the effect of inositol and metformin on hormonal and metabolic and genetic profile in infertile PCOS women resistant to letrozole.

Author contributions SP: data acquisition, conception and design; writing and confirming the final draft. FB: revise, reading, English diting and confirming the final revised manuscript. SAT: Conception and design; writing and confirming the final draft. MAK: Recording the outcomes; providing resources, reading and confirming the final draft

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Compliance with ethical standards

Conflict of interest The authors have any conflict of interest to declare regarding the manuscript.

Ethical approval All procedures in the current study were in accordance with the ethical standards of the Hormozgan University of Medical with the 1964 Helsinki declaration.

Informed consent All the participants provided their informed written consents before inclusion in the study.

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