Combined Insulin Sensitizers as Double-Weapon to detonate PCOS-induced Vicious Circle

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Abstract

Objectives : assessment of the outcomes of women had polycystic ovary syndrome (PCOS) received 6-month therapy of metformin (Met) or combination of myoinositol (MI)/D-chiro-inositol (DCI) or both.

Patients & Methods : 210 PCOS women underwent clinical, US and laboratory work-up to determine baseline T0-data and were divided into 3-equal groups: Met group received met 500 mg tab three times daily, MI-group received MI/DCI combination in 40:1 ratio twice daily and MM-group received both Met and MI therapy and at the end of 6-m (T6) therapy, all patients were re-evaluated to assess gynecological outcomes including resumption of regular menstrual pattern (RMP) and getting pregnant for PCOS-infertile women wishing for pregnancy and metabolic and endocrinal outcomes including impact on body mass index (BMI), glucose tolerance (GT), homeostasis model assessment of insulin resistance (HOMA-IR) index, and hyperandrogenemia.

Results: At T6, 126 women resumed RMP and 15.8% of women got pregnant. T6-BMI was significantly decreased in all women with improved GT and only 15 of 67 insulin resistant women were still resistant. The frequency of women had serum total testosterone (TT) >0.8 ng/ml was decreased from 35.2% to 13.3% with significantly lower levels of TT at T6-samples than T0-samples. The percentages of change in the studied parameters were higher with MM-therapy than either Met or MI-therapy. The rate of resumption of RMP and percentages of change of BMI, HOMA-IR and serum TT were positively correlated but showed negative relation to the use of insulin sensitizer monotherapy. The Receiver Operating Characteristic (ROC) curve analysis defined high percentage of decrease of HOMA-IR index as positive and the use of monotherapy as negative predictor for getting RMP.

Conclusion: Insulin sensitizers' therapy is effective and safe for control PCOS-associated endocrinal, metabolic and gynecological deregulations. Inositol is a synergistic additive to metformin and this combination results in favorable outcomes than monotherapy.

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Introduction

Polycystic ovary syndrome (PCOS) is a common and complex disease affecting women of reproductive age [1] and is characterized by its complex pathological symptoms and mechanisms resulting in endocrine and metabolic dysfunction [2]. Ovulatory dysfunction, increased ovarian volume and/or polycystic ovary morphology with concomitant menstrual abnormalities, chronic anovulation, and decreased fertility or infertility are the characteristic gynecological manifestations of PCOS [3].

PCOS is highly associated with various metabolic and endocrinal disorders, because of the shared common risk factors; PCOS and metabolic-associated fatty liver disease (MAFLD) are concomitant and at the time of PCOS diagnosis, screening for MAFLD is mandatory because it is mostly asymptomatic [4]. The relation between PCOS and epithelial ovarian tumors is biologically plausible because obesity, hyperandrogenemia and fertility disorders, which are inherent to PCOS, are also risk factors for hormone-sensitive tumors [5].

Despite the high prevalence of PCOS, medical treatment is a dilemma because no available pharmacological option can tackle the entire spectrum of PCOS manifestations [6]. Metformin (Met) has pleiotropic actions, but is mainly, used for its glucose-lowering effects for treatment and prevention of type-2 diabetes mellitus (DM), gestational DM and PCOS [7]. Met through decreasing food intake and body weight, and improving lipid profile can influence multiple cardiovascular risk markers, improve MAFLD, modulate inflammatory markers, and possibly reduce cancer risks [8]. Met is widely used because of its positive glycemic control, safety profile, and low costs, but is not well accepted by all patients due to its common gastrointestinal adverse effect [9].

Myo-inositol (MI) is biosynthesized from all MI-containing compounds, by cyclic synthesis and through hydrolysis of phosphatidylinositol [10]. MI protects against MAFLD through reduction of hepatic accumulation of triglycerides [11] and decreases left ventricular stiffness through removal of cholesterol from the myocardium and increasing cardiac function [12]. Hyperglycemia, hypertriglyceridemia and insulin resistance (IR) induces inositol imbalance with deficiency of D-chiro-inositol (DCI) and higher MI levels and DCI administration might improve this imbalance and IR [13].

Objectives

This prospective study compared the effects of MI/DCI combination alone or in conjunction with Met on PCOS-associated endocrinal and metabolic disturbances.

Patients & Methods

All women presented to Gynecology outpatient clinic or Infertility clinic, Zagazig University Hospital with manifestations suggestive of PCOS were clinically evaluated for the presence of at least two of the Rotterdam criteria for diagnosis of PCOS (14, 15) and women had these criteria were evaluated.

Baseline clinical data collection

Collection of clinical data included age, residence, level of education, type of work, marital and fertility statuses, and if infertility was the main complaint. History taking included inquires about the presence of risk factors as sedentary lifestyle, emotional stress and family history of PCOS, obesity-related medical disorders especially DM or MAFLD, history of previous treatment for PCOS and its outcomes. Menstrual pattern such as infrequent menstrual periods with interval between menstrual periods of ≥35 days or amenorrhea which is defined as absence of vaginal bleeding for at least 90 days was discussed

Exclusion criteria

Women had other manifestations of metabolic

syndrome, cardiac manifestations of PCOS, maintained on other therapies or prepared for /received laparoscopic intervention for PCOS, receiving scheduled exercise, lipid-lowering therapies, or maintained on diabetogenic drugs for any other indications were excluded from the study. Also, women had morbid obesity with body mass index (BMI) >35 kg/m2, causes other than PCOS for infertility, manifest DM, hepatic or pancreatic diseases, refused to participate in the study or missed during follow-up were excluded from the study.

Inclusion criteria

Women with diagnostic criteria of PCOS and free of exclusion criteria, accepted to participate in the study and signed the written fully informed consents were included in the study

Study Protocol

The enrolled women were randomly allocated into three groups according to the scheduled therapy for each group. Women were evaluated at time of enrolment (T0) and at the end of 6-m duration of therapy (T6) for:

1. Metabolic disturbances including

- Obesity as judged by BMI which is determined using the equation of weight divided by square height in meter (16).
- Deregulated glucose tolerance as determined using the 75-oral glucose tolerance test (OGTT) that entails estimation of fasting blood glucose (FBG) and postprandial blood glucose (PPBG) at 1-h and 2-h in response to taking 75-g oral glucose and interpreting the results according to the recommendations of the International association of diabetes and pregnancy study groups (IADPSG) (17) as follows: FBG ≥92 mg/dl, 1-h PPBG ≥180 mg/dl and 2-h PPBG ≥153 mg/dl indicates glucose intolerance.
- Insulin resistance was evaluated using homeostasis model assessment of insulin resistance (HOMA-IR) (18) with index ≥2 was indicated IR (19).

2. Clinical hyperandrogenemia was evaluated as

- The presence and severity of hirsutism according to the modified Ferriman-Gallwey (FG) map (20) that divided the body into 9 areas, each area was evaluated using 5-point scale with higher score indicating more extensive hair growth and a score of ≥8 indicates hyperandrogenemia.
- Acne scoring was determined as previously by Adityan et al. (21) as comedones, occasional papules (Grade 1), papules, comedones, few pustules (Grade 2), predominant pustules, nodules, abscesses (Grade 3) and mainly cysts, abscesses, widespread scarring (Grade 4).

3. Ovarian morphology was assessed using either transabdomial or transvaginal ultrasonography (TAU, TVU) for ovarian volume and number of ovarian follicles; ovarian volume >10ml per ovary and/or detection of >12 follicles of 2-9 mm are diagnostic of PCOS.
4. Laboratory evaluations: blood samples were obtained for estimation of FBG & PPBG, fasting serum insulin (FSI), total testosterone (TT) and dehydro-epiandrosterone (DHEA); serum level of TT >0.8 ng/ml indicates biochemical hyperandrogenemia.

Treatment protocol

Patients were divided into three treatment groups; Met-group received metformin hydrochloride (Cidophage tab, Chemical Industrial Development, Cairo Egypt) 500 mg three times daily, MI-group received tablets containing MI/DCI combination in dose of myoinositol 550 mg and DCI 13.8 mg in a 40:1 ratio with 0.2 mg folic acid (Viocyst

tab, Viomix Pharmaceutical Industries, Egypt) twice daily and MM-group received combination of the treatment received by patients of groups Met and MI. Treatment for all groups was continued for six months with no lifestyle changes, dieting regimens or exercise protocols.

Ethical considerations

The study protocol was approved by the departmental committee and discussed freely with women who had PCOS, and those accepted to participate in the study, receive the assigned therapy and attend for follow-up after 6-m had signed a written fully informed consent to undergo the preliminary clinical examination and US and lab evaluations. At the end of 6-m drug therapy and defining the study outcomes, the final approval by the Local Ethical Committee was obtained (ZU-IRB #11195-5/11-23 and the study was registered at Clinical trial.com: NCT06170463. As a reference for lab findings, 16 women who are free of gynecological problems, accepted to give blood samples and meet exclusion criteria were chosen from those attending the family planning clinic.

Randomization & Blindness

Randomization of the enrolled women was conveyed by an assistant who was blinded about the study protocol by computer sequencing system with 1:1:1 sequence and even-numbers dropping to provide the sequence of cases for each group. Patients' sequence was transformed to group title; Met, MI and MM and women were asked to choose an envelope that contained the drug regimen to be followed and scheduled follow-up visit for each woman. The author was blinded about the sequencing process and patients' distribution and drug regimen used. At the 6-m visit and after patients' evaluation, patients' sequence was declared and outcomes were interpreted.

Statistical analysis

One-way ANOVA test and Chi-square test (X2 test) were used to assess the significance of the results. Evaluation of predictability was conducted using the Receiver characteristic curve. The significance of the area under ROC curve (AUC) was assessed in relation to area under the reference curve using IBM® SPSS® Statistics (Version 22, 2015; Armonk, USA). Significance of the results was determined using a P-value at cutoff point of 0.05.

Results

Evaluation process excluded 27 women; 8 women were maintained on PCOS treatment regimens, 4 women had manifest DM, 5 women had bariatric surgery for obesity and eight women had concomitant causes for infertility, while two women refused to participate in the study. Another 15 women did not attend the follow-up visit and 18 women at follow-up assured receiving therapy intermittently and these 34 women were also excluded from the study. At 6-m visit, 70 women per group were evaluated and revision of their enrolment data showed insignificant differences between the three groups (Table 1, Fig. 1).

Table 1: Patients' enrolment data

		Met	MI	MM	P
Age (years)		27.8±4	28.2±4.7	27.5±4.3	0.636
Residence	Urban	38 (54.3%)	31 (44.3%)	43 (61.4%)	0.124
	Rural	32 (45.7%)	39 (55.7%)	27 (38.5%)	
Education	Illiterate	19 (27.1%)	22 (31.4%)	14 (20%)	0.299
	Literate	51 (72.9%)	48 (68.6%)	56 (80%)	

Work	Housewives	26 (37.1%)	18 (25.7%)	24 (34.3%)	0.714
	Officers	17 (24.3%)	22 (31.4%)	19 (27.1%)	
	Workers	15 (21.5%)	19 (27.2%)	14 (20%)	
	Farmers	12 (17.1%)	11(15.7%)	13 (18.6%)	
Menstrual pattern	Infrequent cycles	57 (81.4%)	53 (75.7%)	55 (78.6%)	0.712
	Amenorrhea	13 (18.6%)	17 (24.3%)	15 (21.4%)	
Marital status	Single	16 (22.9%)	10 (14.3%)	12 (17.1%)	0.407
	Married	54 (77.1%)	60 (85.7%)	58 (82.9%)	
Infertility as the main complaint among married women	Yes	32 (45.7%)	28 (40%)	35 (50%)	0.161
	No	22 (31.4%)	32 (45.7%)	20 (28.6%)	

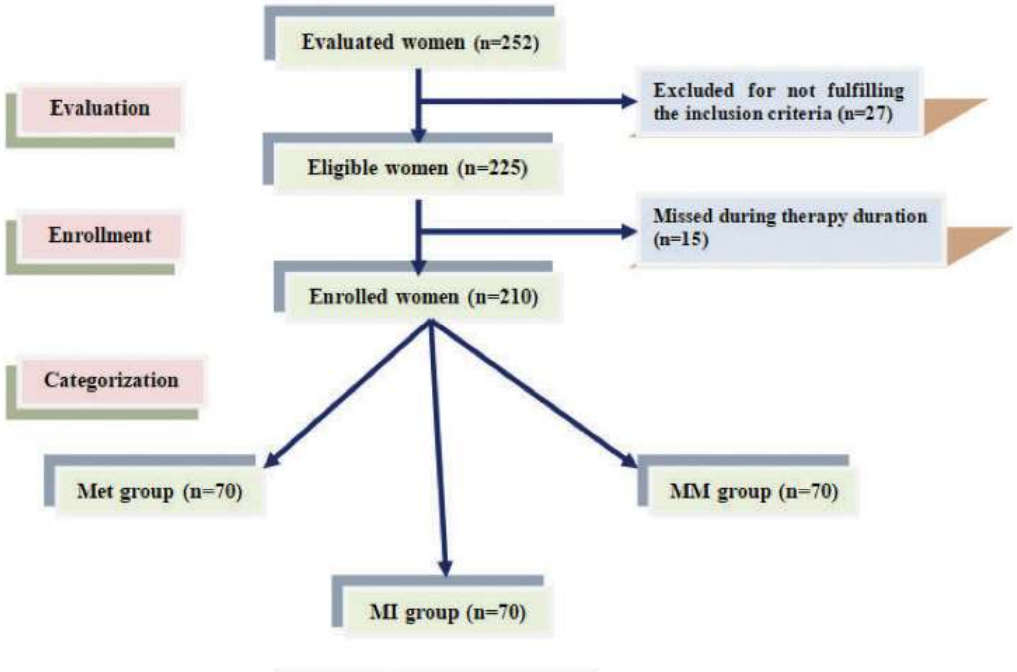


Figure 1: Study Flow Chart

At the end of 6-m therapy (T6), 126 women (60%) resumed regular menstrual cycles and 67 women (31.9%) had infrequent cycles, while 17 women (8.1%) were still had amenorrhea with significantly (P<0.001) reduced deregulated pattern among women of all groups. The frequency of women resumed regular cycles was significantly higher with MM therapy compared to that reported with Met (P=0.041) and MI (P=0.0007) therapies with non-significantly higher frequency with Met therapy (Table 2, Fig. 2).

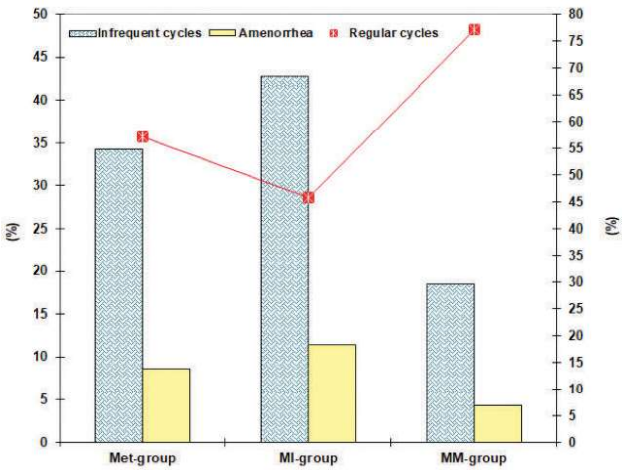


Fig. (2): Patients' distribution according to menstrual outcomes at the end of 6-m therapy

Among women presented with infertility as the main complaint (n=95), 15 women got pregnant for a pregnancy rate of 15.8% that showed insignificant differences between the three groups despite being highest among MM-group (Table 2).

Table 2: Gynecological outcomes

			Met	MI	MM	Total
Menstrual pattern	T0	Regular	0	0	0	0
		Infrequent	55 (78.6%)	53 (75.7%)	57 (81.4%)	165 (78.6%)
		Amenorrhea	15 (21.4%)	17 (24.3%)	13 (18.6%)	45 (21.4%)
	P1			0.687	0.673	
	P2				0.410	
	T6	Regular	40 (57.1%)	32 (45.7%)	54 (77.1%)	126 (60%)
		Infrequent	24 (34.3%)	30 (42.9%)	13 (18.6%)	67 (31.9%)
		Amenorrhea	6 (8.6%)	8 (11.4%)	3 (4.3%)	17 (8.1%)
	P1			0.398	**0.041**	
	P2				**0.0007**	
Pregnancy outcome at T0 for women seeking for pregnancy		Pregnant	5 (15.6%)	3 (10.7%)	7 (20%)	15 (15.8%)
		Not pregnant	27 (84.4%)	25 (89.3%)	28 (80%)	80 (84.2%)
		Total	32 (100%)	28 (100%)	35 (100%)	95 (100%)
		P1		0.577	0.641	
		P2			0.316	

The used therapies significantly (P<0.001) reduced women's BMI at T6 in relation to T0-BMI with non-significant difference between the studied groups both at T0 (P=0.264) and T6 (P=0.209). The percentage of decrease in T6-BMI in relation to T0-BMI was significantly (P<0.001) higher in women of Met and MM groups compared to that of women of MI-group with significantly (P=0.0001) higher in women of MM-group than in women of Met-women (Fig. 3).

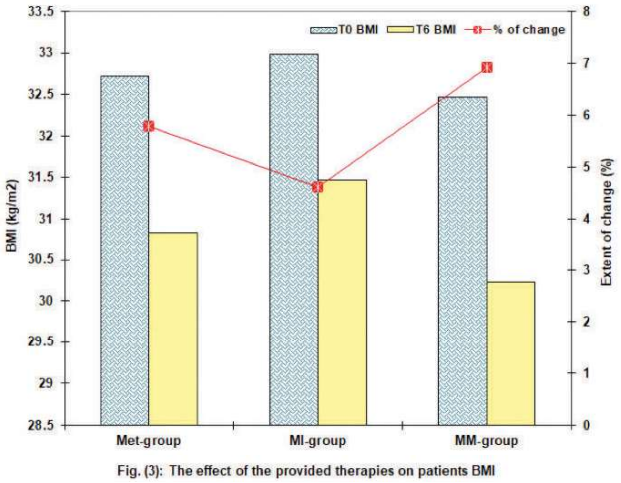


Fig. (3): The effect of the provided therapies on patients BMI

The BG levels estimated at T0 and T6 showed non-significant intergroup differences, apart from FBG that showed significant (P=0.0035) intergroup difference. The estimated BG levels at T6 decreased significantly (P<0.001) in all patients in relation to levels estimated at T0. The percentage of decrease in FBG levels estimated in T6 samples in relation to its T0 levels showed significant (P<0.001, respectively) variance between women of the three groups and was significantly higher with Met- and MM-therapies than MI-therapy (P=0.0009 & <0.001, respectively) with insignificantly (P=0.231) higher percentage of decrease with MM than Met therapies. Despite the significantly lower 1-h and 2-h PPBG levels estimated in T6 than T0 samples of all patients, the intergroup differences were insignificant regarding both the levels and the percentage of decrease.

The results of 75-OGTT conveyed at T0 detected glucose intolerance (GI) of all

women as evidenced by BG levels higher than the diagnostic limits of the IADPSG in all T0 samples; fasting and 1-h and 2-h PP. However, at T6 estimations, 167 patients (79.5%) still had fasting GI, 189 patients (90%) had 1-h GI and 111 patients (52.9%) had 2-h GI with insignificant distribution between the three groups regarding the frequency of women showed FBG level of ≥92 mg/dl or 1-h PPBG level of ≥180 mg/dl. On contrary, the frequency of GI women on 2-h PPBG estimations was significantly lower in MM-group than in MI-group with insignificant difference in distribution of GI women of Met-group and other groups.

Estimated serum insulin levels decreased in all women in T6 samples, but the differences between levels estimated in T0 and T6 samples were insignificant with insignificant intergroup differences both in T0 and T6 samples. However, the percentage of decrease in T6 serum insulin showed significant variance between the three groups and was significantly (P<0.001) higher with MM therapy than Met and MI therapies and significant (P=0.0016) difference in favor of MI therapy. At T0 time, 67 women and at T6 time only 15 women were insulin resistant with HOMA-IR ≥2 with insignificant differences between the distributions of IR women among the three groups. The T6-IR frequency was significantly lower than T0 frequency in the three groups but was lowest with MM therapy. Despite of the insignificant intergroup differences regarding the HOMA-IR index, it was decreased in all patients and the extent of decrease showed significant (P<0.001) intergroup difference and was significantly higher in group MM compared to groups Met (P=0.0004) and MI (P<0.001) with significantly (P=0.027) higher extent of decrease with Met than MI (Table 3, Fig. 4).

Table 3: Metabolic outcomes

Variates Groups			Met	MI	MM	P
BMI (kg/m²)		T0	32.7±1.8	33±2.5	32.5±1.5	0.264
		T6	30.8±1.6	31.5±2.5	30.2±1.5	0.209
		P1	<0.001	<0.001	<0.001	<0.001
		% of change	5.8±1.6	4.62±1.07	6.93±1.65	
		P2		<0.001	0.0001	
		P3			<0.001	
75-OGTT	FBG (mg/dl)	T0	123.7±7.5	125±9.4	124.4±10.6	0.707
		T6	110±12.8	116.2±14.3	109.1±13.2	0.0035
		P1	<0.001	<0.001	<0.001	
		% of change	11.25±7.12	7.27±6.79	12.5±5	<0.001
		P2		0.0009	0.231	
		P3			<0.001	
	1-h PPBG (mg/dl)	T0	187.5±4.6	187.2±5	188.7±5.4	0.177
		T6	166.5±8.8	167.6±8.7	168.58.3	0.389
		P1	<0.001	<0.001	<0.001	
		% of change	11.2±3.9	10.4±4.7	10.7±4.7	0.576
		P2		0.294	0.452	
		P3			0.788	
	2-h PPBG (mg/dl)	T0	165±7	162.6±6.3	164.5±5.1	0.056
		T6	154.9±9.3	151.7±8.9	152.6±7.8	0.083
		P1	<0.001	<0.001	<0.001	
		% of change	6.1±4.1	6.7±3.3	7.2±4.1	0.242
		P2		0.326	0.115	
		P3			0.451	

	Parameter	FBG			1-h PPBG			2-h PPBG		
Glucose intolerance	Group	Met	Met	MM	Met	Met	MM	Met	Met	MM
	Intolerant	56 (80%)	59 (84.3%)	52 (74.3%)	63 (90%)	61 (87.1%)	65 (92.9%)	43 (61.4%)	38 (54.3%)	30 (42.9%)
	Tolerant	14 (20%)	11 (15.7%)	18 (25.7%)	7 (10%)	9 (12.9%)	5 (7.1%)	27 (38.6%)	32 (45.7%)	40 (57.1%)
	P1		0.508	0.421		0.591	0.543		0.392	**0.028**
	P2			0.144			0.255			0.176
HOMA-IR index	Serum fasting insulin levels	T0		5±1.65		4.83±1.7		5.05±1.68		0.737
		T6		4.83±1.63		4.64±1.6		4.74±1.6		0.858
		P1		0.499		0.472		0.265		
		% of change		3.66±1		4.25±1.18		6.26±2		<0.001
		P2				0.0016		<0.001		
		P3						<0.001		
	IR incidence	T0		22 (31.4%)		22 (31.4%)		23 (32.9%)		0.978
		T6		10 (14.3%)		8 (11.4%)		7 (10%)		0.727
		P1		0.016		0.004		0.001		
	Mean index	T0		1.52±0.52		1.5±0.56		1.57±0.59		0.713
		T6		1.31±0.47		1.34±0.51		1.29±0.5		0.886
		P1		0.013		0.075		0.003		
		% of change		14.06±7.64		11.1±6.88		18±4.84		<0.001
		P2				0.017		0.0004		
		P3						<0.001		

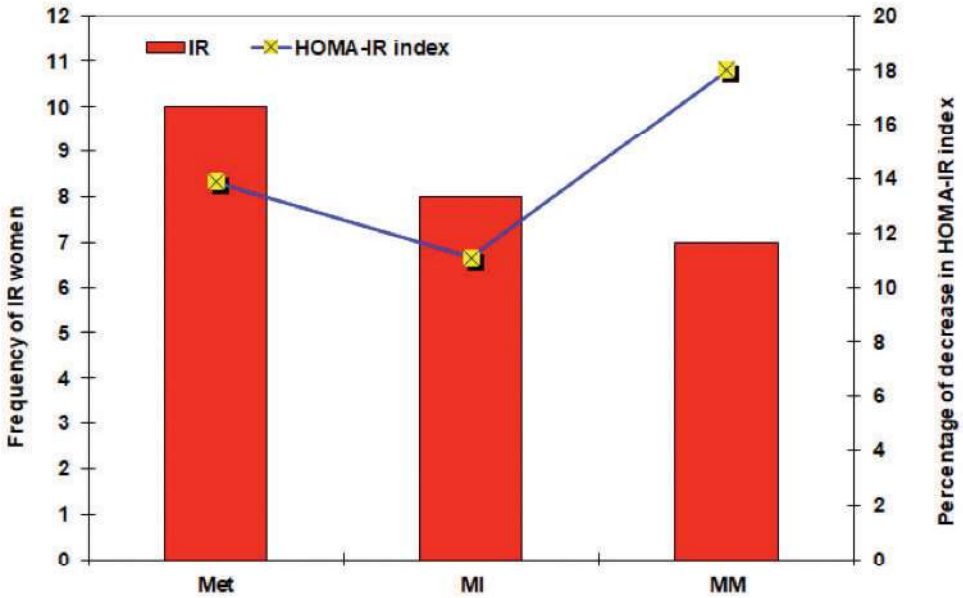


Fig. (4): The frequency of insulin resistant women at T6 evaluation and the percentage of change in HOMA-IR index at T6 in relation to T0

The frequency of women had biochemical hyperandrogenemia, defined as serum TT of ≥0.8 ng/ml, was 35.2% at T0 and was decreased at T6 to 13.3% with significantly lower frequency at T6 than at T0 in all groups. However, the extent of decrease in the frequency of women had hyperandrogenemia showed insignificant differences between the studied groups both at T0 and T6. Moreover, estimated serum TT and DHEA levels were significantly decreased in women of the three groups at T6 in comparison to levels estimated at T0. The inter-group difference was insignificant at T0 for both TT and DHEA, while at T6 the difference was insignificant (P=0.209) in case of TT and was significant (P=0.030) for DHEA levels. The percentage of decrease of serum TT was significantly (P=0.00002) higher in women of MM-group compared to women of other groups, while the percentage of decrease of serum DHEA was significantly higher (P=0.0096) in women of MM-group than in women of Met-group and was insignificantly higher (P=0.151) than in women of MI-group (Table 4).

Clinical hyperandrogenemia manifested as hirsutism was detected in 45 women who showed FG score ≥8 with non-significant distribution among the women of the three groups. Treatment did not improve hirsutism significantly as shown by the insignificant difference in the frequency of women had FG score ≥8 before (21.4%) and after (16.7%) treatment and the non-significant difference between the studied groups. Despite the insignificant differences of mean FG score determined at T0 and T6 between the studied groups, the mean intergroup difference between T0 and T6 FG scores were significant in the three groups (Table 4).

Acne as another manifestation for clinical hyperandrogenemia was frequent among the studied women (68.1%), but unfortunately improved insignificantly with the used therapies and at the end of treatment, the frequency was 61.4%. Regarding the mean acne score, it was decreased insignificantly in patients of groups Met (P=0.205) and MI (0.214) in comparison to mean value of their T0 score, while the difference was significant (P=0.0029) with MM therapy. Further patients' distribution according to the differential items of acne score at T6 showed insignificant difference than that determined at T0 in groups Met and MI (P=0.655 & 0.562, respectively), but the difference was significant (P=0.029) in case of group-MM (Table 4).

Table 4: Endocrinal outcomes

Variates Groups			Met	MI	MM	P
BMI (kg/m²)		T0	25 (35.7%)	22 (31.4%)	27 (38.6%)	0.673
		T6	13 (18.6%)	7 (10%)	8 (11.4%)	0.279
		P1	0.023	0.0018	0.0002	
Hormonal assay	Serum testosterone (ng/ml)	T0	0.77±0.1	0.75±0.09	0.76±0.09	0.264
		T6	0.71±0.09	0.68±0.08	0.69±0.09	0.209
		P1	0.0001	0.0003	<0.001	
		% of change	8.28±2.83	8.42±2.13	10.24±2.46	
		P2		0.739	0.00002	
		P3			0.00002	
	Serum DHEA	T0	296.2±38.8	282.5±29.5	286±38.9	0.066
		T6	273.9±34.5	260.4±28.3	261.5±36.3	0.030
		P1	0.0005	0.00001	0.00018	
		% of change	7.44±2.3	7.82±3.3	8.57±2.75	0.058
		P2		0.425	0.0096	
		P3			0.151	

Hirsutism (FG score)	**Incidence (FG score ≥8)**	T0	19 (27.1%)	13 (18.6%)	13 (18.6%)	0.361
		T6	15 (21.4%)	11 (15.7%)	9 (12.9%)	0.383
		P2	0.403	0.654	0.353	
	Mean FG score	T0	7±1.62	6.9±2.09	6.8±1.16	0.777
		T6	5.7±1.8	6±2	6±1.34	0.494
		P2	0.0001	0.001	0.0002	
Acne score	**Incidence**	T0	43 (61.4%)	47 (67.1%)	53 (75.7%)	0.189
		T6	39 (55.7%)	45 (64.3%)	45 (64.3%)	0.485
		P2	0.674	0.722	0.140	
	Mean score	T0	1.71±0.8	1.79±1	1.85±0.93	0.761
		T6	1.49±0.68	1.56±0.78	1.4±0.54	0.552
		P2	0.205	0.214	0.0052	

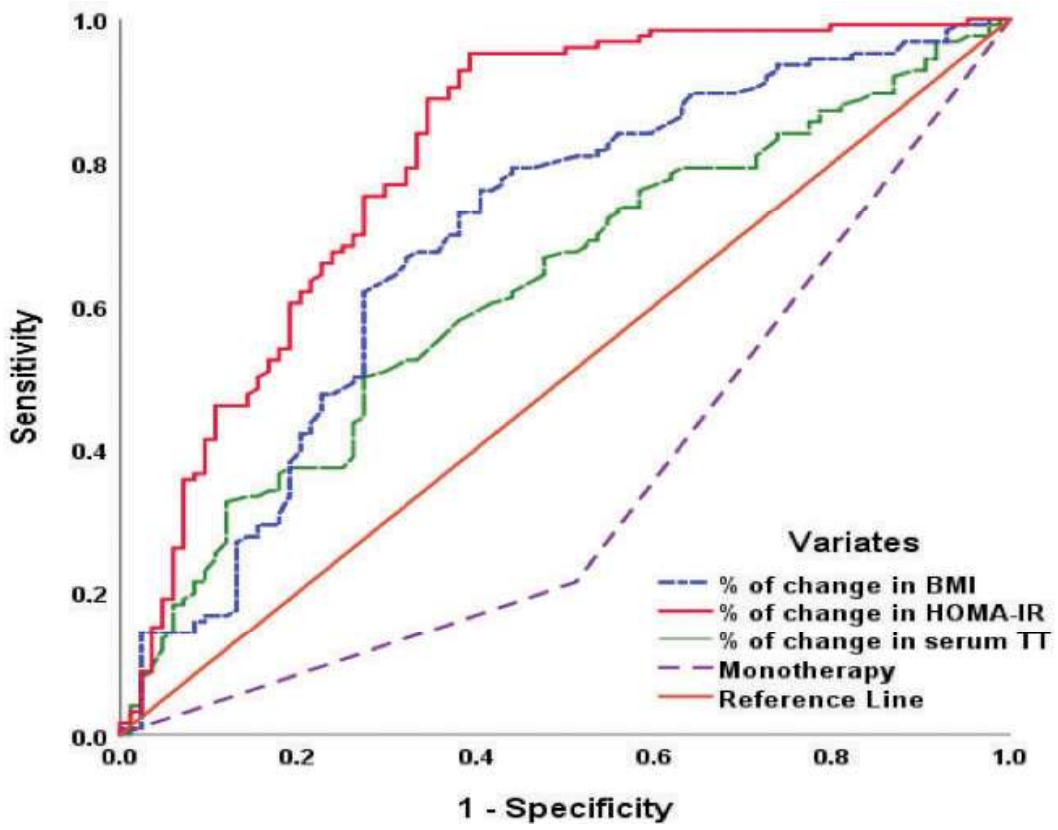
Score items	Time		T0			T6	
	Group	Met	MI	MM	Met	MI	MM
	0	29 (41.4%)	23 (32.9%)	17 (24.3%)	31 (44.3%)	25 (35.7%)	25 (35.7%)
	1	20 (28.6%)	24 (34.3%)	23 (32.9%)	23 (32.9%)	26 (37%)	28 (40%)
	2	15 (21.4%)	13 (18.6%)	19 (27.1%)	14 (20%)	15 (21.4%)	16 (22.9%)
	3	4 (5.7%)	6 (8.6%)	7 (10%)	1 (1.4%)	2 (2.9%)	1 (1.4%)
	4	2 (2.8%)	4 (5.7%)	4 (5.7%)	1 (1.4%)	2 (2.9%)	0

P1	0.609	0.856		
P2		0.655	0.562	0.029

The reported resumption of regular menstrual pattern and percentages of change of BMI, HOMA-IR and serum TT showed negative relation to the use of insulin sensitizer monotherapy. The resumption of regular menstrual pattern was positively related to the percentage of change of HOMA-IR index, BMI and serum TT levels. Also, the percentage of decrease of serum testosterone was positively related to that of HOMAI-IR index and BMI. ROC curve analysis for the predictors for getting regular menstrual pattern showed that the more the control of IR with high percentage of decrease of HOMA-IR index the higher the possibility for getting regular menstrual pattern and defined the use of monotherapy as a negative predictor for such outcome (Table 5, Fig. 5).

Table 5: Statistical analysis for the relation between the study outcomes

	Mono-therapy		Regular menstrual pattern		% of change in serum TT	
	"r"	P	"r"	P	"r"	P
Regular menstrual pattern	-0.309	<0.001	-	-	0.178	0.010
% of change of BMI	-0.474	<0.001	0.295	<0.001	0.145	0.036
% of change of HOMA-IR index	-0.327	<0.001	0.443	<0.001	0.216	0.002
% of change of TT	-0.151	0.029	0.178	0.010		
The Receiver Operating Characteristic (ROC) Curve						
	AUC	**SE**	**P**	**95% CI**		
Monotherapy	0.351	0.040	<0.001	0.274-0.429		
% of change of BMI	0.693	0.038	<0.001	0.618-0.768		
% of change of HOMA-IR index	0.811	0.032	<0.001	0.747-0.874		
% of change of TT	0.626	0.039	0.002	0.550-0.702		



Discussion

Gynecological outcomes of this trial showed that 60% of women resumed regular menstrual pattern and among infertile women wishing to get pregnant a pregnancy rate of 13.7% was reported. Further, resumption of menstrual regularity showed positive relation to the percentage of change in IR-index, BMI and serum androgens. These findings spotlight on the vicious circle of obesity, IR and hyperandrogenemia that deleteriously affects the ovarian function with subsequent menstrual disturbances and subfertility or infertility.

Moreover, the adjustment of menstrual pattern and its underlying pathogenesis factors; obesity, IR and hyperandrogenemia, was significantly higher with combined metformin/inositol combination therapy than with either Met or MI as monotherapy that showed insignificant differences. Also, the ROC curve defined the use of insulin sensitizer monotherapy as a negative predictor for the possibility of resumption of regular menstrual pattern; a finding that illustrated the ability of the applied regimens to detonate this cycle and that better outcomes were obtained with the use of combination therapy as double-weapon to get such improvement.

The reported effects of the used drug regimens on BMI and IR and the relation between resumption of regular menstrual pattern with the extent of decrease in BMI and HOMA-IR assured the assumption that obesity is a hyperinsulinemic state and the relation between IR and ovarian functions. Such relation between IR and hyperandrogenemia was attributed to decreased levels of sex-hormone binding globulin (SHBG) secondary to obesity and IR with subsequently higher levels of free testosterone and free androgen index as evidenced by resumption of serum SHBG levels with reduction of serum free testosterone and decreased free androgen index using various modalities for weight reduction in PCOS obese women including dietary regimens (22), pharmacological interventions (23) or bariatric surgery (24).

Further the comparable effects of both inositol and metformin indicated their efficiency as insulin sensitizers and metabolic adjusting drugs. Similarly, Soldat-Stanković et al. (25) showed comparable effects of MET and MI on BMI, body composition, hormonal profile, metabolism of glucose and insulin, and adiponectin level and concluded that MET and MI, were useful in reducing BMI and improving body composition in PCOS women without significant differences.

Furthermore, the significant differences between women who received combination therapy in comparison to those received monotherapy illustrated the synergism between the effects of each drug to improve outcomes. Similarly, a recent study compared the efficacy of Met as monotherapy versus Met with MI as combination therapy and detected significantly greater improvement of menstrual regularity with combination, but pregnancy rates were comparable and concluded that addition of MI to Met improved menstrual cycle regularity, and QOL in PCOS-women (26).

The reported comparable outcomes of women used Met or MI as monotherapy, go in hand with Rajasekaran et al. (27) who reported comparable effect of MI and Met on ovarian hyperstimulation syndrome in PCOS women prepared to IVF but MI therapy was associated with significantly higher fertilization and cleavage rates and number of good grade embryos and with a systemic review for randomized controlled trials that assured the non-inferiority of inositol compared to metformin regarding effects on BMI, androgen hormonal profile, and insulin action with a risk ratio for getting regular menstrual cycle of 1.79 higher with inositol than placebo (28). Also, Bodepudi et al. (29) detected the comparable effects of Met and MI on clinical, hormonal, and biochemical profiles of PCOS women and documented that the better safety profile and tolerance of MI, due to its minimal side effects, prevents

discontinuation of therapy till getting the desirable effects.

In support of the efficacy of MI as additive to other drugs used for PCOS treatment, Kachhawa et al. (30) compared the effect of using myoinositol and D-chiro-inositol in 3.6:1 ratio versus combined hormonal contraceptive in a series of PCOS women and reported resumed spontaneous menses in about 85% of women, reduction of mean cycle length and these outcomes were continued for three months after stoppage of treatment and concluded that the used inositol combination is effective in regularizing menstrual cycles. Thereafter, Guarano et al. (31) found the addition of MI to alpha-lipoic acid creates synergistic effect that was manifested as improved IR, menstrual regularity and ovulation rhythm of PCOS women especially in obese/overweight patients with T2DM familiarity. Also, Hassan et al. (32) compared resveratrol and MI versus Met and pioglitazone combinations for treatment of PCOS women and reported significant reduction in serum TT, LH and FSH levels with a marked reduction in the ovarian volume with MI combination and significantly higher frequency of menstrual regularity and concluded that combined resveratrol and MI is more effectively ameliorated the altered endocrine, metabolic indices and stress burden especially in high risk group of obese, oligo-anovulatory married PCOS affected women.

The reported beneficial effects of inositol could be attributed to its variant mechanisms of action; experimentally, Bizzarri et al. (33) shown that MI and its epimer D-chiro-inositol (DCI) permits transduction of insulin and improves the complete breakdown of glucose through the citric acid cycle, especially in glucose-greedy tissues, such as the ovary. Also, DCI inhibits generation of reactive oxygen species secondary to the action of NAPH oxidase and improves mitochondrial disruption, (34), MI through inositol triphosphate (IP3) signaling pathway induces calcium ion (Ca2+) release from the endoplasmic reticulum leading to rising of cytosolic Ca2+ levels which in turn activates many enzymes and proteins (35).

Conclusion

Insulin sensitizers' therapy is effective, safe, cheap and in-hand of all PCOS women to control PCOS-associated endocrinal, metabolic and gynecological deregulations. Inositol is a synergistic additive to metformin and this combination results in favorable outcomes than monotherapy. Lack of inositol side effects allowed patients to continue the therapy duration despite of the unpleasant side effects of metformin.

Limitations

The use of minimal acceptable dose of metformin with higher dose of MI/DCO to minimize side effects need to be evaluated. Follow-up after cessation of therapy to detect recurrence of manifestations was mandatory to adjust duration of therapy

Recommendations

Comparative study of the used drug combination versus other treatment regimens for PCOS was required to define the best of which.

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