Combination of Insulin Sensitizer and Omega-3 Fatty acids might minimize the risk for Cardiac events in PCOS women

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Abstract

Objectives: Evaluation of the effect of 3 months therapy of metformin/omega-3 (M/O) combination on body mass index (BMI), insulin resistance (IR), and oxidative and inflammatory milieu in PCOS women at probable cardiac risk (CR) as predicted by the atherogenic index of plasma (AIP).

Patients and methods: 90 PCOS women were randomly allocated into the M group received metformin (500 mg bi-daily) and M/O group received metformin (500 mg bi-daily) and Omega3 (950 mg active omega-3 once daily). Pre- and Post-treatment BMI, Homeostasis model assessment for IR (HOMA-IR), AIP and serum tumor necrosis factor-α (TNF-α), interleukin (IL)-β, superoxide dismutase (SOD), and malondialdehyde (MDA) levels were evaluated. The primary outcome is the effect of provided 3-m therapy AIP.

Results: Pre-treatment AIP defined 15.6% and 58.9% of studied women had high or intermediate cardiac risk (CR), 59 women were obese, 16 women were morbidly obese, and 52 women were insulin resistant with elevated serum levels of TNF-α, IL-1β, MDA, and lower serum SOD levels. Combined therapy allowed a significant decrease in HOMA-IR score, serum TNF-α, IL-1β, and MDA levels with significant elevation of serum SOD. Combination therapy significantly reduced the AIP in comparison to pre-treatment AIP and to that of women of the M group. Moreover, no woman still had high CR after M/O therapy and the frequency of women who had low CR was increased by about 107%.

Conclusion: Insulin sensitizers could improve PCOS-associated disturbances. However, omega-3 adjuvant therapy significantly augmented the effects of insulin sensitizers, minimized the cardiac risk factors, and decreased the risk of probable cardiac events.

Keywords: PCOS, Omega 3, Metformin, Atherogenic index of plasma, Cardiac risk.

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Introduction

Polycystic ovarian syndrome (PCOS) is a complex disorder that affects around 5-10% of women of childbearing age worldwide [1]. PCOS is associated with traits including hyperandrogenemia, irregular menstrual periods, obesity, and insulin resistance [2]. The progress of PCOS women to metabolic syndrome is an important bridge for the development of other diseases especially diabetes mellitus and coronary heart disease [3].

PCOS women frequently have an increased risk for adverse cardiac events secondary to progress to hypertension, atherosclerosis, and vascular disease [3], which gradually lead to endothelial dysfunction and coronary artery calcification with subsequent cardiac events [1].

Obesity is a major risk factor for cardiovascular (CV) disease in the general population and is highly prevalent in PCOS, but the role of hyperandrogenemia is still unclear (4). Moreover, antiandrogenic drugs as combined oral contraceptives usually used in adult women with PCOS carry a low risk of CV or thromboembolic events [5].

Dietary bioactive as omega-3 fatty acids, flavonoids, lutein, and zeaxanthin are food substances that promote health but are not essential to preventing typical deficiency conditions [6]. Omega-3 long-chain, polyunsaturated fatty acids (n-3 PUFA) are essential and had to be provided through the diet due to their limited biological synthesis [7]. The n-3 PUFA can protect against inflammation-related diseases including heart disease [8].

Cardiovascular diseases are defined as conditions involving decreased cardiac muscle blood flow that can lead to heart attacks, stroke, or other disorders [9]. Animal studies showed enhanced cardiac contractile efficiency with attenuation of dysfunction attributable to ischemia on supplementing animal diets with fish oil, in dose equivalent to regular consumption of fish in the human diet, for its high content of omega-3 docosahexaenoic acid [10]. Moreover, an epidemiological study suggested the importance of n-3 PUFAs in preventing ischemic heart disease [11]. Moreover, n-3 PUFA therapy in patients with virus-induced myocardial injury significantly regulated the expression levels of mRNA of and protein synthesis of Toll-like receptor 3 and 4, increased antioxidant gene expression, reduced the secretion of inflammatory factors, alleviated myocardial injury, and improved cardiac function [12].

Hypothesis

This study suggests that combined therapy with 3-n PUFA and metformin might act synergistically to improve PCOS-associated metabolic problems that most probably pave the way for PCOS-associated cardiac diseases.

Objectives

Evaluation of the effect of metformin/omega-3 (M/O) combination therapy on body mass index (BMI), insulin resistance (IR), lipid profile, and oxidative and inflammatory milieu in PCOS women at probable cardiac risk (CR) as predicted by the atherogenic index of plasma (AIP).

Design

Prospective interventional comparative study

Setting

Departments of Obstetrics and Gynecology, and Clinical Pathology, Faculty of Medicine, Benha University

Patients & Methods

This study was conducted from Jan 2020 till Feb 2021 after approval of the study protocol by the Local Ethical Committee to include all women attending the infertility clinic at Benha University Hospital with a picture

suggestive of PCOS for evaluation and those eligible for inclusion were enrolled in the study after signing a written fully informed consent to participate the study and donate blood samples for assigned investigations.

Evaluation Parameters

1. Diagnosis of PCOS

Patients were considered to have PCOS if there were at least two of Rotterdam criteria[13]. Rotterdam criteria included the following items [14]:

(a) Menstrual history: amenorrhea or oligomenorrhea. Oligomenorrhea was defined as having <8 spontaneous menstrual cycles yearly for at least 3 years

(b) Lab findings: Hyperandrogenemia was defined as serum total testosterone level of >0.8 ng/ml

(c) US findings: ovarian volume of >10 ml per ovary on transvaginal ultrasound (TVU) imaging or ovaries containing >12 follicles of varied sizes and ranging between 2 and 9 mm

2. Diagnosis of obesity

Obesity was diagnosed and graded according to BMI which was calculated according to Bray [15] as body weight (in kg) divided by body height (in m^2). BMI was graded according to WHO guidelines [16] as underweight (BMI<18.5 kg/m^2), average weight (BMI=18.5-24.9 kg/m^2), overweight (BMI=25-29.9 kg/m^2), obese-1 (BMI=30-34.9 kg/m^2), obese-2 (BMI=35-39.9 kg/m^2) and obese-3 (BMI>40 kg/m^2).

3. Insulin resistance diagnosis and scoring

Insulin resistance (IR) was evaluated using the homeostasis model assessment (HOMA). The HOMA-IR score was calculated as (fasting serum insulin (μU/ml) x [fasting plasma glucose (mg/ml)/18])/22.5 [17] with HOMA-IR index of >2 indicates IR [18].

4. Atherogenic index of plasma (AIP)

The atherogenic index of plasma (AIP) is defined as the base 10 logarithms of the ratio of plasma triglyceride (TG) to high-density lipoprotein cholesterol (HDL-C) [19]. AIP was employed as a predictor of CR with values of -0.3 to 0.1 are associated with low, values of 0.1-0.24 are associated with the medium, and values above 0.24 with high CR [20].

Laboratory investigations

Blood Sampling

Blood sampling was conducted before starting treatment (Pre-T) and after the end of the 3-m treatment period (Post-T). All enrolled women were asked to attend the hospital lab fasting for 12 hours and gave a blood sample for estimation of blood lipids and to re-attend on the second day fasting 6 hours and gave another blood sample for estimation of fasting blood glucose (FBG) and other parameters. Blood samples were obtained under complete aseptic condition and divided into three parts:

1. The first part was put in a tube containing sodium fluoride (2 mg sodium fluoride/ml blood) to prevent glycolysis for estimation of FBG levels.
2. The second part was put in EDTA containing tube to determine the levels of TG and HDL-c and to calculate the AIP.
3. The third part was collected in a plain tube, allowed to clot, centrifuged at 1500×g for 15 min, and the serum samples were collected in a clean Eppindorff tube and stored at -20°C till be ELISA assayed.

Estimated parameters

Blood sampling was conducted before starting treatment

1. Blood glucose levels were estimated by the glucose oxidase method using

BT1500 Automatic biochemistry analyzer (SPAN Diagnostics, Gujarat India).

2. Plasma levels of triglycerides (TG) and high-density lipoprotein (HDL) were estimated by photoluminescence methods using BT1500 Automatic biochemistry analyzer (SPAN Diagnostics, Gujarat India).
3. Serum levels of insulin, testosterone (T), and sex-hormone-binding globulin (SHBG) using Automatic Immunoassay Analyzer (MAGLUMI 600, Snipe Diagnostic Co., Ltd., China).
4. ELISA estimation of serum levels of tumor necrosis factor-α (TNF-α), interleukin (IL)-β, superoxide dismutase (SOD), and malondialdehyde (MDA).

Exclusion criteria

Menstrual disturbances and/or infertility due to causes other than PCOS, obesity inducing endocrinopathy, ovarian cysts for any cause, hyperprolactinemia, adrenal or ovarian tumor, thyroid dysfunction, Cushing's syndrome, congenital adrenal hyperplasia, current or previous pregnancy within 1 year of enrollment, autoimmune disease, malignancy, chronic inflammatory disorders, current or previous use of oral contraceptives within 6 months of enrollment. Women younger than 30 years were also excluded from the study

Inclusion criteria

Women aged >30 years and had menstrual disorders and/or infertility secondary to PCOS who were free of exclusion criteria and signed fully-informed written consent to participate in the study, receive medications and follow-up visits.

Randomization & Masking

Women who fulfilled the inclusion criteria were randomly divided into two study groups using cards carrying group labels and put in a closed dark envelope. Cards were prepared by an assistant who was blinded about the significance of the label and was chosen by the patient herself. Collection of baseline data and prescription of medications was the duty of one of the authors. Blood samples were obtained and numbered by code numbers and the clinical pathologist was blinded about the baseline data and indications for investigations. Post-treatment data were collected by the 2nd author who was blinded about both the baseline data and results of laboratory investigations.

Groups

Twenty age and BMI-matched fertile females with regular menstrual cycle and free of exclusion criteria were collected as a control group. The enrolled PCOS women were divided into two equal groups:

1. Metformin group (M group): included women who received metformin HCl (Glucophage, Minapharm Pharmaceuticals, Amyria, Egypt) 500 mg film-coated tablets twice daily for three months.
2. Combination group (M/O group): included women who received metformin HCl (Glucophage, Minapharm Pharmaceuticals, Amyria, Egypt) 500 mg film-coated tablets twice daily and 3-n PUFA (Omega 3 Fish oil,) containing 625 mg eicosapentaenoic acid (EPA), 244 mg of docosahexaenoic acid (DHA) with mixed natural tocopherols and fish ingredients. The 3-n OMEGA was provided as soft gel tablets 950 mg active omega-3 and was taken once daily with a meal for three months.

Study outcomes

1. The primary outcome is the effect of provided 3-m M/O therapy in comparison to 3-m metformin therapy on AIP and level of CR in PCOS women.
2. Secondary outcomes included the effects of therapy on BMI, IR, and serum levels of studied biomarkers

Results

During the duration of the study, 127 women were eligible for evaluation, but 37 were excluded for not fulfilling the inclusion criteria and 90 women with a mean age of 35.7 (±2.7) years, were randomly divided into two groups (Fig. 1). Twenty control women of mean age of 35.9 (±2.4) were also included in the study.

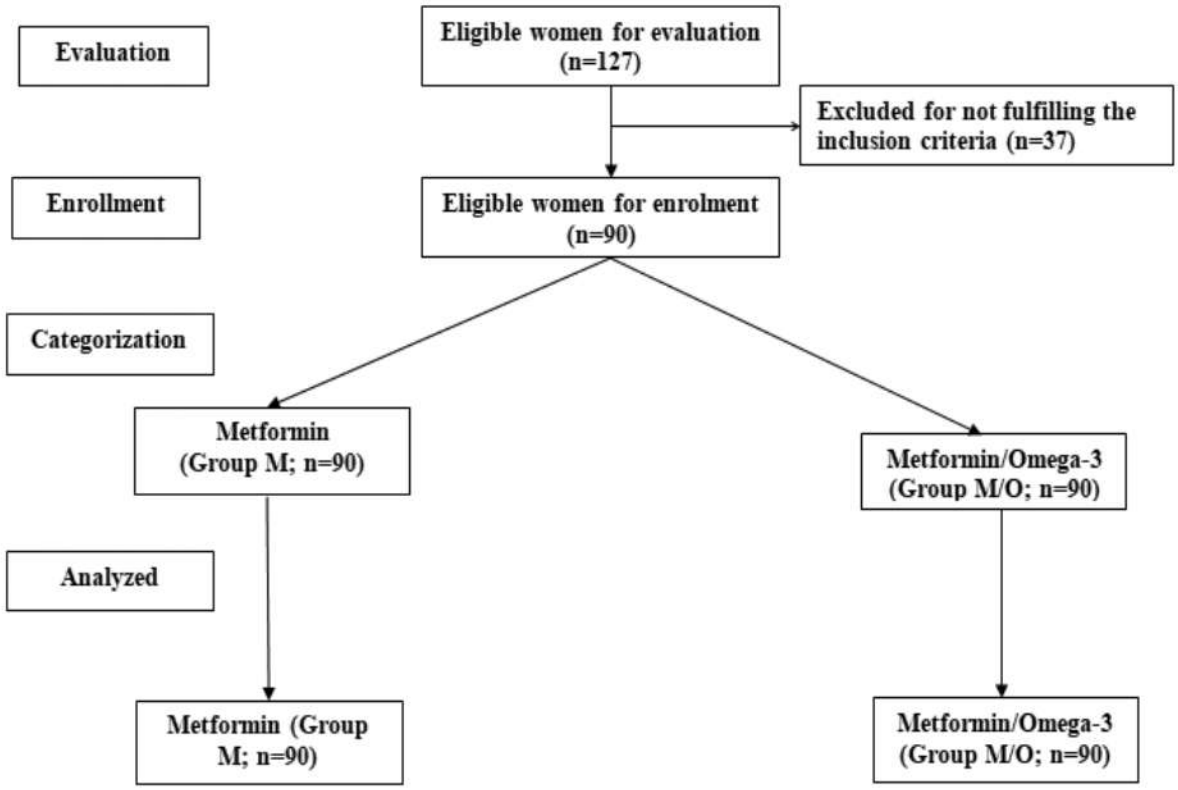

Fig. (1): Study Flow Chart

Pre-treatment BMI (BMI-1) showed a non-significant ($p>0.05$) difference between women of both groups and was non-significantly ($p>0.05$) higher in comparison to BMI of control women. Post-treatment BMI (BMI-2) of women of group M was non-significantly ($p=0.076$) lower in comparison to their BMI-1 with a mean decrease of 2.77 (1.4%), but was still non-significantly higher in comparison to BMI of control women. On contrary, BMI-2 of women of the M/O group was significantly ($p=0.0016$) lower in comparison to their BMI-1 with a mean percentage of decreased BMI of 4.8 (2.4%) and was non-significantly lower than BMI of control and M group women. The percentage of decreased BMI-2 of women of the M/O group was significantly ($p<0.0001$) higher in comparison to that of women of the M group. The distribution of women among BMI strata showed a decreased frequency of morbidly obese and increased frequency of women with average BMI with the non-significant difference between both groups (Table 1, Fig. 2).

Pre-treatment HOMA-IR score was significantly ($p<0.0001$) higher in women of study groups in comparison to control women with non-significant ($p=0.509$) difference between both study groups. Unfortunately, the post-treatment HOMA-IR score of women of both study groups was still significantly ($p<0.0001$) higher in comparison to the score of control women. However, the post-treatment HOMA-IR score was significantly low-

er in both group M (p=0.0002) and M/O (p<0.0001) in comparison to their pre-treatment score with significantly (p=0.0146) lower score of women of M/O group in comparison to that of women of M group. The frequency of IR women was decreased non-significantly (p=0.089) in women of group M but was decreased significantly (p=0.011) in women of group M/O after treatment in comparison to before treatment, despite the non-significant difference between the frequencies of IR women between both groups on both pre-and post-treatment evaluations (Table 1, Fig. 3).

Table 1: BMI and IR data of women of study and control groups and percentage of change after treatment

Variable \ Group		C (n=20)	M (n=45)	M/O (n=45)	P value		
					C vs. M	C vs. M/O	M vs. M/O
BMI (kg/m²)							
Pre-treatment		32 (1.6)	33.3 (2.4)	32.6 (2.4)	0.066	0.57	0.431
Post-treatment			32.4 (2.4)	31 (2.2)	0.911	0.171	0.073
P1 value			0.076	0.0016			
% of change			2.77(1.4%)	4.8 (2.4)			<0.0001
Pre	Av: Ob: MO	4:16:0	6:29:10	9:30:6	0.070	0.222	0.446
Post			9:29:7	12:29:4			0.529
P1 value			0.561	0.673			
HOMA-IR score							
Pre-treatment		0.76±0.19	2.17±0.42	2.07±0.39	<0.0001	<0.0001	0.509
Post-treatment			1.84±0.37	1.59±0.4	<0.0001	<0.0001	0.0146
P1 value			0.0002	<0.0001			
% of change			15.1±5.7	23.3±10.5			0.0001
Pre	IR: IS		29:16	26:19			0.517
Post			21:24	14:31			0.13
P1 value			0.089	0.011			

Data are shown as mean, standard deviation, percentages, and ratios; C: control group; M: Metformin group; M/O: Metformin/Omega 3 group; BMI: Body mass index; AV: Average weight; Ob: obese; MO: morbid obese; HOMA-IR: Homeostasis model assessment of insulin resistance; IR: Insulin resistant; IS: Insulin sensitive; P-value indicates the statistical significance of the difference between the three groups; P1 value indicates the statistical significance of the difference between pre-and post-treatment values; P<0.05 indicates a significant difference; P>0.05 indicates a non-significant difference

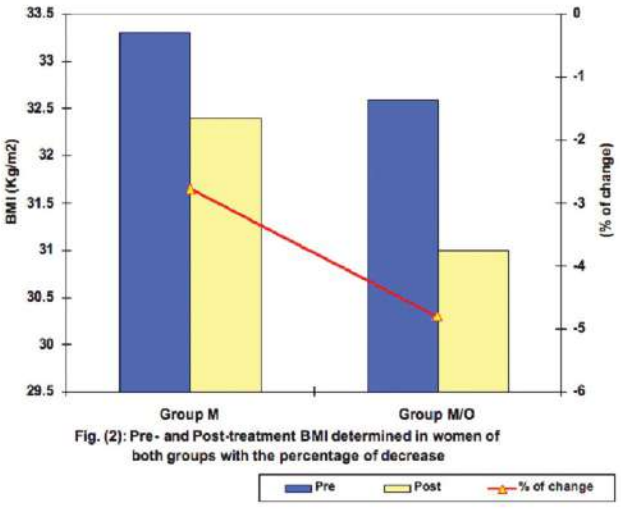

Fig. (2): Pre- and Post-treatment BMI determined in women of both groups with the percentage of decrease

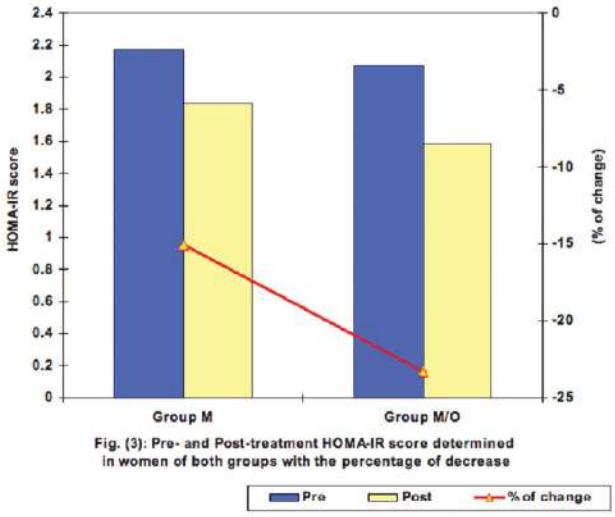

Fig. (3): Pre- and Post-treatment HOMA-IR score determined in women of both groups with the percentage of decrease

Pre-treatment serum TG levels were significantly (p <0.0001) lower, while serum HDL levels were significantly (p <0.0001) higher in control women in comparison to that of women of both study groups. Irrespective of the effect of treatment, post-treatment levels of women of study women were still significantly (p <0.0001) different in comparison to control women. Metformin therapy alone allowed a non-significant (p=0.215) reduction of serum TG with a mean percentage of decrease of 2.59 (±1.24), while it allowed a significant (p=0.038) increase of serum HDL with the percentage of increase of 4.62 (±1.66). On the other hand, combined therapy allowed a significant reduction of serum TG (p=0.0002) with significant elevation of serum HDL (p=0.0008) in comparison to pre-treatment levels. Moreover, post-treatment serum levels of TG and HDL in women of the M/O group showed significant differences (p=0.0194 & 0.031, respectively) in comparison to post-treatment levels estimated in women of the M group. Also, the percentages of change of serum TG and HDL in women of the M/O group were significantly higher in comparison to percentages of change in women of the M group (Table 2, Fig. 4).

Table 2: Atherogenic index of plasma of women of study and control groups and percentage of change after treatment

Variable \ Group	C (n=20)	M (n=45)	M/O (n=45)	P value		
				C vs. M	C vs. M/O	M vs. M/O
TG (mg/dl)						
Pre-treatment	89.8±14.4	122±8.2	123.6±12.3	<0.0001	<0.0001	0.838
Post-treatment		120.4±12	115.3±7.8	<0.0001	<0.0001	0.0194
P1 value		0.215	0.0002			
% of change		2.59±1.24	5.48±0.57			<0.001
HDL-c (mg/dl)						
Pre-treatment	45.4±4.6	38±4	39±3.3	<0.0001	<0.0001	0.552
Post-treatment		39.7±3.8	41.3±3	<0.0001	<0.0001	0.031
P1 value		0.038	0.0008			
% of change		4.62±1.66	6±2.62			0.004
AIP						
Pre-treatment	-0.07±0.11	0.152±0.08	0.136±0.062	<0.0001	<0.0001	0.337
Post-treatment		0.121±0.075	0.084±0.058	<0.0001	<0.0001	0.0085
P1 value		0.066	0.0001			

Cardiac risk							
Pre	Low	19 (95%)	8 (17.8%)	15 (33.3%)	<0.0001	0.00002	0.094
	Intermediate	1 (5%)	27 (60%)	26 (57.8%)			
	High	0	10 (22.2%)	4 (8.9%)			
Post	Low		16 (35.6%)	31 (68.9%)	0.00005	0.021	0.002
	Intermediate		24 (53.3%)	14 (31.1%)			
	High		5 (11.1%)	0			
P1 value			0.105	0.0014			

Data are shown as mean, standard deviation, percentages, and ratios; C: control group; M: Metformin group; M/O: Metformin/Omega 3 group; TG: Triglycerides; HDL-c: High-density lipoprotein cholesterol; P-value indicates the statistical significance of the difference between the three groups; P1 value indicates the statistical significance of the difference between pre-and post-treatment values; P<0.05 indicates a significant difference; P>0.05 indicates a non-significant difference

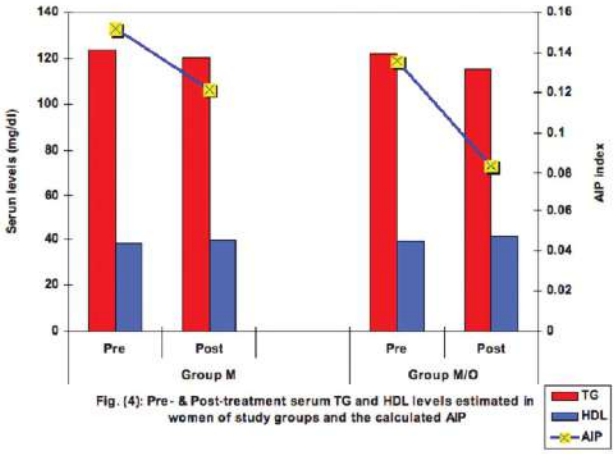

Fig. (4): Pre- & Post-treatment serum TG and HDL levels estimated in women of study groups and the calculated AIP

The calculated pre-and post-treatment AIP of women of study groups was significantly (p<0.0001) higher in comparison to AIP of control women. Pre-treatment AIP of women of group M was non-significantly higher, while post-treatment of group M was significantly higher in comparison to the corresponding AIP of women of group M/O (Fig. 4). Only one of the control women had intermediate CR, while 19 women had low CR. On contrary, pre-treatment CR was high, intermediate, and low in 14 (15.6%), 53 (58.9%), and 23 (25.5%) study women, respectively, with a non-significantly higher frequency of women, had high CR among women of group M. Both lines of therapy, significantly reduced the cardiac risk, but such effect was non-significant (p=0.103) with metformin alone, while was significant (p=0.0014) with combined therapy, in comparison to their respective pre-treatment frequencies. Moreover, at end of 3-m therapy, the frequency of women had mild, intermediate, and high CR was 16 women (35.6%), 24 women (53.3%), and 5 women (11.1%) in group M, while in group M/O, 31 women (68.9%) and 14 women (31.1%) had low and intermediate CR, respectively with a significantly higher frequency of women had low CR among women of group M/O (Table 2; Fig. 5).

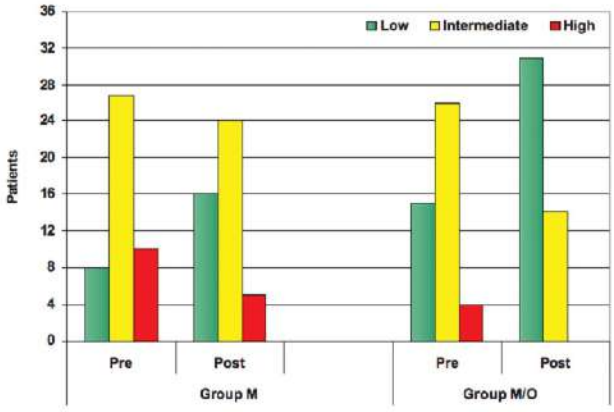

Fig. (5): Pre- and Post-treatment women' distribution according to the predicted cardiac risk by the calculated AIP

Both pre-and post-treatment serum TNF-α and IL-1β levels were significantly higher in comparison to levels estimated in control women with non-significant differences between women of study groups. Post-treatment serum TNF-α and IL-1β levels in women of group M were non-significantly lower in comparison to their pre-treatment levels with percentages of decrease of 3.83 (±2.62) and 3.9 (±2.2) for serum TNF-α and IL-1β levels. On contrary, post-treatment estimated serum TNF-α and IL-1β levels were decreased significantly (p=0.042 & 0.0488, respectively) in comparison to pre-treatment levels. The percentages of decrease of serum TNF-α and IL-1β levels in women of group M/O were significantly (p=0.0003 & 0.0004, respectively) higher in comparison to the percentages of decrease detected in women of group M (Table 3, Fig. 6).

Pre- and post-treatment serum MDA levels and SOD activity levels were significantly (p<0.0001) different in comparison to control levels. Post-treatment MDA levels were significantly decreased in women of groups M and M/O (p=0.0315 & <0.0001, respectively) with significantly (p=0.0019) lower levels in women of group M/O in comparison to women of group M. Moreover, the percentage of decreased MDA levels was (p<0.0001) higher in women of M/O group than in women of M group (Table 3, Fig. 7). Post-treatment SOD activity levels were significantly increased in women of both group M (p=0.0003) and group M/O (p<0.0001) with significantly (p=0.0376) higher activity levels in women of group M/O in comparison to women of group M. Moreover, the percentage of increased SOD activity levels was (p<0.0001) higher in women of M/O group than in women of M group (Table 3, Fig. 8).

Table 3: Pre- and Post-treatment serum levels of studied biomarkers in women of study and control groups and percentage of change after treatment

Variable \ Group	C (n=20)	M (n=45)	M/O (n=45)	P value		
				C vs. M	C vs. M/O	M vs. M/O
TNF-α (ng/ml)						
Pre-treatment	2.014±0.58	3.18±0.63	3.26±0.68	<0.0001	<0.0001	0.866
Post-treatment		3.084±0.6	2.98±0.6	<0.0001	<0.0001	0.763
P1 value		0.463	0.042			
% of change		3.83±2.62	8.05±7.12			0.0003
IL-1β (ng/ml)						
Pre-treatment	12.7±2.3	28.5±7.6	27.3±6.2	<0.0001	<0.0001	0.724
Post-treatment		27.4±7.4	25.1±4.4	<0.0001	<0.0001	0.217
P1 value		0.487	0.0488			
% of change		3.9±2.2	7.2±5.6			0.0004

MDA (nmol/ml)						
Pre-treatment	0.492±0.07	1.62±0.28	1.642±0.28	<0.0001	<0.0001	0.709
Post-treatment		1.5±0.26	1.328±0.25	<0.0001	<0.0001	0.0019
P1 value		0.0315	<0.0001			
% of change		7.51±2.83	18.9±7.25			<0.0001
SOD (IU/ml)						
Pre-treatment	1.9±0.12	1.6±0.16	1.56±0.17	<0.0001	<0.0001	0.578
Post-treatment		1.73±0.17	1.838±0.18	0.0004	0.309	0.0376
P1 value		0.0003	<0.0001			
% of change		8.36±2.2	18.25±8.5			<0.0001

Data are shown as mean, standard deviation; C: control group; M: Metformin group; M/O: Metformin/Omega 3 group; TNF-α: Tumor necrosis factor-α; IL-1β: Interleukin-1β; MDA: Malondialdehyde; SOD: Superoxide dismutase; P-value indicates the statistical significance of the difference between the three groups; P1 value indicates the statistical significance of the difference between pre-and post-treatment values; P<0.05 indicates a significant difference; P>0.05 indicates a non-significant difference

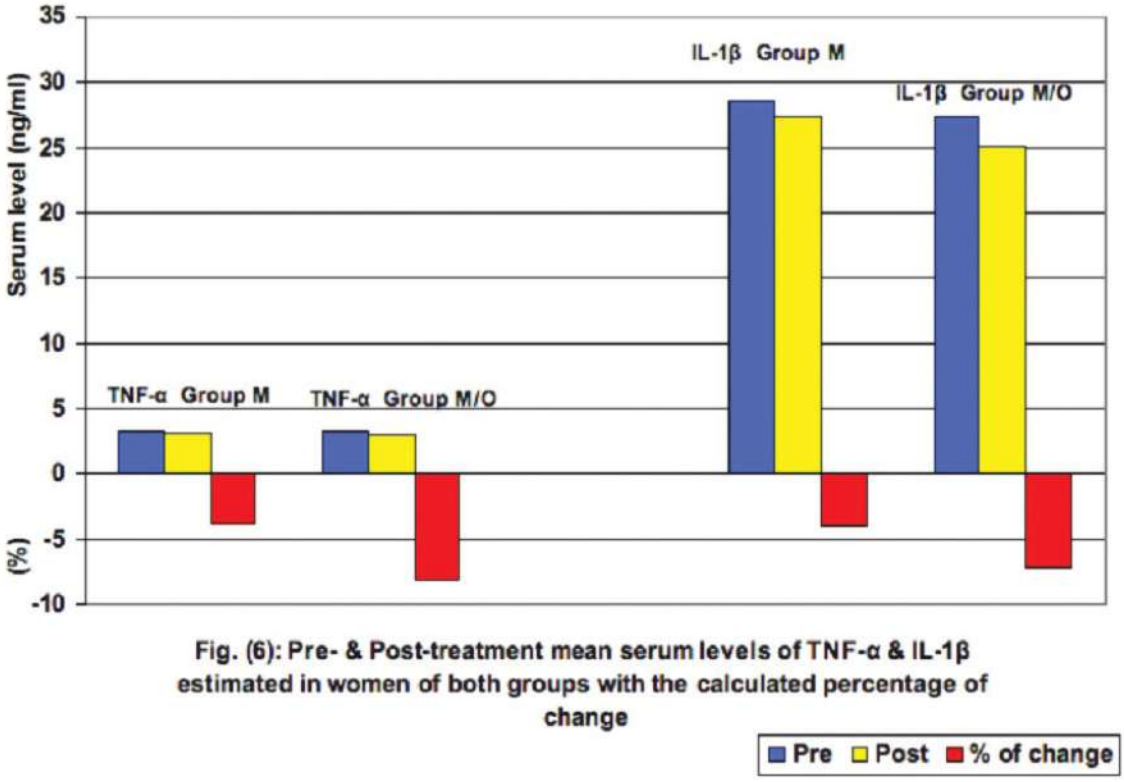

Fig. (6): Pre- & Post-treatment mean serum levels of TNF-α & IL-1β estimated in women of both groups with the calculated percentage of change

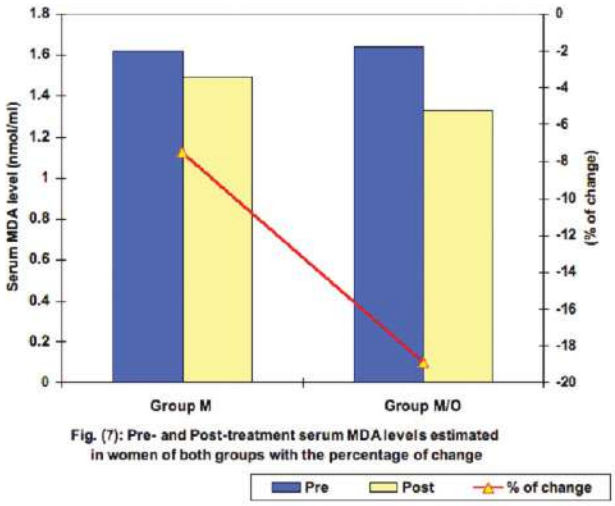

Fig. (7): Pre- and Post-treatment serum MDA levels estimated in women of both groups with the percentage of change

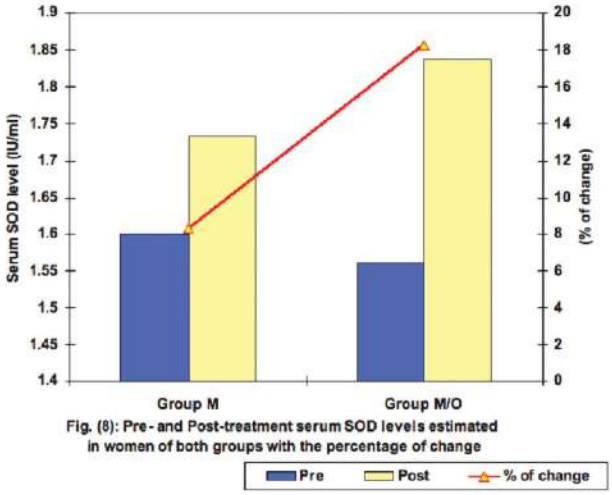

Fig. (8): Pre- and Post-treatment serum SOD levels estimated in women of both groups with the percentage of change

Discussion

The current study included 90 PCOS women with a mean age of 35.7 (±2.7), however, the probable risk for the cardiac event among these women as evaluated using the atherogenic index of plasma (AIP), defined 15.6% and 58.9% of these women had high or intermediate cardiac risk (CR). These findings point to a possible role of PCOS per se, its underlying pathogenic mechanisms, or its associated morbidities for accelerating and accentuating the risk for cardiac events. In support of this assumption, among the studied sample of PCOS women, 59 women were obese and 16 women were morbidly obese, and 52 women were insulin resistant. Moreover, mean serum levels of inflammatory cytokines and lipid peroxidation product malondialdehyde (MDA) were significantly higher in comparison to control fertile women of cross-matched BMI. These findings indicated the fact that PCOS is associated with the disturbed immune milieu in direction of inflammatory arm and redox status in direction of oxidative stress as evidenced by the significantly lower serum levels of SOD in PCOS women than in non-PCOS control women. These disturbances in addition to obesity and IR, both increase the probability of cardiac events, may underlie the reported level of probable CR.

In line with these data, **Ollila et al.** [21] found women at age of 31 years with self-reported PCOS that was manifested as oligo/amenorrhea and hirsutism were found to have higher blood pressure measures than controls and independently of BMI, they will have a higher incidence of cardiovascular morbidity in the premenopausal period. Also, **Mirdamadi et al.** [22] reported that high FBG levels and lipid profiles in obese patients with PCOS are a risk factor for coronary artery disease in PCOS women, but obesity is the more important risk factor and is recommended to assess and monitor CR factors in these women. Moreover, **Duică et al.** [1] documented that PCOS-associated cardiovascular comorbidities gradually lead to endothelial dysfunction and coronary artery calcification, thus posing an increased risk for adverse cardiac events. The applied 3-m therapeutic trial of metformin in combination with omega3 allowed significant improvement of insulin sensitivity with decreased HOMA-IR score, inflammatory status with significant reduction of serum levels of TNF-α and IL-1β, and redox status with a significant decrease of MDA levels with significant elevation of serum SOD. Similarly, **Tosatti et al.** [23] detected a reduction of the inflammatory state in women with PCOS with omega3 supplementation mostly through decreased serum levels of C-reactive protein. In line with obtained results, a meta-analysis to evaluate the effect of omega-3 supplemental therapy on CR

factors in patients with PCOS demonstrated a statistical reduction in serum levels of insulin, total cholesterol, triglyceride, low and very-low-density lipoprotein, and C-reactive protein with the improvement of HOMA-IR score and increased serum levels of high-density lipoprotein [24].

Regarding the probable CR, the metformin/omega 3 combination significantly reduced the AIP in comparison to pre-treatment AIP and to that of women who received metformin alone. Moreover, the effect of adding omega3 to metformin was evident as no woman still had high CR after the end of the therapy, and the frequency of women who had low CR was increased by about 107%. This marvelous effect of omega-3 could be attributed to the reported significant reduction of TNF-α, IL-1β, and MDA levels in comparison to pre-treatment levels and to post-treatment levels in women who received metformin alone.

In support of these findings, a systematic review and meta-analysis revealed that omega-3 and vitamin E co-supplementation have beneficial effects on lipid profile with significantly reduced serum levels of TG and LDL in overweight patients [25]. Another review of the literature reported moderate-certainty evidence suggesting that increasing long-chain omega-3 reduces the risk of coronary heart disease mortality and events with reduction of serum TG and increasing α-linolenic acid slightly reduces the risk of cardiovascular events and arrhythmia [26].

Clinically, **Musazadeh et al.** [27] found omega-3 significantly improvement of serum concentrations of insulin, high-sensitivity C-reactive protein, lipopolysaccharide, total antioxidant capacity, superoxide dismutase activity, MDA, and 8-iso-prostaglandin F2α in patients with non-alcoholic fatty liver in comparison to placebo. Also, **Fazelian et al.** [28] found omega-3 supplementation in chronic kidney disease patients significantly decreased total cholesterol, TG, and MDA levels with a concomitant significant increase of SOD and glutathione peroxidase activities

In line with the effect of omega3 on inflammatory and redox statuses, multiple recent experimental studies detected significant decreases in levels of inflammatory cytokines, improved redox state, and resolution or amelioration of the induced pathologies with the use of omega-3 supplemental therapy [29-31]. Moreover, a recent study had specified this effect to omega-3, not omega-6 where mice fed a diet rich in omega3 showed consistent reductions in serum TNF-α after exposure to 56Fe with no increase in the percentage of osteocytes positive for TNF-α, while this was consistent with the use of omega-6 [32] and another recent study assured a dose-related effect of omega3 on inflammatory cytokines [33].

These effects of omega3 could be attributed to its ability to down-regulate gene expression and mRNA transcription and translation of TNF-α leading to reduction of its serum levels [34]. The positive effect of n-3 PUFAs on lipopolysaccharide-induced inflammatory response was possibly mediated by the nuclear factor-kappa beta (NF-κB) signaling pathway [29]. Moreover, the anti-inflammatory and antioxidant effects of n-3 PUFA could be attributed to resolvin D1 which is the downstream metabolite of docosahexaenoic acid; resolvin D significantly induced higher levels of Bcl-2, SOD, and glutathione, nuclear levels of the nuclear factor erythroid 2-related factor 2 with a significant reduction in reactive oxygen species, MDA, TNF-α, IL-6, NF-κB and expression of cleaved caspase-3 [35].

Conclusion

PCOS is a multifaceted condition possibly induced by or induces obesity, insulin resistance, and systemic pro-inflammatory state and disturbed redox status leading to increased risk of probable cardiac events in these young-aged women. Insulin sensitizers could improve PCOS-associated disturbances. However, omega-3 supplementation as

an adjuvant to insulin sensitizer significantly augmented its effects, minimized the cardiac risk factors, and decreased the risk of probable cardiac events.

Limitations

Evaluation of combined metformin/omega-3 therapy on PCOS-associated hormonal milieu, ovulatory dysfunction, and fertility was also mandatory.

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