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Effect of Metformin on Serum C-reactive protein in Polycystic Ovary Syndrome: A Randomized Controlled Trial

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Abstract

Polycystic ovary syndrome is a chronic low-grade inflammatory state that has an essential role in the future development of insulin resistance and other metabolic sequelae. C-reactive protein has an important role in oxidative stress and chronic low-grade inflammation; its chronic high level is considered one of the causes of PCOS long term consequences. This study was conducted to evaluate the role of Metformin therapy in lowering CRP levels in PCOS. 90 PCOS women were randomized to either 500 mg Metformin tablet 3 times daily for 6 months or placebo. Serum CRP levels decreased significantly in the Metformin group, 3.35 ± 0.99 mg/Litre to 2.58 ± 0.59 mg/Litre (P < 0.0001; 95% CI -1.121 to -0.419), with a significant difference between Metformin and placebo groups, 2.58 ± 0.59 mg/Litre vs 3.27 ± 1 mg/Litre respectively (P = 0.0001; 95% CI 0.346 to 1.034). In conclusion, Metformin is beneficial in lowering serum CRP in PCOS, thus, decreasing the chronic low-grade inflammatory state and decreasing risk of long term sequelae of PCOS.

Keywords

Polycystic Ovary Syndrome, Metformin, Serum C-reactive protein

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I. Introduction

Polycystic ovary syndrome (PCOS) is the commonest endocrine disorder affecting women, it includes; ovulation dysfunction, polycystic ovarian morphology, and hyper-androgenism [1]. Evidence suggests PCOS to be a pro-inflammatory state, even that a chronic low grade inflammatory state has an essential role in its pathogenesis and the future development of insulin resistance and other metabolic sequelae [2-4]. C-reactive protein (CRP) has an important role in oxidative stress and chronic low-grade inflammation [5], its chronic high level is considered one of the causes of PCOS long term consequences [2]. The fact that PCOS women have higher serum levels of CRP compared to control subjects has been supported by some authors [6-9] and has been opposed by others [10, 11], one meta-analysis considered CRP to be the most reliable circulating inflammatory marker in PCOS [12].

Metformin, a biguanide oral anti-diabetic, is one of the suggested treatments for PCOS due to its benefits for hyperinsulinemia and its ability to decrease both total and free testosterone [13, 14]. Metformin is claimed to improve the chronic inflammatory condition associated with PCOS not only by improving hyperglycemia and insulin resistance but also through having a direct anti-inflammatory effect [15, 16]. However, the effect of Metformin treatment on serum CRP in PCOS women has been controversial with some studies supporting its effect [7, 13, 17-22] and others denying it [10, 23-25]. These controversial findings implied the need for further randomized clinical trials to confirm whether the use of Metformin in PCOS reduces serum CRP or not. This randomized study was conducted to clarify the role of Metformin treatment for reduction of serum CRP levels in women with PCOS.

II. Material and Methods

This randomized double-blind placebo controlled trial was conducted in Ain Shams University Hospital during the period from March 2017 to January 2019 after approval of the local ethical committee. Women diagnosed as PCOS were recruited to the study, diagnosis was considered when having 2 of the following criteria; first, a previous history of anovulatory cycles and/ or oligomenorrhea; second, clinical or biochemical hyperandrogenism and finally presence of polycystic ovaries by 2D trans-vaginal ultrasound [26]. Diagnosis by ultrasound depend mainly on the presence of ovarian area >5.5 cm2 or volume >11 ml and/or presence of >12 follicles 2-9 mm in diameter (mean of both ovaries) [27]. Women with thyroid dysfunction, diabetes, hyper-prolactinemia, congenital adrenal hyperplasia, and women receiving any medical or hormonal treatment for PCOS within the last 3 months before entering the study were excluded. A written informed consent was obtained from all subjects before participating in the study. Body mass index (BMI) and Waist/ Hip ratio were obtained for all participants, obesity was considered at a cut off level of > 27 kg/m2.

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Base line serum CRP was obtained for all women using a Roche Hitachi 902 analyzer with Tina-quant CRP high-sensitive assay (Boehringer, Ingelheim, Germany). Lower detection limit was 0.03 mg/L and inter-assay variation coefficient <10%.

Women were then randomized to either; group (M) receiving Metformin 500 mg (Glucophage®, Minapharm, Egypt, under license from Merck Serono, Darmstadt, Germany) 3 tablets daily for 6 months (1500 mg/day), or group (P) receiving 3 placebo tablets daily for 6 months. Randomization was done using computer generated list (MedCalc Software Version 13.2.2, Acacialaan 22, B-8400 Ostend, Belgium) with simple block randomization in ratio 1:1. Blinding was maintained by keeping the drugs in numbered opaque sealed envelopes prepared by a pharmacist not involved in the study thus the randomization key was not revealed till after analysis of the results. BMI, Waist/ hip ratio, and Serum CRP level was obtained again for all participants after the 6 months of therapy.

A previous study suggested reduction of CRP level from 3.08±0.7 mg/liter to 1.52±0.26 mg/liter after 6 months of Metformin therapy, using PASS 11, a sample size of 80 women was required to provide a test of significance of 0.05 and a power of 0.9. Assuming a 10% drop out rate a total of 90 women were included in the study, 45 in each group. Statistical analysis was done by MedCalc© version 12.5 (MedCalc© Software, Ostend, Belgium). Quantitative data were expressed as mean± standard deviation (SD). Qualitative data were expressed as frequency and percentage. Paired sample t-test of significance was used when comparing between related samples. Mann Whitney U test was used for two-group comparisons in non-parametric data. Chi-square (x2) test of significance was used to compare proportions between qualitative parameters. Confidence interval was set to 95% and margin of accepted error was set to 5%. P-value < 0.05 was considered significant.

III. Results

90 PCOS women were randomized equally to both groups, 45 in each group, and there was a total of 7 women dropped out from the study (Figure 1). There was no difference between the two groups regarding age, BMI, Waist/Hip ratio, and pre-intervention CRP level (Table 1). After treatment with Metformin for 6 months there was no significant change in BMI 28 ± 3.3 kg/m2 to 27.02 ± 2.77 kg/m2 (P = 0.1387; 95% CI -2.284 to 0.324) or Waist/ Hip ratio 0.84 ± 0.05 to 0.83 ± 0.04 (P = 0.3081; 95% CI -0.0294 to 0.00939), yet, the difference between Metformin group and placebo became significant (P = 0.0199; 95% CI 0.00324 to 0.0368) (Table 2). There was a highly significant decrease in serum CRP in the Metformin group 3.35 ± 0.99 mg/Litre to 2.58 ± 0.59 mg/Litre (P < 0.0001; 95% CI -1.121 to -0.419), as well as a highly significant difference in CRP between Metformin group and placebo after 6 months (Table 2). Serum CRP was found to be much higher in Obese PCOS (> 27 kg/m2) 3.95 ± 0.75 mg/Litre than in lean PCOS 2.47 ± 0.59 mg/Litre (P < 0.0001; 95% CI -1.189 to -1.771) (Table 3). CRP levels decreased after 6 months of Metformin therapy but this decrease was not statistically significant in lean PCOS unlike obese PCOS where the decrease of CRP levels was highly significant (Table 4).

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IV. Discussion

Being a life-long endocrinopathy and having several long-term sequelae as type 2 diabetes, metabolic syndrome, and cardiovascular events [28], management of PCOS should consider treatment options aiming to prevent these long-term complications. CRP is well known as an inflammatory marker, its sustained elevated levels for periods exceeding 6 years was found to be related to increased risk of diabetes, ischemic stroke, coronary heart disease, heart failure, and mortality [29]. A chronically elevated serum CRP level in women with PCOS has been demonstrated by several authors [6-9]. This study showed that Metformin therapy in PCOS women lowers significantly CRP level especially in PCOS with BMI > 27 kg/m2. The more significant decrease in obese PCOS women might to be attributed to the initially higher CRP levels than in lean PCOS; obesity is known to be associated with elevated CRP levels which usually tend to decrease after weight loss [30]. Weight loss in PCOS women receiving Metformin was noted in this study, but was not statistically significant; still, this weight loss might have a role in lowering the CRP levels.

Metformin treatment for longer durations or higher doses might have caused a significant decrease in the BMI. These results are supported by the conclusion of one meta-analysis studying the effect of Metformin therapy on CRP and interleukin-6 (IL-6) levels in PCOS women, this meta-analysis found that the effect of Metformin therapy on CRP levels was both dose and duration dependent; also it noted a more beneficial effect in obese PCOS, a finding that is consistent with the results of this study [31].

Being a randomized and blinded controlled trial, this study gives a good evidence that the use of Metformin has a beneficial role in lowering serum CRP levels in PCOS women, especially in obese PCOS, thus decreasing the chronic low-inflammatory condition associated with these women which might have an important role in the prevention of long-term sequelae of PCOS like diabetes and cardiovascular insults. Still, it is one of the drawbacks of this study that it did not address the relation between CRP and insulin resistance, neither did it investigate the effect of Metformin therapy on other related inflammatory markers as IL-6. Further studies are needed to investigate the optimal dose and duration of Metformin therapy for prevention of PCOS long-term complications and to investigate whether its role is confined only to obese PCOS or also lean PCOS. Also, long-term follow up studies are needed to study the actual beneficial role of Metformin therapy on reducing the risk of long-term complications of PCOS and not only the theoretical role assumed by decreasing the chronic inflammatory markers associated with PCOS.

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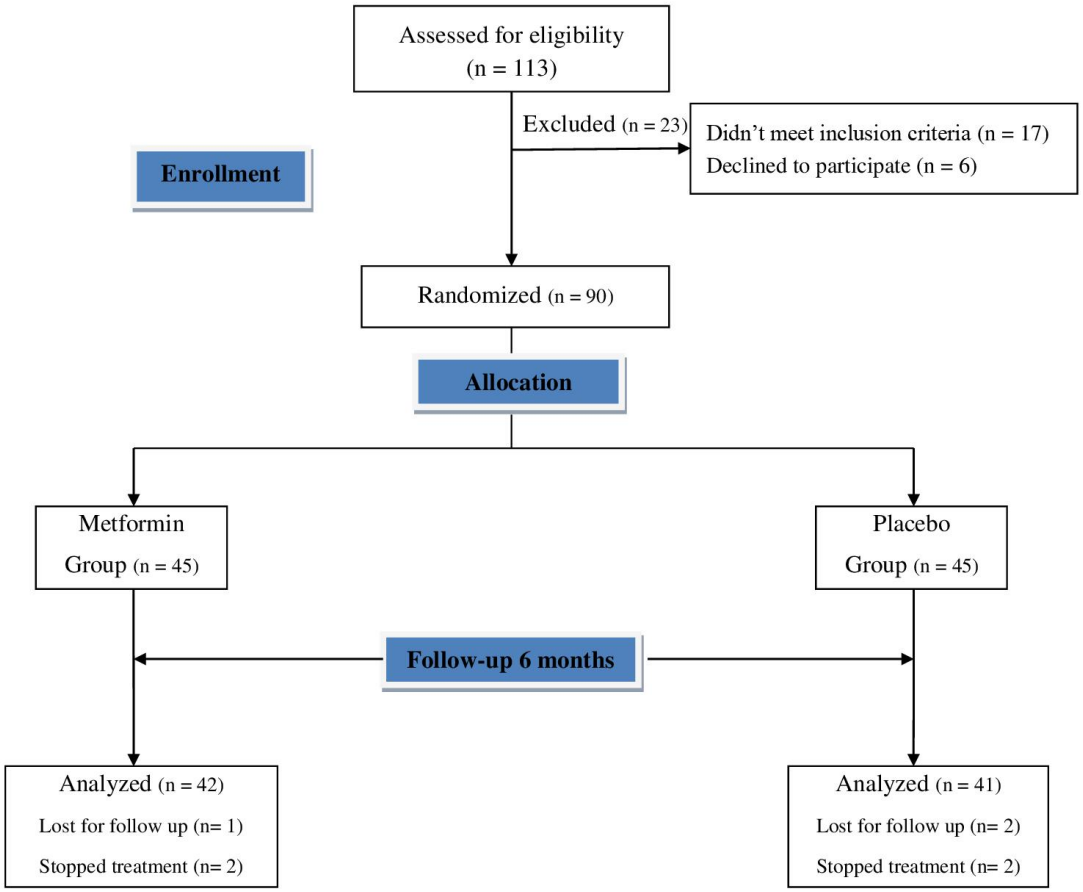

Figure 1. Flow Chart of the study

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Table 1. Pre-treatment characteristics of both groups.

	Metformin (n=45)	Placebo (n=45)	P-value	95% CI
Age, years. (mean ± SD)	28.67 ± 4.35	27.11 ± 4.63	0.103	-3.442 to 0.322
BMI, kg/m^2 (mean ± SD)	28 ± 3.3	27.44 ± 3.38	0.4286	-1.959 to 0.839
Waist/Hip ratio (mean ± SD)	0.84 ± 0.05	0.85 ± 0.04	0.2977	-0.00897 to 0.029
CRP, mg/Litre (mean ± SD)	3.35 ± 0.99	3.3 ± 1.03	0.8149	-0.473 to 0.373

Table 2. Change in BMI, Waist/Hip ratio, and CRP after 6 months

	Metformin	Placebo	P-value	95% CI
Pre BMI, kg/m^2 (mean ± SD)	28 ± 3.3	27.44 ± 3.38	0.4286	-1.959 to 0.839
Post BMI, kg/m^2 (mean ± SD)	27.02 ± 2.77	27.71 ± 3.21	0.2780	-0.566 to 1.946
P-value 95% CI	0.1387 -2.284 to 0.324	0.7057 -1.147 to 1.687		
Pre Waist/Hip ratio (mean ± SD)	0.84 ± 0.05	0.85 ± 0.04	0.2977	-0.00897 to 0.029
Post Waist/Hip ratio (mean ± SD)	0.83 ± 0.04	0.85 ± 0.04	0.0199	0.00324 to 0.0368
P-value 95% CI	0.3081 -0.0294 to 0.00939	1.0000 -0.0172 to 0.0172		
Pre CRP, mg/Litre (mean ± SD)	3.35 ± 0.99	3.3 ± 1.03	0.8149	-0.473 to 0.373
Post CRP, mg/Litre (mean ± SD)	2.58 ± 0.59	3.27 ± 1	0.0001	0.346 to 1.034
P-value 95% CI	< 0.0001 -1.121 to -0.419	0.8915 -0.466 to 0.406		

Table 3. CRP before and after interventioned in Lean and Obese PCOS in both groups.

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	Lean PCO (all) (n=34)	Obese PCO (all) (n=49)	P-value	95% CI
CRP (B.), mg/Litre (mean ± SD)	2.47 ± 0.59	3.95 ± 0.75	< 0.0001	1.189 to 1.771
CRP (A.), mg/Litre (mean ± SD)	2.3 ± 0.49	3.38 ± 0.83	< 0.0001	0.78 to 1.38
P-value	0.1760	0.0004		
95% CI	-0.418 to 0.0779	-0.878 to -0.262		

Table 4. CRP before and after Metformin therapy in Lean and Obese PCOS

	Lean PCO (n=16)	Obese PCO (n=26)	P-value	95% CI
CRP (Before), mg/Litre (mean ± SD)	2.46 ± 0.67	3.89 ± 0.73	< 0.0001	0.991 to 1.869
CRP (After), mg/Litre (mean ± SD)	2.08 ± 0.38	2.89 ± 0.47	< 0.0001	0.538 to 1.082
P-value	0.0577	< 0.0001		
95% CI	-0.773 to 0.0133	-1.342 to -0.658		