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Effect of Metformin Therapy Over Hormone Profile in Newly Diagnosed Polycystic Ovary Syndrome –A Nine Months Randomized Controlled Trial

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Abstract:

Background: Polycystic ovary syndrome (PCOS) is a heterogeneous endocrine disorder affecting women of reproductive age. Treatment by metformin is based on the basis of insulin resistance in PCOS.

Objective: To see the effect of metformin therapy for 9 months on hormonal profiles in PCOS.

Methods: Present double blind placebo-controlled study encompassed 80 PCOS subjects (age, mean±SD: 23.10±4.30; married/unmarried: 44/36; amenorrhea/oligomenorrhea: 90%; abortion: 25%; infertility: 38.6%) who were tested for hormonal profiles (serum testosterone, progesterone and anti-mullerian hormone, AMH) at recruitment and after 9 months of therapy with metformin/placebo having >80% compliance. Among 80, 49 completed the study (metformin=26, placebo=23), 4 became pregnant before reaching the end point (metformin=1, placebo=3), and 27 dropped out (metformin=13, placebo=14). AMH was assayed by ELISA whereas testosterone and progesterone by chemiluminescent method.

Results: Baseline characteristics as well as hormonal and biochemical profiles were statistically similar between metformin and placebo groups. After 9 months, AMH (ng/ml) increased significantly in both metformin (6.10±5.68 vs. 8.67±4.27, p<0.003) and placebo (4.89±3.70 vs. 10.54±3.58, p<0.001) groups. Progesterone (1.55±2.58 vs. 5.16±5.20, ng/ml, p=0.006) increased whereas testosterone significantly decreased (8.11±3.98 vs. 5.96±1.72, ng/ml, p=0.003) in the metformin group. Unlike metformin, testosterone (8.88±3.10 vs. 8.86±3.06, ng/ml, p=0.989) and progesterone (0.94±0.85 vs 0.82±1.34, ng/ml, p=0.670) were unchanged in placebo. Side effect like loose motion, abdominal pain, nausea and vomiting were observed during the initial part of therapy in both groups.

Conclusions: The present double-blind placebo-controlled RCT of nine months revealed that metformin has significant beneficial effect on androgenous activity of PCOS.

Keywords: PCOS, Metformin AMH, Testosterone, Progesterone.

INTRODUCTION

Polycystic ovary syndrome (PCOS) is a heterogeneous endocrine disorder of women with multifactorial causes leading to chronic anovulation associated with androgen excess. It was first described by Stein & Leventhal in 1935 as the commonest endocrine disorder in women of reproductive age leading to menstrual irregularity, which is one of the cardinal clinical features of PCOS.1 Incidence of PCOS in women of reproductive age is

reported to be 5 to 10% which is found to be increasing than assumed.[2] PCOS is characterized by anovulatory menstrual cycle, infertility, hyperandrogenism thus resembling different metabolic syndromes.[3] In 2003 Rotterdam criteria was proposed which requires two of the following characteristics for the diagnosis of PCOS: oligo-ovulation or anovulation, clinical and/or biochemical signs of hyperandrogenism and polycystic ovaries with the exclusion of other etiologies (congenital adrenal hyperplasia, androgen secreting tumors, Cushing syndrome).[4] Women with PCOS may present with multiple manifestation which include cutaneous, reproductive, endocrine and metabolic abnormalities. Cutaneous manifestations including hirsutism, acne and male pattern of baldness are caused by hyperandrogenism. Reproductive manifestations are menstrual dysfunctions like secondary amenorrhea/ oligomenorrhea, infertility, early pregnancy loss and other complication of pregnancy. Metabolic and endocrine abnormalities are increased circulating levels of total and/ or free testosterone, androstenedione, dehydroepiandrostenedione sulfate (DHEAS), decreased sex hormone binding globulin (SHBG), increased insulin level and increased ratio of luteinizing hormone : follicle stimulating hormone (LH/FSH).[5] In addition to the clinical presentation, PCOS poses the long term risk of development of gestational diabetes, type 2 DM, frequent first trimester pregnancy loss and increased risk of cardiovascular disease & endometrial cancer.[6,7,8] Historically, treatment has been directed at ovulation induction for infertility, oral contraceptive or progestins for menstrual irregularity and antiandrogen for hirsutism.[9] This traditional pharmacological therapy for patients with PCOS mainly address the hyperandrogenic state and anovulation, but neglects the associated long time metabolic risk i.e. insulin resistance.

Recently, increasing evidence has shown that the insulin resistance has an important implication in the pathogenesis of PCOS and the use of insulin sensitizing drugs is an effective therapeutic approach. Insulin resistance with resultant hyperinsulinemia, irrespective of excess weight or frank obesity, has been reported in patient with PCOS.[10] A growing body of evidence has suggested that hyperinsulinemia contributes to the excess ovarian androgen secretion observed in women with PCOS.[11] The increase insulin sensitivity by insulin sensitizers may be of therapeutic value in the management of clinical manifestations of hyperinsulinemia and hyperandrogenemia.[12] The improvement of insulin sensitivity by insulin sensitizers e.g. metformin is now thought to be of pivotal therapeutic value in the management of PCOS.

MATERIALS AND METHODS

Subjects

This was a randomized double blind placebo-controlled study comprised of 80 PCOS patients. Inclusion criteria were: age between 18 and 35 years, PCOS diagnosed on the basis of Revised Rotterdam Consensus 2003 criteria. PCOS patients were recruited consecutively who were given either metformin or placebo in a double blind process and were scheduled for five visits within the period of nine months in each subject, whereupon a window period of 14 days was permitted for the 2nd, 3rd, 4th and last visit. Women with PCOS were treated with metformin for nine months in a dose of 1500 mg daily in three divided doses. 49 patients completed the visit in which 23 were in placebo and 26 in metformin group. On the other hand, who failed to fulfill the compliance, were dropped. Thus 27 were dropped, among them 14 were in placebo and 13 in metformin group; 4 got pregnant, 3 in placebo and 1in metformin group.

Assays

AMH was estimated by an enzyme-linked immunosorbent assay, AMH GEN II ELISA kit (Beckman Coulter, Inc. USA) whereas other hormones (FSH, LH and Testosterone) by immunochemiluminometric assay. Values of AMH were presented as nanograms per milliliters (conversion factor to pmol/l = ng/ml × 7.1). AMH was calculated using kc3 biograph with help of the standard supplied with the kit. QC (quality control) was used in each assay run to assess precision of the assay. Intra-assay CV (co-efficient of variance) was 3.4 to 5.4% and inter-assay CV 4.0 to 5.6% for AMH assay.

Statistical Methods

All data are processed by the SPSS program (version-22.0). Values are expressed as frequencies or mean ± SD unless mentioned otherwise. At the end of the study, metformin/placebo was unblinded and subjects were divided into two groups: one metformin and another as placebo. Within each group categorical variables (hirsutism, acne, acanthosis nigricans, menstrual irregularity, infertility) were analyzed by using Chi square. Numerical variables (serum AMH, testosterone, progesterone) were analyzed by paired or unpaired t-test as applicable. P-values ≤0.05 were considered as significant.

RESULTS

Randomized double blind placebo-controlled study was done on PCOS to see the effect of metformin for which 80 subjects (age, mean±SD: 23.10±4.30; married/unmarried: 44/36; H/O amenorrhea/oligomenorrhea: 90%; H/O abortion: 25%; H/O infertility: 38.6%). Characteristics of the subject are shown in Table-I. Metformin or placebo were continued for 9 months in each subjects with intermittent follow-up with periods of one month, two months and three months for last two consecutive visits. Comparison of biochemical parameters were done between the baseline findings and that after nine months by paired model of statistical test within the group and unpaired model for between the groups. Table-II shows the baseline clinical characteristics between metformin and placebo groups [metformin vs. placebo, BMI (kg/m2) 26.18±5.44 vs. 25.30±5.32, p = 0.462; WC (cm): 86.90±12.34 vs. 85.88±11.02, p = 0.696; SBP (mmHg): 110.88±11.43 vs.110.88±8.47, p = 1.0; DBP (mmHg): 74.38±8.56 vs.73.75±6.96, p = 0.721; hirsutism: 82.5% vs.85.0%, p = 0.762; acne: 42.5% vs.40.0%, p= 0.820; acanthosis nigricans: 22.5% vs. 37.5%, p = 0.143]. None of the variables showed any statistical significant difference between the groups. Background hormonal and biochemical profiles (mean±SEM) of the patients are shown in Table-III [metformin vs. placebo, S. prolactin (ng/ml): 14.54±2.78 vs.11.84±1.13, p = 0.373; S.TSH (mIU/L): 2.44±0.20 vs. 2.46±0.22, p = 0.956; S.LH (U/L): 8±0.90 vs. 7.24±0.88, p = 0.547; S. FSH (U/L): 4.75±0.24 vs. 4.61±0.39, p = 0.752; LH/FSH ratio: 1.74±0.18 vs. 2.09±0.33, p = 0.338; S. testosterone (ng/ml): 8.23±0.62 vs. 8.34±0.46, p = 0.893; S.AMH (ng/ml): 5.19±0.79 vs. 5.21±0.64, p = 0.986; S. progesterone (ng/ml): 1.96±0.56 vs. 1.67±0.51, p = 0.707; fasting blood glucose (mmol/L): 4.67±0.06 vs. 4.8±0.07, p = 0.195; SGPT(U/L): 45.23±2.41 vs. 44.90±2.94, p = 0.729; S. creatinine (mg/dl): 0.79±0.02 vs. 0.80±0.03, p = 0.338]. It was also observed in this aspect that there was no statistical difference between the metformin and placebo groups. Fig-1 depicts changes of hormonal profiles (mean±SD) before and after treatment of metformin [AMH (ng/ml): 6.10±5.68 vs. 8.67±4.27, p<0.003; progesterone (ng/ml):1.55±2.58 vs. 5.16±5.20, ng/ml, p = 0.006; testosterone (ng/ml):8.11±3.98 vs. 5.96±1.72, ng/ml, p = 0.003]. It was found that in metformin group AMH level increased significantly as well as progesterone level whereas testosterone significantly decreased. Hormonal profiles before and after treatment by placebo are shown in Table-IV [AMH (ng/ml): 4.89±3.70 vs 10.54±3.58, p<0.001; testosterone (ng/ml):8.88±3.10 vs. 8.86±3.06, ng/ml, p = 0.989; progesterone (mg/ml)0.94±0.85 vs 0.82±1.34, ng/ml, p = 0.670]. AMH (ng/ml) was increased after nine months whereas testosterone and progesterone unchanged statistically.

Comparison between the metformin and placebo groups for the hormonal profiles at the end point of the study is shown Fig-2. Though in both groups, AMH happened to be increased than baseline it was relatively more increased in the placebo group (8.67±4.27 vs. 10.54±3.58,ng/ml, p = 0.106). However increase of progesterone (5.16±5.20 vs. 0.82±1.33, ng/ml, p<0.001) and reduction of testosterone (5.96±1.71 vs. 8.86±3.09, ng/ml, p<0.001) were statistically significant and comparable between metformin and placebo groups.

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Table I. *Characteristics of the study population at baseline (n=80)*

Characteristics	All	Metformin	Placebo	P
n	80	40	40	
Age (m± SD)	23.10±4.30	23.54±5.14	22.67±3.27	0.373
Occupation				
House wife	35(43.8%)	17(42.5%)	18(45.0%)	
Service holder	6(7.5%)	3(7.5%)	3(7.5%)	0.973
Student	39(48.8%)	20(50.0%)	19(47%)	
Marital status				
Married	44(55%)	22(55%)	22(55%)	1.0
Unmarried	36(45%)	18(45.0%)	18(45.0%)	
H/O Amenorrhea/ oligomenorrhea	72(90%)	34(85.0%)	36(90.0%)	0.638
H/O MR/Abortion*	11(25%)	7(31.8%)	4(18.2%)	0.296
H/O Infertility*	17(38.6%)	6(27.3%)	11(50.0%)	0.219

(Within parenthesis are percentages over column total)

Significant values stand for comparison between metformin and placebo by student *t-test and* chi-square *test*

Total dropped cases were = 27, who did not complete study

(metformin = 26, placebo = 23,Primary endpoint due to pregnancy = 4)

m±SD: mean±standard deviation

H/O: history of

*Only married respondents (n = 44)

Table II. *Baseline clinical characteristics of the study subjects*

Variales	All subjects	Metformin	Placebo	p
n	80	40	40	
BMI(Kg/m^2)	25.74±5.37	26.18±5.44	25.30±5.32	0.462
WC(centimeter)	86.39±11.64	86.90±12.34	85.88±11.02	0.696
Systolic BP	110.9±9.99	110.88±11.43	110.88±8.47	1.0
Diastolic BP	74.0±7.76	74.38±8.56	73.75±6.96	0.721
Hirsutism	67(83.8%)	33(82.5%)	34(85.0%)	0.762
Acne	33(41.3%)	17(42.5%)	16(40.0%)	0.820
Acanthosis nigricans	24(30%)	9(22.5%)	15(37.5%)	0.143

(Data were expressed as frequency, percentage, mean±SD)

Independent student *t-test was performed to compare between two groups for quantitative data*

Chi-square test was performed to compare between two groups for qualitative data

BMI: body mass index

Kg/m²: kilogram/meter²

WC : waist circumference

BP: blood pressure

Table III. *Baseline hormonal and biochemical profiles of the study patients*

Hormonal and Biochemical profiles	Total (n = 80)	Metformin (n = 40)	Placebo (n = 40)	p
S. Prolectin(ng/ml)	13.18±1.50	14.54±2.78	11.84±1.13	0.373
S. TSH(mIU/L)	2.45±0.15	2.44±0.20	2.46±0.22	0.956
S. LH(U/L)	7.62±0.63	8±0.90	7.24±0.88	0.547
S. FSH(U/L)	4.68±0.23	4.75±0.24	4.61±0.39	0.752
LH/FSH ratio	1.91±0.18	1.74±0.18	2.09±0.33	0.338
S. Testosterone(ng/ml)	8.29±0.38	8.23±0.62	8.34±0.46	0.893
S. AMH(ng/ml)	4.97±0.47	5.19±0.79	5.21±0.64	0.986
S. Progesterone(ng/ml)	1.82±0.37	1.96±0.56	1.67±0.51	0.707
Fasting blood glucose(mmol/L)	4.73±0.49	4.67±0.06	4.8±0.07	0.195
SGPT(U/L)	45.56±1.89	46.23±2.41	44.90±2.94	0.729
S. Creatinine(mg/dl)	0.79±0.16	0.79±0.02	0.80±0.03	0.338

Data were expressed as mean±SEM

Independent student t-test was performed to compare between two groups

TSH: thyroid stimulating hormone

 LH: luteinizing hormone

FSH: follicle stimulating hormone

AMH: anti-mullerian hormone

Table IV. *Level of AMH, testosterone and progesterone before and after treatment in placebo group (n = 23)*
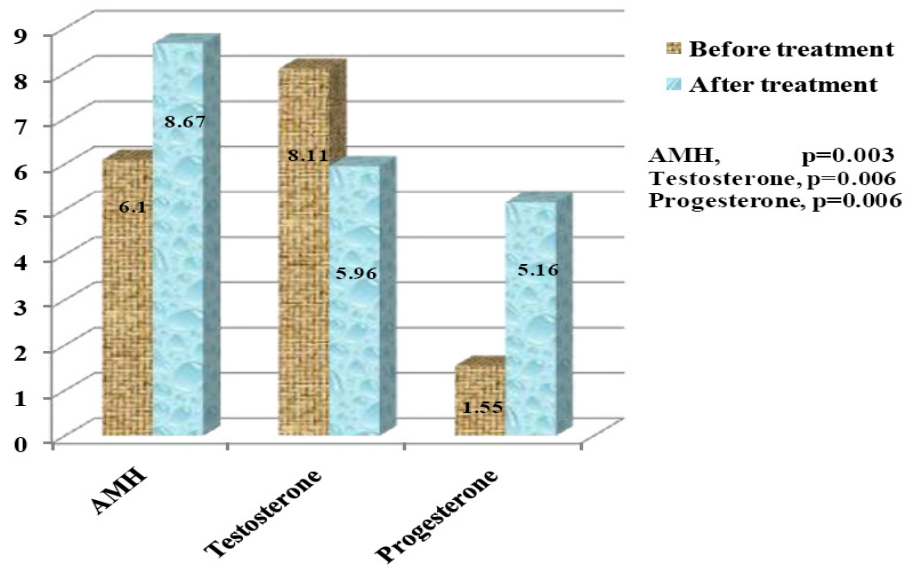
Hormone profile	Before treatment (n =23)	After treatment (n =23)	p
AMH(ng/ml)	4.89±3.70	10.54±3.58	< 0.001
Testosterone(ng/ml)	8.88±3.10	8.86±3.06	0.989
Progesterone(ng/ml)	0.94±0.85	0.82±1.34	0.670

Data were expressed as mean±SD

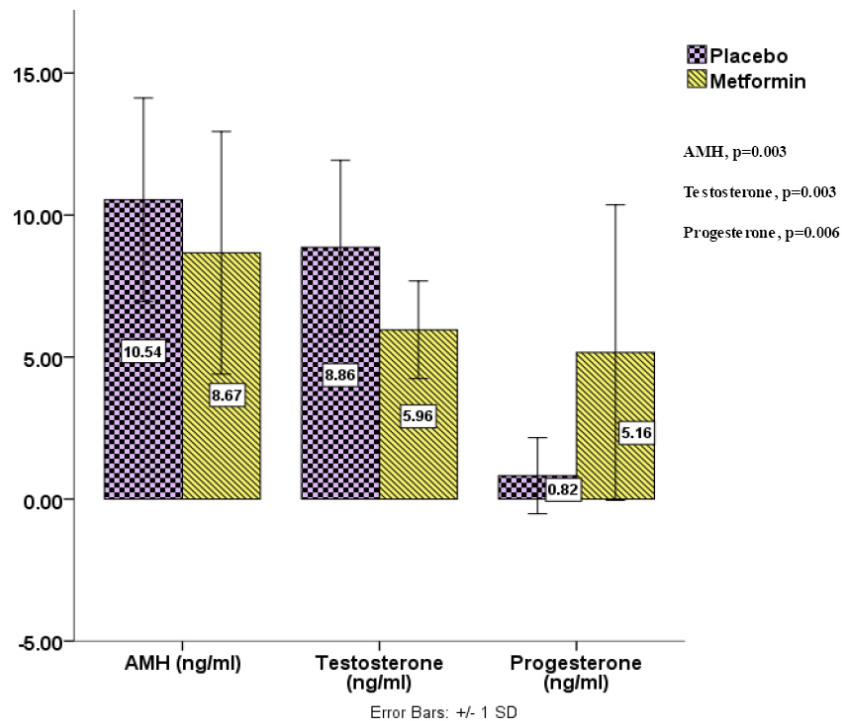*Paired sample t-test was performed to compare within group*

AMH = anti-mullerian hormone

Fig1. *Level of AMH (ng/ml), testosterone (ng/ml) and progesterone (ng/ml) before and after treatment in metformin group (n=26)*

AMH: anti-mullerian hormone

Fig 2. *Level of AMH, testosterone and progesterone after last visit between placebo and metformin groups*

AMH: anti-mullerian hormone

DISCUSSION

PCOS is not an uncommon disorder affecting many women in reproductive age. Recognition of insulin resistance as an important factor in the pathogenesis of PCOS instigated a wide use of insulin sensitizing agents for the treatment of the problem.[13] Among the insulin sensitizers, metformin is the most commonly used agent which is preferred for the treatment of PCOS.[14] Though the beneficial effect of metformin has been proposed and advocated by many authorities, it has not been substantiated in wide scale by RCT.

In this study a similar baseline clinical and biochemical characteristics are present between metformin and placebo groups. Similar to the baseline clinical characteristic, biochemical and hormonal profiles were statistically non-different between the two groups at initiation of the study. Comparing within group, significant reduction of androgenic activity was observed in the metformin group but not in the placebo after nine months of treatment. Likewise, mean progesterone level in the third week of cycle or nearby time in the last month improved significantly in the metformin group but not in placebo. Contrary to the common belief and unlike androgen, in both groups, AMH increased significantly which was more in placebo. Menstrual regulation significantly improved after treatment with metformin as well as placebo but more with metformin. Though not significantly different from the placebo group, the adverse events were more frequent with the metformin group.

Significant heterogeneity in the hormonal response after metformin was observed. [15,16,17,18] The present study observed reduction of testosterone whereas increase of progesterone and AMH level in metformin group; but in placebo there was no significant change in testosterone and progesterone level. However, AMH level raised significantly and with higher magnitude than that of metformin in the placebo group. Velazquez and colleagues (1994)[14] reported in an observational study a significant improvement in menstrual regularity and reduction of circulating androgen levels as well as significant reduction of body weight. Yu Ng et al. (2001) [19] found that serum testosterone concentration was significantly reduced in the metformin group after 3 months and there was a significant increase of serum testosterone in placebo group.[20] This view is supported by others also. [8, 14, 15,19, 21]

In the present study, despite the improvement of metabolic parameters and reduction of androgen levels, AMH levels increased after treatment, more with placebo than metformin group. Tomava et al. (2011)[16] found discrepant levels of AMH level after metformin therapy; some showing decrease and some having increased or no change. Nascimento et al. (2013) found no alteration in AMH levels after treatment.[17] The dose and duration of metformin therapy needs to be optimized for getting the benefit.

Regarding the side effects, as expected metformin was more associated with minor gastrointestinal events. However, neither in the metformin group nor in the placebo group, there were any serious adverse events.

In conclusion, the present double blind placebo-controlled RCT of nine months period by metformin on PCOS revealed that the drug has significant beneficial effect on the androgenic activity. There was no remarkable adverse event caused by the drug. AMH was not found to be reduced, rather increased after nine months treatment by metformin in a dose of 1500 mg/day or by placebo.

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