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Research Article

EFFECT OF METFORMIN THERAPY IN FEMALE VISITING DERMATOLOGIST FOR ACNE VULGARIS HAVING ENDOCRINE AND SONOGRAPHIC CHARACTERISTICS OF POLYCYSTIC OVARY SYNDROME (PCOS)

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ABSTRACT

The objectives of the present investigation were to study the effect of metfomin on acne vulgaris (and other hyperandrogenism) associated with polycystic ovary syndrome (PCOS). Females of reproductive age suffering from the acne vulgaris &/or other hyperandrogenism were included in the study. Physical and clinical parameters like age, weight, body mass index (BMI), waist to hip ratio (WHR), acne score, hirsutism score, presence of androgenic alopecia, menstrual irregularity pre- and post-treatment were noted. Family history of hyperandrogenism, diabetes, hypertension and thyroid dysfunctions were taken. Subjects were called on 3rd to 5th day of menstrual cycle for ultrasonography and hormonal estimations like insulin, TSH, Testosterone, Progesterone, DHEAS, Prolactin, LH and FSH. From 33 females, 25 met the Rotterdam criteria of PCOS and 17 females completed metformin 1-1.5 g/day (and/or minoxidil 2% in case of severe androgenic alopecia) therapy for 3 months. After 3 months of metformin therapy improvement in physical and clinical signs like weight, waist to hip ratio, acne (before treatment 2.37 ± 0.17 and after treatment 1.68 ± 0.19), hirsutism (before treatment 14.3 ± 1.7 and after treatment 12.8 ± 1.8), androgenic alopecia (6 out of 13 subjects showed hair growth) and menstrual irregularities (50% had regular cycle) were observed along with normalization of hormones (Insulin, Testosterone, LH, FSH and LH/FSH ratio) and ultrasonographic changes (ovarian volume, number of follicles, ovarian- and uterine- resistance index). It is thus concluded that patients visiting dermatologist for acne vulgaris must be considered for the possibility of PCOS and can be treated with the metformin.

Keywords: Metformin, PCOS, Acne, Hirsutism, Insulin, Testosterone.

INTRODUCTION

Acne vulgaris is a chronic inflammatory dermatosis which is notable for open and/or closed comedones (black heads and white heads) and inflammatory lesions including papules, pustules, or nodules (Strauss et al., 2007). Acne vulgaris is treated depending on its severity. For patients with only comedonal disease, topical comedolytic agents are appropriate; tretinoin 0.01% to 0.05%, adapalene 0.1%, or tazarotene 0.1% applied nightly on the dry skin is effective. Sunscreens may prevent sun sensitivity caused by any of these agents. Patients with superficial inflammatory papules require topical antimicrobial agents. These may be combined with comedolytics. Benzoyl peroxide containing gels, creams, or washes should be applied once daily**.** Clindamycin 1% or erythromycin 2% in combination with benzoyl peroxide is more effective than either product alone. Azelaic acid 20%, an antibacterial and comedolytic, has been shown to be effective and well tolerated. For moderately severe inflammatory acne, systemic antibiotics must be used for at least 1 to 3 months. Choices and dosages include tetracycline 500 mg twice daily, doxycycline 50 to 100 mg twice daily, minocycline 100 to 200 mg daily, erythromycin 250 mg four times daily, or erythromycin ethylsuccinate 400 mg two to three times daily. In patients with severe nodular acne, in recalcitrant cases, or when there is scarring, isotretinoin is the drug of choice. The standard dosing is 1 to 2 mg/kg daily for 4 to 6 months (Frankel, 2006).

To date there has been only one published trial addressing use of metformin in acne in women having PCOS as a specific endpoint. Kolodziejczyk et al., (2000) found a significant decrease in acne score from 1·45 to 1·14 (p<0·001). However, the clinical significance of the decrease is uncertain. More data are required.

The precise objectives of the present investigation were to study the effect of metformin and/or minoxidil treatment for three months in patients of acne vulgaris associated with PCOS.

Subjects and methods

It was prospective, open-labelled, unblind, randomized single centre clinical study carried out in the outpatient department of dermatology at Samarpan Medical and Research Organization, Modasa, and Dr. Rasiklal Shah Sarvajanik Hospital, Modasa (Gujarat). Females of reproductive age suffering from the acne vulgaris and/or other hyperandrogenism manifestations (hirsutism, androgenic alopecia and irregular menstrual cycle) were included in the study. Patients were analysed clinically and graded according to the severity of the condition. Diagnosis of acne vulgaris associated with the PCOS was based on Rotterdam criteria. Womens with congenital adrenal hyperplasia, androgen-secreting tumors, Cushing's syndrome with diabetes mellitus, smokers, on steroid hormones, on drugs known to have effects on lipid metabolism during the past 2 years, with bilateral tubal block or tubercular endometritis or male factor infertility were excluded from the study. Thirty-three females of reproductive age suffering from the acne vulgaris and/or other hyperandrogenism manifestations (hirsutism, androgenic alopecia and irregular menstrual cycle) were included in the study. Eleven unrelated normal controls were selected randomly were also included.

The study protocol was approved by institutional review board of Dr. Rasiklal Shah Sarvajanik Hospital, Modasa. All the female patients were given information regarding the study and those ready to sign the consent form were included in the study.

At the first visit, menstrual pattern (past and present), presence of acne, hirsutism scoring (Ferriman and Gallwey 1961), androgenic alopecia and acanthosis nigricans were noted. Acne severity was rated as follows, Acne score 0 - for no acne at all, Acne score 1 - ≤ 15 comedones + ≤ 10 papules + ≤ 5 pustules (Mild acne), Acne score 2 - >15 comedones or > 5 papules or > 5 pustule and < 5 nodulocystic lesions (Moderate acne) and Acne score 3 - ≥ 5 nodulocystic lesions (Severe acne) as per Charoenvisal et al., 1996. Anthropometric measurements were made by using World Health Organization (WHO) techniques. Height (meters) was measured bare foot to within 0.5cm. Weight (kilograms) was measured to within 100gm in light clothes using digital scale. Circumferences were measured to within 1 mm using soft measuring tape in the standing position. Waist circumference was measured midway between the lowest rib margin and iliac crest. Hip circumference was taken over the widest

part of the gluteal region. Body mass index (BMI) and waist hip ratio (WHR) were calculated. Physical examinations of all the subjects were performed at the beginning before the treatment and after 3 month of the treatment. Family histories of all the subjects included were taken to confirm the hereditary factor.

After clinical diagnosis of acne vulgaris and other hyperandrogenism manifestations, all the subjects were called on the 3rd to 5th day of menstrual cycle for ultrasonography. In all unmarried female patients transabdominal pelvic ultrasonography were performed using Envisor Phillips with 2-5 MHz convex electronic probe, while in all married female patients transvaginal colour doppler ultrasonography were performed using Wipro GE Notebook Colour Doppler with 5.5 to 7.5 MHz electronic probe. Ultrasonography was performed on the subjects before and after the treatment by an authorized Radiologist. Ultrasonographic data from the right and left side of the genital system were evaluated, as no statistical significant difference existed in right sides and left sides, the value used was the sum of both the sides divided by two.

Venous blood samples were collected on the 3rd to 5th day of menstrual cycle. Blood samples were collected in the morning hours during empty stomach for estimation of various hormonal profiles like FSH, LH, Prolactin, DHEAS, Progesterone, Testosterone, Insulin and Thyroid stimulating hormone and RBC, WBC, Platelets, Heamoglobin and SGPT prior to the treatment. 5 ml of blood was collected in a clean glass vials. RBC, WBC, Platelets and Heamoglobin counts were performed using whole blood, while rests of the estimations were done using serum. Blood was allowed to clot at room temperature. Serum was collected by centrifuging the blood sample. Sampled sera were stored at -20°C until analysis was performed. Hormones estimations were performed before and after treatment. Serum level of Insulin, Prolactin, LH, Thyroid stimulating hormone, Testosterone, FSH, DHEAS and Progesterone were determined by Radioimmunoassay (RIA) method (Board of Radiation and Isotope Technology, BARC Vashi complex, vashi, Navi Mumbai, India).

Metformin 1-1.5 g/day (and/ or minoxidil 2% in case of severe androgenic alopecia) was started to all 25 subjects after the diagnosis of acne vulgaris associated with PCOS. Patients were asked to continue treatment for three months and called for the follow-up after three months.

Results have been presented as means ± SEM. Statistical analysis was performed using paired or unpaired Student's *t*-test as appropriate for all quantitative variables. Two tailed significance was calculated with 95% confidence interval. For qualitative variables Chi-squared test were mainly used. And differences were considered statistically significant at $p<0.05$. All the statistical analysis was done using Graph pad prism version 5.02 and Medical Calculator version 11.1.0.0.

RESULTS

Among 25 female patients suffering from acne vulgaris associated with PCOS, eight subjects discontinued the treatment later on so they were not included in the follow-up. Complete clinical information was available in 17 subjects who completed successful treatment with metformin 1-1.5 g/day (and/or minoxidil 2% in case of severe androgenic alopecia) therapy with 3 months of follow-up and did not show any serious adverse effects. Results were analysed for these 17 women. Of these women, 10 were unmarried women and 7 were married women. Subjects were grouped into obese PCOS and Non-obese PCOS as per there Body mass indices (BMI), subjects having BMI >24.99 kg/m^2 where considered as obese PCOS while subjects having BMI <24.99 kg/m^2 where considered as non-obese PCOS.

Physical parameters like weight, body mass indices and waist to hip ratio of obese PCOS subjects and non-obese PCOS subjects were compared with normal subjects as shown in Table 9. Mean age of normal subjects, obese PCOS and non-obese PCOS were 21.36 ± 0.65, 23.8 ± 2.56 and 23.5 ± 1.86 respectively. Significant difference in weight and BMI of obese PCOS subjects were observed as compared to normal subjects while no significant change observed in weight and BMI of non-obese subjects as compared to normal subjects

(Table 1). Waist to hip ratio (W/H) of obese PCOS subjects and non-obese PCOS subjects showed no significant difference as compared to normal subjects (Table 1).

Three months of treatment with metformin produced significant reduction in weight and waist to hip (W/H) ratio of obese PCOS subjects (Table 2). While no significant change was observed in BMI of obese PCOS subjects after treatment. However, treatment with metformin did not produce any statistical significant reduction in weight, waist to hip (W/H) ratio or BMI in non-obese PCOS subjects (Table 3).

An improvement in clinical aspects of hyperandrogenism, acne, hirsutism**,** androgenic alopecia and menstrual regularity was observed after treatment with Metformin 1-1.5 g/day (and/ or minoxidil 2% in case of severe androgenic alopecia) in patients with Acne vulgaris associated with PCOS. All 17 subjects who completed therapy for 3 months, presented themselves at an earlier age with severe symptoms— acne (87.5%), hirsutism (69.2%), Acanthosis nigricans (23.52%), obesity (35.2%), and menstrual irregularity (82.35%). Family history of hyperandrogenism was included in the study revealed that 9 subjects had family history of acne vulgaris, 2 subjects had family history of Androgenic alopecia and irregular menses (Table 4).

After 3 months of treatment with metformin there was a reduction in acne lesions (from 2.37 ± 0.17 to 1.68 ± 0.19) (Table 5 and Fig. 1).

Thirteen subjects were sufferring from hirsutism severe to moderate they where graded as per the Ferriman and Gallwey (1961) showed mean hirsutism score which was 14.3 ± 1.7 was significantly reduced to 12.8 ± 1.8 after the treatment of metformin (Table 5).

Androgenic alopecia in frontal and occipital region was observed in 13 (76.47%) subjects before treatment and after treatment with metformin 1-1.5 g/day and minoxidil 2% androgenic alopecia was observed only in 7 (41.17%) subjects and 6 (35.3%) subjects started showing hair growth (Table 5 and fig. 2).

The menstrual irregularity that was observed in 14 (82.35%) women got reduced to 4 (23.52%) after treatment with metformin for 3 months. 10 (58.82%) women showed regular menstrual cycle (Table 5)**.**

Thyroid stimulatin hormone (TSH), lutinizing hormone (LH) and LH/FSH ratio of obese and non-obese PCOS subjects showed significant difference as compared to normal subjects. Testosterone and DHEAS levels were not significantly different in obese and non-obese PCOS as compared to normal subjects. Insulin and 17-OH Progesterone levels were significantly higher only in obese PCOS patients as compared to normal subjects. Prolactin and Follicle stimulating hormone (FSH) were found significantly higher only in non-obese PCOS patients as compared to normal subjects (Table 6).

After the three months of treatment with metformin no statistically significant changes were seen in TSH, prolactin, DHEAS and 17-OH progesterone in either obese (Table 7) or non-obese PCOS subjects (Table 8). However insulin, testosterone and LH/FSH ratio were significantly decreased in both obese (Table 7) and non-obese PCOS subjects after treatment (Table 8). LH level showed no significant difference in Obese PCOS subjects after treatment, while it was significantly reduced in non-obese PCOS after the treatment. FSH showed no significant difference in Non-Obese PCOS subjects after treatment (Table 8), while it was significantly increased in Obese PCOS after the treatment (Table 7).

Transvaginal colour doppler ultrasonography of 7 married PCOS womens before treatment showed, rise in ovarian resistance index (RI) 0.76 ± 0.02, uterine resistance index (RI) 1.08 ± 0.02 and ovarian volume 13.19 ± 0.53. After the three months treatment of metformin there was significant reduction in ovarian resistance index (RI) 0.39 ± 0.052, uterine resistance index (RI) 0.71 ± 0.014 and ovarian volume 7.04 ± 0.73 (Table 9).

Transabdominal ultrasonography of ovaries in 10 unmarried PCOS subjects revealed mean number of follicles was 9.55 ±0.91 and mean ovarian volume was 14.58 ± 0.66 which were significantly higher as compared to normal subjects. After the three months treatment of metformin there was significant reduction in mean number of follicles and mean ovarian volume (Table 10). Patient with polycystic appearance of ovary showed improvement after treatment of metformin.

Table: 1 Physical parameters of Normal subjects compared to PCOS subjects before treatment

Parameters	Normal (n=11)	Obese PCOS (n=6)	p value[a]	Non-obese PCOS (n=11)	p value[b]
Weight	53.5 ± 0.0195	64.1 ± 1.3	0.0019*	50.54 ± 2.06	0.3029
BMI	20.22 ± 0.72	26.4 ± 1.08	0.0002*	19.16 ± 0.72	0.3144
W/H ratio	0.81 ± 0.0117	0.84 ± 0.018	0.1243	0.81 ± 0.018	0.7829

All the values are expressed in Mean ± SEM.
a = Obese PCOS vs. Normal subjects
b = Non-obese PCOS vs. Normal subjects.
***p<0.05 shows significant difference.**

Table: 2 Physical parameters of subjects before and after treatment of Obese PCOS

Parameters	Obese PCOS (n=6)	Obese PCOS after 3 months (n=6)	p value
Weight	64.1 ± 1.3	54.16 ± 1.8	0.0124*
BMI	26.4 ± 1.08	24.85 ± 2.74	0.4189
W/H ratio	0.84 ± 0.018	0.78 ± 0.11	0.0415*

All the values are expressed in Mean ± SEM.
***p<0.05 shows significant difference**

Table: 3 Physical parameters of subjects before and after treatment of Non-obese PCOS

Parameters	Non-obese PCOS (n=11)	Non-obese PCOS after 3 months (n=11)	p value
Weight	50.54 ± 2.06	49.72 ± 2.15	0.8153
BMI	19.16 ± 0.72	18.85 ± 0.74	0.8065
W/H ratio	0.81 ± 0.018	0.78 ± 0.016	0.3300

All the values are expressed in Mean ± SEM.
***p<0.05 shows significant difference**

Table: 4 Family history of hyperandrogenism

Hyperandrogenism	No. of subjects
Acne Vulgaris (%)	9(52.94)
Androgenic alopecia (%)	2 (11.76)
Irregular menses (%)	2 (11.76)
Total (%)	**12 (70.58)**

Table: 5 Clinical manifestations in subjects before and after the treatment

Parameters	Before treatment (n=17)	After treatment (n=17)	Chi square	P value
Acne Vulgaris Score	2.37 ± 0.17	1.68 ± 0.19	-------	0.0004*
Hirsutism Score	14.3 ± 1.7	12.8 ± 1.8	-------	0.0468*
Androgenic alopecia	13 (76.47%)	7 (41.17%)	1.250	0.2636
Acanthosis Nigricans	3 (17.64%)	3 (17.64%)	0.167	0.6831
Menstrual Irregularity	14 (82.35%)	4 (23.52%)	4.500	0.0339*

***p<0.05 shows significant difference between before treatment vs. after treatment**

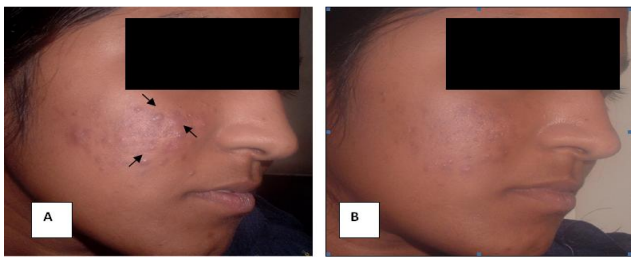

Fig. 1 (A) Papules with active lesions. (B) Reduced disease activity after treatment with metformin.

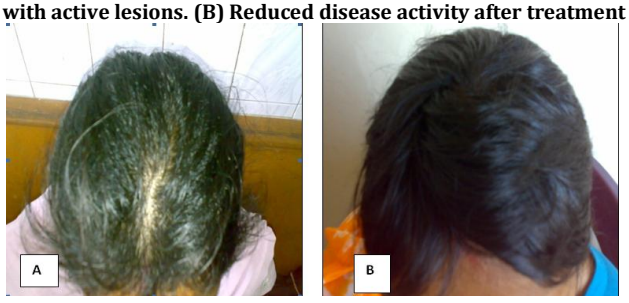

Fig.2 (A) Significant Androgenic alopecia (B) same patient after treatment with minoxidil and metformin shows hair growth

Table: 6 Hormonal profile of the Normal subjects compared to PCOS subjects before treatment

Parameters	Normal (n=11)	Obese PCOS (n=6)	p- value[a]	Non-obese PCOS (n=11)	p- value[b]
TSH (µIU/ml)	1.8082 ± 0.2663	3.9 ± 0.67	0.0138*	5.1 ± 1.19	0.0141*
Inuslin (µU/ml)	18.94 ± 2.734	43.7 ± 5.58	0.009*	25.9 ± 2.4	0.0696
Prolactin (ng/ml)	7.74 ± 1.4	12.48 ± 3.44	0.1508	14.6 ± 2.77	0.0382*
Testosterone (ng/ml)	1.469 ± 0.271	2.06 ± 0.42	0.2304	1.9 ± 0.3	0.2569
DHEAS (µg/dl)	225.5 ± 21.21	210 ± 25.16	0.6587	204.5 ± 19.2	0.4736
17-OH Progesterone(ng/ml)	0.7518 ± 0.1394	1.88 ± 0.24	0.0006*	3.53 ± 1.46	0.0731
LH (mIU/ml)	6.29 ± 0.9	9.38 ± 0.56	0.0307*	11.34 ± 0.74	0.0003*
FSH (mIU/ml)	6.84 ± 0.67	4.85 ± 0.43	0.0582	4.92 ± 0.62	0.0499*
LH/FSH ratio	0.97 ±0.15	2 ± 0.18	0.0001*	2.58 ± 0.26	0.0011*

All the values are expressed in Mean ± SEM.
a = Obese PCOS vs. Normal subjects
b = Non-obese PCOS vs. Normal subjects.
*** p<0.05 shows significant difference.**

Table: 7 Effect on Hormonal profile before and after treatment of Obese PCOS

Parameters	Obese PCOS (N=6)	Obese PCOS after 3 months (N=6)	P value
TSH (µIU/ml)	3.9 ± 0.67	3.58 ± 0.73	0.7131
Inuslin (µU/ml)	43.7 ± 5.58	24.5 ± 2.7	0.0328*
Prolactin (ng/ml)	12.48 ± 3.44	9.23 ± 1.7	0.4158
Testosterone (ng/ml)	2.06 ± 0.42	1.385 ± 0.24	0.0466*
DHEAS (µg/dl)	210 ± 25.16	119.16 ± 19.76	0.4489
17-OH Progesterone (ng/ml)	1.88 ± 0.24	1.71 ± 0.164	0.6238
LH (mIU/ml)	9.38 ± 0.56	9.28 ± 0.56	0.3632
FSH (mIU/ml)	4.85 ± 0.43	7.23 ± 1	0.0474*
LH/FSH ratio	2 ± 0.18	1.35 ± 0.11	0.0371*

All the values are expressed in Mean ± SEM.
*** p<0.05 shows significant difference**

Table: 8 Effect on Hormonal profile before and after treatment of Non-obese PCOS

Parameters	Non-obese PCOS (N=11)	Non-obese PCOS after 3 months (N=11)	P value
TSH (µIU/ml)	5.1 ± 1.19	3.18 ± 0.61	0.1996
Inuslin (µU/ml)	25.9 ± 2.4	17.1 ± 2.75	0.0399*
Prolactin (ng/ml)	14.6 ± 2.77	13.2 ± 1.76	0.3277
Testosterone (ng/ml)	1.9 ± 0.3	1.2 ± 0.1	0.0426*
DHEAS (µg/dl)	204.5 ± 19.2	203.81 ± 25.38	0.5884
17-OH Progesterone (ng/ml)	3.53 ± 1.46	2.31 ± 0.4	0.4571
LH (mIU/ml)	11.34 ± 0.74	8.76 ± 0.85	0.011*
FSH (mIU/ml)	4.92 ± 0.62	5.35 ± 0.7	0.5459
LH/FSH ratio	2.58 ± 0.26	1.71 ±0.155	0.0082*

All the values are expressed in Mean ± SEM.
*** p<0.05 shows significant difference**

Table: 9 Colour doppler ultrasonography changes in married women before and after treatment

Parameters	Before treatment (n=7)	After treatment (n=7)	p value
Ovarian RI (<0.49)	0.76 ± 0.02	0.39 ± 0.052	0.0464*
Uterine RI (<0.79)	1.08 ± 0.02	0.71 ± 0.014	0.0053*
Ovarian Volume (<6.5 cm^3)	13.19 ± 0.53	7.04 ± 0.73	0.0494*

All the values are expressed in Mean ± SEM.
*** p<0.05 shows significant difference**

Table: 10 Ultrasonography changes in Unmarried female before and after treatment

Parameter	Normal (n=11)	Before treatment (n=10)	After treatment (n=10)
Number of follicles (<6)	4.81±0.23	9.55 ±0.91*	7.15±0.62#
Ovarian Volume (<6.5 cm^3)	4.58 ± 0.37	14.58 ± 0.66*	11.4 ± 0.93#

DISCUSSION

Decrease in waist to hip ratio is very important because tissue redistribution may consequently affect insulin sensitivity independently from body weight. Fatty tissue redistribution by metformin alone independent from weight has been demonstrated previously by Nestler at al., (1998). Ovarian dysfunction in obese oligomenorrheic women with PCOS can be improved by weight loss alone. Without metformin weight loss (through calorie restriction and increased exercise) in PCOS patients is exceptionally difficult to achieve and maintain (Crave et al., 1995 and Kiddy et al., 1992) owing to countervailing, weight-preserving, anabolic effects of high insulin (Glueck et al., 1999c), androstenedione, testosterone and DHEAS. These findings are consistent with our study, we also found significant reduction in weight and waist to hip ratio as compared to normal control, and this may be due to insulin sensitizing effect of metformin.

While no significant decrease in BMI as compared to normal control but significant improvement in menstrual cyclicity.

Ovarian dysfunction in obese oligomenorrheic women with PCOS can be improved by weight loss alone that leads to significant metabolic and clinical improvements for PCOS women. (Crave et al., 1995; Kiddy et al., 1992). Women losing over 5% of their body weight had significant regulation of their menses. This finding is consistent with present study.

In the present study we observed all acne patients had had hirsutism (69.2%), Acanthosis nigricans (23.52%), and menstrual irregularity (82.35%). Many authors have reported Acne, hirsutism, seborrhea, and androgenic alopecia are a common manifestation of hyperandrogenism (Rittmaster, 1997; Michelmore et al., 1999; Silfen et al., 2003).

Administration of metformin for 3 months reduces the acne score by 71%. It may be due to a decrease of insulin levels indicating that insulin-sensitizing therapy has effect on acne.

Many authors had examined the treatment of hirsutism with metformin (1·5–1·7 g daily). Three of the studies showed no changes, (Crave et al., 1995; Morin-Papunen et. al., 1998; Moghetti et al., 2000) and five (Ciampelli et al., 1999; Ibanez et. al., 2000; Ibanez et. al., 2001; Kolodziejczyk et al., 2000 and Pasquali et al., 2000) showed significant reductions in the Ferriman-Gallwey score after metformin treatment. Our study support this finding that metformin causes reduction in hirsutism.

In our study, Metformin treatment regularize menstrual cycle which may be due to reduction in ovarian cytochrome P450c-17α enzyme which has been identified in PCOS involved in androgen production in insulin resistant state (Nestler et al., 1998). In adult women with PCOS, Metformin has restored menstrual cyclicity in 44%–96% (Glueck et al., 1999b) and ovulatory cycles in 34% (Nestler et al., 1998) and 79% (Moghetti et al., 2000).

DeVillez et al., (1994) reported that topical minoxidil either arrest hair loss or induce mild to moderate hair regrowth in approximately 60% of women. This data is consistent with our study.

Several studies have investigated the heritability of PCOS and determined that some phenotypes of the disorder appeared in first degree relatives of the probands (Cooper et al., 1968; Recabarren et al., 2008; Givens, 1988; Sam et al., 2005; Leibel et al., 2006). However, the genetic component of this disease remains controversial. Many authors had reported that the female relatives with male pattern alopecia of patients with PCOS had higher androgen levels than female relatives without alopecia (Diamanti-Kandarakis et al., 1998; Gambineri et al., 2004; Franks, 1991). Hu et al., 2010 study suggests that a family history of the symptoms is associated with the clinical symptoms of patients with PCOS, and indicates the involvement of inheritance in the pathogenesis of PCOS. In our study we observed, chances of PCOS and hyperandrogenism is higher in patients with strong family history, this may be due to some genetic factor.

Insulin can increase androgen production in several ways (Gambineri et al., 2002). It interacts with both its own receptor (willis and franks, 1995) and insulin growth factor receptor type I (IGF-I) in the ovary (El-Roeiy et al., 1994), leading to stimulation of the ovarian cytochrome P450c17α (Nestler and Jakubowicz, 1996). In women with PCOS, there is evidence to suggest that peripheral hyperglycemia is due to postreceptor defects specifically leading to reduced tyrosine autophosphorylation and increase in serine phosphorylation of the beta chain of insulin receptor (Dunaif and Xia, 1995). This increase is serine phosphorylation has also been shown to augment both adrenal and ovarian CYP17 activity, with subsequently an increase in androgen production (Zhang et al., 1995). Furthermore, clinical studies have demonstrated a reduction in CYP17 activity in women with PCOS treated with metformin (Nestler and Jakubowicz, 1997; Marca et al., 2000). Insulin also directly decreases serum SHBG concentration, which increases the levels of free testosterone, the active fraction of this androgen (Nestler and Jakubowicz, 1996; Nestler et al., 1991). In our study, acne associated with PCOS patients had insulin resistance and high testosterone level with metformin treatment there was increase in insulin sensitivity and reduction in testosterone level.

Hyperinsulinism and resultant hyperandrogenism in PCOS chronically alter gonadotropin secretion, increasing LH (Moghetti et al., 2000; Dunaif et al., 1996), disrupting the normal pituitary-ovarian axis, and leading to oligoamenorrhea and infertility. Hyperinsulinemia in conjunction with hyperandrogenemia also may lead to morbid obesity, hirsutism, acne, frequent hypertension and hyperlipidemia which together increase risk for myocardial infarction and stroke later in life (Dunaif et al., 1996; Cibula et al., 2000). The paradigm assuming the central role of hyperinsulinemia in PCOS has provided an impetus toward the development of treatments aiming at lowering insulin levels. Regardless of the etiopathogenesis that determine insulin resistance, it is logical to consider a drug that decreases intestinal glucose absorption, suppresses hepatic glucose production, and promotes glucose uptake and utilization by peripheral tissue at the postreceptor level. Metformin has these properties and is well tolerated with minimal side effects. Metformin use in adult women with PCOS has been safe, without reported lactic acidosis (Moghetti et al., 2000). The mode of action of metformin is still incompletely understood; however, it appears that its major effects involve decreasing hepatic glucose output and thus lowering the insulin requirement (Inzucchi et al., 1998; Moghetti et al., 2000). Previous studies using Metformin (1.5 g/day) in adult women with PCOS (Morin-Papunen et al., 1998) have reported significant improvements in insulin sensitivity, reduction of hyperinsulinemia, and, to varying degrees, weight loss, and reductions in hyperandrogenemia.

In Nestler and Jakubowicz, (1997) reduction in the levels of testosterone, androstenedione and an increase in levels of SHBG levels are expected to ameliorate acne/seborrhea observed in women treated with metformin. These effects were independent of changes in body weight was subsequently confirmed. Reduction in LH, FSH and LH/FSH ratio after metformin treatment was previously seen by Aruna et al., 2004. In Onalan et al., 2005 study FSH levels significantly increased in all hyperinsulinemic groups after metformin treatment. This finding is consistent with our study. Increase in FSH may be a result of reduction in serum estradiol levels either by decreased availability of androgens for conversion to estrogens or decreased ovarian aromatase activity. We observed metformin therapy increase FSH significantly in Obese PCOS patients, decrease LH significantly in non-obese PCOS patients and decreased LH/FSH ratio significantly in both obese and non-obese PCOS subjects as compared to normal control.

In the present study, DHEAS and progesterone levels were normal so chances of hyperandrogenism due to non classic form of congenital adrenal hyperplasia is ruled out.

Silfen et al., (2003) observed polycystic appearance of ovary in most of the PCOS subjects, also observed mean ovarian volume in PCOS subjects was higher than the normal control but not statistically significant. Ovarian size has been shown to be largely dependent on LH, insulin has independent effects of LH. Silfen et al., (2003) observed comparable ovarian volumes seen in the non-obese and obese PCOS groups, because the former had higher LH and the later greater fasting and stimulated insulin levels. This data is consistent with our findings, that significant rise in ovarian volume in PCOS patients as compared to normal control this may be due to higher LH level in non-obese PCOS patients and Insulin resistance in obese PCOS patients. The appearance of PCO on ultrasound scanning is common. Only a fraction of those with PCO appearance, however, have the clinical and/or endocrine features of polycystic ovary syndrome (Michelmore et al., 1999; Asuncion et al., 2000). In our study we observed ultrasound PCO as well as clinical and/or endocrine features of polycystic ovary syndrome.

The study by Aruna et al., (2004) showed 96% of patients in had enlarged ovaries, metformin showed significant decrease in stromal thickness, ovarian volume, and number of follicles. This report is consistent with our findings.

Hyperinsulinemic insulin resistance is a cardinal finding in the pathophysiology of PCOS, and hyperinsulinemia plays a key role by increasing circulating ovarian androgen concentrations and impeding ovulation (Dunaif, 1997). Endothelial dysfunction and increased arterial stiffness were reported in insulin resistant states

(Dunaif, 1997; Shinozaki et al., 2003). Thus, metformin reduces circulating ovarian androgen and causes improvement in blood flow of uterine artery and ovarian artery. Experimental and preliminary data have shown that insulin promotes angiogenesis and vasodilatation in ovarian stromal tissue. The mechanism by which insulin exerts this action is not completely understood but may be related to relaxation of vascular smooth muscle, activation of vascular endothelium, or IGF receptor stimulation (Steinberg et al., 1994). High local androgen concentration in the ovaries of women with PCOS may be responsible for greater vascularization of the ovarian stroma (Agrawal et al., 1998). An explanation for the present findings may be the paracrine action of growth factors in the pathophysiology of PCOS (Resende et al., 2001). Several studies have shown a correlation between uterine and ovarian stromal artery blood flow parameters and testosterone (Chekir et al., 2005).

Furthermore, elevated LH level and LH/FSH ratio, important pathophysiological features of PCOS, are associated with hyperplasia of the ovarian thecal and stromal cells, and may be responsible for the increased ovarian stromal vascularization by acting via catecholaminergic stimulation, neoangiogenesis, and leukocyte and cytokine activation (Battaglia et al., 1998; Findlay, 1986). PCOS is likely to be associated with increased uterine arterial blood flow resistance independent of obesity (Chekir et al., 2005). There are some overlapping pathophysiological relationships between reduced insulin sensitivity and obesity. Insulin resistance may be the pathogenetic factor linking increased vascular impedance and obesity (Battaglia et al., 1996). Failure of implantation and spontaneous abortion commonly occur in women with PCOS (Glueck et al., 1999a). The specific pathological mechanisms have not been elucidated. In women with PCOS, the increased impedance of the uterine arteries, by reducing uterine perfusion, may interfere with implantation and increase the risk of spontaneous abortion. Moreover, increased androgen levels in women with PCOS may block the effects of estrogen at the endometrium. Thus, endometrial receptivity and implantation may be affected by the increased androgen levels and impaired uterine perfusion (Chekir et al., 2005). Women with PCOS have a high risk for future cardiovascular disease and pre-eclampsia, in which vascular dysfunction is involved (Kashyap and Claman, 2000). Our finding is consistent with above data, there was increased in LH and LH/FSH ratio responsible for increase in ovarian and uterine artery resistance index and in long term may causes infertility, cardiovascular diseases and pre-eclampsia due to reduction in estrogen level. Metformin regularizes hormonal level and prevent all these long term consequences.

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