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THE ROLE OF METFORMIN IN INDUCTION OF OVULATION IN OBESE INFERTILE PATIENTS WITH POLYCYSTIC OVARY SYNDROME

Fouad Hamad Al-Dahhan[#], Huda Moosa Omran[*]

[#]FRCOG Assistant Professor, [*]MB,ChB, Department of Obstetrics and Gynaecology, College of Medicine, University of Basra, Basra-Iraq

Abstract

This study aimed to find the effective method of induction of ovulation; in obese infertile patients with polycystic ovary syndrome. It is a prospective case-control study done at Infertility clinic of Basra Maternity and Child hospital.

Sixty obese, hirsute infertile patients for more than two years; with ultrasound findings of polycystic ovary syndrome, subjected to the following investigations: Serum LH, FSH, Testosterone, Prolactin &fasting blood sugar. All investigations were carried on early follicular phase of the cycle. Patients were divided randomly into two groups: Group A and group B. All patients received clomiphene citrate 50-150mg for five consecutive days beginning on day 5 of the cycle. The patients with group B also received 500mg of Metformin tablet three times daily for 6 months. Ovulation- which assessed by transvaginal folliculometry, and ovarian artery Doppler velocimetry; triggered with hCG when one or more follicle measuring ≥ 18 mm in diameter, and blood flow indices of the active ovary; showing the dominant follicles were good; on ultrasonic examination. Ovulation response and pregnancy rate were assessed in both groups.

Results obtained from this study pointed out the beneficial effect of Metformin on ovulation induction in obese hirsute women with PCOS.

It is concluded that PCOS remains an enigmatic disease. Once considered relatively benign, PCOS is implicated in medical disorders related to hyperinsulinism and hyperandrogenemia. Restoring fertility and treating abnormal hair growth remain important considerations in the physical and psychological health of reproductive-age women. Metformin has shown great promise in the treatment of insulin-resistant PCOS, but whether it would benefit all women who have PCOS remains unclear. Weight loss is the most important primary recommendation that can be made in the treatment of PCOS.

Introduction

For many decades, the coexistence of hirsutism, amenorrhea, and infertility has been recognized as a common syndrome in women. The first formal description of what is now called polycystic ovary syndrome (PCOS) was made in 1935 by Stein and Leventhal, who noted an association between amenorrhea, hirsutism, and enlarged multicystic ovaries[1]. PCOS is the most common endocrinopathy in women of reproductive age, with 4% to 10% affected worldwide.The main recent advance in the agreed definition of PCOS is the recent definition agreed at the Rotterdam ESHRE/ASRM sponsored PCOS consensus workshop2 that PCOS was a primarily condition of ovarian dysfunction whose cardinal features were hyperandrogenism and polycystic mor-

phology on ultrasound. The workshop agreed that two of the following three criteria(oligomenorrhoea and/or anovulation, clinical and/or biochemical sign of hyperandrogenism, polycystic ovaries morphology on ultrasound) were required in order to diagnose the condition after exclusion of other causes of androgen excess. Ultrasound morphology defined as the presence of 12 or more follicles in each ovary (with one ovary is sufficient for diagnosis) measuring 2-9 mm in diameter, and/or increased ovarian volume (> 10 ml)[2]. However in North America, the definition was based on an NIH consensus definition agreed in 1992 which required that PCOS be diagnosed in women with chronic anovulation and evidence of androgen excess for which there is no other cause[3]. Hirsutism and menstrual irregularities are the most common presenting symptoms and may be found in as many as 90% of women who have PCOS[4]. Infertility is the third most common presenting complaint, with an incidence as high as 75% in some populations with PCOS[5]. Other common findings include acne (2%-25%) and obesity (40%-80%)[6].Acanthosis nigricans suggests hyperinsulinemia, particularly in the obese patient, and is seen in as many as 50% of women who have PCOS[7].Insulin resistance has been detected in more than 50% of women with hyper-androgenic anovulation[8,9]. Treatment focused on weight loss, hair removal, contraceptives, GnRh agonists, anti-androgens, and insulin sensitizers[10]. Metformin is an oral biguanide; anti-hypoglycemic drug used in type two diabetes for lowering blood glucose, mainly by decreasing hepatic glucose production and intestinal absorption of glucose and improve insulin sensi tivity[10].The study was carried on to compare the effectiveness of clomiphene citrate alone with that of a combination of metformin and clomiphene citrate in obese infertile patients with PCOS.

Materials and Methods

A case-control study conducted in the infertility clinic at the department of obstetric and gynecology in cooperation with department of Radiology in Basra Maternity and Child hospital during the period from October 2003 to October 2004.

We enrolled obese hirsute women; who were 18-35 years of age, who attended the clinic because of infertility more than 2 years, they referred by gynecologists with a provisional diagnosis of polycystic ovarian syndrome .The patients were informed clearly of the nature and the purpose of the study, and the potential benefit and risks of clomiphene citrate, metformin. Sixty women who fulfilled the criteria of inclusion were recruited to share in prospective study. The inclusion criteria were, no pelvic factors of infertility(i.e. tubal factor), normal values of semen analyses according to WHO criteria and normal postcoital test. Women are euthyroid having normal serum Prolactin & blood glucose level. The exclusion criteria are diabetic patients & other factors of infertility with anovulation.

Each patient was assessed clinically, biochemically and ultrasonographically aiming for a definitive diagnosis of polycystic ovarian syndrome.

The patients; were randomly divided into two groups: group A and B. All patients had received clomiphene citrate, the initial dose of was 50 mg per day for 5 consecutive days ,starting on the fifth day of spontaneous or induced bleeding, if no response was obtained ,the dose was successively increased in subsequent treatment until 100 or 150 mg per day were given. The smallest effective clomiphene dose was continued for the

next cycles. The patients of group B received also co-treatment 500 mg of metformin three times daily for 6 months. Each woman in group B was asked to initiate metformin therapy once daily for first 2-3 days, then increasing the dose to twice daily for another 2-3 days and to three times daily for the remaining period of the course. This stepwise progression helps patients adjust to drug to minimize the gastrointestinal side-effect often associated with it. Transvaginal ultrasound examination in early follicular phase, the diagnostic criteria of polycystic ovaries defined by Adam's et al[7] .

Ovarian size was measured in three dimensions, ovarian volume was estimated using the formula for a prolate ellipsoid : volume=0.5233D1D2D3, were D1, D2, and D3 are the three maximal longitudinal, anteroposterior and transverse diameters [11] .

The monitoring was started by Transvaginal 3D folliculometry on day 10 of the cycle on an every other day basis until the dominant follicle reached 14mm in diameter .Then after colour equipment was added for monitoring the ovarian blood flow on the dominant side. 10,000IU of hCG was given to the patients intramuscularly, when the blood flow indices of the active ovary; showing the dominant follicle were good i.e. around.± 0.54 RI, ± 0.9 for PI, .± O.16 for Peak systolic velocity. All the patients followed by combined 3D Transvaginal ultrasound and colour Doppler imaging, therefore lay in the range of 14-20mm follicular diameter. Transvaginal ultrasound scan was obtained on the second day after hCG administration to establish whether ovulation had occurred, or not. On the ninth day after hCG administration, blood was obtained for the determination of the progesterone level. Also on the same day, an ultrasound examination; was performed to detect the presence of ovarian hyperstimulation syndrome(OHSS). In this study, ovulation was considered to have occurred if the serum progesterone level was above 5ng/ml on the ninth day after hCG administration or if a pregnancy occurred. Serum pregnancy test; was performed 14 days after ovulation. Clinical assessment of hirsutism scored according to Gallway & Ferriman score[8,12] ;weight, height and body mass index (BMI). Waist to hip ratio(WHR) & the degree of acne was evaluated. All the selected patients were obese with BMI >28kg/sequar meter. Biochemical assessment was made with venous sample drawn between 8-10 am after an over night fasting in early follicular phase of the menstrual cycle in eumenorrhoic or oligomenorrhoic women or at a random time in amenorrhoic.

Serum was obtained by centrifugation for 15 minutes within one hour after venipuncture and stored at -20C until assayed. Glucose estimation was done by the glucose oxidase method using automated analysis.

Serum level of FSH, LH, Prolactin and testosterone were determined by double antibody radio immunoassay (CIS bio-international-France).

The intra-assay and inter-assay coefficient of variation were <10% of all assay performed. Serum progesterone level at day nine of the use of (hCG) was assayed.

The results were compared statistically by using Z test $P<0.05$ is significant. Statistical calculations were performed by using SPSS software.

Results

Sixty patients with a mean age of years (18-35years) were allocated in this therapeutic trial. All were known cases of PCOS, with a mean duration of their chief compliant 5.8 years (range 2-14years),

the onset of the disease was range from 15-29. The infertility was either primary, as in 23 of the patients of both group A and B, or secondary as in 7 of the patients of both groups. All the patients are overweight (BMI>28kg/m2), as shown in table I). There was no statistically difference between the two groups as regard the age, BMI, duration of infertility, and ovarian volume.

Table II shows the clinical features that found in both groups. Both groups had menstrual irregularity; in group A, 15 had secondary amenorrhea(50%), 10 had oligomenorrhoea (33.33%) and 3 had dysfunctional uterine bleeding(10%) ,but some had regular cycle . while in group B 18 had secondary amenorrhea(60%), 8 had oligomenorrhoea(26.66%) and 2 had dysfunctional uterine bleeding(6.67%) ,and some had regular cycle. With Ferriman-Gallwey score of hirsutism>8, in group A, 17 had hirsutism (56.67%) while in group B, 21 had hirsutism (70%). Regarding acne in group A, 20 had acne(66.67%) , ranging from mild acne in 11(36.67%), moderate acne in 7(23.33%), and severe acne in 2(6.67%). While in-group B, 18(60%) had acne, ranging from mild acne in 12 (40%), moderate acne in 4(13.33%), and severe acne in 2(6.67%). There was no statistically difference between the two groups as regard the clinical features.

Table III shows the hormonal features of the two groups .All the patients were euglycemic; serum level of TSH, and Prolactin were within normal range, Progesterone low, and Testosterone was high, there was inverted FSH/LH ratio in both groups. There was no statistically difference between the two groups regarding the hormonal features that found in both groups. Table IV shows the serum fasting blood sugar in group A and group B before and after treatment. In group A there was no significant difference in fasting blood sugar during the course of treatment, while in group B the level of fasting blood sugar was insignificantly decrease after treatment with metformin. Its mean level was (4.63±0.246) and became (4.01±0.23). This means that; metformin insignificantly reduced the level of fasting blood sugar in euglycemic patient after 6 months course.

Table V shows the level of serum progesterone in group A and group B before and after treatment. In group A there was no significant change in the level of serum progesterone as its mean level, was before treatment (0.6±0.49) while after treatment was (0.9±0.31). While in group B the level of serum progesterone was significantly elevated during the treatment course. Its mean level, was (1.6±0.39) before treatment and became (13.9±19.23) after treatment with combination of metformin and clomiphene citrate. There was statistically difference between group A and group B; the level of serum progesterone was significantly higher in group B than in group A; this indicate better ovulation induction in group B.

Table VI shows compares of ovarian response to clomiphene alone (group A) and clomiphene combined with metformin (group A). In group A the number of cycles with premature ovulation was (18) while in group B (4).

The diameter of the mature follicle at the time of ovulation was larger in group B than group A. The ovulation rate in-group A was (62%) while in group B was (78%).

There was statistically difference between group A and group B in the ovarian response and it indicate more ovulation in group B.

In addition, the incidence of OHSS was more in group A, as (6) developed mild OHSS and one developed moderate

OHSS; while in group B only (2) developed mild OHSS.

Table VII shows, that there was no significant difference in the occurrence of pregnancy between group A and B in the first three months of treatment later on the pregnancy occurred more frequent in group B as compare to group A in the next three month. Table VIII shows the pregnancy rats in group A and B it was low in both groups, in group A the pregnancy rate per patient was (10%) and per cycle was (1.73%) while in group B the pregnancy rate per patient was (23%) and per cycle was (4.16%).

Discussion

PCOS is a common and heterogeneous disorder of women of reproductive age, typically seen with symptoms of infertility, menstrual irregularity, and androgen excess. The fundamental pathophysiological defect is unknown, but the key features include insulin resistance, androgen excess and abnormal gonadotrophins dynamics[5] .There is more emerging evidence that insulin resistance has a primary etiological role in PCOS and it is the cause of hyperandrogenism and not consequence increase androgen[12]. Obese women with PCOS have consistently been shown to be insulin resistant to a greater degree than their weight-matched controls. It appears that obesity and PCOS have synergistic effect on the degree and severity of insulin resistant and subsequent hyper-insulineamia[12]. At least five different modalities have been used to lower insulin levels in PCOS one of them recently is metformin more recently used.

Life style modification is very important in the treatment for PCOS, as weight loss and exercise show a striking improvement in ovulatory function and features of hyperandrogenism. Specifically, dietary modification, moderate exercise, cessation of smoking and moderate alcohol and caffeine intake are lifestyle modifications recommended in women with PCOS[10].

The effect of increased physical activity alone (no change in dietary intake) in obese women with PCOS was investigated by Randeva et al who reported a significant reduction in WHR, and plasma homocysteine concentrations (a cardiovascular risk marker) but no significant change in BMI amongst 12 obese women with PCOS who adhered to a 6 month exercise program[13].

Hyperinsulinemia is a key component in the pathogenesis of PCOS. This realization has provided the basis for advances in treatment strategies for women with the disorder. Weight reduction, when it can be achieved, is still an important component in the treatment of PCOS. However, not all women with PCOS are obese, and because the etiology of obesity in PCOS in not known, there is currently no effective manner to target this problem in PCOS. Pharmacologic reduction in insulin levels appears to offer another therapeutic modality for PCOS and is one that may ameliorate the sequel of both hyperinsulinemia and hyper-androgenemia[14].

These finding give a theoretical basis for the administration of insulin sensitizing agents such as metformin, trigolitazone, diazoxide and somatostatin to improve the endocrinal abnormalities associated with PCOS and thus facilitate the ovulation[15]. Metformin is a biguanide antihyperglycemic drug used to treat non-insulin dependent diabetes mellitus, it lowers blood glucose production, it also enhance s insulin mediated glucose disposal but does not stimulate insulin secretion. Although there is some modest improvement in glucose disposal rate with Metformin, the primary mechanism of action appears to be its effect on

reducing hepatic glucose output. It may cause minor gastrointestinal adverse effects, abdominal discomfort and very rare lactic acidosis[15].

The first study in which metformin was administered to test the hypothesis that androgen reduction follows from insulin reduction was that of Velazquez et al[16]. In that study, metformin was administered to 26 women with PCOS (500 mg three times daily for 8 weeks) and resulted in a significant reduction in total testosterone, free testosterone, and free androgen index as well as a significant rise in SHBG in comparison with pretreatment levels. These changes were associated with reductions in insulin responses to oral glucose administration and in the "insulogenic index" (defined as the ratio of insulin to glucose response after oral glucose administration). It is important to note that the subjects in this study lost weight, which was a likely contributor to the reduction in insulin secretion on repeated oral glucose tolerance test (OGTT). As a result, the effect of metformin upon insulin secretion could not be clearly separated from that of weight loss.

In this study of women who were obese hirsute, with definitive diagnosis of PCOS we chose to use metformin because several previous studies had documented its ability to decrease serum insulin in women with PCOS.

This is similar to our results; after 4-6 months of treatment there was a significant induction of ovulation in patients of group B (those who received metformin plus Clomiphene citrate).

We performed this study to determine whether decreasing insulin resistance with metformin in obese women with PCOS will facilitate the ovulation induction by clomiphene citrate. We had used transvaginal sonography and colour Doppler imaging in the assessment of follicular development and ovarian blood flow (and hence follicular vascularity) because it is direct, quick and non – invasive method of monitoring ovulation[17].

In this study the patients of group B who received combined therapy had ovulatory rate of (78%) as compared with group A had ovulatory rate (62%). the size of follicle at time of hCG administration in group B is larger than A .These finding support the idea that hyperinsulineamia impedes ovulation in obese, hirsute women with PCOS and decreases in the insulin resistance facilitates and regulates the induction of ovulation[15].The study showed that the best ovulation rate can achieved with administration of hCG to the patient with 18 mm dominant follicle showing good Doppler indices. In this study the pregnancies occurred in group B were 7/30, in contrast in group A were 3/30($P<0.01$).

In the first few cycles there was no significant difference in pregnancy frequency later on there was a significant difference in pregnancy frequency between the two groups ($P<0.002$) which could attribute to decrease of insulin resistant in group B.

Insulin resistance has been found to increase the likelihood of OHSS[18]; this is similar to our results since in group A, 6 cases showed evidence of mild OHSS and 1 showed evidence of moderate OHSS, while 2 cases of group B showed evidence of mild OHSS. These results are consistent with the hypothesis that hyperinsulinemia and insulin resistance are important in the pathogenesis of PCOS[19].

Table I: The characteristics of Patients

	Group A	Group B	P value
Age(year)			
At admission to study:			
Range	18-35	18-35	
Mean ±SE	25±1.22	24.7±1.21	
At onset of disease:			**NS**
Range	15-29	15-29	
Mean ±SE	20.562±1.68	20.541±1.54	
Duration of infertility			**NS**
Range	2-14	3-14	
Mean ±SE	5.8±1.45	6.1±1.55	
Primary no.	23	23	
Secondary no.	7	7	
BMI(kg/m^2) **Mean ±SE**	29.6±0.8	29.57±0.8	**NS**
Ovarian volume(cm3)	11.986±1.8	12.45±1.36	**NS**

Values are given as Mean ±SD. NS =No significant

Table II: The clinical features that found in the patients of group A and B.

	Group A	Group B
Menstrual cycle		
Regular	**n=2(**6.67%).	**n=2**(6.67%).
Oligomenorrhea	**n=10(**33.33%)	**n=8**(26.66%)
Secondary amenorrhea	**n=15**(50%)	**n=18**(60%),
DUB	**n=3**(10%)	**n=2**(6.67%).
Hirsutism Ferriman-Gallwey score >8	**n=17**(56.67%)	**n=21**(70%).
Acne total	**n=20**(66.67%)	**n=18**(60%)
Mild	**n=11**(36.67%)	**n=12**(40%)
Moderate	**n=7**(23.33%)	**n=4**(13.33%)
Severe	**n=2**(6.67%)	**n=2(**6.67%)

Table III: The hormonal features of the patients of group A and B.

	Group A	P-value	Group B
FBS mmol/L	**4.55±3.84**	**NS**	**4.63±0.246**
LH MIU/ml	**15.2±0.89**	**NS**	**15.3±0.79**
FSH MIU/ml	**7.84±3.5**	**NS**	**8.65±3.4**
TSH µIU/ml	**1.01±0.068**	**NS**	**1.01±0.81**
Testosterone ng/ml	**2.18±0.7**	**NS**	**3.21±0.36**
Prolactin ng/ml	**9.4±4.5**	**NS**	**9.7±3.9**
Progesterone ng/ml	**0.6±0.49**	**NS**	**1.6±0.39**

Values are given as Mean ±SD. NS =No significant.

Table IV: Serum fasting blood sugar mmol/L, of group A&B before, and after Metformin.

	Group A	P-value	Group B
Before treatment Mean ±SD	4.55±3.84 SE(0.702)		4.63±0.246 SE(0.045)
After3months of Treatment Mean ±SD	4.2±4.93 SE(0.901)	NS	4.21±5.2 SE(0.95)
After6months of treatment Mean ±SD	4.4±0.26 SE(0.047)	NS	4.01±0.23 SE(0.042)

Values are given as Mean ±SD. NS =No significant

Table V: Serum progesterone level(ng/ml) of group A and B; before and after treatment.

	Group A	P-value	Group B
Before treatment Mean ±SD	**0.6±0.49 SE(0.089)**		**1.6±0.39 SE(0.071)**
After3 months of Treatment Mean ±SD	**0.7±0.27 SE(0.049)**	**<0.0005***	**2.08±0.44 SE(0.081)**
After6months of treatment Mean ±SD	**0.98±0.31 SE(0.057)**	**<0.0001***	**13.9±19.23 SE(3.51)**

Values are given as Mean ± SD. *Statistically significant difference compared with the base line.

Table VI: Comparison of the ovarian response to Clomiphene alone (Group A) and Clomiphene combined with Metformin (Group B).

	No. of mature follicles Preovulatory*	Diameter of largest follicle at the time of hCG administration*	Ovulation rate	No. of cycles with premature ovulation	Ovarian hyper stimulation syndrome			
					Mild	Moderate	Severe	Total
Group A	**1.92±0.89**	**20.63±1.02**	**62%**	**18**	**6**	**1**	**0**	**7**
Group B	**3.12±0.70**	**21.98±2.04**	**78%**	**4**	**2**	**0**	**0**	**2**
P value	**<0.0001**	**<0.001**	**<0.05**	**<0.001**	**<0.001**			

P-Value was determined by the Z-test. *Values are given as Mean ±SD.

Table VII: Pregnancies that occurred in each cycle of the two groups.

Cycle	No. of pregnancies/No. of cycles for each group		
	Group A	Group B	P-Value
1	0/30	1/30	NS
2	1/30	0/29	
3	1/29	1/29	
4	0/28	2/28	0.002*
5	0/28	1/26	
6	1/28	2/25	
total	3/173	7/167	0.01§

P-Value was determined by Z-test for two proportions. NS=no significant.

*P-Value for the last 3 cycles. SP-Value for all cycles.

Table VIII: Results of ovulation induction in the two groups.

	Group A	Group B
No. of patients	30	30
No. of cycles	173	167
No. of pregnancies	3	7
Pregnancy rate per patient	10%	23%
Pregnancy rate per cycle	1.73%	4.16%

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