

The use of oral thiazolidinediones in correction of hormonal abnormalities among unmarried women with resistant PCO .

Wisam Akram F.I.C.O.G.

Inas Taha F.I.C.O.G.

Waffaa N Saied MSc Pharmacology,

Summary:

Background; Polycystic disease of the ovary is complex of symptoms with various clinical, hormonal and biochemical abnormalities.

Setting; AL-Elwya Maternity Teaching Hospital

Aim; to test the ability of pioglitazone in correcting biochemical and hormonal changes among unmarried women with PCO.

Design; Prospective

Methods: 23 women with PCO in whom previous treatment with metformin has failed were recruited to participate in the study. All the women were single with chronic anovulation and menstrual abnormalities and hirsutism. They were put on pioglitazone 30 mg daily for 6 months. FSH, LH, LH/ FSH, fasting insulin level, free testosterone, estradiol, and serum sex binding globulin as well as mid luteal progesterone were assessed prior to treatment and six months later. **Results;** there was significant reduction in the mean serum fasting insulin level [53.08+12.75 vs. 22.43+4.29: $P < 0.001$]. In addition there was significant reduction in the mean serum free testosterone, LH and LH/ FSH ratio [3.21+0.36 vs. 1.68+0.43: $P < 0.001$], [15.19+4.43 vs. 10.72+3.08: $P < 0.001$], [2.41+0.23 vs. 1.71+0.12: $P < 0.001$] respectively. In addition mean serum progesterone at mid luteal phase increased significantly [2.44+1.11 vs. 18.61+2.28: $P < 0.001$]. No woman during the treatment course has shown any sign of liver impairment or toxicity.

Conclusion; Pioglitazone is an insulin sensitizing drug which may be useful among women with PCO in whom previous treatment with metformin has failed. Yet, caution should be practiced in prescribing the drug until further studies confirm its safety and efficacy.

Key words; polycystic ovary, pioglitazone, insulin resistance.

Introduction:

In the 1930s, Stein and Leventhal first described a complex of symptoms associated with ovulation dysfunction and hyperandrogenism (clinical hirsutism or elevated serum testosterone and androstenedione), as polycystic ovarian syndrome (PCOS). PCOS affects approximately 5 to 7% of reproductive age women, (1). These clinical manifestations are due to persistent ovulation dysfunction resulting from numerous causes including ; insulin resistance, hyperinsulinemia, and hyperandrogenism (2). The familial patterns of PCOS suggests a genetic component with a possible X-linked dominant transmission and autosomal dominant mode of inheritance (3). Insulin resistance

Over the last several years, the association between insulin resistance and PCOS has become more evident and is one of the most important relationship , affecting both obese and thin women (4).

Insulin resistance (IR) is defined as: reduced glucose response to a given amount of insulin. There are several clinical and laboratory criteria to define insulin resistance, but none is accepted by all. These include; $MRI > 27 \text{ kg/M}^2$, waist/hip ratio > 0.85 , presence of acanthosis nigricans, an elevated fasting insulin concentration, and decreased glucose/insulin ratio(5). Resistance to insulin-stimulated glucose uptake is relatively common, often referred to as Syndrome X.. Several mechanisms have explained insulin resistance: peripheral target tissue resistance, decreased hepatic clearance, or increased pancreatic sensitivity, (6,7). Overweight is defined as; having a BMI 25-30 kg/M^2 ; obesity is defined as having a BMI $> 30 \text{ kg/M}^2$. Ovulation dysfunction occurs with a BMI $> 25 \text{ kg/M}^2$. Generally speaking, all obese women and most overweight women are insulin resistant. Obese, anovulatory women with hyperandrogenism have a characteristic distribution of body fat known as android (central body) obesity, as in older men. This fat distribution is associated with hyperinsulinemia, impaired glucose tolerance, diabetes mellitus, and an increase in androgen production resulting in decreased levels of sex hormone-binding globulin (SHBG) and increased levels of free testosterone and Estradiol (8).

*AL- Kindy Medical College, Baghdad's University, Senior In Gynecology And Obstetrics, AL- Elwya Maternity Teaching Hospital

**AL- Elwya Maternity Teaching Hospital

***AL- Mustansyria Medical College, AL-Mustansyria University

Hyperinsulinemia:

Hyperinsulinemia produces hyperandrogenism in PCOS patients (9), by reducing the number of functional insulin receptors or by blocking them (10). Circulating insulin then binds to IGF receptors, which are structurally similar to the insulin receptors and results in androgen production by theca cells. In addition, Hyperinsulinemia inhibits hepatic synthesis of SHBG and hepatic production of IGFBP-1. Lower levels of SHBG allows for increased circulating levels of androgens and estrogens. Lower levels of circulating IGFBP-1 increases IGF activity resulting in increased theca cell androgen production. Finally, insulin may directly increase the LH secretion in obese, anovulatory women. By the age of 40, up to 40 % of PCOS patients develop impaired glucose tolerance or clinical diabetes, while during reproductive years, these women are more likely to experience spontaneous abortions or gestational diabetes and they are at increased risk of developing hyperandrogenism and hyperinsulinemia later in life (11).

As there is a strong evidence that excess insulin plays a role in the development of PCOS, it is reasonable to assume that reducing circulating levels of insulin may help restore normal reproductive function. This may be accomplished by weight loss, improved nutrition, and exercise, which should be the first line of therapy for an overweight woman with PCOS (12).

Insulin sensitizing agents and PCO

These new drugs, approved by the FDA for the treatment of Type 2 diabetes and known as insulin sensitizing agents, have shown to improve body's response to insulin, thereby reducing the need for excess insulin and restoring their levels to normal. The best studied insulin sensitizing agent available for women with PCOS is metformin (Glucophage®), a biguanide. Metformin reduces circulating insulin and androgen levels and restores normal ovulation in women with PCOS. Even if metformin alone does not restore ovulation, it may improve a woman's response to fertility drugs. Gastrointestinal irritation, especially diarrhea, is a common side effect. These symptoms usually improve after few weeks. Lactic acidosis is a rare but serious adverse effect of metformin. Metformin is not recommended for patients with kidney, lung, liver, or heart disease (13).

Rosiglitazone (Avandia™) and pioglitazone (Actos(V)), which belong to the thiazolidinedione group of antidiabetic agents, reduce hyperandrogenism and restore ovulation in PCOS patients. Liver toxicity is the main concern with these agents. Liver tests should be performed every two months for the first year and

periodically thereafter. The new insulin sensitizing agents have not been linked to birth defects in animals or humans, but are not recommended for use during pregnancy.

Unlike ovulation induction drugs, insulin sensitizing agents have little or no risk of multiple pregnancies. Metformin is used as the first insulin sensitizing agent; thiazolidinediones may be considered if metformin is ineffective or not tolerated by the patient.

Because these medicines correct the underlying metabolic abnormalities associated with PCOS, their long-term use may delay the likelihood of developing Type 2 diabetes and cardiovascular disease. Since data are lacking however, long-term use of insulin sensitizing agents for this purpose cannot be recommended at present (14).

Aim of the study:

The aim is to test the ability of the new insulin sensitizing drug "pioglitazone" in correcting biochemical and hormonal changes among unmarried women with PCO whose previous treatment with metformin has failed to correct their menstrual abnormalities and hirsutism.

Patients and methods:

This was a prospective clinical trial, conducted over two-years in AL-Elwyia Maternity Teaching Hospital, from January 2003 till June 2005. Twenty three unmarried women were recruited into the study. Inclusion criteria were ;unmarried women with hirsutism and hypooligomenorrhea due to chronic unovulation. , though metformin , in a dose of 2 g/day for at least one course of six continuous months , have already been used but failed to induce their ovulation or correct their hypooligomenorrhoea or amenorrhoea . Polycystic ovary syndrome was essentially diagnosed by detection of an early follicular phase LH/FSH ratio>2, with characteristic ultrasound findings of unilateral or bilateral ovarian polycystic pattern. Each patient was subjected to complete history and thorough physical examination.

On examination; height in meters and weight in kilograms were recorded. Thereafter; body mass index was calculated as weight (kg)/height (m) ². Patients were subgrouped into four groups as regard to BMI: (normal group) with BMI >19 <25Kg/m², (overweight group) with BMI 25-<30 Kg/m², (obese group) with BMI 30- <35K g/m² while(severely obese group) has BMI 35 Kg/m² or more. The clinical evidence of androgen excess (acne or hirsutism) was looked for in areas of hair distribution. Hirsutism was graded into; none, mild, moderate or severe according to Raj et al. Mild degree is scored when dark hair is detected on the upper lip or around the areola. Moderate

hirsutism is assigned when dark hair is also present on the chest as hair removal is needed at frequent intervals (<3 months). When hirsutism affects abdomen, back or limbs; the severe form is recognized. Breasts were examined and excluded for clinical evidence of galactorrhoea.

In the month prior to pioglitazone use, two blood samples were obtained, one in the early follicular phase (days 3-5) where hormones been assessed by R(A procedure (those include; LH, FSH, Free Estradiol, testosterone; sex hormone binding globulin, fasting serum insulin fasting serum sugar and HDL-Cholesterol levels.) and the second one was collected at mid-luteal phase, for progesterone level estimation. All hormonal assays were conducted in AL-Kindy Center for Diabetes and Endocrinology. Then; patients were put on pioglitazone tablets (Actos) in a dose of 30 mg daily for 6 months course. All women were strictly instructed to come back to the hospital for at least two visits throughout the treatment period, to be reviewed by a colleague physician for liver toxicity. Fortunately, no woman throughout the treatment course showed any sign or symptom of liver impairment, as reported by the colleague physician in charge upon examination and by necessary investigations for liver enzymes. At the end of the course, another two samples were collected as the previous two, for assessment of the same hormones and biochemical analysis. Data were analyzed using Microsoft Excel 2003 software after their collection. Continuous data were expressed as mean and standard deviation while numerical data were expressed as number and percent. Continuous data were compared with t student test. P values less than 0.05 were considered as significant.

Results:

Table (1): The physical characteristics of the 23 women who have participated in the study

The physical Characteristic	Mean ±SD	Range
Age (years)	27.78+ 4.47	20-36
Weight (Kg)	85.13+11.92	55-102
Height (m)	1.71+0.094	1.5-1.9
BMI (Kg/m ²)	28.98+3.51	22.5-38.7
Duration of symptoms (years)	6.52+2.17	3-11

After collection of data, results were processed in representative tables. In table number 1 the over all epidemiological characteristics of women who have participated in the study were presented as mean of age, weight, height, body mass index and the period of overall symptoms. Despite all the 23 women were unmarried, yet; the mean symptom duration was 6.52 years which indicate a long

period of previous non fruitful treatment for their menstrual disturbances. In table number 2, the distribution of menstrual disturbances and the U/S appearance are shown. In table number 3, the distribution of women in the whole study group is shown according to their body mass index. The maximum number lies in the over- weight group (65.21 %), while only one woman fell within normal group. In addition, 2 women only were severely obese, one with 101 kg while the other was 102 kg.

Table (2): The presenting symptoms and ultrasonographic picture of the 23 women who have participated in the study

The symptom and Ovarian U/S appearance	Number affected	Percentage
The cycle pattern		
Normal cycle pattern	3	13.04 %
Oligomenorrhoea	20	86.95 %
Amenorrhoea	0	0%
Hirsutism		
None	1	3.34 %
Mild	5	21.73%
Moderate	15	65.21 %
Severe	2	8.69 %
Ultrasonography finding		
Typical	21	91.30%
Atypical	2	8.69%

Table (3): The distribution of women in the whole study group according to their Body Mass Index

BMI (Kg/m ²)	Number of women
<25	1(4.34%)
25 - <30	15(65.21%)
30 - <35	5(21.73%)
35 and more	2(8.69%)

In table number 4 hormonal, biochemical and menstrual profiles before and 6 months after treatment course with pioglitazone are shown. It shows progressive and significant reduction in mean serum LH, LH/ FSH ratio [15.19+4.43 vs. 10.72+3.08: P < 0.001] and [2.41+0.23 vs. 1.71+0.12: P<0.001] respectively. It also shows a progressive reduction of free testosterone 6 months after starting pioglitazone course which was statistically significant [3.21+0.36 vs. 1.68+0.43: P<0.001]. In addition there is a significant rise in the mean serum progesterone at the mid luteal phase [2.44+1.11 vs. 18.61+2.28: P<0.001]. As far as fasting serum insulin level is concerned, reduction in the mean value before and after treatment course which has reached statistical significance [53.08+12.75 vs. 22.43+4.29: P< 0.001]. On the other hand, there was significant increase in the serum sex hormone binding globulin [7.9 ±2.5 vs. 31.4±13.1: P<0.001]. Despite statistically significant reduction in the mean serum fasting glucose level, yet no reading

was reported to be in the 1 ypglycemic range [4.61+0.59 vs. 4.43+0.50: P=0.00134]. However the mean serum high density lipoprotein was significantly increased after six months of pioglitazone treatment 0.75+0.40 vs. 2.077+0.34: P<0.001]. The mean menstrual cycle in days was normalized among all women in the study group [44.47+9.20 vs. 25.95+3.67: P<0.001]. Unfortunately we didn't measure the differences in regard to the improvement of hirsutism from clinical point of view, as it was not planned initially in the study protocol, though all women reported a remarkable improvement of hirsutism.

Table (4): The pre and post course treatment of the various hormonal, clinical and biochemical profiles in the study group as expressed as mean and SD

Hormone	Pre-course Mean + SD	Post-course Mean + SD	P
FSH (IU/L)	6.30+1.76	6.26+1.71	NS; P=0.29
LH (IU/L)	15.19+4.43	10.72+3.08	P<0.001
LH/ FSH Ratio	2.41+0.23	1.71+0.12	P<0.001
Estradiol(pmol/l)	173.91+10.59	120.17+9.02	P<0.001
Progesterone(nmol/l)	2.44+1.11	18.61+2.28	P<0.001
Testosterone(nmole/l)	3.21+0.36	1.68+0.43	P<0.001
Insulin(IU/ l)	53.08+12.75	22.43+4.29	P<0.001
SHBG(nmol/l)	7.9 ±2.5	31.4±13.1	P<0.001
Fasting Glucose (mmol/ l)	4.61+0.59	4.43+0.50	P<0.05 P=0.00134
HDL-Cholesterol (mmol/l)	0.75+0.40	2.077+0.34	P<0.001
Mean menstrual cycle (days)	44.47+9.20	25.95+3.67	P<0.001

Discussion:

Treatment with insulin-sensitizing agents is a relatively recent therapeutic strategy in women with polycystic ovary syndrome (PCOS) Checa et al (15). In our study a total of 23 women with previously failed treatment for PCO by metformin were shown to have improvement in lowering their high levels of LH and testosterone in addition to SHBG. The results were consistent with those obtained by Nora Brettenthaler et al (16), who investigated forty premenopausal women with PCOS, allocated to treatment with either pioglitazone (30 mg/d) or placebo, for 3 months. Pioglitazone showed significant improvement in insulin sensitivity, hyperandrogenism, and ovulation rates in those women, thereby providing both metabolic and reproductive benefits.

The reduction of mean serum insulin shown in

our study led to enhanced response of granulosa cells in the ovary to the circulating FSH in the form of Estradiol production, ultimately correcting hypooligomenorrhea which was also shown in the study. The above mentioned results are consistent with results obtained by Micky et al (17). He admitted PCOS women to the General Clinical Research Center (GCRC) at the University of California, San Diego (UCSD), on two separate occasions during midfollicular phase, before and during pioglitazone treatment and FSH infusion. The response of follicular cells to FSH infusion was significantly higher 6 months after pioglitazone treatment in the form of higher Estradiol production. The response of women with PCO to pioglitazone who have failed to previous metformin treatment shown in our study was consistent with results obtained by Charles et al (18). He studied a total of 13 women with polycystic ovary syndrome (PCOS) not optimally responsive to metformin, assessed for the efficacy and safety to the added pioglitazone. He concluded that those women, when pioglitazone was added; insulin, glucose, insulin resistance, insulin secretion, and DHEAS fell, while HDL-cholesterol and sex hormone-binding globulin rose. The occurrence of expected menses was two folds higher than on metformin alone, and menstrual pattern improved without any adverse side-effect.

D. Romualdi et al (19) did a study on women with PCO whether they were hyperinsulinemic or normo-insulinemic. An improvement was observed in lipid assessment of both groups. Therapy was well-tolerated, and he suggested that there was a selective effect, partially independent of insulin secretion, of pioglitazone on the clinical and hormonal disturbances of PCOS. Our study have shown that mean serum HDL-cholesterol, which is the most sensitive marker for free testosterone among lipid profile's blood component, was significantly increased 6 months after pioglitazone treatment. The increase of total HDL- Cholesterol can be linked to the significant reduction in the mean serum free testosterone 6 months after starting the pioglitazone course. This result is consistent with those obtained by the study of Wiebke et al (20) which has shown that troglitazone inhibits two key enzymes reggired for the production of testosterone, increasing free cholesterol and LDL and reducing HDL-Cholesterol. Androgen biosynthesis requires 3 β -hydroxysteroid dehydrogenase type II (3 β HSDII), 17 α -hydroxylase and 17,20-lyase activities of cytochrome P450c17. Thiazolidinedione and biguanide drugs, which are used to increase insulin sensitivity in type 2 diabetes, lower serum androgen concentrations in women with polycystic ovary syndrome. However, it is unclear whether this is secondary to increased insulin sensitivity or

to direct effects on steroidogenesis. To investigate potential actions of these drugs on P450c17 and 3 β HSDII, he used "humanized yeast" to express these steroidogenic enzymes in microsomal environments. The biguanide metformin had no effect on either enzyme, whereas the thiazolidinedione troglitazone inhibited 3 β HSDII (K₁ = 25.4 ± 5.1 μM) and both activities of P450c17 (K₁ for 17 α -hydroxylase, 8.4 + 0.6 μM; K₁ for 17, 20-lyase, 5.3 ± 0.7 μM). The action of troglitazone on P450c17 was competitive, but it was mainly a noncompetitive inhibitor on 3 β HSDII. The thiazolidinediones: rosiglitazone and pioglitazone exerted direct but weaker inhibitory effects on both P450c17 and 3 β HSDII. These differential effects of the thiazolidinediones do not correlate with their effects on insulin sensitivity, suggesting that distinct regions of the thiazolidinedione molecule mediate these two actions. Thus thiazolidinediones inhibit two key enzymes in human androgen synthesis contributing to their androgen-lowering effects, whereas metformin affects androgen synthesis indirectly, probably by lowering circulating insulin concentrations.

Conclusion:

Pioglitazone is a new insulin sensitizing agent, useful among women with resistant PCO who failed to respond to previous metformin course. We didn't record any complication in our series, though we still advice our colleagues to be cautious in putting their patients with PCO on pioglitazone, unless failure to initial courses with metformin is well documented. Larger multi-center trials are needed to confirm further; safety and efficacy of this new drug in treating women with PCO.

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