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Original Studies

Effects of Pioglitazone on Menstrual Frequency, Hyperandrogenism and Insulin Resistance in Adoloscents and Young Adults with Polycystic Ovary Syndrome

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Abstract. *Study Objective:* To study the clinical, metabolic and adverse effects of pioglitazone over a period of 6 months in obese adolescent and young adults with polycystic ovary syndrome.

Design: This was an open labeled study. Each patient served as her own control.

Setting: Outpatient department of a university affiliated teaching hospital.

Participants: Unmarried women (age 15−25 yrs) with chronic anovulatory cycles and obesity, and with clinical evidence of hyperandrogenism.

Interventions: Pioglitazone at a dose of 30 mg once daily for a period of 6 months along with dietary advice and exercise.

Main Outcome Measures: Resumption of normal menstrual cycles, clinical improvement in hyperandrogenism and changes in insulin resistance measured by fasting glucose insulin ratios.

Results: Twenty-two women were enrolled. At the end of the study period 91% of the subjects had regularization of menstrual cycles. There was no change in the modified Ferriman-Gallwey hirsutism scores. Decline in fasting insulin levels at the end of the study was 45.6% from baseline along with significant increase in the fasting glucose/insulin ratio from baseline.

Conclusion: Administration of pioglitazone for 6 months along with advice about diet and physical activity in obese adolescents and young adult women with polycystic ovary syndrome results in significant improvements in menstrual frequency. There is a significant improvement in insulin resistance using the G/I ratio (<7.5 mg/10^{-4} U) as the biochemical marker.

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Introduction

Polycystic ovary syndrome (PCOS) is one of the most common endocrine problem affecting adolescent girls. Adolescents present mainly symptoms related to chronic anovulation (amenorrhea and oligomenorrhea) and hyperandrogenism. In addition majority of the girls are obese, insulin resistant, and have hyperinsulinism. This combination predisposes these adolescents to an increased risk of diabetes mellitus, cardiovascular disease, and infertility later in life.[1]

The pathogenesis of this syndrome is still unclear. Over 60% of patients with PCOS are insulin resistant and obese. Furthermore 20−40% of non obese PCOS patients have evidence of insulin resistance.[2] Chronic exposures to high insulin levels leads to development of acanthosis, increased body fat and finally glucose intolerance. Current data suggest that lowering insulin resistance with the use of sensitizers can ameliorate menstrual abnormalities, improve ovulation rates, lower circulating androgen levels, and improve metabolic parameters.

Metformin was first used in 1994 in PCOS.[3] Significant improvements in free testosterone levels, spontaneous ovulation rates, and improvement in metabolic parameters were noted in various studies.[3−6] Thiazolidinediones as a new class of insulin sensitizers were introduced in 1998 and initial studies with Troglitazone, one of the thiazolidinedione class, suggested responses similar to the use of metformin.[7−9]

Pioglitazone is a newer thiazolidinedione developed for the treatment of type 2 diabetes. The drug

is well tolerated in patients with diabetes in clinical studies. Common adverse events include edema, weight gain, and headache.[10] In 2003 Romualdi et al used pioglitazone for the first time in adult women with PCOS. The 6-month study showed improvements in menstrual frequency, hirsutism, and insulin sensitivity.[11]

This present study was designed to study the clinical (menstrual frequency, weight/body mass index, and hyperandrogenism), metabolic (fasting glucose and insulin resistance) and adverse effects of pioglitazone over a period of 6 months in obese adolescent and young adult patients with PCOS. To the best of our knowledge, no previous studies have looked at the use of pioglitazone in this important subgroup of patients with PCOS.

Materials and Methods

Type of Study

This was an open labeled study. Each patient served as her own control and baseline characteristics were compared to those observed at the end of study.

The Institutional Ethics committee cleared the study protocol and an informed consent was obtained from each subject before entry into the study.

Subjects

Unmarried young women in the age group of 15−25 years with clinical features suggestive of polycystic ovary syndrome were screened on presentation at the Endocrinology Out patient department of a university affiliated teaching hospital.

Patients meeting the following inclusion criteria were entered into the study.

1. Unmarried women (age 15−25), who were not planning to get married in the following 12 months of the study period.
2. Chronically anovulating defined as less than 6 menstrual cycles in the past 12 months.
3. Obese (defined as having a Body Mass Index (BMI) > 25 kg/m^2).
4. Clinical evidence of hyperandrogenism (defined as a modified Ferriman-Gallwey[12] hirsutism score ≥ 7).

Study Protocol

On entry into the study, clinical details including detailed menstrual history including age of onset of menarche and menstrual pattern over the past year were recorded. Fasting Insulin levels, fasting plasma glucose, and total serum testosterone values were measured. Hyperandrogenism was assessed by the modified Ferriman-Gallwey (mFG)[12] and acanthosis nigricans was graded according to Burke et al.[13]

All patients were given dietary advice, encouraged to increase physical activity and asked to maintain a menstrual calendar. All patients were started on pioglitazone at a dose of 30 mg once daily per oral after breakfast. The medication was continued for a period of 6 months. Patients were reviewed after 3 and 6 months. At each visit compliance of treatment was checked with a pill count and subjective evaluation of drug tolerability was done.

At 6-month repeat visit, laboratory assessment of fasting insulin, glucose and total testosterone was done. Menstrual frequency over a 6-month period was noted from the menstrual calendar. Modified Ferriman-Gallwey hirsutism score, grade of acanthosis nigricans, and anthropometry was rechecked at the end of the study.

Hormonal Assays

Fasting serum insulin was measured by radioimmunoassay using the Coat-A-Count kits (DPC, Los Angeles, CA). The intraassay coefficient of variation (CV) in our lab for serum insulin estimation is 9.3%, 5.1%, 3.5%, and 5% at mean concentration of 17, 39, 80,117 and 278 μIU/ml. The interassay CV is 10%, 7.1%, 7.2% and 4.9% at mean concentration of 16, 35, 76, and 9 μIU/ml, respectively.

Serum total testosterone was measured on Immulite 2000 analyzer (DPC, Los Angeles, CA) by a competitive chemiluminescence immunoassay. The normal range for women in our laboratory was 0.5−1.2 ng/ml. The intraassay CV is 27%, 10.5%, 10% and 9.5% at mean concentrations of 0.5, 1.0, 2.0, and 4.0 ng/ml. The interassay CV is 13.8% at a mean concentration of 0.94 ng/ml.

Fasting glucose was measured using glucose oxidase method on Hitachi 912 auto analyzer (Boehringer Mannheim). The intraassay CV at a mean concentration of 160 mg/dl was 1% and the interassay CV was 3.2% and 4.2% at mean concentrations of 99 and 264 mg/dl respectively.

Determination of Insulin Resistance

Fasting glucose and insulin ratio (G/I ratio) were calculated. A value of <4.5 mg/10^{-4} U was a strong predicator of Insulin resistance in adult women and a value <7.5 mg/10^{-4} U was a predictor of insulin resistance in adolescents.

Sample Size and Statistical Analysis

The sample size was based on the previous study with troglitazone where a 7.8 μIU/ml decrease in fasting Insulin and a decrease in 0.4 ng/dl of total testosterone was noted.[9] The sample size required to detect a similar post treatment difference with 99% ($P < 0.01$) confidence and 80% power was computed with the True Epistat statistical software. This worked out

to 22 subjects. A Student *t* test was used to assess statistical significance. For the proportion of patients with insulin resistance (IR) before and after intervention, the significance was assessed using the chi-square test.

Results

Twenty-two women fulfilled the study criteria and were enrolled in the study. All patients received 30 mg of pioglitazone for 6 months and completed both baseline and end of study assessments. The mean age of the study participants was 19.4 yrs (range 15−24 yrs) and the mean age of menarche was 13 years (range 10−17 yrs). All patients were obese with a mean BMI of 29.5 ± 7.9 kg/m^2 (mean ± SD). All patients had severe menstrual irregularities. Average number of cycles in the last 6 months was 1.4 ± 0.5 (mean ± SD) with mean duration of menstrual symptoms of 44.6 ± 30.2 months (mean ± SD) (range 12−120 months). Table 1 shows clinical and biochemical features before and after treatment with pioglitazone for 6 months.

Clinical Outcomes

Though there was no significant change in weight or BMI from baseline (Table 1), twelve subjects gained weight ranging from 1 to 6 kg during the course of this study. At the end of the study period 20 of 22 (91%) of the women had regularization of menstrual cycles. There was, however, no change in the grade of acanthosis and in the mFG hirsutism scores at the end of 6 months of therapy.

Metabolic Profile

Changes in the mean fasting plasma glucose (FPG) levels were significant at the end of study (Table 1). None of the subjects experienced any hypoglycemic symptoms on therapy with pioglitazone. Impaired fasting glucose (defined as FPG >110 mg %) was seen in only one subject at initial evaluation and this persisted at the end of study. Decline in fasting insulin levels at the end of the study was 45.6% from baseline. There was a significant increase in the fasting glucose/insulin ratio (G/I ratio) from baseline. Using a value of G/I ratio < 4.5 mg/10^{-4} U as significantly associated with IR in adolescents there were 10 (45.4%) subjects with IR at baseline and after treatment this declined to 6 (27.2%) subjects. This difference was not statistically significant ($P = 0.23$). However, if a fasting G/I ratio of < 7.5 mg/10^{-4} U was taken as a predictor of insulin resistance, 18 (81.8%) subjects had IR at baseline. Only 11 (50%) subjects continued to have IR at the end of the study period. This was statistically significant ($P = 0.02$)

Total serum testosterone was elevated (> 1.2 ng/ml) in 32% of subjects. There was a minimal increase in the mean post treatment total testosterone values which was not statistically significant.

Pioglitazone was well tolerated by all subjects during the period of study. There were no drug-related adverse events.

Discussion

The major manifestations of PCOS in adolescence are chronic anovulation characterized by oligomenorrhea and amenorrhea and by symptoms related to hyperandrogenism. Most adolescent girls with PCOS seek medical help for the menstrual irregularities, obesity, and/or hirsutism. Pioglitazone has been studied in adult women with PCOS and has shown remarkable improvements in insulin resistance, ovulatory rates and hyperandrogenism. The aim of our study was to look at clinical and metabolic outcomes with the use of pioglitazone in adolescent girls and young adults with PCOS over a period of 6 months.

For the purpose of the study we recruited a subset of young women with PCOS who had clinically significant manifestations of the disease. All patients had significant oligomenorrhea (less than 2 cycles in

Table 1. Details of Important Clinical and Biochemical Variables Seen at Baseline and after 6 Months of Therapy with 30 mg Pioglitazone Once Daily

Variables	Baseline (n = 22)		End of Study (n = 22)		*P*- value
	Mean ± SD	Range	Mean ± SD	Range	
Weight (kg)	69.4 ± 10.3	52−89	70.3 ± 10	58−90	NS
BMI (kg/m^2)	29.5 ± 3.9	24.7−41.8	29.7 ± 4.17	25.0−43.3	NS
Number of menstrual cycles in previous six months	1.4 ± 0.5	1−2	5.27 ± 1.45	2−7	0.0001*
Fasting Serum Insulin levels (μIU/ml)	26.4 ± 22.5	5.8−98	14.3 ± 10.2	2.3−36.9	0.01*
Fasting plasma Glucose (mg/dl)	92.2 ± 11.2	77−120	87.6 ± 11.2	68−121	0.03*
Serum Total Testosterone values (ng/dl)	0.96 ± 0.6	0.3−2.6	1.21 ± 0.5	0.5−2.4	NS
Fasting Glucose/Insulin ratio (mg/10^{-4} U)	5.54 ± 3.0	1.1−14.4	10.3 ± 8.0	2−36	0.01*

Abbreviation: NS, not statistically significant
*Statistically significant

the preceding 6 months), were obese (BMI $>$ 25 kg/m^2), had clinical evidence of insulin resistance (presence of acanthosis nigricans), and had evidence of hyperandrogenism (mFG score of ≥ 7). In this subgroup of patients with PCOS the use of pioglitazone 30 mg daily for a period of 6 months in addition to dietary and exercise advice lead to significant improvements in menstrual frequency, fasting insulin levels, and insulin resistance calculated by the fasting G/I ratio. There was a tendency for weight gain that was not statistically significant, with no clinical improvement in hyperandrogenism. There was no decrease in total testosterone values.

Our clinical findings are in agreement with previous studies carried out in adult women with PCOS.[11] Ninety-one percent of the study subjects had regularization of menstrual cycles at the end of 6 months of pioglitazone therapy. This was much higher than has been obtained in studies with adult women, probably because all patients had severe menstrual irregularities at baseline when they entered the study. Romualdi et al[11] documented regularization of menstrual cycles in 83% of adult women (18−36 yrs) treated with pioglitazone for 6 months and Brettenthaler et al[14] showed cycle regularization in 43% of adult women at the end of 3 months of therapy with pioglitazone. Both these studies have shown some increase in weight but as in our study, the results were not statistically significant.

Chronic insulin resistance and hyperinsulinemia are implicated in the development of reproductive abnormalities and hyperandrogenism in patients with PCOS. Insulin resistance is also documented as a risk factor for clinical pathologies associated with PCOS, such as gestational and type 2 diabetes mellitus, hypertension and cardiovascular disease.[15] In addition the elevated insulin levels may increase adrenocorticotrophic hormone-stimulated steroidogenesis from the adrenal glands, while impairing 17,20-lyase activity.[16]

In this study we used a simple fasting G/I ratio to objectively document insulin resistance in study subjects and studied the effect of therapy with pioglitazone for 6 months. Dunaif et al had suggested that a fasting G/I ratio of less than 4.5 is a highly sensitive and specific test for the diagnosis of insulin resistance in adult women with PCOS.[16] All subjects in our study had obesity and acanthosis nigricans. However, using the above criteria (G/I ratio $<$ 4.5 in mg/10^{-4} U), only 46% (10/22) of our study group had objective insulin resistance. There was also no significant change in the percentage of patients with insulin resistance using the above criteria after 6 months of pioglitazone therapy. Macut et al first compared adolescent PCOS with adult PCOS and they found that fasting plasma glucose is significantly lower and fasting G/I ratio is significantly higher in adolescent girls with PCOS.[17] The mean G/I ratio in adolescents was 7.52 $\pm$ 2.54 (mean $\pm$ SD) compared to 4.28 $\pm$ 0.44 (mean $\pm$ SD) in adult women with PCOS. This study suggests that there is worsening insulin resistance with advancing age and a different cut-off should be used in adolescent girls (G/I ratio $<$ 7.5 mg/10^{-4} U). Applying these criteria 82% (18/22) of subjects in our study had objective evidence of insulin resistance at the beginning of the study. After 6 months of therapy with pioglitazone, the number of subjects with objective insulin resistance declined to 50% (11/22). This difference in proportion was statistically significant ($P = 0.02$).

Though some authors have questioned the relationship of insulin resistance and hyperinsulinism with oligomenorrhea, in our study we have shown significant association between fasting insulin levels and fasting G/I ratio with menstrual frequency. This is particularly true when an objective G/I ratio of $<$ 7.5 mg/10^{-4} U was used to document insulin resistance. It seems reasonable to use this simple test for the diagnosis of insulin resistance with a cut off of $<$ 7.5 mg/10^{-4} U in adolescents to document insulin resistance and initiate therapy with sensitizers.

For the purpose of assessing response of treatment with pioglitazone on hyperandrogenism, we used the mFG hirsutism score to clinically grade the degree of hyperandrogenism and total testosterone levels. There was no significant change in the clinical scores or in the total testosterone levels. This may be a reflection of the short duration of the trial and high interindividual variability in total testosterone and sex hormone binding globulin (SHBG) levels. The lack of availability of reliable free testosterone and SHBG assays in our present setting limited us from documenting any change in the free hormone levels. Romualdi et al were, however, able to document significant changes in FG scores at the end of 6 months of pioglitazone therapy in adult women which we could not reproduce in younger women.[11] Brettenthaler and colleagues did not observe any significant change in FG scores at the end of 3 months of therapy with pioglitazone in adult women.[14]

There were no significant adverse events which lead to the discontinuation of the drug in any patient during study period. Weight gain, though not statistically significant, was observed in more than half of the patients enrolled in the study. Subjects gained up to 6 kilograms with 23% (5/22) of patients gaining more than 3 kg of weight during the course of the study. In a study from Mexico that randomized obese adult women with PCOS to either pioglitazone or metformin therapy for over 6 months, an average of 4.7 kg weight gain was seen in the pioglitazone arm.[18] In contrast the weight gain appears to be to a lesser extent in adolescents with PCOS treated with pioglitazone.

Recent reports about a possible increase in cardiovascular morbidity and mortality associated with the use of rosiglitazone in type 2 diabetes mellitus have queered the pitch for all drugs in this class.[19] Larger studies focusing on cardiovascular outcomes are underway. Unlike rosiglitazone, pioglitazone has been studied in a large prospective, randomized trial of cardiovascular outcomes called PROACTIVE. The primary end point, which was a broad composite that included cardiovascular and peripheral vascular events, showed a trend towards benefit with pioglitazone. A secondary endpoint of myocardial infarction, stroke and death from any cause showed a significant effect favoring pioglitazone.[20]

In summary our study shows that administration of pioglitazone for 6 months along with advice about diet and modest increase in physical activity in obese adolescents and young women with PCOS resulted in significant improvements in menstrual frequency and in over 90% of subjects it established cyclical menstruation. There is a significant improvement in insulin resistance using the G/I ratio (<7.5 mg/10^{-4} U) as the biochemical marker. Weight gain though present appears to be much lesser than what was seen in adult women with PCOS. No significant adverse outcomes were noted in 6 months of therapy. No changes were noticed clinically in hyperandrogenism and no change was noticed in the total testosterone values at the end of 6 months. Long term studies are required to assess whether these short term improvements in insulin resistance lead to reduction in new onset type 2 diabetes and cardiac events on the long term.

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