Human Reproduction Vol.18, No.2 pp. 299–304, 2003 DOI: 10.1093/humrep/deg105

Sequential treatment of metformin and clomiphene citrate in clomiphene-resistant women with polycystic ovary syndrome: a randomized, controlled trial

Susan Shanti George[1,5], Korula George[1], Christina Irwin[1], Victoria Job[2], R.Selvakumar[2], Visalakshi Jeyaseelan[3] and M.S.Seshadri[4]

[1]Reproductive Medicine Unit, [2]Department of Clinical Biochemistry, [3]Department of Biostatistics and [4]Department of Endocrinology, Christian Medical College, Vellore, Tamil Nadu, India

[5]To whom correspondence should be addressed at: Reproductive Medicine Unit, Christian Medical College, Vellore, Tamil Nadu, India 632004. E-mail: rmu@cmcvellore.ac.in

BACKGROUND: Recognition of the importance of insulin resistance in clomiphene-resistant women with polycystic ovary syndrome (PCOS) has led to the use of insulin sensitizers. METHODS: A randomized, controlled trial was conducted to compare efficacy of sequential treatment with metformin and clomiphene citrate with conventional gonadotrophins. Sixty clomiphene-resistant women with PCOS were randomized to two groups (n = 30 each), using computer-generated tables. Group I received metformin for 6 months, followed by ovulation induction with clomiphene citrate; group II received hMG for ovulation induction. Hormonal profiles were evaluated at the onset and after completion of treatment. RESULTS: There was no significant difference in pregnancy rates between the two groups (16.7 versus 23.3%). In group I, there was a significant improvement in menstrual function and ovulation after treatment (40%, $P < 0.001$; and 46.7%, $P < 0.001$), with a significant decrease in fasting insulin levels ($P < 0.05$). There were no changes in other biochemical parameters. The ovulation rate in group II was 43.3%, with a high drop-out rate. The cost-effective analysis for medications per pregnancy in group I was US$ 71 ± 3 versus US$ 277 ± 171 in group II. CONCLUSIONS: Sequential treatment with metformin and clomiphene citrate is an effective and safe option for clomiphene-resistant women with PCOS.

Key words: insulin resistance/insulin sensitizer/metformin/PCOS

Introduction

Polycystic ovary syndrome (PCOS) is a condition comprising variable clinical features of infertility, menstrual disturbances, hyperandrogenism, polycystic ovaries, together with biochemical abnormalities of raised serum levels of LH, androgens and insulin (Adams *et al*., 1986). PCOS is the most common endocrine disorder in women of reproductive age (Franks, 1995). Clomiphene citrate is the treatment of choice for the anovulatory infertile woman with PCOS, but only 75% of patients respond positively (Polson *et al*., 1989). Ovulation induction with gonadotrophins is the standard treatment for clomiphene-resistant women; however, this is expensive and has added risks of ovarian hyperstimulation and multiple pregnancies. Clomiphene resistance is associated with obesity (Polson *et al*., 1989), when weight reduction is beneficial (Clark *et al*., 1995). Insulin resistance is also linked with clomiphene resistance (Murakawa *et al*., 1999), and the suggested treatment includes the use of insulin sensitizers such as metformin (Velazquez *et al*., 1994) and troglitazone (Mitwally *et al*., 1999).

Metformin lowers elevated blood glucose levels in patients with diabetes mellitus, but does not reduce blood glucose values in non-diabetics. Insulin resistance in PCOS involves a post-receptor defect in the insulin signal transduction chain between the receptor kinase and glucose transport (Ciaraldi *et al*., 1992). As metformin increases the binding of insulin to its receptor and enhances the post-receptor action, women with PCOS might benefit from metformin therapy. In addition to improving insulin sensitivity, metformin has weight-reducing effects and has also been shown to be safe during embryogenesis and pregnancy (Coetzee and Jackson, 1979, 1984; Denno and Sadler, 1994).

A randomized, controlled trial was conducted to determine whether sequential treatment with metformin and clomiphene citrate would be as effective as hMG in improving ovulation and pregnancy rates in clomiphene-resistant PCOS women. The pre- and post-treatment hormonal levels of these women were also monitored in order to determine whether metformin treatment improves the biochemical milieu.

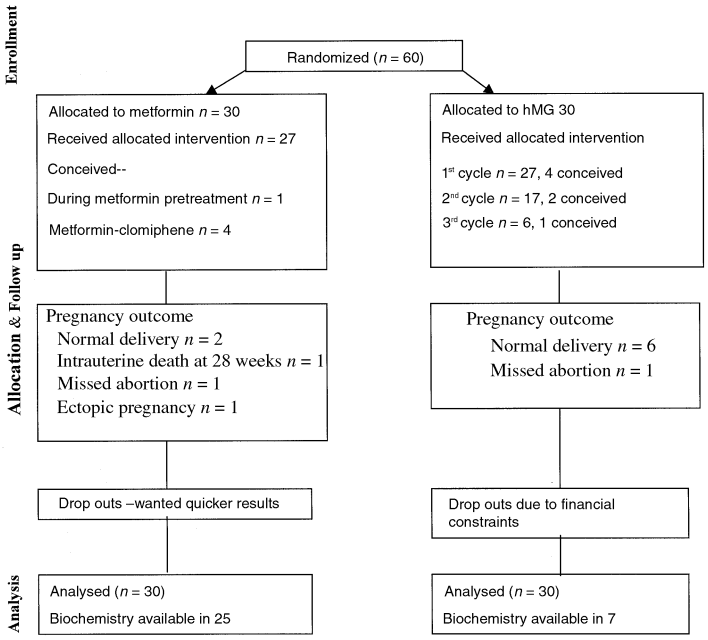

Figure 1. Flow chart of randomized, controlled trial of metformin and clomiphene citrate versus hMG.

Materials and methods

The present clinical trial was conducted in the Reproductive Medicine Unit of Christian Medical College in southern India between 1999 and 2001, with approval of the institutional review board. Infertile women with PCOS who were also resistant to clomiphene were included. A diagnosis of PCOS was based on clinical features of oligomenorrhoea and hyperandrogenism, along with either biochemical abnormalities of a raised LH/FSH ratio or LH or ultrasound features of polycystic ovary. Clomiphene resistance was defined as failure to ovulate to a dose schedule of 200 mg/day for 5 days. Laboratory investigations showed that all women had normal liver, renal and thyroid function, glucose tolerance and prolactin levels. Women with associated tubal or male factor infertility and those with a body mass index (BMI) >35 kg/m^2 were excluded from the study. At time of entry to the study, BMI and waist-to-hip ratio were documented. Serum levels of FSH, LH, dehydroepiandrosterone sulphate (DHEAS), testosterone, sex hormone-binding globulin (SHBG) and insulin sensitivity were measured during the follicular phase.

Insulin sensitivity was measured using the short insulin tolerance test (Bonora *et al*., 1989). In brief, an intravenous bolus injection of actrapid insulin (0.05 U/kg) was given. Blood samples were drawn from the contralateral arm at 5 min before and at 3, 5, 7, 9, 12 and 15 min after insulin injection. Glucose was then injected i.v. at 15 min to prevent a fall in plasma glucose level. The rate of fall of blood glucose (%) was calculated from the linear regression of all values between 3 min and 15 min; this rate of fall (in % per min) was taken as the index of insulin sensitivity. Women in both the groups were instructed to maintain their usual lifestyle and eating habits during the study. All women provided their informed consent to participate in the study.

After being enrolled into the trial, women were randomized (see Statistical analysis) to group I or II (Figure 1). Those women in group I received metformin 1500 mg/day in three divided doses for 6 months. Each woman was given sufficient tablets for 30 days of treatment, and reviewed at 30-day intervals to monitor compliance and record any side effects. After 6 months of metformin treatment, the clinical and biochemical parameters were rechecked. Patients were asked to maintain a menstrual calendar throughout the trial. Clomiphene citrate (150 mg/day) was restarted along with metformin after 6 months. Follicular monitoring was carried out using ultrasound scan to study the ovulatory response. When the leading follicle reached >20 mm, hCG (5000 IU) was given to induce ovulation. The clomiphene citrate dose was increased to 200 mg if the women did not ovulate on the 150 mg/day schedule. Three cycles with ovulatory dose were planned.

Group II women underwent ovulation induction with hMG by use of a low-dose, step-up regimen, with an intention to treat for at least three cycles. In brief, treatment with hMG was commenced on day 5 of a spontaneous or induced cycle. The starting dose was 75 units, and this was increased by increments of 75 units every 7–10 days if there was no evidence of an ovarian response on ultrasound, i.e. no follicle >10 mm in diameter. When follicular development had started, the same dose was continued until the leading follicle size was >18 mm and hCG (5000 IU) was given to induce ovulation. This group also underwent clinical and biochemical evaluation after treatment if there was no conception.

Hormone assays

SHBG levels were measured using an immunoradiometric assay based on ligand-coated tubes and monoclonal antibodies, one of which was ^{125}I-labelled and the other ligand-labelled. Testosterone, DHEAS and insulin were also estimated using solid-phase competitive radio-immunoassays (all kits from Diagnostic Product Corporation, USA). FSH and LH were measured using a two-site sandwich immunoassay with direct chemiluminometric technology on a Bayer ACS-180 analyser.

Statistical analysis

The accepted pregnancy rate with hMG is ~40%, and the expected pregnancy rate in the metformin group was 25%. However, it was decided to favour metformin even if the difference was 20%. By keeping alpha and beta errors at 5 and 20% respectively, the calculated sample size was 30 in each arm of the trial.

Block randomization was carried out using RALLOC software, with concealment of treatment allocation by use of opaque envelopes.

The statistical analyses were performed using an intention-to-treat (per protocol) method. The pregnancy rate between the two arms was compared by a proportion test using the concept of equivalence trial, in which the δ* was considered as 20% (Blackwelder, 1982). The 95% confidence interval for the difference in pregnancy rate was calculated. A test of proportion was used for within-group analysis in group I. The baseline parameters between the two groups were compared using an independent *t*-test, while the baseline and post-treatment hormone levels were compared using a paired *t*-test. Comparisons between responders and non-responders were made using the Mann–Whitney *U*-test.

Results

Sixty women were enrolled into the trial, of whom 30 were randomized to the metformin group (I) and 30 to the hMG group (II). The clinical and hormonal variables of the women are listed in Table I. The groups did not differ with respect to either anthropometric variables or biochemical values (Table I).

In the metformin group, 27 women completed the trial and three discontinued medications at 3 months. Three women reported mild side effects of transient nausea and vomiting, which subsided with a reduced dose of 1000 mg/day for 2 weeks. There was a significant improvement in menstrual cycles ($P < 0.001$) with metformin, and in ovulation ($P < 0.001$) with metformin-clomiphene. Twelve women (40%) resumed regular menstrual cycles in 3 months, and one conceived thereafter. Fourteen women (46.7%) ovulated, and four conceived with clomiphene citrate at a dose level of either 150 or 200 mg/day. The overall pregnancy rate in this group was 16.7%.

In the hMG group, three women defaulted therapy in the first cycle due to financial constraints. The remaining 27 patients had 50 cycles of ovulation induction with hMG, and seven of them conceived, to give a cumulative pregnancy rate of 23.3%. Only 39 cycles had ovulation (43.3%), and in 11 cycles women discontinued treatment during the cycle due to poor response and inability to continue hMG. The mean number of hMG ampoules (75 units) used was 19.2 ± 9.4; total dosage ranged from three to 44 ampoules. There was no significant difference in the age, BMI and basal serum levels of FSH, LH, insulin and testosterone in those patients who discontinued compared with those who continued. The requirement of hMG also was not significantly different between the women who continued and those who did not (number of hMG ampoules required by discontinued versus continued groups: second cycle 16.7 ± 14.5 and 8.6 ± 6.2; and third cycle 8.6 ± 5.5 versus 6.7 ± 3.3). Hormonal evaluation after failed treatment was possible in seven patients.

The pregnancy rates were 23.3 and 16.7% in hMG and metformin groups respectively. Although the difference between the two arms was not significant, the power of 50% suggested the need for additional subjects in each arm. However, the 95% CI for the difference between the two arms was –13% and 26.2%. This suggested that the difference in pregnancy rate between the metformin and hMG groups might be as low as 13%. As this estimate was lower than the hypothesized value of 20%, and the 95% CI also included the null value 0% between the two limits, there was evidence that the pregnancy rate in the metformin group was as good as that in the hMG group.

The pre-treatment and post-treatment anthropometric and biochemical variables were compared in both groups (Table II).

Table I. Baseline characteristics of women in the metformin and hMG groups

Characteristic	Metformin group (n = 30)	hMG group (n = 30)
Age (years)	25.1 ± 3	26 ± 2.9
BMI (kg/m^2)	25.5 ± 3.7	24.6 ± 2.6
Waist-to-hip ratio	0.8 ± 0.04	0.8 ± 0.1
Serum FSH (mIU/ml)	6.1 ± 2.1	6.1 ± 2.3
Serum LH (mIU/ml)	12.9 ± 5.6	14.7 ± 7.9
LH/ FSH ratio	2.3 ± 1.2	2.6 ± 1.3
Fasting glucose (mg/dl)	101.1 ± 18.3	98.1 ± 8.1
Fasting insulin (μU/ml)	15.4 ± 10.7	20.0 ± 18.7
Fasting glucose/insulin ratio	12.7 ± 13.0	9.3 ± 9.0
Insulin sensitivity (%/min)	2.2 ± 1.0	2.2 ± 1.2
DHEAS (μg/dl)	203.0 ± 174.2	200.5 ± 182.7
SHBG (nmol/l)	10.6 ± 12.9	10.6 ± 11.5
Testosterone (ng/dl)	70 ± 40	70 ± 50

Values are mean ± SD.
BMI = body mass index; DHEAS = dehydroepiandrosterone sulphate; SHBG = sex hormone-binding globulin.

Table II. Variables in the metformin and hMG groups before and after treatment

Characteristic	Metformin group (n = 25)		hMG group (n = 7)	
	Pre-treatment	Post-treatment	Pre-treatment	Post-treatment
BMI (kg/m^2)	25.7 ± 3.9	24.9 ± 2.9[a]	25.9 ± 2.9	25.9 ± 2.9
Waist-to-hip ratio	0.8 ± 0.1	0.81 ± 0.04	0.8	0.6 ± 0.4
Serum FSH (mIU/ml)	5.9 ± 2.1	6.8 ± 2.7	6.1 ± 2.0	4.4 ± 4.5
Serum LH (mIU/ml)	12.4 ± 5.4	12.7 ± 8.1	14.2 ± 6.4	7.5 ± 5.5[b]
LH/FSH ratio	2.5 ± 1.3	2.1 ± 1.0	1.4 ± 0.6	2.5 ± 5.5
Fasting glucose (mg/dl)	102.1 ± 19.4	96.3 ± 14.6	103.9 ± 9.9	99.3 ± 8.4
Fasting insulin (μU/ml)	16.4 ± 10.3	12.3 ± 8.4[b]	33.1 ± 30.1	11.4 ± 7.2
Fasting glucose/insulin ratio	11.7 ± 12.8	14.6 ± 19.0	4.8 ± 2.7	11.6 ± 6.5
Insulin sensitivity (%/min)	2.1 ± 1.0	2.2 ± 1.6	2.9 ± 0.7	2.4 ± 1.2
SHBG (nmol/l)	10.1 ± 13.3	9.0 ± 9.1	6.5 ± 12.0	10.1 ± 11.4
Testosterone (ng/dl)	63 ± 30	55 ± 30	70 ± 60	70 ± 30

Values are mean ± SD.
BMI = body mass index; SHBG = sex hormone-binding globulin.
[a]$P < 0.001$ versus pre-treatment; [b]$P < 0.05$ versus pre-treatment.

Table III. Characteristics of the metformin group in terms of responders and non-responders

Characteristic	Responders (n = 14)	Non-responders (n = 13)
Age (years)	25.86 ± 2.9	24.4 ± 2.9
BMI (kg/m^2)	25.9 ± 3.5	25.3 ± 4.2
Waist to hip ratio	0.8 ± 0.1	0.8 ± 0.1
Serum FSH (mIU/ml)	6.1 ± 1.8	6.1 ± 2.4
Serum LH (mIU/ml)	13.0 ± 4.8	12.0 ± 5.8
LH/ FSH ratio	2.4 ± 1.4	2.2 ± 1.1
Fasting glucose (mg/dl)	97.7 ± 6.4	105.9 ± 26.8
Fasting insulin (μU/ml)	14.8 ± 11.5	17.2 ± 9.2
Fasting glucose/insulin ratio	14.7 ± 14.7	9.5 ± 9.2
Insulin sensitivity (%/min)	2.4 ± 1.1	1.8 ± 0.8
SHBG (nmol/l)	11.4 ± 14.9	9.7 ± 11.9
Testosterone (ng/dl)	60 ± 30	70 ± 40

Values are mean ± SD.
BMI = body mass index; SHBG = sex hormone-binding globulin.

In the metformin group there was a significant decrease in BMI ($P < 0.001$) and fasting insulin levels ($P < 0.05$), but all other parameters did not change significantly. Although the change in BMI was statistically significant, it was not clinically relevant. A decrease in BMI was seen in 13% of women, whereas insulin was decreased in 27%. The BMI ranged from 15 to 32 kg/m^2, and only three women had a BMI >30 kg/m^2. In the hMG group there was no significant change in any of the variables, except serum LH level.

In the metformin group, there was no significant difference in pre-treatment variables between responders (those women who ovulated) and non-responders (Table III). The BMI was also similar for both pregnant and non-pregnant women (data not shown).

The ovulatory response in the metformin group could not be predicted using receiver operating characteristic (ROC) curves of fasting insulin, glucose-insulin ratio and insulin sensitivity.

Discussion

Insulin resistance with resultant hyperinsulinaemia is a prominent feature of PCOS, and it is seen both in obese and normal-weight women (Chang *et al*., 1983; Dunaif *et al*., 1989; Nestler *et al*., 1989). Moreover, obese women develop a greater degree of insulin resistance as their body mass increases (Rittmaster *et al*., 1993).

Hyperinsulinaemia plays a key role in development of ovarian hyperandrogenism (Barbieri *et al*., 1988; Barbieri, 1991). Insulin stimulates androgen synthesis in the ovary (Barbieri *et al*., 1986) and inhibits SHBG synthesis in the liver (Nestler *et al*., 1991; Rajkhowa *et al*., 1994), with the result being an increased bioavailability of free androgens. This increased intra-ovarian androgen production leads to altered gonadotrophin secretion and impaired folliculogenesis (Barbieri *et al*., 1986; Olson *et al*., 1995), and these women present with anovulation and infertility. Ovulation induction with clomiphene citrate is the treatment of choice, though about 20% of these women do not respond. Obesity and hyperinsulinaemia are well correlated with clomiphene citrate resistance (Parsanezhad *et al*., 2001).

Ovulation induction with gonadotrophins is the standard treatment for clomiphene-resistant women. However, this approach is associated with complications and has the added disadvantage of high cost and need for careful monitoring. Hence, there is a clear need for an alternative, less expensive therapy.

Metformin has been shown to have beneficial effects on ovarian function and hormonal milieu. In an uncontrolled study PCOS women were treated with metformin and showed improved menstrual cycles and hormonal parameters (Velazquez *et al*., 1994). Subsequently, other studies using metformin in PCOS women have shown improved clinical parameters and variable changes in hormonal levels. Placebo-controlled, randomized trials with metformin have shown improved menstrual function (Nestler *et al*., 1998; Moghetti *et al*., 2000) and improved insulin levels and insulin sensitivity in anovulatory PCOS women (Unluhizarci *et al*., 1999; Moghetti *et al*., 2000; Ng *et al*., 2001). The improved insulin levels were associated with variable changes in testosterone, SHBG and BMI (Morin-Papunen *et al*., 1998; Unluhizarci *et al*., 1999; Kolodziejczyk *et al*., 2000). Two studies reported no significant change in hormonal levels with metformin therapy (Acbay and Gundogdu, 1996; Ehrmann *et al*., 1997).

Two randomized, placebo-controlled trials in clomiphene-resistant women showed improved ovulatory rates with sequential therapy of metformin and clomiphene citrate (Vandermolen *et al*., 2001; Kocak *et al*., 2002). However, another study in a similar group of women could not demonstrate better ovulatory rates in spite of improved hormonal levels (Ng *et al*., 2001).

In the present study, metformin pre-treatment followed by clomiphene citrate was compared with standard hMG therapy in clomiphene-resistant PCOS patients. It is preferable—but clinically difficult—to select only those women who are insulin-resistant, as there is no ideal screening test to detect insulin sensitivity. Use of the euglycaemic clamp is possible only in a research setting, and the insulin tolerance test is also difficult to conduct in clinical practice. A single fasting insulin level is unreliable, and monitoring the fasting glucose to insulin ratio is useful mainly in obese women (Legro *et al*., 1998). Consequently, all clomiphene-resistant women were included in these investigations.

The results of the present study demonstrated that sequential therapy with metformin significantly improves ovarian function, as shown by regular menstrual cycles (40%) and also ovulation rates (46.7%) in clomiphene-resistant women with PCOS. Metformin treatment resulted in a significant decrease in fasting insulin levels, even though there was no change in testosterone and SHBG levels. However, only total testosterone was measured. Previously, an improved total testosterone level after 2 months of metformin therapy had been reported, but this had returned almost to starting levels by 6 months (Morin-Papunen *et al*., 1998). The free testosterone decreased significantly in 4–6 months. The sequential therapy of metformin and clomiphene citrate also improves pregnancy rates in these women. Even if all the women in the hMG group had completed the three cycles of hMG as had been planned, and a better pregnancy rate had been achieved, this would not

have had any considerable influence on the confidence interval of difference in pregnancy rate. However, the pregnancy rates in both groups were lower than predicted. The power of the study warrants a larger trial to compare the two treatment protocols. Based on the current pregnancy rates between the two groups—that is, 23.3% versus 16.7% with type I and II errors at 5 and 20% respectively—and expecting the difference between the two arms within 20%, a sample size of 128 in each arm is required for an equivalence trial.

Although the pregnancy rate was 23.3% in the hMG-treated women, only 50 cycles achieved ovulation induction among the 90 expected cycles. Even among those women who started the treatment cycle, 11 discontinued after partial treatment (though financial constraints led to the high discontinuation rate in this group). It is clear from Figure 1 that the majority of women were happy to undergo the first cycle with hMG, although as the treatment progressed, the number of drop-outs increased. This was in fact due to financial strain with added cycles, although in the metformin group three women also discontinued therapy after 3 months as they requested ovulation induction without delay.

Higher fasting insulin levels and lower serum androstenedione have been reported as predictors of clinical improvement of improved menstrual cycles after metformin (Moghetti *et al*., 2000). Others observed baseline fasting insulin and testosterone as the predictors of insulin and testosterone changes (Kolodziejczyk *et al*., 2000). In the present study, none of the baseline hormone levels predicted the response to metformin.

The duration of pre-treatment with metformin is an unresolved issue. In several studies, this period has ranged from 1 month (Nestler *et al*., 1998; Kocak *et al*., 2002) to 8–12 weeks (Unluhizarci *et al*., 1999; Vandermolen *et al*., 2001), and to 6 months in some cases (Morin-Papunen *et al*., 1998; Moghetti *et al*., 2000). In the present trial, 40% of the women achieved regularized menstrual cycles on metformin, and 25% of them showed this clinical change by 3 months. Hence, pre-treatment with metformin should be given for at least 3 months.

Those women who did not conceive on metformin and clomiphene citrate were later advised to undergo hMG treatment. Metformin may improve their ovulatory response to hMG. Indeed, metformin pre-treatment in hMG cycles has been shown to result in fewer mature follicles, lower estradiol levels and lower cancellation rates (De Leo *et al*., 1999). There was also a trend towards a lower requirement for hMG. By reducing insulin levels, metformin brings about a reduction in activity of cytochrome p450c-17α in both obese and lean PCOS women (Nestler and Jakubowicz, 1996, 1997). This leads to a decrease in intra-ovarian and plasma levels of androgen, which in turn favours a reduction in estradiol levels and orderly follicular growth. Thus, metformin pre-treatment would benefit not only women who fail to conceive with clomiphene citrate but also those who would need hMG therapy.

The cost–efficacy analysis of the two treatment protocols showed the cost of medications per pregnancy in the metformin group to be US$ 71 $\pm$ 3, compared with US$ 277 $\pm$ 171 in the hMG group. The indirect cost of therapy was calculated for both groups. In comparing the metformin and hMG groups, the number of visits for ultrasound scans was 1.7 $\pm$ 0.7 and 3.7 $\pm$ 2.3 respectively ($P = 0.0001$), while the duration when frequent visits to the hospital were required was 2.8 $\pm$ 1.6 and 7.0 $\pm$ 5.5 days respectively ($P = 0.0001$). This indirect calculation of days lost from work indicated a major expense considering that the per capita gross national product (GNP) in the country is only US$ 440.

The requirement for hMG is unpredictable, and multiple cycles of treatment are often needed before a successful outcome is achieved. The cost of hMG per patient ranged from US$ 80 to 700. If hMG therapy were to be selected as the first option in women with clomiphene resistance, it would result in many women discontinuing therapy, especially if they were self-financing their treatment. It is clear that metformin sequential therapy is a less expensive option for clomiphene-resistant women, and it is logical to offer this for clomiphene resistance in the step-wise treatment protocol for PCOS women. Therefore, hMG therapy would be best offered to those women who do not conceive with metformin-clomiphene treatment. If this step-wise protocol were to be practised, it would reduce the burden on both funding agencies and self-financing clients. However, the metformin-clomiphene protocol is time consuming and may not be ideal for women aged >35 years and who require more rapid treatment protocols. On balance, metformin-clomiphene therapy represents a viable interim option for young clomiphene-resistant PCOS women, and is also a valuable treatment alternative in developing countries where patients have financial constraints that preclude ovulation induction with hMG.

Acknowledgements

The authors thank the nursing staff of the Reproductive Medicine Unit, Dhanasundari Bhasker and Anita Solomon for nursing assistance, and USV Ltd for financial support of the trial.

References

Acbay, O. and Gundogdu, S. (1996) Can metformin reduce insulin resistance in polycystic ovary syndrome? *Fertil. Steril.*, **65**, 946–949.

Adams, J., Polson, D.W. and Franks, S. (1986) Prevalence of polycystic ovaries in women with anovulation and idiopathic hirsuitism. *Br. Med. J.*, **293**, 355–359.

Barbieri, R.L. (1991) Polycystic ovarian disease. *Annu. Rev. Med.*, **42**, 199–204.

Barbieri, R.L., Makris, A., Randall, R.W., Daniels, G., Kistner, R.W. and Ryan, K.J. (1986) Insulin stimulates androgen accumulation in incubations of ovarian stroma obtained from women with hyperandrogenism. *J. Clin. Endocrinol. Metab.*, **62**, 904–910.

Barbieri, R.L., Smith, S. and Ryan, K.J. (1988) The role of hyperinsulinemia in the pathogenesis of ovarian hyperandrogenism. *Fertil. Steril.*, **50**, 197–211.

Blackwelder, W.C. (1982) ''Proving the Null Hypothesis'' in clinical trials. *Controll. Clin. Trials*, **3**, 345–353.

Bonora, E., Moghetti, P., Zancanaro, C., Cigolini, M., Querena, M., Cacciatori, V., Corgnati. A. and Muggeo, M. (1989) Estimates of *In vivo* insulin action in man: comparison of insulin tolerance tests with euglycemic and hyperglycemic glucose clamp studies. *J. Clin. Endocrinol. Metab.*, **68**, 374–378.

Chang, R.J., Nakamura, R.M., Judd, H.L. and Kaplan, S.A. (1983) Insulin resistance in nonobese patients with polycystic ovarian disease. *J. Clin. Endocrinol. Metab.*, **57**, 356–359.

Ciaraldi, T.P., El-roeiy, A., Madar, Z., Reichart, D., Olefsky, J.M. and Yen, S.C. (1992) Cellular mechanisms of insulin resistance in polycystic ovarian syndrome. *J. Clin. Endocrinol. Metab.*, **75**, 577–583.

Clark, A.M., Ledger, W., Galletly, C., Tomlinson, L., Blaney, F., Wang, X. and Norman, R.J. (1995) Weight loss results in significant improvement in pregnancy and ovulation rates in anovulatory obese women. *Hum. Reprod.*, **10**, 2705–2712.

Coetzee, E.J. and Jackson, W.P. (1979) Metformin in management of pregnant insulin independent diabetics. *Diabetologia*, **16**, 241–245.

Coetzee, E.J. and Jackson, W.P. (1984) Oral hypoglycaemics in the first trimester and fetal outcome. *S. Afr. Med. J.*, **65**, 635–637.

De Leo, V., Marca, A., Ditto, A., Morgante, G. and Cianci, A. (1999) Effects of metformin on gonadotropin induced ovulation in women with polycystic ovary syndrome. *Fertil. Steril.*, **72**, 282–285.

Denno, K.M. and Sadler, T.W. (1994) Effects of biguanide class of oral hypoglycemic agents on mouse embryogenesis. *Teratology*, **49**, 260–266.

Dunaif, A., Segal, K.R., Futterweit, W. and Dobrjansky, A. (1989) Profound peripheral insulin resistance, independent of obesity, in polycystic ovary syndrome. *Diabetes*, **38**, 1165–1174.

Ehrmann, D.A., Cavaghan, M.K., Imperial, J., Sturis, J., Rosenfield, R.L. and Polonsky, K.S. (1997) Effects of metformin on insulin secretion, insulin action and ovarian steroidogenesis in women with polycystic ovary syndrome. *J. Clin. Endocrinol. Metab.*, **82**, 524–530.

Franks, S. (1995) Polycystic ovary syndrome. *N. Engl. J. Med.*, **333**, 853–861.

Kocak, M., Caliskan, E., Simsir, C. and Haberal, A. (2002) Metformin therapy improves ovulatory rates, cervical scores and pregnancy rates in clomiphene citrate-resistant women with polycystic ovary syndrome. *Fertil. Steril.*, **77**, 101–106.

Kolodziejczyk, B., Duleba, A.J., Spaczynski, R.Z. and Pawelczyk, L. (2000) Metformin therapy decreases hyperandrogenism and hyperinsulinemia in women with polycystic ovary syndrome. *Fertil. Steril.*, **73**, 1149–1154.

Legro, R.S., Finegood, D. and Dunaif, A. (1998) A fasting glucose to insulin ratio is a useful measure of insulin sensitivity in women with polycystic ovary syndrome. *J. Clin. Endocrinol. Metab.*, **83**, 2694–2698.

Mitwally, M.F., Kuscu, N.K. and Yalcinkaya, T.M. (1999) High ovulatory rates with use *of* troglitazone in clomiphene-resistant women with polycystic ovary syndrome. *Hum. Reprod.*, **14**, 2700–2703.

Moghetti, P., Castello, R., Negri, C., Tosi, F., Perrone, F., Caputo, M., Zanolin, E. and Muggeo, M. (2000) Metformin effects on clinical features, endocrine and metabolic profiles and insulin sensitivity in polycystic ovary syndrome: a randomized double-blind, placebo controlled 6-month trial, followed by open long term clinical evaluation. *J. Clin. Endocrinol. Metab.*, **85**, 139–146.

Morin-Papunen, L.C., Koivunen, R.M., Ruokonen, A. and Martikainen, H.K. (1998) Metformin therapy improves the menstrual pattern with minimal endocrine and metabolic effects in women with polycystic ovary syndrome. *Fertil. Steril.*, **69**, 691–696.

Murakawa, H., Hasegawa, I., Kurabayashi, T. and Tanaka, K. (1999) Polycystic ovary syndrome. Insulin resistance and ovulatory responses to clomiphene citrate. *J. Reprod. Med.*, **44**, 23–27.

Nestler, J.E. and Jakubowicz, D.J. (1996) Decreases in ovarian cytochrome p450c17α activity and serum free testosterone after reduction in insulin secretion in polycystic ovary syndrome. *N. Engl. J. Med.*, **335**, 617–623.

Nestler, J.E. and Jakubowicz, D.J. (1997) Lean women with polycystic ovary syndrome respond to insulin reduction with decreases in ovarian p450c17α activity and serum androgens. *J. Clin. Endocrinol. Metab.*, **82**, 4075–4079.

Nestler, J.E., Clore, J.N. and Blackard, W.G. (1989) The central role of obesity (hyperinsulinemia) in the pathogenesis of the polycystic ovary syndrome. *Am. J. Obstet. Gynecol.*, **161**, 1095–1097.

Nestler, J.E., Powers, L.P., Matt, D.W., Steingold, K.A., Plymate, S.R., Rittmaster, R.S., Clore, J.N. and Blackard, W.G. (1991) A direct effect of hyperinsulinemia on serum sex hormone-binding globulin levels in obese women with the polycystic ovary syndrome. *J. Clin. Endocrinol. Metab.*, **72**, 83–89.

Nestler, J.E., Jakubowicz, D.J., Evans, W.S. and Pasquali, R. (1998) Effects of metformin on spontaneous and clomiphene induced ovulation in the polycystic ovary syndrome. *N. Engl. J. Med.*, **338**, 1876–1880.

Ng, E.H., Wat, N.M. and Ho, P.C. (2001) Effects of metformin on ovulation rate, hormonal and metabolic profiles in women with clomiphene-resistant polycystic ovaries: a randomized, double blinded placebo-controlled trial. *Hum. Reprod.*, **16**, 1625–1631.

Olson, B.R., Scott, D.C., Wetsel, W.C., Elliot, S.J., Tomic, M., Stojilkovic, S., Nieman, L.K. and Wray, S. (1995) Effects of insulin like growth factors I and II and insulin on the immortalized hypothalamic GTI-7 cell line. *Neuroendocrinology*, **62**, 155–165.

Parsanezhad, M.E., Alborzi, S., Zarei, A., Dehbashi, S. and Omrani, G. (2001) Insulin resistance in clomiphene responders and nonresponders with polycystic ovarian disease and therapeutic effects of metformin. *Int. J. Gynaecol. Obstet.*, **75**, 43–50.

Polson, D.W., Kiddy, D.S., Mason, H.D. and Franks, S. (1989) Induction of ovulation with clomiphene citrate in women with polycystic ovary syndrome: the difference between responders and nonresponders. *Fertil. Steril.*, **51**, 30–34.

Rajkhowa, M., Bicknell, J., Jones, M. and Clayton, R.N. (1994) Insulin sensitivity in women with polycystic ovary syndrome: relationship to hyperandrogenemia. *Fertil. Steril.*, **61**, 605–612.

Rittmaster, R.S., Deshwal, N. and Lehman, L. (1993) The role of adrenal hyperandrogenism, insulin resistance and obesity in the pathogenesis of polycystic ovarian syndrome. *J. Clin. Endocrinol. Metab.*, **76**, 1295–1300.

Unluhizarci, K., Kelestimur, F., Bayram, F., Sahin, Y. and Tutus, A. (1999) The effects of metformin on insulin resistance and ovarian steroidogenesis in women with polycystic ovary syndrome. *Clin. Endocrinol.*, **51**, 231–236.

Vandermolen, D.T., Ratts, V.S., Evans, W.S., Stovall, D.W., Kauma, S.W. and Nestler, J.E. (2001) Metformin increases the ovulatory rate and pregnancy rate from clomiphene citrate in patients with polycystic ovary syndrome who are resistant to clomiphene citrate alone. *Fertil. Steril.*, **75**, 310–315.

Velazquez, E.M., Mendoza, S., Hamer, T., Sosa, F. and Glueck, C.J. (1994) Metformin therapy in polycystic ovary syndrome reduces hyperinsulinemia, insulin resistance, hyperandrogenemia and systolic blood pressure, while facilitating normal menses and pregnancy. *Metabolism*, **43**, 647–654.

Submitted on September 24, 2002; accepted on November 16, 2002