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Do the Dose And the Starting Time of Clomiphene Citrate in Induction Protocol Affect the Clinical and Endocrinological Response ?

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OBJECTIVE – To compare the standard ovulation induction protocol with the protocol of starting clomiphene citrate on the first day of cycle and for only 3 days. **METHODS** – Fifty-nine women between 20 and 32 years of age, who were diagnosed as anovulatory by the criteria of World Health Organization Class II and had not received any medications for infertility were included in this prospective randomised study. Group I received clomiphene citrate 50 mg / day for 3 days starting on the first day of the cycle and group II 50 mg / day for 5 days starting on the fifth day. Estradiol level on the 11th day and estradiol and progesterone levels on the 22nd day, endometrial thickness, ovulation rate, pregnancy rate and miscarriage rate were studied. **RESULTS** – In group I, serum levels of estradiol and progesterone and the rate of ovulation were found to be lower and the rate of abortion higher. **CONCLUSION** – Starting therapy with Clomiphene citrate on the first day of the cycle with short duration does not give any benefit to endocrinological and clinic outcomes.

Key words : clomiphene citrate, ovulation induction, pregnancy rate, early abortion

Introduction

An evolution has occurred in reproductive endocrinology with the clinical use of clomiphene citrate (CC) . Its mechanism of action and various effects on different parts of the reproduction axis are not completely known and its dose and starting time of treatment are not standardised

Clomiphene citrate is the most widely used drug in ovulation induction. It is well established that pregnancy rate of 40% obtained with CC therapy are less than expected which are based on 80% rates of successful ovulation[1]. The discrepancy between the rate of ovulation after the use of CC and relatively low pregnancy rate is explained in part by the adverse effect of CC on the uterine cervix[2,3], vaginal epithelium[2,3] and the endometrium[4-6] during the implantation period. Some studies have shown that use of ethynyl estradiol during the follicular phase can reverse these effects of CC, but the results are far from satisfactory.

The administration, dose and timeframe of CC has been determined empirically. It may be reasonable to decrease the dose and / or to start the drug earlier in order to decrease the desired antiestrogenic effect especially during sensitive pre-ovulatory period.

In this study, we started CC on the first day of the cycle with the idea of probable maintenance of satisfactory levels of FSH during follicular recruitment and selection and thinking that because of low levels of clomiphene citrate in the pre-ovulatory phase the antiestrogenic effect can be lowered.

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Materials and Methods

Patient selection

Seventy-six patients admitted to infertility clinic were enrolled in the study. This study was carried out between April 1998 and December 2001. All couples diagnosed as infertile underwent complete serum hormonal evaluation, hysterosalpingography, laparoscopy and sperm analyses. From these patients, the ones who had not received any infertility treatment and who had ovarian dysfunction according to World Health Organization Class II were taken into the study

Interventions

All those having cause for infertility other than anovulation (WHO Class II) were excluded from the study. The start of menses either spontaneous or induced by progesterone was designated as day of the treatment cycle. All patients were randomly enrolled in one of the two groups according to computer generated randomization table. In Group I (n=30), stimulation began on day 1 with administration of 50 mg clomiphene citrate daily for 3 days. This group of patients had 72 treatment cycles.

In Group II (n=29), induction began on day 5 with administration of 50 mg CC daily for 5 days. This group of patients had 64 treatment cycles. If ovulation and a

normal luteal phase could not be achieved in the first cycles, the dose of CC was increased by 50 mg / day to a maximum of 150-200 mg/day.

Plasma levels of estradiol were measured on day 11 and 22 and plasma progesterone levels were measured on day 22 by radio immunosorbent assay (RIA) (Diagnostic Products Corp, LA, USA).

Transvaginal ultrasonography was performed on day 9, 11 and 13 of the cycle for assessment of follicular growth and endometrial thickness that was measured from the echogenic interface of the endometrial-myometrium junction in transverse fundal sections. All sonographic evaluations were performed with 5 MHz vaginal probe by the same author of the study (SK).

The number of follicles with a diameter of 18 mm or greater were recorded before human chorionic gonatotrophin (hCG) administration. When the diameter of the leading follicles was 18 mm or greater, 10.000 IU of hCG was administered to the patients for the exact timing of ovulation. The collapse of the dominant follicle and presence of free fluid in the pouch of Douglas during sonographic scans and serum progesterone levels greater than 5 ng/ml were regarded as positive signs for ovulation[7].

Durations of follicular and luteal phases were measured with respect to the day of ovulation and uterine bleeding.

After one day menstrual retardation or after 7 days of progesterone measurement patients were tested for the presence of a possible pregnancy by measuring β -hCG levels by RIA method (Diagnostic Corp., LA, USA). Results were expressed in mIU/ml. When serum β-hCG levels were high, the presence of a clinical pregnancy was confirmed later by means of transvaginal ultrasonography. The patients who achieved pregnancy were followed to assess early pregnancy outcomes until 22 weeks of their pregnancy.

Statistics

All statistical analysis of demographic properties and calculations were performed with SPSS software program for Windows. In the analyses of continuous responses, groups were compared by t test; for discrete data this was done by means of the chi-square test. P value of 5% or less was considered statistically significant.

Results

A total of 76 anovulatory women were enrolled into the study. Three women who did not fulfill inclusion criteria and seven women who refused to participate to the study were excluded. Four women in Group I and three women in Group II were lost of follow-up.

In both groups mean age, mean infertility duration, gravidity and body mass index were similar as shown in Table I.

Table I : Characteristics of Patients.

Parameters	Group I (n=30) Mean	Group II (n=29) mean
Age (year)	25.6	25.7
Body Mass Index (kg/m^2)	23.7	24.0
Duration of infertility (months)	42.1	36.9
Gravidity	0.65	0.54

Table II : Plasma Hormone Levels in the Treatment Cycles.

Parameters	Group I (n=72)	Group II (n=64)	P value
Estradiol at day 11 (pg/ml)	265.59±7.57	255.46±8.86	0.073
Estradiol at day 22 (pg/ml)	228.70±7.14	247.75±8.54	0.032
Progesterone at day 22 (ng/ml)	10.99±0.76	13.36±0.75	0.028
β-hCG at day 29 (mIU/L)	166.90±11.71	145.50±13.12	0.081

Table III : Mean Follicular Phase, Mean Luteal Phase, Number of Follicle and Endometrial Thickness.

Parameters	Group I	Group II	P value
Mean follicular phase in days	12.26±0.11	12.96±0.14	0.043
Mean luteal phase in days	13.69±0.14	13.54±0.13	0.246
The number of follicle ≥18mm	0.88±0.075	0.95±0.078	0.321
Endometrial thickness(mm)	10.91±0.206	10.75±0.166	0.471

Table IV : Ovulation and Pregnancy rates

Parameters	Group I	Group II	P value
Ovulation rate (%)	63.88	78.11	0.028
Pregnancy / ovulatory cycle (%)	23.90	16.00	0.048

Table V : Treatment Cycle wise Pregnancies

Treatment Cycle number	Group I (n=30)	Group II	No. of Pregnancies (Group I / Group II)
1	30	29	5/4
2	25	25	4/3
3	17	10	2/1
Total	72	64	11/8

Estradiol levels were similar in both the groups on day 11. But on day 22 estradiol and progresterone levels were significantly higher in Group II (P=0. 032 and P=0.028 respectively). Plasma βhCG levels were similar in both the groups (Table II).

The mean luteal phase, the numbers of follicles with diameter 18 mm or greater and the endometrial thickness measured on day 13 were similar in both the groups, but the mean follicular phase of Group I patients was significantly shorter than that of Group II patients (P=0.043) (Table III).

The ovulation rate was 63.88% in the Group I, but 78.11% in Group II. The difference was statistically significant (P=0.028). At the same period, the percentage of pregnancy was significantly greater in Group I (Table IV). Eleven pregnancies were achieved in the Group I patients, however eight pregnancies were achieved in Group II patients (P=0.0532). The miscarriage rates were 36.36% in group I and 25% in Group II. There was a statistically significant difference between two Groups in terms of miscarriage rates (P=0.029).

Table V gives the treatment cycle wise pregnancies in the two groups.

Discussion

It is shown here that the starting of ovulation induction with clomiphene citrate on the first day of the cycle and also short-term therapy is barely significantly superior to the standard ovulation induction protocol as judged by pregnancy rates (P=0.048) although day 22 progesterone levels are significantly superior (P=0.028). In Group I, 11 pregnancies were achieved by 19 treatment cycles as against 8 pregnancies in 13 treatment cycles in Group II. The difference is statistically significant (P=0.214)

The discrepancy between the ovulation rate and pregnancy rate induced by CC has attracted the attentions of many researchers. This discrepancy was attributed to the anti-estrogenic effect of CC and researches were concentrated on adding estrogen to the treatment in order to eliminate this undesired effect[8]. In a recent study to decrease the peripheral antiestrogenic effect of CC, 50 mg of CC was used for 3 days only and some remarkable results were obtained[9].

In standard ovulation protocols, no differences have been observed in the rates of ovulation, pregnancy or spontaneous abortion whether CC was started on day 2, 3, 4 or 5 [10]. In our study when CC was started on the first

day and continued for 3 days follicular phase shortened, ovulation rate decreased and abortion rate increased.

The ovulation induction effect of CC is secondary to increased gonadotropin secretion[11]. There is a rapid increase in serum FSH and LH levels on the second and third day of the therapy and those begin to decrease after the last dose of CC treatment. The increase in serum FSH levels observed 2-3 days after CC is sufficient for initiating folliculogenesis. Once it is initiated, it progreses to ovulation as in spontaneous cycles[12].

According to current physiology, FSH should have increased in the follicular recruitment period in Group I patients[13]. The plasma half-life of CC is long and estimated to be 5 days[14]. If the long half-life of CC is taken into account, increase in the FSH is continuing during the follicle selection period of the cycle. The discrepancy between ovulation rate and pregnancy rate cannot be explained only by the antiestrogenic effect of CC. An early increase of FSH and following poor folliculogenesis and probable premature LH peak may help to explain this discrepancy.

The levels of progesterone and estradiol on day 22 in Group I patients were found to be significantly low. This finding can also be attributed to the poor folliculogenesis or the negative effect of CC on corpus luteum[15].

The relation between abortion and plasma progesterone level could not be proven in researches. The succesful pregnancy outcomes were reported with even low progesterone levels[16]. In our study, higher rate of abortion in Group I patients can be explained with desynchronized endometrial proliferation due to lack of harmony in the levels of estradiol and progesterone rather than progresterone levels only[17].

Though the ovulation rate was lower in Group I patients (P=0.028), the pregnancy rate per ovulatory cycles is barely better (P=0.048). As a result, starting ovulation induction with CC on first day of cycle and only 3 days treatment in order to eliminate the peripheral antiestrogenic effect is not much superior to standard ovulation induction by CC. But the number of treatment days in Group I (72 cycles x 3 days = 216) was significantly less than that in Group II (64 cycles x 5 days = 320).

Lastly, we calculated a regression line concerning body mass index and dose of CC and it showed a significant positive slope with increasing body mass index (r=0.54, P 50.01).

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