International Journal of Biomedical And Advance Research

ISSN: 2229-3809 (Online)
Journal DOI:10.7439/ijbar
CODEN:IJBABN

Research Article

Supplementation with estradiol valerate and gonadotropins in clomiphene citrate stimulated IUI cycles

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Abstract

Introduction: Clomiphene, a selective estrogen receptor modulator, has been used to induce ovulation in patients suffering from chronic oligo-anovulation and ovulatory dysfunction with ovulation rates of 60-85% and pregnancy rates of 15-50% per woman. Anti-estrogenic effects of clomiphene on the endometrium are likely to be one of the causes of suboptimal pregnancy rates in spite of good ovulation rates.
Methodology: The study comprised of one hundred and fifteen infertile women who were divided into three groups- group 1 receiving clomiphene (CC 100 mg) from day 3-7, group 2(CC+estradiol valerate 2 mg TDS from day 8 onwards till next menstruation or through 12 weeks of pregnancy) and group 3(CC+HMG 75 IU from day 7-9). The patients were monitored by ultrasonological follicle and endometrial thickness measurement and serum estradiol levels. A single IUI was performed 24-36 hours after the administration of HCG trigger.
Results: Clomiphene is a reasonably good agent for ovulation induction with overall ovulation rate of 91.3% and pregnancy rate of 17.14%, 17.14% in clomiphene group, 14.28% in estradiol valerate group and 20% in gonadotropin group.
Conclusion: Supplementation with estradiol valerate or gonadotropins in CC stimulated IUI cycles did not have any positive effect on the endometrium on the dayof trigger. The study failed to show statistical significant difference in pregnancy rates, however abortion rates were decreased marginally in estradiol or gonadotropin supplemented group.
Keywords: Clomophene citrate, estradiol valerate, gonadotropins

1. Introduction

Clomiphene, a selective estrogen receptor modulator (SERM), having both estrogen agonist and antagonist properties[1] has been used to induce ovulation in patients suffering from chronic oligo-anovulation and ovulatory dysfunction. Clomiphene citrate, a competitive antagonist of 17 β estradiol competes with endogenous estrogen for nuclear estrogen receptors at sites throughout the body. However, unlike estrogen, clomiphene binds to nuclear estrogen receptors for an extended interval of time and thereby depletes receptor concentrations by interfering with receptor cycling.[2] Reduced negative estrogen feedback triggers normal compensatory mechanisms that alter the pattern of gonadotropin-releasing hormone secretion and stimulate increased pituitary gonadotropin release which, in turn, drives ovarian follicular development.[3] At the pituitary level, clomiphene might also increase the sensitivity of gonadotrophs to GnRH.[4] In anovulatory women with WHO Type II anovulation, clomiphene has been reported to induce ovulation in 60-85% of patients and achieve a pregnancy rate of 15-50% per woman.[5]

Anti-estrogenic effects of clomiphene on the endometrium are likely to be one of the causes of suboptimal pregnancy rates in spite of good ovulation rates. In addition to desirable central actions, clomiphene can exert less desirable anti-estrogenic effects at peripheral sites in the reproductive system. Estrogen plays a critical role in the formation of endometrium in natural cycles. Apart from its major role in proliferative phase, it also primes the endometrium for the luteal phase by the further proliferation of the basal cell layer and the induction of P- receptors[6], thereby ensuring the capacity of the endometrium to become secretory. There are studies that have shown that adverse effects of CC on endometrium can be prevented by administering estrogen together with or after clomiphene[7,8,9,10]. Hence to counteract anti-estrogenic effects of clomiphene on endometrium, estrogen supplementation was initiated in the proliferative phase in clomiphene citrate stimulated IUI cycles in the present study. Studies using clomiphene for IVF cycles on the contrary do not appear to reduce the implantation rates in presence of the concomitant use of gonadotropins, due to more sustained E2 production, which may have positive effect on the endometrium and normal implantation rates. A sequential medication regimen, in which HMG is taken after clomiphene, has also been found to improve pregnancy rates in clomiphene cycles.

Therefore the current study aims at evaluation of endometrium in clomiphene stimulated IUI cycles with addition of either estradiol valerate or gonadotropins.

2. Materials and Methods:

A total of consecutive one hundred and fifteen infertile women attending the infertility & IVF clinic in the Department of Obstetrics and Gynaecology, Maulana Azad Medical College and associated Lok Nayak Hospital during period from September 2010 – January 2012 were recruited. Women having infertility with ovulatory dysfunction, anovulation, unexplained infertility, male factor or endometriosis, in the age group of 20-40 years, with regular cycles/oligomenorrhoea or amenorrhoea associated with positive progesterone challenge test were included in the study. Women with Bilateral tubal block, severe male factor (total motile count <5 million) not willing for donor semen or with uncontrolled medical disorders like hypothyroidism, hyperprolactinemia, diabetes mellitus, active tuberculosis were excluded. A total of 10 patients failed to ovulate and were excluded from the final analysis. A complete infertility workup was done for the patients. Laparohysteroscopy was performed in patients with tubal block on hysterosalpingogram or in cases of prolonged infertility. The patients were then randomly divided into three groups using computerised random number table. On day 2/3 of the cycle, serum FSH, serum LH and serum estradiol were done in fasting blood samples. Baseline trans-vaginal sonography was performed to look for endometrial thickness and follicle size and number. In study group 1,

clomiphene citrate 100 mg orally once daily was given from the third day of cycle for five consecutive days. In study group 2, estradiol valerate was added in the doses of 2mg tablet thrice a day orally simultaneously with clomiphene citrate from day 3 and continued till next menstruation or till pregnancy was confirmed. In study group 3, human menopausal gonadotropin in the doses of 75 IU by intramuscular route was given from day 7 to day 9 in addition to clomiphene citrate. Trans-vaginal sonography was performed starting from day 8 every alternate day or as required to note follicular size and number and endometrial thickness. Injection HCG 10,000 IU was given intramuscularly when the size of leading follicular diameter was approximately 18-20mm to trigger ovulation. Serum estradiol levels were measured when there was at least one follicle with a minimum diameter of 18mm in fasting blood samples in all patients. All ultrasound examinations were performed by a single experienced ultrasonographer, blinded to the patient group assignment, by USG machine MEDISON with 7.5 MHz transducer. A single IUI was performed 24-36 hours after the administration of HCG trigger. IUI with donor semen was performed for patients with severe male factor, voluntarily willing for the use of donor semen. From the day of IUI, all patients received progesterone at doses of 200 mg twice a day by vaginal route.

Treatment was maintained till next menses or through 12 weeks of pregnancy.

Serum β HCG levels were evaluated 2 weeks after IUI to confirm pregnancy. A clinical pregnancy was defined by visualisation of gestational sac at the first planned transvaginal ultrasound examination performed at 5-6 weeks of pregnancy. Ultrasounds were done as and when required. On-going pregnancy was gestations that reached 12 weeks of gestation. Abortion rates and ectopic rates were also noted.

2.1 Statistical analysis

Student's unpaired t test and Mann-Whitney test was applied depending on data distribution, wherever applicable. If p value was less than 0.05, the difference was considered to be statistically significant.

3. Results

The epidemiological factors like mean age, mean duration of infertility, type of infertility and basal hormonal parameters were comparable in the three groups. There was no pregnancy achieved in age group ≥ 35 years though the difference between pregnant and non pregnant group was not statistically significant. *The mean duration of infertility varied significantly between the pregnant and the non pregnant group (p value = 0.049 Student's unpaired t test).* Higher FSH levels (> 10) are associated with poor pregnancy outcome. Maximum pregnancy rates are obtained between day 2 estradiol levels of 20 – 80 pg/ml.

Table 1.Distribution of patients according to causes of infertility

	CC	CC+Estradiol	CC+HMG	Total	Percentage
Ovulatory dysfunction	12	14	17	43	40.7%
Tubal	12	10	9	31	29.5%
Male Sperm count (<20million/ml)	8	4	11	23	22%
Uterine	3	4	6	13	12.4%
Endometriosis	2	0	0	2	1.9%
Unexplained	6	6	5	15	14.3%

(There was more than one cause in many patients)

The results for endometrial thickness(using Mann-Whitney test) on day 8 were near significant difference in CC and CC + estradiol group (p value = 0.053 group 1 vs. group 2). There was statistically significant difference in the endometrial thickness on day 8 and day 10 in the CC and CC + gonadotropin group (p value = 0.001 group 1 vs. group 3). There was no difference in the endometrial thickness on the day of trigger in the three groups (p value = 0.779 group 1 vs. group 2, p value = 0.276 group 1 vs. group 3). The endometrial thickness on day 10 did reach near significant statistical difference in the pregnant and the non pregnant group (p value = 0.053) and varied significantly on the day of trigger (p value = 0.012). There is significant difference in estradiol levels on the day of HCG trigger between CC and CC + gonadotropin (p value = 0.000(using Mann-Whitney test)). The estradiol levels on the day of HCG trigger did not differ significantly between CC and CC + estradiol group.

Good ovulation and pregnancy rates were achieved with CC with IUI in the current study. The overall ovulation rate was 91.3%. The overall pregnancy rate was 17.14%. The pregnancy rate in CC group was 17.14%, CC+ estradiol group was 14.28% and in CC+ gonadotropin group was 20%. The pregnancy rates did not differ significantly. The abortion rates in CC group was 50 %(3/6), CC+ estradiol group was 20 %(1/5) and 14.28 %(1/7) in CC + gonadotropin group. One quadruplet pregnancy occurred in CC+gonadotropin group. Embryo reduction was done at 8 weeks for two embryos. The pregnancy went on successfully and twin pregnancy was delivered by caesarean section at 38 weeks.

4.Discussion

There is a significant reduction in efficacy of ovulation induction with clomiphene with IUI with advancing maternal age. The present study demonstrated a pregnancy rate of 18.95% in women aged < 35 years, whereas there was no pregnancy achieved in women of age >35 years. Studies evaluating prognostic indicators for success in IUI cycles have indicated that duration of infertility is an important prognostic indicator.[11] The mean duration of infertility varied significantly between the pregnant and the non pregnant group (p value = 0.049). FSH levels greater than 10 IU/L have high specificity(80-100%) for predicting poor response to stimulation, but their sensitivity to identify such women is low and decreases with threshold value.[12] When the basal FSH level is normal and the estradiol concentration is elevated (>60-80 pg/ml), the likelihood of poor response to stimulation is increased and the chance of pregnancy is decreased.[13] The present study shows no pregnancy was achieved in women with basal FSH levels > 10 m IU/ml. Maximum pregnancy rates were achieved in women with basal E2 levels between 20-80 pg/ml.

A varied difference was observed in the endometrial thickness in the early proliferative phase in the three groups, reaching statistical significance in CC and CC+gonadotropin on day 8 and day 10, but reaching near significance for CC and CC+estradiol on day 8 only. The present study was in agreement with other studies that demonstrated poor endometrium in clomiphene cycles in the late proliferative phase but these changes no longer existed on the day of LH surge.[14,15] This can be explained by a decline in blocking effect of CC late in the cycle or by significantly elevated E2 circumventing E2 receptor blockade. Hence the anti-estrogenic effects of clomiphene were more pronounced in the proliferative phase and were improved by either addition of estradiol valerate or gonadotropins. These anti-estrogenic effects were no longer present on the day of trigger and hence addition of either estradiol valerate or gonadotropins did not affect the endometrial thickness on the day of trigger. During the present study, it was shown that addition of estradiol valerate elicited no favourable response on the endometrium on the day of trigger. The results reached near statistical significance in the proliferative phase on day 8 like in a study by Yagel *et al*., but the results did not reach statistical significance on the day of trigger as shown in a study by Ben-Ami *et al*. and Elkind-Hirsch *et al*. Our study differed from a study carried out at Apollo group of hospitals which obtained a significant difference in the endometrial thickness on the day of trigger in the estradiol treated group when compared with clomiphene alone but the dose and duration differed from the current study(ethinyl estradiol 2mg twice daily from eighth day of cycle till the day of HCG administration).[8,9,16,17]

Addition of gonadotropins also elicited favourable response on the endometrium but not reaching statistical significance on the day of trigger. Apparently, clomiphene regimen used in the current study does not have pronounced anti-estrogenic effects on the endometrium. A study carried out by Dickey *et al*.[18] concluded that increased fecundity when HMG is administered after clomiphene compared to clomiphene alone is related to doubling of implantation rate per follicle. The estradiol level per follicle also nearly doubled for clomiphene-HMG compared to clomiphene alone. Higher estradiol levels may improve the endometrium in addition to quality of oocyte, number of follicles, tubal milieu for fertilization which contributes to higher pregnancy rates. Endometrial thickness is an important predictor of pregnancy outcomes in IUI/IVF cycles. The current study demonstrated a statistical significant difference in the endometrial thickness on the day of trigger in the pregnant and the non pregnant group (p value = 0.012). There is a significant difference observed in estradiol levels on the day of HCG trigger between CC and CC+gonadotropin group (p value = 0.000) in the current study. This is in agreement with other studies which show nearly doubling of estradiol levels per follicle in CC+HMG group compared with CC alone.[18]

Good ovulation and pregnancy rates were achieved with CC with IUI in the current study. The overall ovulation rate was 91.3%. The overall pregnancy rate was 17.14%. The pregnancy rate in CC group was 17.14%, CC+ estradiol group was 14.28% and in CC+ gonadotropin group was 20%. The pregnancy rates did not differ significantly. Our study did not have higher pregnancy rate in the estrogen supplemented group probably because of small sample size as the results did not vary greatly in the two groups (14.28% vs. 17.14%) or there might be other reasons for estradiol valerate to fail to deliver. Our study demonstrated a slightly higher pregnancy rate in CC+HMG (20%) group compared with CC (17.14%) but the results were not statistically significant. The abortion rates in CC group was 50 %(3/6), CC+ estradiol group was 20 %(1/5) and 14.28 %(1/7) in CC + gonadotropin group.

There is a controversy regarding supplementation of estradiol in CC stimulated IUI cycles. Those who favour supplementation with estradiol, consider it to be helpful in improving endometrial thickness which may or may not improve pregnancy rates.[7,10,19], while others have found no beneficial effect.[16,17] There is a concern regarding whether addition of estradiol interferes with ovulation induction by clomiphene. Therefore, some authors recommend estrogen administration from day 8 of cycle [8,9,16,19] but others have found no effect on ovulation rates hence prefer using estradiol early in the cycle.[7] In the present study, we did not find a beneficial effect of estradiol supplementation of clomiphene cycles, the dose and duration used in current study decreased pregnancy rates marginally. Larger trials are still warranted to establish efficacy of estradiol supplementation in CC stimulated IUI cycles. There is also apprehension regarding E2 supplementation in early pregnancy and when to stop.

There is also a controversy regarding supplementation with gonadotropins in CC stimulated IUI cycles, few authors have found increased pregnancy rates[18] while others have found no beneficial effect.[20,21] The current study attained higher pregnancy rates in gonadotropin supplemented groups but the results were not statistically significant. There is added advantage of lesser abortion rates though marginally. Our study showed few unpleasant side effects with clomiphene. These were not increased with addition of estradiol valerate. Addition of gonadotropins produced a single case of mild ovarian hyperstimulation syndrome and one case of quadruplet pregnancy.

5. Conclusion

Clomiphene citrate is a reasonably good agent for ovulation induction in IUI cycles with overall ovulation rate of 91.3% and pregnancy rate of 17.14%. The present study illustrates that supplementation with estradiol valerate or gonadotropins in CC stimulated IUI cycles did not have any positive effect on the endometrium on the day of trigger. The study failed to show statistical significant difference in pregnancy rates, however abortion rates were decreased marginally in estradiol or gonadotropin supplemented group.

Acknowledgement

With great pride and pleasure, I hereby thank all those who gave me the wisdom and strength to write this thesis. First and foremost, I would like to thank my parents who have dedicated their lives to the sole purpose of my success and prosperity. They are an inspiration for all my efforts and rightful owners of all the success I achieve in life. I feel proud to sincerely express my gratitude to my teacher and guide, Dr. Anjali Tempe who kept on encouraging me to positively go ahead with this work. Her mammoth experience in Obstetrics and Gynaecology has been a great help to me in my project. I am obliged to Dr. Latika Sahu, for her superb guidance and constant encouragement. I am extremely thankful to Dr. Chandan, Dr. Pushpa who has always been kind, supportive and approachable even for the most trivial of my problems. Working under them has always made me feel extremely protected and cared. I would like to thank Dr. Alpana Saxena for timely availability of evaluation of serum estradiol levels which were critical for the conduction of the study. Special thanks to the Biochemistry department for their help in completing thesis work smoothly and systematically. Last but not the least, I wish to thank all the 105 patients who participated in this study. I request the almighty to be merciful to them and grant them the peace and comfort they require.

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