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A Study of Ovulation Induction Using Combined Letrozole and Clomiphene Citrate in Patients Previously Resistant To Clomiphene and Letrozole When Used Alone

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ABSTRACT: Disorders of ovulation accounts for 40% of female infertility. Successful ovulation induction is the only mode of treatment in these patients. Multiple treatments have been recommended for infertility in these patients. Among these clomiphene citrate and letrozole are most common drugs used worldwide as 1st line of therapy and most cost effective. But some patients are found to be resistant to these drugs when used individually. So there is always a need for another cost effective method for these patients. Patients with anovulation or oligovulation who had failed ovulation and positive pregnancy test with use of clomiphene and letrozole used individually, but did not have other significant causes of infertility were given both these two drugs simultaneously for 3 cycles in an out-patient basis and results observed. A total of 80 patients between 20 – 35 years of age were studied during 1 year and 2 months period who were resistant to clomiphene and letrozole when used individually. They were divided in three age groups and each group were sub-divided in 2 sub-groups – normal weight and overweight as per BMI. USG evidence of ovulation and positive pregnancy test were used as outcome. Overall ovulation occurred on 81% patients, and positive pregnancy test found in 43% patients. Result clearly showed benefit of combined regimen used hereamong these two drugs resistant patients if other factors are corrected. So, these two drus regimen can be used as an alternative to other costly and elaborate methods and even can be used as 1st line of treatment in selected patients.

KEYWORDS: anovulation, oligovulation,, letrozole, clomiphenes.

I. INTRODUCTION

Disorders of ovulation accounts for about 40% of female infertility [1]. These disorders are most easily diagnosed and treatable, most common cause of oligo and anovulation among general population and women presenting with infertility is PCOS (6.8%-18%) [2,3]. Ability to induce ovulation and attain pregnancy in anovulatory infertile women remain one of the greatest achievements today. When anovulation is the only factor, prognosis for pregnancy is generally good.

All women with anovulation can be classified as per WHO in four groups [4]. Group 1: hypogonadotrophichypogonadal anovulation- accounts for 5-10% and includes Kallmann syndrome, stress etc, Group 2: eugonadotrophiceuestrrogenic anovulation – accounts for 75-85%, mainly includes PCOS, Group 3: hypergonadotrophic anovulation – accounts for 10-20%, includes premature ovarian failure, Turner's syndrome etc, and Group 4: hyperprolactinemic anovulation. So causes of anovulation are varied. Therefore, few initial investigations are required in every case to rule out specific pathology and other associated factors. Women with irregular menstrual (oligo-ovulatory) cycles or no menstrual periods (amenorrhea or anovulation) are likely to have ovulatory dysfunction. In these women, medications can be used to cause regular ovulation. Before medicines are given, determining the cause of the problem with ovulation should be done. Some possible reasons for ovulation problems include polycystic ovary syndrome (PCOS), low production ofLH and FSH by the pituitary, ovaries that do not respond to normal levels of LH and FSH, thyroid disease, increased levels of the hormone prolactin

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(hyperprolactinemia), obesity, eating disorders, or extreme weight loss and/or exercise. Sometimes the cause cannot be identified for certain. Women with ovulatory dysfunction typically benefit from ovulation induction with fertility drugs. Ovulation induction with fertilitydrugs is also used in patients without ovulatory dysfunction. The goal is to stimulate the ovaries to produce more than one follicle per cycle leading to the release of multiple eggs in the hope that at least one egg will be fertilized and result in a pregnancy. This is called controlled ovarian stimulation (COS), or superovulation, and may be accomplished with medicines taken by mouth or by injection. The most commonly prescribed ovulation drugs are clomiphene citrate (CC), aromatase inhibitors (such as letrozole), and gonadotropins (FSH, LH, human menopausal gonadotropin (hMG), chorionic gonadotropin (hCG)). Other medicines used in ovulation induction include bromocriptine, cabergoline, GnRH, GnRH analogs, and insulin-sensitizing agents.Anovulation or oligoovulation are important characteristics of PCOS. Oligoovulation manifests as irregular menstrual bleeding and is seen in 70% of patients. In PCOS patients with a complaint of infertility, the treatment of choice is induction of ovulation. Multiple treatments have been recommended for infertility in patients with PCOS, including weight reduction, clomiphene citrate, metformin, gonadotropins, pulsed gonadotropin-releasing hormone, gonadotropin-releasing hormone agonists, ovary cauterization, ovarian wedge resection, letrozole, and assisted reproductive technology, such as in vitro fertilization.

Clomiphene is the most commonly prescribed ovulation-induction drug used to stimulate ovulation in women with infrequent ovulation or amenorrhea and it is still considered as first line of therapy for ovulation induction in PCOS. The drug works primarily by competitively inhibiting the binding of estradiol to its receptor in the hypothalamus, thereby releasing the hypothalamus from negative inhibition and allowing increased release of follicle stimulating hormone (FSH) from the pituitary gland. This increase in FSH release enhances follicular growth, increasing the chances of ovulation. The drug has also proven useful for producing multiple ovulation in couples with unexplained infertility. It also is used to induce more than one follicle to develop in conjunction with IUI as a treatment for unexplained infertility and for those who are unable or unwilling to pursue more aggressive therapies. The standard dosage of CC is 50-100 milligrams (mg) of clomiphene per day for five consecutive days. Treatment begins early in the cycle, usually starting on the second to fifth day after menstruation begins although it can also be started without a period if the woman is anovulatory. If ovulation does not occur at the 50-mg dose, CC is increased by 50-mg increments in immediate or subsequent cycles until ovulation happens.Although ovulation rates are in the range of 70-80% the actual pregnancy rates are significantly lower at around 30-40% [5,6] . Clomiphene resistance together with side effects like multi-follicular development and cyst formation are areas of concern. More than 200 mg each day for five days is usually not helpful, and women who do not ovulate on a clomiphene dosage of 200 mg tend to respond better to a different treatment, such as injections of gonadotropins.

Aromatase inhibitors are medicines that temporarily decrease estradiol levels, which cause the pituitary gland to make more FSH. Two medicines, letrozole and anastrozole, are currently FDA-approved to treat breast cancer that occurs after menopause, but mostly letrozole has also been used to induce ovulation in women with ovulatory problems [7,8]. Treatment begins early in the cycle, usually starting on the second to fifth day after menstruation begins although it also can be started without a period if the woman is anovulatory. The typical dose is 2.5–5 mg daily for five days. Studies show that pregnancy rates with aromatase inhibitors are similar to CC rates, and may be better in certain ovulation disorders such as polycystic ovary syndrome (PCOS). It causes ovulation in 60%–80% of patients in clomiphene-resistant patients, it caused ovulation in 62% of cases, and pregnancy occurred in 14.7% of patients [9]. Letrozole does not have any adverse effects on the fetus and is safe [10,11] .Letrozoledecreases the secretion of estrogen both in the brain and in the periphery, Similar to CC, it can be used to cause more than one follicle to develop for fertility treatments with superovulation-IUI, with similar success rates with CC combined with IUI.In women undergoing ovulation induction for the treatment of oligoanovulation, clomiphene citrate has long been the initial drug of choice for first-line therapy, male factor infertility and other disorders where controlled ovarian hyperstimulation has been deemed of value. Though it is in use for more than 40 years, clomiphene has some significant limitations. First, only75–80% of anovulatory women respond to the medication with appropriate follicular growth [12]. Furthermore, sideeffectsof the drug can be psychologically

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difficult to endure (hot flashes and mood swings) and detrimental to fertility (impaired endometrial development and abnormal cervical secretions). The drug has a lengthy half-life, and adverse effects may be cumulative over time [13].Aromatase inhibitors also has the potential to enhance FSH release, not by the inhibiting estradiol-receptor interaction, but rather by inhibition of estradiol synthesis. One such inhibitor, letrozole, was approved for use in 1997 for the treatment of breast cancer. By 2001, it had been used in anovulatory women with great success, and at present the drug is extremely popular among physicians and patients in the treatment of both ovulation dysfunction and for controlled ovarian hyperstimulation because it has fewer side-effects than clomipheneand less chance of multiple gestation. Letrozole is an orally active, nonsteroidal, selectivearomatase inhibitorand hence an antiestrogen. It prevents aromatase from producingestrogens by competitive, reversible binding to the heme of its cytochrome P450 unit. The action is specific, and letrozole does not reduce production of corticosteroids: the drug has a half-life of only 45 hours, and side effects, while similar to those of clomiphene, are far milder and less frequent [14,15]. The most common side effects are sweating, hot flashes, arthralgia (joint pain), and fatigue.Generally, side effects include signs and symptoms of hypoestrogenism. There is concern that long term use may lead to osteoporosis, which is in certain patient populations such as post-menopausal women or osteoporotics, bisphosphonates may also be prescribed[16].

Gonadotropins are recommended in the event of resistance to clomiphene and letrozole. PCOS patients resistant to clomiphene need a lower dose of gonadotropins to stimulate ovulation. Further, the therapeutic index is very narrow because a suboptimal dose will not cause ovulation, and a small increase in dose can potentially cause ovarian hyperstimulation syndrome. Therefore, treatment with gonadotropins requires precise and continuous monitor-ing and serial hormonal and sonographic evaluation.PCOS patients have an increased risk of ovarian hyperstimulation syndrome due to formation of numerous follicles. Different methods have been proposed to prevent ovarian hyperstimulation syndrome in PCOS, but none of them can completely prevent it. Even with all these modalities, patients may still develop ovarian hyperstimulation syndrome that could be fatal [17]. Multiple pregnancies are another adverse effect of these drugs and are considered to be an adverse effect of infertility treatments in general [18,19].

Considering the side effects of gonadotropins, the need for continuous monitoring, risk of ovarian hyperstimulation syndrome, and even risk of death, in this study it was decided to use a combination of letrozole and clomiphene in clomiphene-resistant or letrozole-resistant PCOS patients prior to gonadotropins, to evaluate the effect of this combination on ovulation and pregnancy in such patients.

II. MATERIALS AND METHODS

This study is conducted in my OPD clinic in a Sub-Division town near Kolkata among patients attending for infertility. A total of 80 patients studied during a period of 1 year and 2 months from January 2019 to February 2020. All of the couples have normal male factor; normal HSG and absence of any other significant ill-health in females. Females were aged between 20 – 35 years. All of them have either regular mense or oligomenorrhoea, with or without features of PCOD (ultrasonographic and hormonal), otherwise essentially normal USG, with no other hormonal disturbances e.g thyroid, prolactin etc and all of them have been suffering from infertility for more than 1year with history of anovulation with treatment of both clomiphene citrate and letrozole individually. No gonadotropin will be used, it was predetermined. 5mg of letrozole and 50 mg of clomiphene daily were given daily from day 3 to day 7 of each cycle for upto3 cycles or till she gets pregnants. As these patients already received letrozole and clomiphene separately, so not more than 3 cycles tried, if ovulation failed to occur in 3 cycles it is considered as failure of this protocol. In patients with features of PCOD metformin was added separately along with dietary advice and advice for life style changes. All of them had BMI between 19-24 and 25-29. Ovulation weredetermined by serial folliculometry supplemented by day 21 progesterone level. Pregnancy was confirmed by urinary pregnancy test and presence of heart beat by TVS at 6-7 weeks. In 36 patients 1 or 2 cycles of IUI done when ovulation occurred.

As aim of this study was to induce ovulation in patients resistant to clomiphene and letrozole when used alone, in a cost effective way, patients had been selected carefully after excluding other factors of infertility. Only normal weight and over-weight patients between 20-35 years of age had been included in this study just to give emphasis on resistance factor.

All the steps of the study were explained to the patients, and informed consents were taken from

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them. All patients' information remained confidential. The cost of combination therapy with letrozole and clomiphene was less than that of gonadotropins and surgery, so there were no extra costs to the patients.

III. RESULTS

A total of 80 patients have been taken for study. All have been given the same protocol and continued for 3 cycles unless pregnancy test becomes positive. They have been divided in 3 groups according their age and each group again subdivided into 2 subgroups according to their BMI. According to a 2014 study published the New England Journal of MedicineSuccess rate of clomiphene citrate in ovulation induction is between 48.3%%, successful pregnancy outcome is about 19.5%% and multiple pregnancies between 7.4%,whereas success rate of letrozole in ovulation induction is 61.7%, successful pregnancy outcome is 27.5% and multiple pregnancy rate is 3.2%20. Result of my study shows that success rate of ovulation induction and positive pregnancy test is better with combined therapy than individual use of clomiphene and letrozole, even in those patients who were previously resistant to clomiphene and letrozole. The results relate to the study are shown in Table. 1.

IV. DISCUSSION

Ovulation failure is a major cause of subfertility in both PCOS and non-PCOS patients. Induction of ovulation in the mainstay treatment in these patients except in patients with premature ovarian failure. Several medications and regimens have been used for induction of ovulation, but none has had a significant outcome. Examples of such treatments include clomiphene, letrozole, metformin, gonadotropins, gonadotrophin-releasing hormone agonists, cauterization and wedge resection of the ovaries, and assisted reproductive technologies. Each of them has its own, success rate, failure rate, side-effects and cost factor. Clomiphene is still considered as 1st line of treatment, followed by Letrozole. Clomiphene resistance is defined as three cycles of failure to ovulate or six cycles of ovulation without pregnancy. Recently, letrozole has been proposed as the most effective infertility medication and is being used for induction of ovulation in PCOS21,22. This drug has been recommended as a substitute for clomiphene as first-line treatment to induce ovulation in PCOS. In recent practice, letrozole isconsidered an alternative to clomiphene in ovulatory and nonovulatory infertile women22. This drug can induce ovulation in 62% of clomiphene-resistant patients, and can result in pregnancy in 14.7% of these cases 23. It also does not has any detrimental effect on endometrium, Gonadotropins are also recommended for PCOS patients resistant to clomiphene. Suboptimal doses of gonadotropins can result in no response and high doses can cause ovarian hyperstimulation syndrome, multiple pregnancy and even death. Gonadotropins are costly and it requires intense USG monitoring and hormonal assay, thereby increasing cost further. Laparoscopic ovarian drilling can be an effective treatment in clomiphene-resistant patients, but due to its temporary effects, risk of adhesions, and risk of poor ovarian reserve, its use is still a matter of debate. This technique is more appropriate for patients who fail to ovulate after gonadotropins, or do not accept the cost and adverse effects of these drugs. Considering the extent of adverse effects and costs associated with gonadotropins and surgery, we decided to use a combination of letrozole and clomiphene in our patients, who were resistant to letrozole and clomiphene used alone. The results of our study show that in PCOS patients resistant to clomiphene and letrozole alone, a combination of the two drugs resulted in formation of dominant follicles in 81% of cases and pregnancy in 43% of cases without any incidence of hyperstimulation.

Different studies using letrozole and clomiphene in combination has shown better result than individual drug used alone. Masomeh Hajishafiha et al24 in their study with 100 ladies over 257 cycles found formation of dominant follicle in 82.9% cases with a positive pregnancy rate of 42%, this is clearly superior outcome than when drugs used alone. In another study Mejia RB et al25 taking 70 patients between 18-40 years of age found significantly higher ovulation rate with combined therapy than with letrozole alone

So far few studies using combined letrozole and clomiphenewhich are available compared their outcome with individual use of these 2 drugs and found significant benefit of combined therapy. But study of use of combined therapy among individual drug resistant patients has not been found yet. If other factors of infertility are eliminated, only anovulation and oligovulation in non-obese but clomiphene and/or letrozole resistant patients are concerned the combined therapy showed significant better result.

According to the results of this study, it can be advised that, in PCOS patients resistant to clomiphene and letrozole alone, a combination of

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the two drugs can be tried prior to treatments having more severe adverse effects, or surgery. This combination can also be used as 1st line of therapy in severe PCOS to save time and money.

TABLE :1 Data related to Patients ovulation and positive pregnancy

Age group	BMI	No. of patients	Successful Ovulation	Pregnancy positive	FHS in TVS	Pregnancy Completed 1ST Trimester	Spontaneous pregnancy loss	% of ovulation	% of positive pregnancy	% of pregnancy completed 1st trimester	OHSS	Multiple pregnancy
20–25	18.5 – 24.9 25–29.9	15 18	14 16	10 07	09 06	09 05	01 02	93 89	67 39	0 28	0 0	2 1
26–30	18.5 – 24.9 25–29.9	11 16	09 12	06 05	06 04	05 03	01 02	81 75	55 31	45 19	0 0	1
31–35	18.5 – 24.9 25 – 29.9	08 12	06 08	03 03	03 02	03 02	00 01	75 67	38 25	38 16	0 0	
Total		80	65	4	30	27	07	81	43	34	0	4

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