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Original Research Article

Efficacy and Safety of Letrozole Add-on Injection HMG on Ovulation and Pregnancy: Comparison with Letrozole Alone

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Abstract: ***Background:*** Nowadays, Infertility is increasing worldwide. Many regimens are used in the treatment of infertility. Among them, a combination of oral aromatase inhibitor drugs and gonadotropin is recommended as a very effective regimen for inducing ovulation. But there is a scarcity of related data in the Bangladesh context. The present study was designed to compare the effect of letrozole and the combination of letrozole with low-dose intramuscular injection of HMG on ovulation and pregnancy. ***Methods:*** This randomized controlled trial was conducted at the department of Obstetrics and Gynaecology in Rajshahi Medical College Hospital from June 2020 to May 2021. A total of 95 patients with a history of infertility were selected as the study population according to inclusion and exclusion criteria. Written informed consent was obtained from each patient. All of them were randomly distributed into two groups, group-A, and group B, by the flipping coin method, where group-A (Experimental group) patients received letrozole with low dose intramuscular injection of HMG (45 patients) and group B (Control group) patients received Letrozole only (50 patients). Relevant investigations were done, along with scheduled follow-ups. ***Results:*** Mean age of the study patients was 26.86±3.71 (SD) years, whereas the majority were ≤30 years (74.7%). Age was statistically similar among the patients of both groups. The endometrial thickness, follicular diameter, and a number of mature follicles were statistically higher among patients who received letrozole with low dose intramuscular injection of HMG than the patients who received only letrozole. Maximum follicular diameter ≥18mm (91.1%) in group-A among them (60%) achieved in 1st Cycle. Maximum endometrial thickness was achieved in the first Cycle in both groups (60% vs. 32%) among group-A patients. The positive pregnancy rate was also higher among group-A patients (37.8% vs. 22.0%) in 1st Cycle. Ovarian Hyperstimulation Syndrome was observed only in group-A patients. ***Conclusion:*** The study concluded that a combination of letrozole and low dose HCG was more successful than only letrozole therapy in inducing ovulation and pregnancy. But extensive studies should be conducted before validating.

Keywords: Letrozole, Ovulation, Pregnancy, Polycystic Ovary Syndrome.

INTRODUCTION

Infertility is defined by the International Committee for Monitoring Assisted Reproductive Technology (ICMART) and WHO as the 'failure to achieve a pregnancy after 12 months or more of regular unprotected sexual intercourse. According to the Centers for Diseases Control (CDC), 1.5 million women in the US (6%) are infertile [1]. Based on a survey performed in developed countries, the World Health Organization (WHO) estimates that ovulatory disorders account for 25% of infertility among women (Weiss and Clapauch 2014). The most common cause of female infertility is ovulatory dysfunction, in which various hormonal factors interfere with the complex sequence of hormonal events required to trigger ovulation. Ovulation is a physiologic process defined by the rupture and release of the dominant follicle from the ovary into the fallopian tube, where it has the potential to become fertilized.

Ovulation is the third phase of menstrual Cycle and is triggered by the surge of luteinizing hormone (LH) surge from the pituitary. The ovulation process is regulated by fluxing gonadotropic hormone (FSH/LH) levels. LH acts on preovulatory follicles to stimulate specific molecular and cellular events that mediate the release of a mature female germ cell, the oocyte (egg) [2]. Follicular release occurs around 14 days prior to menstruation in a cyclic pattern if the hypothalamic-pituitary-ovarian axis function is well regulated. Problems can occur at any point in this pathway (hypothalamus, pituitary, and ovary) and can lead to failure to ovulate. The most common cause of chronic ovulatory dysfunction is a polycystic ovarian syndrome,

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or PCOS, which interferes with ovulation at multiple points [3].

Clomiphene citrate (CC) was once considered the drug of choice for first-line treatment of anovulatory dysfunction [4]. CC had many problems- antiestrogenic effects on the endometrium, cervical mucus, and prolonged accumulation in tissues leading to prolonged depletion of estrogen receptors. This could result in hot flushes and perimenopausal symptoms, in addition to the above side effects. Letrozole, a selective, reversible third-generation aromatase inhibitor, was introduced into infertility practice in the year 2000 and has been used for ovulation induction with no deleterious effect on the endometrium. It acts by inhibiting the conversion of androgens to estrogen, creating a hypoestrogenic environment similar to the central reduction of negative feedback done by CC [5].

Letrozole may be superior or at least equal to CC in ovulation (60-80%) and pregnancy rates in anovulatory PCOS women. Also, it induces ovulation in 62%, with a pregnancy rate of 14.7% of patients with improper response to clomiphene or CC resistance [6]. In addition, Letrozole is rapidly eliminated from circulation due to a shorter half-life with monofollicular growth and lower preovulatory estradiol (E2) level with thicker endometrium. Letrozole does not deplete estrogen receptors and has no antiestrogenic peripheral actions.

Therefore, it has no detrimental effect on endometrial receptivity or cervical mucus quality with safety for the fetus. Human menopausal gonadotropin (HMG) was first successfully extracted from the urine of post-menopausal women in 1950. Serono registered the first HMG preparations in Italy in 1950, but these were impure in terms of protein content and did not have standardized proportions of FSH and LH. Subsequent preparations contained equal proportions of FSH and LH [7]. Human menopausal gonadotropin (HMG) can secrete gonadotropin to promote follicle maturation, stimulate ovulation, and accelerate the development of the corpus luteum [8].

So far, few studies have been comparing letrozole and a combination of letrozole with low dose intramuscular injection of human menopausal gonadotropin on ovulation and their pregnancy outcome in Bangladesh. Thus, the study aimed to see a comparison.

Objectives

General objective:

To determine the comparative superiority of letrozole to letrozole with low dose intramuscular injection of HMG in patients' ovulation and their pregnancy outcome

Specific objectives:

- To assess the follicle number, size, endometrial thickness, and pregnancy rate in both group
- To compare pregnancy rate and outcome of pregnancy between the two groups
- To observe pregnancy outcomes, including multiple pregnancy rates, ovarian hyperstimulation syndrome, and miscarriage
- To assess the socio-economic condition of the participants

LITERATURE REVIEW

Fertility is a key element of reproductive health, and infertility is recognized as a global public health issue.

Infertility:

Infertility is defined by the International Committee for Monitoring Assisted Reproductive Technology (ICMART) and WHO as the 'failure to achieve a pregnancy after 12 months or more of regular unprotected sexual intercourse. The evaluation may be initiated sooner in patients with risk factors for infertility or if the female partner is older than 35. The Centers for Disease Control and Prevention of the United States emphasizes that infertility is more than a quality-of-life issue, with considerable public health consequences, including psychological distress, social stigmatization, economic strain, and marital discord. The etiology of female infertility is as follows:

- Ovulatory disorders - 25%
- Endometriosis - 15%
- Pelvic adhesions - 12%
- Tubal blockage - 11%
- Other tubal/uterine abnormalities - 11%
- Hyperprolactinemia - 7%

Cervical factors are also considered minor, although they are rarely the sole cause [9].

Treatment for ovulatory dysfunction:

Depending on the etiology of ovulation disorders, treatment can vary. Polycystic Ovarian Syndrome or PCOS is the most common cause of ovulation disorder, and it can be treated with lifestyle changes (diet and exercise), pharmacological therapies (oral agents such as clomiphene citrate, letrozole or metformin, or injectable agents such as gonadotrophins), surgical therapy (laparoscopic ovarian surgery) or In Vitro Fertilization (IVF) [10].

Below discussed are the common drugs used for ovulation induction [11].

Estrogen antagonists:

Clomiphene Citrate

Clomiphene citrate (CC) is a nonsteroidal estrogen-receptor modulator that indirectly induces

ovulation. It has been used for decades in ovulation induction and assisted reproduction. It is most commonly used as a first-line ovulation induction treatment in PCOS. The mechanism of action of CC is that it antagonizes the negative feedback of estrogen at the hypothalamus with a consequent increase in ovarian stimulation by endogenous gonadotropin and subsequent rises in circulating follicle-stimulating hormone (FSH) and luteinizing hormone (LH) levels. Clomiphene has drawbacks, including its overall poor efficacy and an undesirable side-effect profile, including hot flashes, mood swings, headaches, and visual disturbances. It also has genotoxic, cytotoxic, embryotoxic, and teratogenic effects.

Letrozole

Letrozole, an aromatase inhibitor, has been demonstrated to be an effective ovulation induction agent in patients who exhibit CC resistance and in obese patients with PCOS. This drug reduces serum estradiol secretion from the ovary and releases negative feedback of estrogen secretion from the hypothalamus and pituitary gland. The reduced circulatory estradiol increases the stimulation of FSH, so folliculogenesis leads to ovulation. The use of letrozole is associated with significantly higher ovulation and live birth rates in obese women with PCOS than the use of CC. For this reason, letrozole has become the first-line agent for many providers when treating obese patients with PCOS with anovulatory infertility. It is also effective in patients with CC resistance.

Gonadotropins

- Urinary FSH
- Recombinant FSH and LH (Human Menopausal Gonadotropin or HMG)
- Urinary hCG (Chronic human Gonadotropin)
- Urinary HMG
- Pure FSH
- Urinary hCG

Human Menopausal Gonadotropin (HMG)

HMG is a combination of recombinant FSH and LH. It was initially extracted from the pituitary gland and then urine of post-menopausal women. Since then, it has been used for anovulatory treatment in patients with hypogonadotropic amenorrhoea and those with polycystic ovary syndrome (PCOS). However, a major problem with gonadotropin induction of ovulation has been the high incidence of multiple follicle development with the attendant increased risk of multiple pregnancies and of ovarian hyperstimulation syndrome. These risks can be limited by the use of a low-dose 'step-up' regimen, particularly in patients with PCOS. Low-dose gonadotropin regimens are now accepted as best practice in the case of clomiphene-resistant PCOS.

Comparison between letrozole and combination of letrozole with low dose HMG in ovulatory disorders [12].

Clomiphene Citrate (CC) is usually the drug of choice for ovulation induction. However, when it comes to PCOS, the most common disease where ovulation induction is necessary, these patients tend to become CC resistant or show stunted follicular growth. Unwanted pregnancy outcomes and pregnancy rates are also common with CC use.

These patients are then treated with continuous HMG or gonadotropin. But, this treatment can become expensive, multi follicular development leading to multiple pregnancies, OHSS. Gonadotropins used in combination with CC or letrozole decrease the dose required for optimum stimulation and make it more cost-effective. It has been found that ovulation induction with sequential CC - gonadotropins results in a fecundity double that of CC alone and equal to gonadotropins alone or concurrent with clomiphene. This, therefore, reduces the requirement for gonadotropin. At the same time, the use of letrozole induces folliculogenesis by releasing the Hypothalamo-Pituitary axis from the tonic inhibitory effect of estrogen & by augmenting gonadotropin secretion. It helps follicular development without adversely affecting the peripheral estrogen-sensitive tissues. Adding a few ampoules of gonadotropin at intervals along with letrozole increases FSH at the follicular receptor level & produces good-quality oocytes. Combining Letrozole with gonadotropins for controlled ovarian stimulation not only reduces the dose of gonadotropin required for optimal follicle development but also improves the ovarian response to the same in poor respondents. The results of a prospective pilot study have shown that letrozole has a significantly higher pregnancy rate than CC in gonadotropin-combined IUI cycles. These researchers believed their favorable outcomes could be attributed to a thicker endometrium and a lower estradiol level.

Relevant studies regarding the "Efficacy and Safety of Low Dose Intramuscular injection of Human Menopausal Gonadotropin on Ovulation and Pregnancy Outcome for the Treatment of Infertility: A Comparison with Letrozole":-

The effects of letrozole and human menopausal gonadotropin (HMG) in the treatment of patients with polycystic ovary syndrome (PCOS) resistant to clomiphene citrate. A total of 96 subjects with included. They observed that there was no significant difference in the number of ovulation cycles between the 2 groups (53.6% vs. 64.7%). The number of mature follicular cycles in the HMG group was higher than that of the letrozole group and abortion rate (6.2% vs. 10.4%). There was no significant difference in the endometrial thickness between the 2 groups on the day of HCG injection [(9.1±0.2)mm vs. (10.7±1.6) mm]. The incidence of ovarian cysts was lower than

that of the HMG group, and ovarian hyperstimulation was found in 12.5% of the subjects receiving HMG. The study concluded that Letrozole-induced ovulation could obtain ovulation rate and pregnancy rate similar to gonadotropin but reduce the risk associated with treatment [13].

Yu *et al.,* (2019) evaluated the effects of Letrozole plus human menopausal gonadotropin (HMG) on ovarian stimulation (OS) of intrauterine insemination (IUI) cycles. A total of 1,005 IUI cycles were included in this study. Couples underwent natural cycle (NC) IUI (n=150) or IUI after OS with letrozole (n=207) or IUI after OS with letrozole + HMG (n=648). The results showed the clinical pregnancy rates were 9.0%, 13.0%, and 17.0%, and the live birth rates were 7.0%, 9.0%, and 14.0% in the NC, letrozole, and letrozole + HMG IUI groups, respectively. Twin pregnancy rates were higher in letrozole+HMG IUI groups than in the other two groups. They concluded that The letrozole + HMG protocol of OS in IUI could improve follicular development, increase the thickness of the endometrium, and significantly increase the live birth rate, but not significantly increase the multiple pregnancy rate [8].

Carried out a study to evaluate the clinical outcome of using letrozole alone or with gonadotropin as first-line ovulation induction in anovulatory infertile polycystic ovary women. A total of 80 infertile polycystic ovarian syndromes (PCOS) women were recruited. The women are sorted into two groups according to the dominant follicle size on day 7 or 8: Group A (letrozole only group) and Group B (letrozole plus gonadotropin). In our study, the overall pregnancy rate was (67.5%), and the ovulation rate was 91.3%. The ovulation rate was significantly higher in Subgroup A than in B (97.9% vs. 81.3%). The pregnancy rate was higher in Subgroup A (72.9% vs. 59.4%), but it was statistically insignificant. The number of follicles was significantly higher in subgroup B.

However, pregnancy outcome was similar in both groups. They suggested that both Letrozole alone or in combination with gonadotropin as a first-line treatment in PCOS may be reasonable since this approach in patients stimulated for Intra-Uterine Insemination. Ovulation induction was done by letrozole in one group and combined letrozole and gonadotropins in another group. Monitoring was done by TVS for 3 cycles. They found Mean follicular number was 1.28±0.94 in letrozole and 3.37±0.87 in combined letrozole and gonadotropins. No. Of follicles >18mm 12 patients in letrozole and 50 patients in combined letrozole and gonadotropins. Pregnancy/cycle was 12/77 (5.19%) in letrozole and 28/65 (14.3%) in combined letrozole and gonadotropins. They concluded that the number of mature follicles and pregnancy rate is higher in the combined letrozole and gonadotropins group than in letrozole alone [14].

MATERIALS AND METHODS

Study design: Randomized Clinical Trial.

Study place: Rajshahi Medical College Hospital, Rajshahi.

Study period: 12 Months (June 2020 to May 2021).

Study population: Infertile women attending Gynae and Obs Department (Indoor & Outdoor) of Rajshahi Medical College.

Sample method: Convenient sampling was done in this study.

Sample size

The sample size was calculated by using the following formula:

$$n = \frac{\{P_1(100-P_1)+P_2(100-P_2\}}{(P_1-P_2)^2} \times (Z_\alpha + Z_\beta)^2$$

Where,
n = estimated sample size
Zα = Standard normal variate for level of significance: 1.96 Zβ = standard normal variate for power: 1.64 at 90% power
P1 = Proportion of patients having clinical pregnancy after letrozol therapy = 30.8% (Chen *et al*., 2016).
P2 = Proportion of patients of having clinical pregnancy after combined letrozol and HMG therapy =55.7% (Chen *et al*., 2016).

Therefore,

$$n = \frac{\{30.8(100-30.8)+55.7(100-55.70)\}}{(30.8-55.7)^2} \times (1.96+1.64)^2$$

n= 96.12=97 (approx.)

So, the calculated sample size was 97 in each group, with a total of 194. But due to resource constraints and the pandemic situation, a total of 95 patients were included in this study- one group containing 50 patients and another group of 45.

Inclusion Criteria

- Age: < 40 years
- History of infertility for at least 2 years and with either of the following problems (unexplained infertility, polycystic ovarian syndrome)
- Normal uterus and patent tubes on the saline infusion sonography
- Normal semen analysis of husbands

Exclusion Criteria

- Female with bilateral tubal block
- Severe endometriosis

- Patients with a history of ovarian surgery
- Pelvic adhesion
- Patients complicated with liver, kidney, or thyroid dysfunction
- Patients who will not receive treatment after enrollment according to the established regimen or who gave up amid treatment.
- Male with sperm count <10 million / ml

Data processing and analysis

Following data collection, the collected data were assessed for completeness, accuracy, and consistency before the commencement of the analysis. Data analysis was carried out by using SPSS version 23 (IBM Corp., Armonk, NY). Exploratory data analysis was carried out to describe the study population where categorical variables were summarized using frequency tables while continuous variables were summarized using measures of central tendency and dispersion, mean and standard deviation. Qualitative or categorical variables were described as frequencies and proportions. To determine the association between categorical variables, a chi-square test was done. To determine the difference between continuous variables, an independent student t-test was done. A level of $p< 0.05$ was considered statistically significant.

Ethical consideration

The researcher was duly concerned about the ethical issues related to the study. In this study, the following criteria were followed to ensure maintaining ethical values.

- Formal ethical clearance was taken from the ethical review committee of the Rajshahi Medical College Hospital for conducting the study
- Confidentiality of the person and the information was maintained, observed and unauthorized persons didn't have any access to the data
- Informed written consent was taken from the subject
- The content of the consent requirements was as such:

i. Explanation of the nature & purpose of the study
ii. Explanation of the procedure of the study
iii. Explanation that they have the right to refuse, accept & withdraw to participate in the study

- The participants didn't gain financial benefit from this study

RESULT

The randomized clinical trial was conducted among infertile women attending Gynae and Obs. Department of Rajshahi Medical College Hospital. A total of 95 patients diagnosed with infertility were taken in this study. Patients were divided into group A (Experimental group) and group B (Control group). Group A patients received Letrozole with HMG for 3 months, and group B received Letrozole for 3 months.

Table 1: Age distribution of study population (n=95)

Age	Total n (%)	Group A n (%)	Group B n (%)	P value
Mean age (years)	26.86±3.71	27.56±3.78	26.24±3.57	0.085*****
Age group				
≤30 years	71 (74.7%)	31 (68.9%)	40 (80.0%)	0.213†
>30 years	24 (25.3%)	14 (31.1%)	10 (20.0%)	
Total	**95 (100.0%)**	**45 (100.0%)**	**50 (100.0%)**	

Group A: Letrozole with HMG
Group B: Letrozole
*Student's t-test done to measure the significance
†Chi-square test was done to measure significance

The mean age of the study population was 26.86±3.71 years. The majority lies in ≤30 years of age (74.7%). There was no significant difference between groups regarding age.

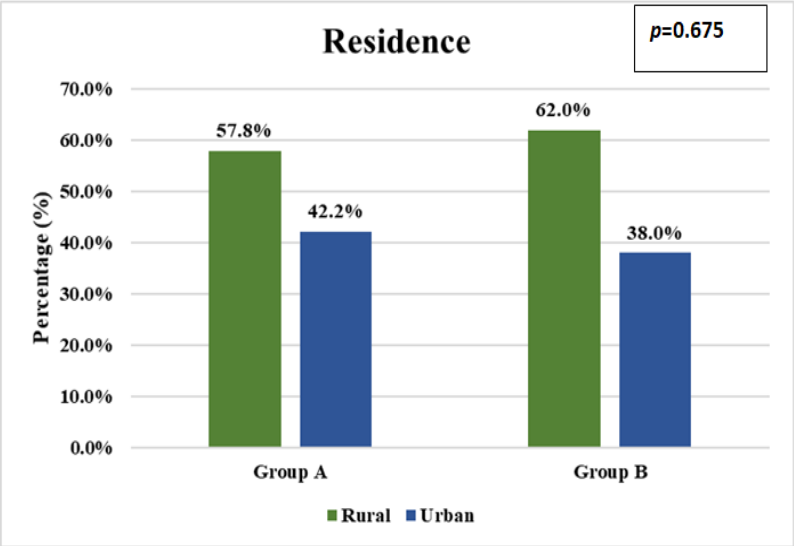

Figure 1: Distribution of study population according to residence (n=95)
Group A: Letrozole with HMG
Group B: Letrozole

The majority of participants in both groups were from rural areas (57.8% in group A and 62.0% in group B). The difference was statistically non-significant (p=0.675). The chi-square test determined *P* value.

Table 2: Demographic characteristics of the study population (n=95)

Demographic characters	Total (n=95)	Group A (n=45)	Group B (n=50)	*P* value*
Height (m)	1.56±0.05	1.53±0.05	1.58±0.04	<0.001
Weight (Kg)	63.66±8.16	66.46±9.41	61.13±5.86	0.001
BMI (Kgm^{-2})	26.24±3.17	28.35±3.03	24.34±1.78	<0.001
Infertility duration (years)	3.18±1.07	3.42±1.06	2.96±1.05	0.035

Group A: Letrozole with HMG
Group B: Letrozole
***p* value measured by Student's t-test**

The mean BMI of the study population was 26.24±3.17 Kgm-2 with infertility for 3.18±1.07 years.

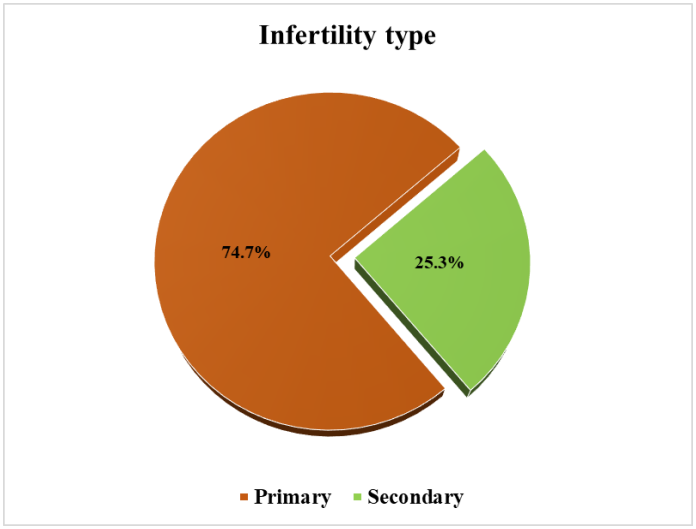

Figure 2: Type of infertility in study population (n=95)

About 74.7% of participants had primary infertility, and the rest, 25.3% had secondary infertility.

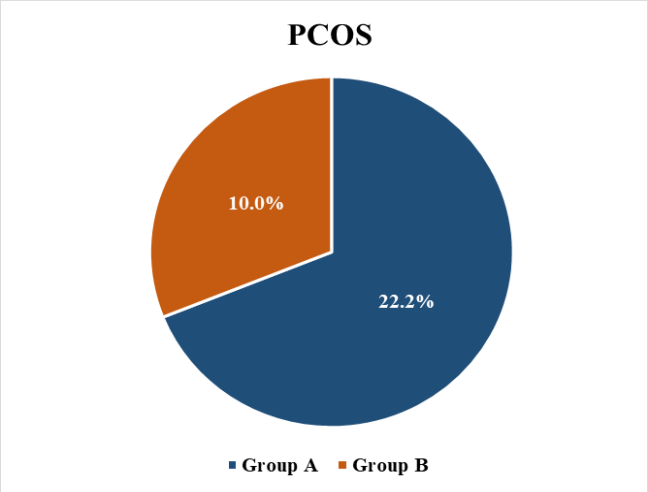

Figure 3: Presence of Polycystic ovary syndrome in study population (n=95)
Group A: Letrozole with HMG
Group B: Letrozole

About 22.2% of group A patients and 10.0% of group B patients had polycystic ovary syndrome in the study.

Table 3: A comparison of follicular and endometrial conditions of women receiving different induction protocols in the study (n=95)

Variables	Group A (n=45)	Group B (n=50)	*P* value
Mean follicular number	2.04±1.13	1.02±0.74	<0.001†
Follicular diameter ≥18 mm	41 (91.1%)	35 (70.0%)	0.002*
Maximum follicular diameter (mm)	19.44±1.70	18.39±2.03	0.012†
Endometrial thickness (mm)	8.88±0.45	8.47±0.87	0.006†
OHSS	2 (4.45%)	0	0.132*

Group A: Letrozole with HMG
Group B: Letrozole
***p value measured by chi-square test**
†p value measured by Student's t-test OHSS: Ovarian hyperstimulation syndrome.

On HCG injection day, both the endometrial thickness (8.88±0.45), follicular diameter (19.44±1.70), and the number of mature follicles (2.04±1.13) of Group A were significantly higher than those of Group B ($p<0.05$) Group A suffered from OHSS, incidence rate (0.132).

Table 4: Maximum endometrial thickness achieved in different Cycles of therapy (n=95)

Cycle	Group A (n=45)	Group B (n=50)	*P* value*
1st Cycle	27 (60.0%)	16 (32.0%)	0.006
2nd Cycle	7 (15.6%)	14 (28.0%)	0.873
3rd Cycle	2 (4.4%)	7 (14.0%)	0.407

Group A: Letrozole with HMG
Group B: Letrozole
***P value measured by chi-square test**

A comparison of responses regarding maximum endometrial thickness achieved in different Cycles showed that 60% in group A and 32.0% in group B reached maximum endometrial thickness after 1st Cycle, which is statistically significant (p=0.006).

Table 5: Effects of different regimens on pregnancy (n=95)

Pregnancy outcome	Group A (n=45)	Group B (n=50)	*P* value*
Single pregnancy	20 (44.4%)	13 (26.0%)	0.059
Multiple pregnancies	4 (8.9%)	3 (6.0%)	0.590
Miscarriage	2 (4.4%)	0	0.132

Group A: Letrozole with HMG
Group B: Letrozole
***P value measured by chi-square test**

A comparison of pregnancy outcomes between the groups was made. Single and multiple pregnancy rate was found to be statistically similar in group A and B (40.0% vs 22.0%; p=0.057 and 8.9% vs 6.0%; p=0.590). There were 2 miscarriages in group A.

DISCUSSION

Throughout history, human societies have been preoccupied with procreation, which is the major aspect of maintaining the survival and perpetuation of the human race. Infertility has been a well-known cause of disharmony and disgrace among couples (mark of displeasure, grounds for divorce, and in the extremes, suicide) since time immemorial. Infertility is one year of unprotected intercourse without pregnancy [15]. Out of all infertility cases, 30% are unexplained [16]. The therapy for unexplained infertility due to the absence of a correctable abnormality is usually empiric. The proposed treatment regimens include intrauterine insemination (IUI), ovarian stimulation with oral or injectable medications, a combination of IUI with ovarian stimulation, and assisted reproductive technologies (ARTs) [12]. Over the last years, letrozole, an orally active potent aromatase inhibitor, has been widely used for ovulation induction. It has a very good effect on women with failure or resistance to clomiphene citrate. Moreover, Gonadotropin preparations, either urinary or recombinant follicle-stimulating hormone (rFSH), have been used to stimulate ovulation in women who failed to respond to CC (Clomiphene citrate) [17]. But it may lead to the following drawbacks: cost, the risk of multiple pregnancies, hyperstimulation, and cycle cancellation [18]. Taking into consideration the drawback effect of CC versus the benefits of letrozole mentioned above on one hand, and on the other hand, the previous studies showed that cotreatment with letrozole significantly reduced the FSH dose required during controlled ovarian stimulation (COH) in women with unexplained infertility; we proposed the possibility of using it alone or in combination with gonadotropins for ovarian induction for a productive approach in women with infertility.

The randomized clinical trial of 95 infertile women attending the Department of Gynae and Obs., Rajshahi Medical College Hospital, was studied. Among them, 45 (47.4%) received Letrozole with low dose intramuscular injection of HMG (Group A), and the rest, 50 (52.6%), received Letrozole only (group B). There is no significant difference in the age, where the mean age is 27.56±3.78 years in Group A versus 26.24±3.57 years in Group B (p=0.085). Furthermore, 68.9% in group A and 80% of patients in group B were aged ≤30 years. Fadia J Alizzi studied a statistically similar age group of patients between group A and group B (24.4±2.0 years and 28.6±4.5 years; p<0.001) in their similar study [19]. In the study of Chen et al., the mean age of the LE (letrozole) group was 26.4±4.2 years, and the LE+HMG groups were 27.7±5.2 years (Chen et al. 2016). The majority in both groups hailed from rural areas (57.8% in group A and 62% in group B). A majority believe the predominant religion of participants in group A (88.9%) and group B (92.0%). The major religion in Bangladesh is Islam (90.4%). The majority of patients were housewives (72.6%), followed by 16.8% businessmen, and most had a monthly family income of 10,001-15,000 BDT (41.1%).

About 74.7% of infertility was categorized as primary and 25.3% secondary. Mean height, weight, and BMI were 1.56±0.05 m, 63.66±8.16 Kg, and 26.24±3.17 Kgm-2. The mean infertility duration of the study population was 3.18±1.07 years with a range of 1 to 5 years which is 3.42±1.06 years in group A, and 2.96±1.05 years in group B. A similar previous study in India showed the mean duration in the LE group as 3.24±1.0 years, while among the LE+HMG group, it was 3.4±1.0 years (Chen et al. 2016). In China's study, the mean duration was respectively 2.6±1.9 years and 2.5±1.8 years, and they also found primary infertility to be the most common type of infertility in all the groups [20].

On HCG injection day, a number of mature follicles≥18 mm (2.04±1.13), follicular diameter (19.44±1.70), and the endometrial thickness (8.88±.45) of the LE+HMG group (group A) were significantly higher than those of the other group (p<0.05). Only group A (4.45%) suffered from OHSS. Malhotra *et al*., reported that the mean number of dominant follicles was higher in the letrozole-HMG group (group B) (3.22±0.33) in comparison to the letrozole group (group A) (2.89±0.23) (p<0.001) (Malhotra et al. 2012). They also reported that the mean endometrial thickness was significantly higher in letrozole-HMG combination (group B) versus letrozole group (group A). In this study, letrozole was associated with fewer development of mature follicles (70.0%) compared to the LE+HMG combination (91.1%), but it is comparable with the study that showed ovulatory rate by letrozole was 75% and 87.5%, respectively [12]. Maximum follicular diameter 60% in group A and 42% in group B in 1st Cycle (P=0.080). Maximum endometrial thickness achieved in different Cycles showed that 60% in group A and 32.0% in group B reached maximum endometrial thickness after the 1st Cycle, which is statistically significant (p=0.006). Another study by Mitwally showed that treatment with LE+gonadotropins required less amount of gonadotropins than gonadotropins alone, and the number of follicles≥18mm was higher in the combined LE+gonadotropins group (3.3 vs. 1.9, respectively) [21].

The clinical pregnancy rate was highest in the LE+HMG group (group A) (37.8%) than in LE only (group B) (22.0%) in the first Cycle with statistical, not significance (p=0.092). Malhotra et al. also found a similar kind of result in their study, with the pregnancy rate in letrozole+HMG combination being significantly

higher (35.48%) than letrozole alone group (10.81%) (p=0.013). A previous study suggests that adding letrozole to FSH can reduce the dose of FSH and medication expense without detrimental effects on endometrial thickness and pregnancy [14]. Pregnancy outcomes showed that single pregnancy was observed in 44.4% of patients in group A and 26.0% in group B, and multiple pregnancies in 8.9% in group A and 6% of group B. There were two early miscarriages in group A. No statistical significance was seen regarding miscarriage and single or multiple pregnancies in between groups in a current study like previous one [8]. In a China study, 12% LE group and 15% LE+HMG group had single pregnancy after induction [20]. In ovulating women, letrozole combined with HMG can significantly improve the live birth rate [22]. In summary, the regimen using LE in combination with a low-dose injection of HMG every other day had a satisfactory effect on ovulation, and a high clinical pregnancy rate, which provides a promising option for the treatment of patients with infertility. The regimen has many advantages, but ultrasound and related laboratory examinations are needed to monitor various indices during the treatment, thus increasing the cost of treatment.

CONCLUSION

This study observed that outcome of ovulation induction was significantly more successful through a combination of letrozol and low-dose HCG than only letrozole. Endometrial thickness, follicular diameter, and the number of mature follicles were statistically higher among patients who received letrozole with low-dose HMG. Moreover, the pregnancy occurrence rate was also significantly higher. However, before concluding, further clinical study is recommended.

LIMITATIONS

- All samples were collected from a single site
- The sample size was not representative of generalizing the findings
- Follow-up ultrasound and related laboratory examinations are needed to monitor various indices during the treatment, so treatment cost is higher.

RECOMMENDATION

- Further RCT trials with a larger sample size are recommended

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