Original Article

DOI: 10.7860/JCDR/2021/45786.15138

Effectiveness of Letrozole and Human Chorionic Gonadotropin Injection in Ovulation Induction: A Cohort Study

Obstetrics and Gynaecology Section

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ABSTRACT

Introduction: Ovulation stimulation followed by timed intercourse or Intrauterine Insemination (IUI) is widely used for treatment of anovulatory infertility.

Aim: To compare the effectiveness of Letrozole (LE) alone versus LE and human chorionic gonadotropin injection in ovulation induction and pregnancy rates in women undergoing ovulation induction/follicular tracking in Enugu, Nigeria.

Materials and Methods: The longitudinal cohort study was carried out in University of Nigeria Teaching Hospital and Livingston Specialist Gynaecological Hospital in Enugu, Nigeria. Study population were women coming for ovulation stimulation/follicular tracking. Ovulation was confirmed by ultrasound evidence of ovulation with a progesterone level of greater or equal to 25 nmol/L on day 21, positive pregnancy test/ultrasound detection of a gestational sac. Patients were consecutively assigned to group A or B. Group A received LE only for the induction while group B received 10000 IU of human chorionic gonadotropin injection in addition to LE. A total of 5 mg of LE was given daily from day 3 to day 7. The primary outcome measured was the number of ruptured/crenated follicles on either arm while the secondary outcomes were the number of Luteinised Unruptured Follicles (LUF) and pregnancy rates. The Statistical analysis was performed using the Statistical Package for the Social Sciences version 21.0 software (SPSS Inc., Chicago, IL, United States).

Results: A total of 50 women were in each arm of the study. There was no significant difference in age and parity between the two arms. There was no significance difference between the period of infertility and the number of the previous cycles of ovulation stimulation. (p=0.444 and 0.526, respectively). Ovulation was significantly associated with HCG injection (p=0.001). However, there was no statistical significance between both arms regarding the number of LUF (p=0.216). HCG injection was significantly associated with pregnancy. Subjects who took HCG injection were over two times more likely to become pregnant than those without HCG injection (OR=2.488, 95% CI for OR=1.057-5.857, p=0.037).

Conclusion: This study showed that both the ovulation rate and pregnancy rate are significantly improved when human chorionic gonadotrophin injection is given after ovarian stimulation.

Keywords: Infertility, Polycystic ovary, Pregnancy

INTRODUCTION

Infertility is commonly defined as the failure to achieve conception after a minimum of 12 months of unprotected intercourse [1]. Polycystic Ovary Syndrome (PCOS) is the most common condition that leads to anovulatory infertility in young women. There has been a significant increase in the number of women that present with PCOS and leading to increased scientific examination towards understanding the syndrome [2]. The syndrome has several clinical manifestations, and anovulatory infertility is one of the most common [3].

Ovarian stimulation followed by timed intercourse or IUI is used for the treatment of various forms of infertility, like anovulatory infertility, unexplained infertility, male-factor infertility, and other disorders of infertility [4]. This treatment modality is used when the female partner has one or both tubes patent and some ovarian function, and the male partner has active and motile sperm cells [5]. For infertile couples that meet these criteria, combining ovarian stimulation with IUI can be an effective means of achieving pregnancy.

Clomiphene Citrate (CC) was the first commonly used ovulation induction drug in clinical practice. It has structural similarities to oestrogen and is a Selective Oestrogen Receptor Modulator (SERM). It is a competitive inhibitor of oestrogen, and its actions results in increased pituitary gonadotropin hormones secretion [6]. Use of CC in ovulation induction gives a 60-85% ovulation rate, and results in a 10-20% pregnancy rate per cycle [7]. CC has anti-oestrogenic effects on the endometrium; this results in a poor pregnancy rate and can lead to a significant rate of early pregnancy loss due to Oestrogen Receptor (ER) depletion [8]. The long half-life of CC also leads to its accumulation in the body and prolongs these adverse effects [6].

More recently, LE, an aromatase inhibitor, used for the treatment of breast cancer, has been widely used for ovulation induction. LE prevents the conversion of androgen to oestrogen by inhibiting aromatase activity in-vivo. This releases the negative feedback effect of oestrogen on the hypothalamus and pituitary leading to release of gonadotrophins; causing follicular growth and subsequent ovulation [9]. LE induces ovulation in about 60-80% of patients, and more rapidly excreted from the body than CC [10]. LE has been shown to have good ovulation rate of about 62% in CC-resistant PCOS women, and leads to pregnancy in 14.7%; and, does not have any adverse effects on the fetus and is safe [11].

There are numerous parameters which can affect treatment result after ovarian stimulation and timed-intercourse or IUI [12]. Parameters are age of the woman, semen parameters and method of semen preparation [13], the quantity of inseminations done [14,15] as well as the quantity of preovulatory follicles, period of infertility, and kind of infertility and quantity of preceding management cycles [16]. It has been recommended that the timing of insemination in IUI cycles with ovulation is most likely the most significant variable affecting the success of treatment [16].

This study aimed at investigating the effectiveness of LE versus LE and human chorionic gonadotrophin on ovulation and pregnancy rates in a cohort of women in Enugu, Southeast Nigeria.

MATERIALS AND METHODS

This was a prospective longitudinal cohort study. The study was carried out in University of Nigeria Teaching Hospital, Enugu and Livingston specialist Gynaecological Hospital, in Enugu between May 2019 to April 2020. The study was conducted after obtaining ethical clearance from the Ethics committee of the University of Nigeria Teaching Hospital, Enugu, Nigeria (NHREC/05/01/2008B-FWA00002458-1RB00002323). The study population was drawn from women coming for ovulation stimulation/follicular tracking who met the eligibility criteria.

Inclusion criteria: Women with ovulation factor infertility (primary or secondary), Women with normal HSG result, women whose husband had a normal semen analysis, women below 40 years of age were included.

Exclusion criteria: All those who did not meet the inclusion criteria were excluded.

Sample size calculation: The sample size was calculated to detect a difference of 5% in ovulation rate between the two groups with a standard deviation of 0.6 and a power of 90% assuming two-tailed alpha error of 0.05. 50 patients were recruited in each arm of the study.

Study Procedure

Patients seen in clinic, who consented to the study, were consecutively assigned to group A or B. Both groups received preconception 5 mg folic acid daily. All women who were confirmed pregnant were given Dydrogesterone (Duphaston®) 10 mg twice daily for 12 weeks. Group A received LE only for the induction while group B received, 10,000 IU of human chorionic gonadotropin injection by route in addition to LE. A 5 mg of LE was given daily from day 3 to day 7. Transvaginal ultrasound was commenced on day 7 of the cycle and continued every other day until dominant follicle or follicles of about 18 mm or above were obtained. The transvaginal scan was done by one or a maximum of two radiologists to reduce measurement bias. The power of the transvaginal probe used is 7.5 MHz. Those that received HCG injection were given the injection when follicle/s of about 18 mm diameter was obtained while those without were not given the injection. Transvaginal ultrasound was continued on alternate days as above to confirm ovulation. Crenation of the follicle and the presence of fluid in the pouch of douglas was considered to be the signs of rupture of the follicle. Follicles that attained dominant size but yet unruptured on day 21 or 7 days after HCG injection or those with a diameter of up to 30 mm were considered luteinised follicles. Confirmation of ovulation was done by:

- Ultrasound evidence of ovulation with a progesterone level of greater or equal to 25 nmol/L on day 21
- Positive pregnancy test/ultrasound detection of a pregnancy.

The primary outcome was the number of ruptured/crenated follicles on either arm while the secondary outcomes were the number of LUFs and pregnancy rates. Data obtained including demographics were extracted using a proforma.

STATISTICAL ANALYSIS

The Statistical analysis was performed using the Statistical Package for the Social Sciences version 21.0 software (SPSS Inc., Chicago, IL, United States). The results were expressed as mean±standard devaition (SD). Odds ratios and 95% confidence intervals were calculated. Chi-squared test and Fisher's-exact test was used at an appropriate place to calculate the p-value. The p-value was considered significant at <0.05.

RESULTS

Fifty women completed the study in each arm of the study. [Table/Fig-1] shows the age and parity distribution of the women. There was no significant difference between the two arms. [Table/Fig-2] shows the general clinical characteristics of the women. There was no significance difference between the period of infertility and the number of the previous cycles of ovulation stimulation (p=0.444 and 0.526 respectively). [Table/Fig-3] shows the association between ovulation and HCG injection. The table shows that ovulation is significantly associated with HCG injection. While 40 (80%) of the HCG arm ovulated, 25 (50%) of the no HCG arm ovulated (p=0.001). However, there was no statistical significance between both arms as regards the number of LUF (p=0.216). [Table/Fig-4] shows that HCG injection is significantly associated with pregnancy. Subjects who had HCG injection were over two times more likely to become pregnant than those without HCG injection (OR=2.488, 95% CI for OR=1.057-5.857, p=0.037). [Table/Fig-5] shows the mode of insemination among ovulation subjects between HCG and no HCG groups.

Variable	HCG n (%)	No HCG n (%)	χ^2, p-value
Age (years)			
25-29	27 (54)	23 (46)	0.640, 0.424
30-34	23 (46)	27 (54)	
Parity			
0	23 (46)	25 (50)	0.160, 0.689
1 and above	27 (54)	25 (50)	

[Table/Fig-1]: Demography of the respondents.
p-value was calculated using chi-square test

Variable	HCG n (%)	No HCG n (%)	χ^2, p-value
Period of infertility (years)			
1-4	42 (51.9)	39 (48.1)	0.585, 0.444
5-9	8 (42.1)	11 (57.9)	
Number of previous cycles of ovulation stimulation			
0	2 (33.3)	4 (66.7)	4.164, 0.526
1	7 (46.7)	8 (53.3)	
2	24 (57.1)	18 (42.9)	
3	11 (57.9)	8 (42.1)	
4	3 (30)	7 (70)	
5	3 (37.5)	5 (62.5)	
Number of follicles up to 18 mm follicle			
0	7 (38.9)	11 (61.1)	1.087, 0.581
1	30 (52.6)	27 (47.4)	
2	13 (52)	12 (48)	
Day 18 mm follicle achieved			
0	7 (38.9)	11 (61.1)	7.703, 0.103
12	3 (37.5)	5 (62.5)	
13	15 (41.7)	21 (58.3)	
14	22 (62.9)	13 (37.1)	
15	3 (100)	0 (0)	

[Table/Fig-2]: General clinical characteristics.
p-value was calculated using chi-square test

Ovulation status	HCG n (%)	No HCG n (%)	OR	95% CI, p-value
No ovulation	7 (14)	22 (44)	referent	
Ovulation (Ruptured)	40 (80)	25 (50)	5.029	1.875-13.485, 0.001*
LUF	3 (6)	3 (6)	3.143	0.513-19.248 0.216**

[Table/Fig-3]: Association between ovulation and HCG injection.
*Chi-squared test; **Fisher's-exact test; LUF: Luteinised unruptured follicles

Pregnancy	HCG n (%)	No HCG n (%)	OR	95% CI, p-value
Yes	22 (44)	12 (24)	2.488	1.057-5.857, 0.037#
No	28 (56)	38 (76)		

[Table/Fig-4]: Association between HCG injection and pregnancy.
#Chi-squared test

Mode of conception	HCG n (%)	No HCG n (%)	χ^2	p-value
Timed intercourse	27 (67.5)	15 (60)	0.378	0.538
IUI	13 (32.5)	10 (40)		

[Table/Fig-5]: Comparing of mode of insemination among ovulation subjects between HCG and No HCG groups.
p-value was calculated using chi-square test

[Table/Fig-6] shows the mode of conception amongst the pregnant women in the two arms with timed intercourse contributing to more pregnancies in the HCG arm (54.5%) and IUI in the no HCG arm (83.3%).

Mode of conception	HCG n (%)	No HCG n (%)	χ^2	p-value
Timed intercourse	12 (54.5)	2 (16.7)	4.600	0.032
IUI	10 (45.5)	10 (83.3)		

[Table/Fig-6]: Comparing of mode of conception among pregnant subjects between HCG and no HCG groups.
IUI: Intrauterine insemination; p-value was calculated using chi-square test

DISCUSSION

Infertility has become the commonest reason for gynaecological clinic attendance in our environment [17]; and with an increase in the age of marriage amongst women, anovulatory infertility is on the rise. Infertility due to anovulation affects all age groups and parity as there is no statistical difference among the two study groups as regards age and parity. Ovulation rate was obviously higher in the group that received HCG injection compared to those that did not receive the injection. The ovulation rate of 80% was very significantly higher than the ovulation rate of 50% in the LE only group. A similar study amongst women with polycystic ovary had an ovulation rate of 52.4% amongst the LE group; but, when LE was combined with Human Menopausal Gonadotrophin, ovulation rate increased to 65.3% [18].

Despite the assumed superiority of LE to CC in ovulation induction, CC was shown to have ovulation rate of 60-85% when used with HCG [6] which is comparable to the ovulation rate of 80% for our study where LE was used. Our study despite higher ovulation rate showed no statistical difference in LUF rate among those that received or didn't receive HCG injection. Previous study showed increased LUF in cycles in which HCG injection was not given; though this was had women with Polycystic Ovarian Syndrome [18]. The pregnancy rate from our study was 44%. This is statistically higher than the rate in no HCG group. This was also higher than the rate in two other studies where pregnancy rates were 14.4% [6] and 26.51% [19]. The high rate may be as a result of stricter patient selection in our study. The 44% pregnancy rate was however lower than the 55.7% in another study [19].

Most of the pregnancies in the HCG group was due to timed intercourse (54.5%), whereas, in the no HCG group it was as a result of IUI (83.3%). The clinical significance of this is not clear. However, this study with a large number of women contributes to the body of knowledge especially from the perspective of an African population.

Limitation(s)

The present study was limited by it's non randomised study design.

CONCLUSION(S)

This study showed that both the ovulation rate and pregnancy rate are significantly improved when human chorionic gonadotrophin injection was given after ovarian stimulation with LE. It is recommended that HCG injection can be added in cycles of ovarian stimulation with LE to improve ovulation and pregnancy rates.

REFERENCES

[1] Dankert T, Kremer JA, Cohlen BJ, Hamilton CJ, Pasker-de Jong PC, Straatman H, et al. A randomised clinical trial of clomiphene citrate versus low dose recombinant FSH for ovarian hyperstimulation in intrauterine insemination cycles for unexplained and male subfertility. Hum Reprod. 2007;22(3):792-97. Doi: 10.1093/humrep/del441.

[2] Kar S. Clomiphene citrate or letrozole as first-line ovulation induction drug in infertile PCOS women: A prospective randomised trial. J Hum Reprod Sci. 2012;5(3):262-65. Doi: 10.4103/0974-1208.106338.

[3] Guzick D. Polycystic ovary syndrome: Symptomatology, pathophysiology, and epidemiology. Am J Obstet Gynecol. 1998;179(6 Pt 2):S89-93. Doi: 10.1016/s0002-9378(98)70238-8.

[4] Mitwally MF, Abdel-Razeq S, Casper RF. Human chorionic gonadotropin administration is associated with high pregnancy rates during ovarian stimulation and timed intercourse or intrauterine insemination. Reprod Biol Endocrinol. 2004;2:55. Doi: 10.1186/1477-7827-2-55.

[5] Allen NC, Herbert CM 3rd, Maxson WS, Rogers BJ, Diamond MP, Wentz AC. Intrauterine insemination: A critical review. Fertil Steril. 1985;44(5):569-80. Doi: 10.1016/s0015-0282(16)48969-7.

[6] Brown J, Farquhar C, Beck J, Boothroyd C, Hughes E. Clomiphene and anti-oestrogens for ovulation induction in PCOS. Cochrane Database Syst Rev. 2009;(4):CD002249. Doi: 10.1002/14651858.CD002249.pub4. Update in: Cochrane Database Syst Rev. 2016 Dec 15;12 :CD002249.

[7] Fisher SA, Reid RL, Van Vugt DA, Casper RF. A randomised double-blind comparison of the effects of clomiphene citrate and the aromatase inhibitor letrozole on ovulatory function in normal women. Fertil Steril. 2002;78(2):280-85. Doi: 10.1016/s0015-0282(02)03241-7.

[8] Nakamura Y, Ono M, Yoshida Y, Sugino N, Ueda K, Kato H. Effects of clomiphene citrate on the endometrial thickness and echogenic pattern of the endometrium. Fertil Steril. 1997;67(2):256-60. Doi: 10.1016/S0015-0282(97)81907-3.

[9] Casper RF, Mitwally MF. Use of the aromatase inhibitor letrozole for ovulation induction in women with polycystic ovarian syndrome. Clin Obstet Gynecol. 2011;54(4):685-95. Doi: 10.1097/GRF.0b013e3182353d0f.

[10] Mitwally MF, Casper RF. Use of an aromatase inhibitor for induction of ovulation in patients with an inadequate response to clomiphene citrate. Fertil Steril. 2001;75(2):305-09. Doi: 10.1016/s0015-0282(00)01705-2.

[11] Ganesh A, Goswami SK, Chattopadhyay R, Chaudhury K, Chakravarty B. Comparison of letrozole with continuous gonadotropins and clomiphene-gonadotropin combination for ovulation induction in 1387 PCOS women after clomiphene citrate failure: A randomised prospective clinical trial. J Assist Reprod Genet. 2009;26(1):19-24. Doi: 10.1007/s10815-008-9284-4.

[12] Hughes EG. The effectiveness of ovulation induction and intrauterine insemination in the treatment of persistent infertility: A meta-analysis. Hum Reprod. 1997;12(9):1865-72. Doi: 10.1093/humrep/12.9.1865.

[13] Burr RW, Siegberg R, Flaherty SP, Wang XJ, Matthews CD. The influence of sperm morphology and the number of motile sperm inseminated on the outcome of intrauterine insemination combined with mild ovarian stimulation. Fertil Steril. 1996;65(1):127-32. Doi: 10.1016/s0015-0282(16)58039-x.

[14] Ransom MX, Blotner MB, Bohrer M, Corsan G, Kemmann E. Does increasing frequency of intrauterine insemination improve pregnancy rates significantly during superovulation cycles? Fertil Steril. 1994;61(2):303-07. Doi: 10.1016/s0015-0282(16)56522-4.

[15] Khalifa Y, Redgment CJ, Tsirigotis M, Grudzinskas JG, Craft IL. The value of single versus repeated insemination in intra-uterine donor insemination cycles. Hum Reprod. 1995;10(1):153-54. Doi: 10.1093/humrep/10.1.153.

[16] Tomlinson MJ, Amissah-Arthur JB, Thompson KA, Kasraie JL, Bentick B. Prognostic indicators for intrauterine insemination (IUI): Statistical model for IUI success. Hum Reprod. 1996;11(9):1892-96. Doi: 10.1093/oxfordjournals.humrep.a019513.

[17] Ugwu EO, Onwuka CI, Okezie OA. Pattern and outcome of infertility in Enugu: The need to improve diagnostic facilities and approaches to management. Niger J Med. 2012;21(2):180-84. PMID: 23311187.

[18] Chen Z, Zhang M, Qiao Y, Yang J. Effects of letrozole in combination with low-dose intramuscular injection of human menopausal gonadotropin on ovulation and pregnancy of 156 patients with polycystic ovary syndrome. Pak J Med Sci. 2016;32(6):1434-38. Doi: 10.12669/pjms.326.11391.

[19] Pourali L, Ayati S, Tavakolizadeh S, Soleimani H, Teimouri Sani F. Clomiphene citrate versus letrozole with gonadotropins in intrauterine insemination cycles: A randomised trial. Int J Reprod Biomed (Yazd). 2017;15(1):49-54.

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PLAGIARISM CHECKING METHODS: [Jain H et al.]
- Plagiarism X-checker: Jul 07, 2020
- Manual Googling: May 03, 2021
- iThenticate Software: Jun 10, 2021 (17%)

ETYMOLOGY: Author Origin

AUTHOR DECLARATION:
- Financial or Other Competing Interests: None
- Was Ethics Committee Approval obtained for this study? Yes
- Was informed consent obtained from the subjects involved in the study? Yes
- For any images presented appropriate consent has been obtained from the subjects. NA

Date of Submission: **Jul 06, 2020**
Date of Peer Review: **Sep 08, 2020**
Date of Acceptance: **May 04, 2021**
Date of Publishing: **Jul 01, 2021**