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Predictors for Response to Letrozole as an Ovulation Induction in Anovulatory Infertile Polycystic Ovarian Syndrome Women

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Abstract

To assess the predictive value of different clinical, laboratory, and ultrasound parameters in Letrozole when used as an ovulation induction in anovulatory infertile PCOS women. The current study was done in the secondary-referral infertility clinic in AL-Yarmouk teaching hospital, and Al-Mustansiriyah medical college. Sixty-seven anovulatory infertile women with the polycystic ovarian syndrome. Letrozole was given orally on day 2 or 3 of the menstrual cycle for five days and repeated for three consecutive cycles. The primary outcome measures were to evaluate the response rate in the form of successful ovulation and clinical pregnancy. The ovulation rate was (64.2%), with clomiphene naïve vs. previous clomiphene use; it was 87.5%, 51.2% respectively, while pregnancy rate was (32.8%), 41.6% with clomiphene naïve vs. 27.9% with previous clomiphene use. Clinical (age, BMI, Waist circumference, cycle length and days between cycle, infertility period and type, previous reproductive outcome, androgen symptom and m-FG score), laboratory (E_2, FSH, LH, testosterone, FAI, FBS, fasting insulin, HOMA – IR, and AMH) and ultrasound (mean ovarian volume, mean AFC and antral follicular diameter) parameters were founded to affect ovulation and pregnancy in different extent. After putting all variables in a scoring system, it was found that if the patients had > 26 points for the score, it's more likely that the woman becomes pregnant. A predictive pregnancy score was developed from basic clinical, laboratory, and ultrasound parameters. It may help the clinician to individualized ovulation induction protocol in PCOS women; however, external validation of this system is recommended in a more extensive prospective study.

Keywords: Letrozole, Polycystic ovarian syndrome (PCOS), infertility.

1. Introduction

Polycystic ovarian syndrome (PCOS) is a very common heterogeneous ovarian endocrinopathy affecting 6–8% of women, leading to several health complications, including menstrual dysfunction, hirsutism, acne, obesity, and metabolic syndrome, and it is the most frequent cause of anovulatory infertility accounting for >80% of all cases (Ding, et al [1], Norman, et al [2], The Thessaloniki ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group [3]). Induction of ovulation safely is essential for women with WHO group II-PCOS who wish to conceive. there are many medical options which can be used to treat ovulation disorders and infertility, including estrogen receptor modulators (such as clomiphene and tamoxifen), aromatase inhibitors (such as letrozole), insulin-sensitizing drugs (such as metformin), and direct hormonal stimulation of the ovaries (gonadotropins) (Wang, et al [4]). The majority of the studies done on letrozole were on patients with clomiphene resistance (Mitwally, et al [5], Kamath, et al [6], Quintero, et al [7]), and because of its short history in this respect, concepts like letrozole resistance and failure were not addressed. As the first line of treatment, letrozole can be used because it is ovulation, pregnancy, and live birth rate are higher as well as lower multiple pregnancy rates, although the reluctance to adopt such new therapy is frequent in clinical practice (McCartney, et al [8], Legro, et al [9], Casper, RF [10], Alizzi, FJ [11]). Taken in considerations that the presence/ absence of all PCOS features whether clinical or biochemical will help the clinicians to manage these patients correctly and that the recognition of predictors of treatment response to OI is essential for the success of a therapy because their identification could lead clinicians to the best-individualized treatment to improve the efficacy of the therapy, while optimizing its safety profile (Rausch, et al [12]). Several types of research had studied predictors of patient responses to ovulation induction with clomiphene citrate in anovulatory infertile

women due to PCOS (Ellakwa, et al [13], Mahran, et al [14], Imani, et al [15]). In comparison, few researchers studied letrozole predictors to response (Palihawadana, et al [16]). The aim of current study is to investigate whether clinical, biochemical, endocrine, and Sonographic characteristics during initial assessment of anovulatory infertile women due to PCOS may predict the ovarian response to letrozole treatment whether it is used as a first-line or as a second line after clomiphene resistant or failure and to develop a possible applicable scoring system to help clinician for OI individualization.

2. Materials and Methods

i. Materials

A prospective cohort study conducted in the infertility unit at Al-Yarmouk teaching hospital. The institutional review board of the hospital approved the protocol; all participants were given written informed consent and enrollment began from May 2017 and was completed in July 2018. Eligible participants who were medically fit infertile PCOS women aged 18-35 years and their Body mass index (BMI) of 19-35 kg/m² were enrolled in the study. Diagnosis of PCOS depending on modified Rotterdam criteria, which include two out of three criteria, abnormal ovulatory function (amenorrhea or oligomenorrhea), clinical and or biochemical hyperandrogenism, and ultrasound features of polycystic ovaries (Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group [17]). All participants had hysterosal-pingography that proved patent fallopian tubes, and their partners had normal semen analysis parameters according to the modified criteria of the World Health Organization. Clinical, laboratory (biochemical &endocrine), and Sonographic screening was carried out before initiation of letrozole treatment.

a. Clinical Screening

Full history and examination questioner formula fulfilled and include: age, menstrual cycle history (regular,

oligomenorrhea, and amenorrhoea) and days between cycles, period and type of infertility (primary or secondary with previous reproductive outcome), previous medication use (as hormonal contraceptives, insulin-sensitizing agents and other OI- pre clomiphene citrate use for the last three months) and surgery. The BMI was calculated, androgen symptoms were assessed, and hirsutism was scored according to the modified Ferriman-Gallwey score (m-FG score) (Hatch, et al [18]).

b. Laboratory (biochemical & endocrine) screening

Baseline laboratory testing was performed after an overnight fasting on 2nd –3rd day of menstrual cycle using Immulite 2000 XPi immunoassay system /Siemens to test hormones: FSH, LH, plasma levels of estradiol (E_2), S. Testosterone, sex-hormone-binding globulin (SHBG), S. fasting insulin, FBS, S. Prolactin, TSH and AMH. Free androgen index (FAI) measured using the formula: FAI= total testosterone (nmol/ L)) x 100 /sex hormone-binding globulin (SHBG) (nmol/ L)) and HOMA – IR was measured using the formula= Fasting Glucose (mg/dl) x Fasting Insulin (μU/ml) / 405). Less than 1.0 means insulin-sensitive which is optimal, above 1.9 indicates early insulin resistance and above 2.9 indicates significant insulin resistance. All patients had normal serum prolactin, thyroid-stimulating hormone (TSH) and 17-OH progesterone. a participant with elevated prolactin level, congenital adrenal hyperplasia, thyroid problem, Cushing's syndrome, and androgen-secreting tumors were excluded. Although there is no accepted national or international clinical standard for determining the accuracy of a testosterone assay, hyperandrogenemia was diagnosed either clinically (acne/hirsutism) or biochemically (testosterone ≥2.5 nmol/l or free androgen index [FAI] ≥5) (Rosner, et al [19]).

c. *Transvaginal Ultrasound Screening*

All the participants are seen on their 2nd or 3rd spontaneous menstrual cycle day or after progesterone-induced withdrawal bleeding where baseline transvaginal ultrasound TVU were done using 7.5 MHz vaginal probes (R7-Samsung-Korea). Polycystic ovaries are present when one or both ovaries demonstrate 12 or more follicles measuring 2–9 mm in diameter or the ovarian volume exceeds 10 cm^3. Only one ovary meeting either of these criteria is sufficient to establish the presence of polycystic ovaries (Balen, et al [20]). Ovarian volume; Antral Follicular Count (AFC), Antral Follicular Size (AFS) and endometrial thickness assessed and measured. Ovarian volume measured according to the formula: 0.5 × lengths × width × thickness of the ovary (Orsini, et al [21]). AFS categorized estrogen two groups: group one (2-5 mm antral follicles size) and group two (6-9 mm antral follicles size).

ii. *Methods*

letrozole (Femara, Novartis Pharmaceuticals) 5mg were given orally from the 2nd-3rd-day cycle and for five continuous days followed by TVU tracking of follicular growth and endometrial thickness from 9 days of the menstrual cycle and repeated every alternate day till dominance was confirmed or excluded 2 weeks after the end of treatment. when one follicle size ≥17 mm, Recombinant human chorionic gonadotropin (hCG) alpha (ovidrel, 250 mcg, Merck Serono Pharmaceutical) was given subcutaneously but should be prevented if patients have >3 follicles (15–18) mm. Patients were advised to have intercourse 24 to 36 hours after the hCG injection. Midluteal serum progesterone was assayed one week after hCG injection, levels 7.9 ng/ml (>25 nmol/L) indicate ovulation. The level of serum hCG was measured 14 days after the hCG administration in the absence of menstruation for the detection of biochemical pregnancy and clinical

pregnancy then after confirmed by TVU. If ovulation occurred, the regime repeated unaltered for three consecutive cycles unless pregnancy occurred. Once pregnancy occurred, letrozole respond is achieved, failure to achieve pregnancy termed letrozole failure and failure to achieve ovulation termed letrozole resistant. Primary outcome measures were to evaluate the response rate in the form of successful ovulation and clinical pregnancy and secondary outcome measures were to assess clinical, biochemical, endocrine and ultrasound parameters as a predictor to response to letrozole.

iii. Statistical Analysis

SPSS 22.0.0 (Chicago, IL), MedCalc Statistical Software version 14.8.1 (MedCalc Software bvba, Ostend, Belgium; 2014), a software package used to make the statistical analysis, if the p-value less than 0.05 considered being significant. For assessment of the normal distribution and if continuous variables follow it we use Anderson darling test, for analyzing the differences in means between two groups we use two samples t-test, for calculating the odd ratio (OR) and their 95% confidence intervals we use Binary logistic regression analysis. Receiver operator curve used to see the validity of different parameters in separating active cases from control (negative cases) and area under the curve i.e. AUC and its p-value prescribe this validity (if AUC $\geq$ 0.9 mean excellent test, 0.8 – 0.89 means good test, 0.7 – 0.79 fair test otherwise unacceptable).

3. Results

At the end of the study period, a total of 67 infertile PCOS women were enrolled. Table (1) shows the demographic and clinical characteristics of the eligible women that were included in the study. The mean age of them was (27.9 ± 5.5 years), mean BMI was (27.6 ± 3.5 Kg/m^2) with mean WC (86.2 ± 16.4 cm), around two-thirds of the participants had primary infertility, hirsutism was seen in 83.4% of

the women and the m-FG score was 8-15. Forty-three women (64.2%) had the previous history of clomiphene citrate use for three cycles before the enrollment in this study and ended with either failure or resistant. Table 2 shows the related laboratory and ultrasonic characteristics of the study group. Regarding ultrasound, the mean ovarian volume and mean AFC for both ovaries was 10.8 ± 0.5 cm^3 and 14.4 ± 2.4 cm^3 respectively. The antral follicles size was 2-5 mm in around 30% of participants, while 70% had a size of 6-9 mm. Table 3 and figure 1 display the primary outcome measures of the study (ovulation and pregnancy rate) where the ovulation rate in the whole participants was 64.2% and the pregnancy rate was 32.8%. The ovulation rate was higher in the clomiphene naïve group than those who used clomiphene (87.5% vs. 51.2%); also there was a difference in the rate of pregnancy between the two groups (41.6% vs 27.9%). In Table (4) we can see that most of the clinical, ultrasound and hormonal variables are significantly different between the women who achieved success ovulation vs. those who

Table (1): *Demographic and clinical characteristics*

Variables	Value
Number	67
Age (years), mean ± SD	27.9 ± 5.5
BMI (kg/m^2), mean ± SD	27.6 ± 3.5
Waist circumference (cm) , mean ± SD	86.2 ± 16.4
Cycle length: N (%)	
Regular	10 (14.9%)
Oligomenorrhea	52 (77.6%)
Amenorrhea	5 (7.5%)
Days between cycles: N (%)	
23-34	8 (11.9%)
35 – 45	23 (34.3%)
46 – 55	22 (32.8%)
56 – 65	10 (14.9%)
>65	4 (6.0%)
Infertility period (months), mean ± SD	22.1 ± 9.0
Type of infertility: N (%)	
Primary	44 (65.7%)
Secondary	23 (34.3%)
Previous reproductive outcome:	
Parity: N (%)	
Null	44 (65.7%)
Single	18 (26.9%)
Multiple	5 (7.5%)
Abortion: N (%)	
Null	51 (76.1%)
Single	14 (20.9%)
Twice	2 (3.0%)
Androgen symptoms: N (%)	
Hirsutism	56 (83.6%)
Acne	9 (13.4%)
Seborrhea	9 (13.4%)
m-MF score, n (%); N (%)	
<8	11(16.4%)
8-15	56 (83.6%)
Previous use of clomiphene citrate: N (%)	
Clomiphene naïve	24 (35.8%)
Used with Failure	18 (26.9%)
Used with Resistance	25 (37.3%)

failed ovulation, except the age, insulin level, and HOMA-IR where statistically there was no significant difference. The significant difference in most of the variables where existed also between the women who achieved pregnancy vs. the failure, as it is showed in Table (5). The univariate analysis (logistic regression test) showed that the increases in the following variables predict decrease in the odds for having successful ovulation: days between cycle, infertility period, BMI, waist circumference, mean ovarian volume, modified FG score, androgen symptoms, LH, testosterone, free androgen index, FBS, AMH, and mean AFC, while the increases in the following variables predict increase in the odds for having successful ovulation: E_2, SHBG, endometrial thickness and antral follicular diameter as illustrated in table 7. The increases in the following variables predict decrease in the odds for having successful pregnancy: days between cycle, infertility period, BMI, waist circumference, mean ovarian volume, modified FG score, LH, testosterone, free androgen index, insulin, HOMA-IR, AMH, dominant follicle size, and mean AFC, while the increases in the following variables predict increase in the odds for having successful ovulation: E_2, SHBG, endometrial thickness and antral follicular diameter, as illustrated in table 7. Patients with secondary infertility (compared to primary) had a 2.8-fold increase odd of achieving ovulation and it was significant, and a 2.5-fold increase the odds of achieving pregnancy but it was not statistically significant. Previous use of clomiphene with failure in pregnancy had 4.2 folds increased odd of achieving successful pregnancy compared to those used clomiphene and had with resistance outcome (it was statistically significant), also those that did not use previously clomiphene had 3.75 folds increased odd of achieving successful pregnancy (but it was not statistically significant). Patients with previous pregnancy had 3.75 folds increase odd of achieving ovulation and 8 folds increased odd of achieving pregnancy (compared to those who

Table 2: Ultrasound and laboratory characteristics of the study group

Variables	Value
Ultrasound predictors	
Mean ovarian volume(cm^3) ± SD	10.8 ± 0.5
Mean AFC ± SD	14.4 ± 2.4
Antral follicular diameter	N (%)
2 – 5 mm	20 (29.9%)
6 – 9 mm	47 (70.1%)
Laboratory predictors	
$E_{2\ (pmol/l)}$, mean ± SD	124.8 ± 27.0
FSH (IU/L), mean ± SD	5.1 ± 0.9
LH(IU/L), mean ± SD	9.7 ± 3.6
SHBG(nmol/l), mean ± SD	39.9 ± 7.7
Testosterone(nmol/l), mean ± SD	1.7 ± 0.2
Free androgen index, mean ± SD	4.4 ± 1.3
FBS(mg/dl), mean ± SD	93.0 ± 7.9
Insulin(miu/ml), mean ± SD	5.04 ± 1.8
HOMA – IR, mean ± SD	1.16 ± 0.43
AMH(ng/ml), mean ± SD	6.6 ± 0.8

Table 3: Response to letrozole in the study group

Variables	Value
Cycle number: N (%)	
2	16 (23.9%)
3	51 (76.1%)
Total cycles number	185
Mean diameter of the dominant follicle. (mm) ± SD	20.1 ± 1.4
The number of dominant follicles: N (%)	
1	41 (95.3%)
2	2 (4.7%)
Endothelial thickness(mm)±SD	8.0 ± 0.8
Progesterone(nmol/l)	27 ± 2.3
Ovulation, n (%)	43 (64.2%)
Pregnancy, n (%)	22 (32.8%)
Ovulation in clomiphene naive, n/total (%)	21/24 (87.5%)
Pregnancy in clomiphene naïve, n/total (%)	10/24 (41.6%)
Ovulation in previous use of clomiphene, n/total (%)	22/43 (51.2%)
Pregnancy in previous use of clomiphene, n/total (%)	12/43(27.9%)

Table 4: The difference in characteristic variables between the ovulation successes vs. failure

Variables	No ovulation	Ovulation	p-value
Number	24	43	-
Age	29.1 ± 5.7	27.3 ± 5.3	0.186
BMI	30.8 ± 3.9	25.9 ± 1.6	<0.001
WC	100.1 ± 18.3	78.4 ± 8.1	<0.001
Infertility Period	30.3 ± 9.1	17.6 ± 4.8	<0.001
Mean Ovarian Volume	11.4 ± 0.4	10.4 ± 0.2	<0.001
Mean AFC	17.3 ± 0.8	12.7 ± 0.9	<0.001
ET	7.1 ± 0.1	8.5 ± 0.6	<0.001
m-FG score	10.1 ± 1.6	6.3 ± 3.5	<0.001
E2	96.0 ± 11.7	140.9 ± 18.2	<0.001
FSH	5.4 ± 1.1	5.0 ± 0.8	0.115
LH	13.7 ± 2.4	7.5 ± 1.6	<0.001
SHBG	35.0 ± 6.3	42.6 ± 7.2	<0.001
Testosterone	1.9 ± 0.2	1.6 ± 0.1	<0.001
Free Androgenic Index	5.5 ± 1.3	3.8 ± 0.8	<0.001
FBS	99.3 ± 4.2	89.5 ± 7.4	<0.001
Insulin	4.8 ± 1.6	5.2 ± 2.0	0.340
HOMA-IR	1.2 ± 0.4	1.2 ± 0.4	0.859
AMH	7.3 ± 0.6	6.2 ± 0.5	<0.001

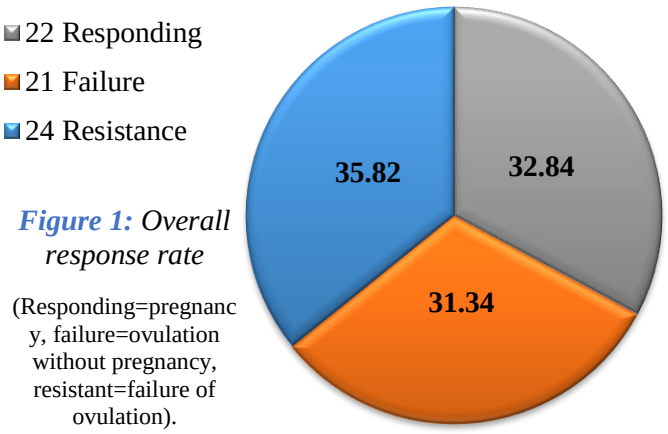

Figure 1: Overall response rate

(Responding=pregnancy, failure=ovulation without pregnancy, resistant=failure of ovulation).

Table (5): *The difference in characteristic variables between pregnancy success vs. failure*

Variables	No pregnancy	Pregnancy	p-value
Number	45	22	-
Age	28.8 ± 6.0	26.2 ± 3.7	0.031
Infertility Period	25.2 ± 9.1	15.9 ± 4.5	<0.001
BMI	28.4 ± 3.9	26.0 ± 1.8	0.001
WC	90.9 ± 17.2	76.5 ± 8.9	<0.001
Mean Ovarian Volume	10.9 ± 0.5	10.4 ± 0.1	<0.001
Dominant Follicle Size	20.9 ± 1.6	19.4 ± 0.9	0.001
ET	7.5 ± 0.6	8.9 ± 0.3	<0.001
m-FG score	8.3 ± 3.3	6.3 ± 3.5	0.026
E2	109.4 ± 18.2	156.5 ± 6.6	<0.001
FSH	5.2 ± 1.0	5.0 ± 0.8	0.603
LH	10.9 ± 3.7	7.3 ± 1.8	<0.001
SHBG	36.9 ± 6.0	45.9 ± 7.5	<0.001
Testosterone	1.8 ± 0.2	1.6 ± 0.1	<0.001
Free Androgenic Index	4.9 ± 1.2	3.5 ± 0.8	<0.001
FBS	93.8 ± 8.5	91.4 ± 6.6	0.254
Insulin	5.4 ± 2.0	4.2 ± 1.0	0.002
HOMA-IR	1.3 ± 0.5	1.0 ± 0.3	0.002
AMH	6.7 ± 0.8	6.2 ± 0.5	0.001
Mean AFC	15.1 ± 2.5	12.8 ± 0.8	<0.001

Table (6): *ROC (receiver operator characteristic) analysis of various predictors for Ovulation*

Variables	AUC	Cut point	SN	SP
Clinical predictors				
Days between cycles	0.905	≤ 35 – 45	70%	96%
Infertility period (months)	0.875	≤30	100%	58%
BMI	0.837	≤28.9	100%	75%
Waist circumference	0.826	≤92	100%	75%
Modified FG score	0.904	≤8	84%	83%
Ultrasound predictors				
Mean ovarian volume	0.997	≤10.7	93%	100%
Mean AFC	1.0	≤15	99%	99%
Endometrial thickness	0.995	>7.2	98%	100%
Laboratory predictors				
E2	0.976	>110	98%	92%
LH	0.970	≤13.85	100%	83%
SHBG	0.810	>35	79%	67%
Testosterone	0.912	≤1.7	93%	83%
Free androgen index	0.869	≤4.57	81%	83%
FBS	0.887	≤92	72%	100%
AMH	0.948	≤6.8	91%	92%

ROC: receiver operator characteristic, AUC: area under the curve. SN: sensitivity, SP: specificity

Table (7): Regression analysis (logistic regression) of possible predictors of ovulation and pregnancy

Variables	Ovulation		Pregnancy	
	OR (95% CI)	p-value	OR (95% CI)	p-value
Clinical predictors				
Age	0.939 (0.856 – 1.030)	0.185	0.907 (0.817 – 1.008)	0.070
Days between cycles	0.067 (0.016 – 0.276)	<0.001	0.377 (0.195 – 0.727)	0.004
Infertility period	0.775 (0.683 – 0.879)	<0.001	0.788 (0.686 – 0.905)	0.001
BMI	0.567 (0.432 – 0.744)	<0.001	0.786 (0.647 – 0.954)	0.015
Waist circumference	0.892 (0.845 – 0.942)	<0.001	0.924 (0.878 – 0.972)	0.002
Androgen symptoms	0.192 (0.059 – 0.623)	0.006	0.547 (0.155 – 1.930)	0.348
Modified FG score	0.108 (0.033 – 0.355)	<0.001	0.850 (0.732 – 0.987)	0.033
Infertility (secondary)	2.812 (1.002 – 7.895)	0.049	2.449 (0.839 – 7.150)	0.101
Previous CC use				
Not used	Can't be estimated		3.750 (0.980 – 14.355)	0.054
Failure			4.200 (1.018 – 17.322)	0.047
Resistance			Reference	
Previous reproductive outcome				
Previous pregnancy	3.750 (1.019 – 13.795)	0.047	8.0 (2.237 – 28.605)	0.001
Abortion	2.062 (0.579 – 7.347)	0.264	0.229 (0.026 – 2.047)	0.187
No past pregnancy	Reference		Reference	
Ultrasound predictors				
Mean AFC	1.9×10^{-14} (4.2×10^{-15} – 4.3×10^{-8})	0.001	0.549 (0.382 – 0.788)	0.001
Antral follicular diameter	1.7×10^{10} (8.1×10^{9} – 7.8×10^{10})	0.001	1.4×10^{9} (8.1×10^{8} – 2.5×10^{9})	0.001
Mean ovarian volume	<0.001	<0.001	0.013 (0.001 – 0.177)	0.001
Laboratory predictors				
E2	1.204 (1.088 – 1.331)	<0.001	38.037 (11.291 – 48.231)	0.001
FSH	0.647 (0.374 – 1.119)	0.119	0.858 (0.486 – 1.514)	0.597
LH	0.425 (0.294 – 0.616)	<0.001	0.637 (0.477 – 0.850)	0.002
SHBG	1.220 (1.087 – 1.369)	0.001	1.212 (1.096 – 1.341)	<0.001
Testosterone	5×10^{-8} (1×10^{-11} – 2×10^{-4})	<0.001	2.2×10^{-4} (1.6×10^{-7} – 5.9×10^{-3})	0.001
Free androgen index	0.160 (0.062 – 0.413)	<0.001	0.198 (0.085 – 0.464)	<0.001
FBS	0.761 (0.666 – 0.870)	<0.001	0.963 (0.902 – 1.027)	0.251
Insulin	1.158 (0.858 – 1.563)	0.338	0.528 (0.295 – 0.946)	0.032
HOMA – IR	0.899 (0.282 – 2.861)	0.857	0.062 (0.006 – 0.669)	0.022
AMH	0.014 (0.001 – 0.132)	<0.001	0.333 (0.151 – 0.735)	0.006

OR: odds ratio, CI: confidence interval

Patients with no ovulation had no active follicle (so odd ratio for dominant follicle size can't be calculated)

did not have previous pregnancy (it was significant), those with previous abortion had no statistical difference compared to those without previous pregnancy, Patients with Antral follicular diameter 6-9 mm significantly correlated with successful ovulation and pregnancy compared to those with 2-5 mm, as illustrated in table 7. The internal validation of different variables was done using ROC-AUC, which revealed that the following markers had an excellent ability to predict ovulation: days between cycles, mean ovarian volume, modified FG score, E_2, LH, testosterone, AMH, mean AFC, and endometrial thickness. Also, the following variables had a good ability: infertility periods, BMI, waist circumference, SHBG, FBS, and free androgen index, as clarified in Table 6. Regarding the predictors of pregnancy, the following markers had excellent ability to predict pregnancy: endometrial thickness and E_2 levels, while the following markers had a good ability to predict pregnancy: infertility period, mean ovarian volume, LH, SHBG, free androgen index, and dominant follicle size. Also, the following markers had a fair ability to predict pregnancy: days between cycles, waist circumference, modified FG score, testosterone, insulin, HOMA-IR, AMH, and mean AFC. As illustrated in table 8. After putting all variables in a scoring system we can see that the individual points of the summary score were calculated based on their odd ratio (different weighing according to the magnitude of the OR), and for each subject we calculate their score based on the cut point calculated in the ROC analysis (of the above the threshold than we give the respective point; and add for each domain to calculate the score), this summary score than analyzed using ROC to find the optimal cut point to predict pregnancy. Summary score

1. If days between cycles is $\leq 35 - 45$ days, then give three points
2. If infertility period ≤ 18 months than giving one point

3. If BMI ≤ 28.9 kg/m^2 than give one point
4. If waist circumference ≤92 cm than give one point
5. If m-FG score ≤ 8.0 than give one point
6. If mean antral follicular count ≤13.0 give two points
7. If mean ovarian volume ≤ 10 give five points
8. If E_2 > 142 give four points
9. If LH ≤ 7.0 gives one point
10. If SHBG >44 give one point
11. If total testosterone ≤ 1.6 give five points
12. If free androgenic index ≤ 4.21 give four points
13. If fasting insulin ≤ 4.17 give two points
14. If HOMA-IR < 0.99 give four points
15. If AMH ≤ 6.8 gives three points
16. If it has a previous pregnancy without abortion than give four points otherwise zero.

17. If the previous use of clomiphene leads to ovulation without pregnancy give four points, if the patients did not use clomiphene previously gives two points, else zero.

18. If the patient with antral follicular diameter 6 – 9 mm give 5 points, else zero

Finally, If the patients had > 26 points for the score that is highly likely to become pregnant (score range from 0 – 50), as illustrated in Table 9 and Figure 2.

4. Discussion

The primary outcome measure for letrozole as an OI in the study shows that the overall clinical response in the form of ovulation and pregnancy was 64.2% and 32.8%, respectively, and these results were lower than those seen in other studies (Alizzi FJ [11], Amer, et al [22], Palomba, et al [23]). The lower ovulation and pregnancy rate seen could be due to the

Table (8): *ROC (receiver operator characteristic) analysis of the predictors for pregnancy*

Markers	AUC	Cut point	SN	SP
Clinical predictors				
Days between cycles	0.730	≤ 35 – 45	68%	64%
Infertility period	0.837	≤18	86%	69%
BMI	0.684	≤28.9	100%	40%
Waist circumference	0.747	≤92	100%	40%
Modified FG score	0.712	≤8	82%	51%
Ultrasound predictors				
Mean ovarian volume	0.802	≤10.5	100%	69%
Mean AFC	0.744	≤13	91%	64%
Dominate follicle size	0.801	<19.9	64%	76%
Endometrial thickness	0.985	>8.3	100%	91%
Laboratory predictors				
E2	1.0	≥142	99%	99%
LH	0.809	≤7	82%	80%
SHBG	0.831	>44	64%	91%
Testosterone	0.796	≤1.6	73%	73%
Free androgen index	0.844	≤4.21	82%	76%
Insulin	0.724	≤4.17	95%	49%
HOMA – IR	0.749	≤0.99	95%	67%
AMH	0.732	<6.8	100%	58%

ROC: receiver operator characteristic, AUC: area under the curve. SN: sensitivity, SP: specificity

Table (9): *Assessment of the score as a predictor of pregnancy*

AUC	Cut point	SN	SP	PPV	NPV
0.967	>26	100%	82%	73%	100%

AUC: area under the curve. SN: sensitivity, SP: specificity, PPV: positive predictive value., NPV: negative predictive value

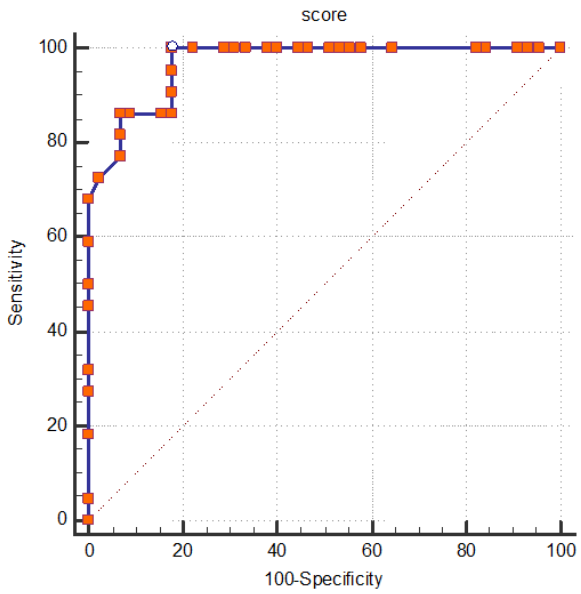

Figure (2): *ROC (receiver operator characteristic) curve of the score to predict pregnancy*

inclusion of 64.2% of the study population who used clomiphene previously and encountered clomiphene failure (26.9%) and clomiphene resistant (37.3). (Morad and Farag [24]) showed that the pregnancy rate was 20% in letrozole use after clomiphene failure while ovulation and pregnancy rate after clomiphene resistant was 44.24% and 23.89% respectively in (Rahmani, et al [25]) study vs. 51.2% and 27.9% respectively in our study; on the other hand, (Mitwally and Casper [26]) study showed ovulation rate of 75% and the pregnancy rate of 25%. In clinical practice searching for predictors to response to OI treatment in women with PCOS may help the clinicians to manage these patients appropriately. In the current study, we took different clinical, laboratory and ultrasonographic parameters to show its effect on clinical response in the form of improving ovulation and increasing pregnancy rate. Regarding clinical predictors to response to letrozole, we found that increases in the variables: days between cycle, infertility period, BMI, waist circumference, modified FG score and androgen symptoms, predict decrease having successful ovulation and pregnancy. Ultrasonographic predictors to response in our study show that an increase in mean ovarian volume and AFC and a decrease in antral follicular diameter (group one 2-5 mm) decrease in the odds of having successful ovulation and pregnancy. Laboratory predictors to response in the study show that increase in LH, testosterone, free androgen index, AMH predicts the decrease in the odds for having successful ovulation and pregnancy while an increase in insulin and HOMA-IR reduction in the odds for having successful pregnancy while an increase in both E_2 and SHBG enhance both ovulation and pregnancy. Our study goes with Mary E. Rausch et al. Study who established samples to predict successful ovulation, conception, pregnancy, and live birth in a participant with PCOS have induction of ovulation, and they find that the factors that were persistently significant in all

models were baseline BMI, FAI, proinsulin level, and duration of infertility (Rausch, et al [12]). On the contrary, the age in our study was not a predictor for the response, and this may be due to the exclusion of women above 35 years of age. Also, in our research, the existence of hirsutism was found to have an adverse prognosis for both ovulation and pregnancy, while in (Rausch, et al [12]) Study the presence of hirsutism was noted to have an adverse prognosis on conception, pregnancy, and live birth, but no ovulation. Obesity also shows negative impacts on stimulation in ovulation induction cycles in both studies. Some studies found that resistance to clomiphene is more common in women with insulin resistant, obese, and hyperandrogenism (Imani, et al [27]) whether these agents predispose to letrozole resistance, too, is still not well known. Our study showed insulin resistant, obesity, and hyperandrogenism does negatively affect pregnancy rate in women using letrozole as an OI. As it is seen in most of the studies that assess the response to different ovulation protocols, there were many trials to have a scoring system or module to put different clinical, laboratory, and ultrasound features to predict the response. Statistical analysis with logistic regression test, followed by internal validation using ROC-AUC analysis to clarify the cutoff point of different variables, this assessment has brought a scoring system from 0-50 points, and those who above 26 have a high probability of success of ovulation and pregnancy. It is agreed that such scoring systems or postulated modules need further validation by external validation through a prospective study that should include a large sample with similar inclusion criteria (Fauser, et al [28]). Limitations of the current study may possibly be due to a small sample size and using pregnancy as the primary outcome measure rather than LB, although it is of significant value in clinical practice and used as a primary outcome measure in many studies. Another possible limitation of the study is the exclusion of women

above 35 years of age since, after this age, the chance of pregnancy diminished; also, women with BMI above 35 were excluded to encourage the women to decrease weight before starting OI as recommended by WHO.

5. Conclusions

A predictive pregnancy score was developed from basic clinical, laboratory, and ultrasound parameters. It may help the clinician to individualized ovulation induction protocol in PCOS women; however, external validation of this system is recommended in a more extensive prospective study.

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Author Contribution

All authors certify that they have participated sufficiently in work to take public responsibility for the content, including participation in the concept, design, analysis, writing, or revision of the manuscript.

Conflict of Interest

The authors report no conflict of interest.

Ethical Clearance

The study was approved by the Ethical Approval Committee.

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