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Clinical and Laboratory Evaluation of Women with Abnormal Clomiphene Citrate Challenge Test

ABSTRACT

Background:

About one of each ten couples is subfertile. Ovulation disorders underline about 30% of female subfertility. Ovarian follicles depletion and poor oocyte quality is known as diminishing ovarian reserve.

This study was conducted with the aim to determine the clinical and laboratory attributes of women with abnormal clomiphene citrate challenge test and diminished ovarian reserve.

Patients and methods: One hundred eight women of 22-50 years old were studied including those with primary, secondary infertility or recurrent pregnancy loss. All women were tested by Clomiphene Citrate Challenge (CCC) test after estimation of day 3 serum FSH, then the patients received 100 mg clomiphene citrate on day 5 to 9 and again serum FSH level was measured on day 10 of the cycle. Serum progesterone was also evaluated on cycle day 21 .

RESULTS: From a total of 108 women involved in the study, 72 women had normal CCC test and 36 abnormal CCC test. Out of 36 women, 11 had abnormal CCC test depending on day 10 of the cycle alone. Among women with abnormal CCC test, (91.6%) were over the age 35 years versus (41.7%) in the normal group. The discrepancy in the onset of menarche was statistically insignificant. Finally the pregnancy rate was 25% in normal group compared to 5% for abnormal group.

CONCLUSIONS: This study revealed that women with certain clinical and laboratory attributes need to be evaluated for ovarian reserve including: Women older than 35 year, Menstrual cycle span under 26 days especially in patients older than 35 years, Unexplained infertility, endometriosis or previous ovarian surgery.

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Introduction

About one of each ten couples is subfertile. Ovulation disorders underline about 30% of female subfertility..(1) Ovarian follicles depletion and poor oocyte quality is known as diminishing ovarian reserve (DOR). (2)

When approaching an infertile couple scheduled for induction of ovulation and intrauterine insemination, the infertility specialist has few criteria to determine the chance of success. Although the patient age and duration of barrenness are acknowledged determinants of success of infertility treatment, further criteria required for more appropriate selection of therapeutic plan. (3,4)

Several diagnostic tests have been designed for assessment of ovarian reserve and proper selection of patients for assisted reproductive approach. Day 3 FSH, AMH, and, the clomiphene citrate challenge (CCC) test are the three commonly used diagnostic tests .

Navot et al (1987) was the first who set the clomiphene citrate challenge (CCC) test to assess ovarian reserve.(5) . Several studies have shown that the CCC is a useful indicator of ovarian reserve and a good predictor of IVF outcome (6,7), including women below 37 years of age who show low pregnancy rate with diminished ovarian reserve as revealed by an abnormal CCC response. (8)

It has been found that older age (>35 years) predicts an abnormal CCC test. Fecundity declines with ageing ovaries, which coincides with advanced chronologic age. (9) It has been found that the likelihood of conception in women consulting IVF centers is correlated with ovarian reserve. Poor response to gonadotropin therapy, previous pelvic surgery, cigarette smoking, and perhaps an inherited accentuated follicle diminution are key determinants of the ovarian reserve. Patients with an abnormal CCC test were found to have decreased fertility despite normal baseline follicle-stimulating hormone (FSH), luteinizing hormone (LH), and regular menstruation. (10,11)

Diminished ovarian reserve poses a therapeutic challenge for infertility expert, and its management is a time-dependent, hence diagnosis must be established as early as possible. Thus, a simple, sensitive screening test for ovarian reserve is clearly mandated.

This study was conducted with the aim to determine the clinical and laboratory attributes of patients who have abnormal clomiphene citrate challenge test and diminished ovarian reserve.

Patients and Methods

A retrospective study was carried out in Basrah maternity and children

hospital from the period of first of October 2004 till October 2005. It included those women between the age of (22-50) years who attended infertility center of Basrah maternity and children hospital including those women with primary and secondary infertility and those with recurrent pregnancy loss defined as three or more consecutive abortions. They were checked properly for other factors of subfertility as tubal factors and male factors (only those with ovulatory problem were included in our study.)

On presentation, history was taken from those women including: age, date of presentation, age of menarche, duration of menstrual cycle, gravidity, parity, history of recurrent miscarriage, history of smoking, any family history of early menopause (mothers age of menopause), history of presence and duration of grey hair. Women height and weight were measured. Body mass index (BMI) was calculated (by dividing the patient weight in Kg by the height in meter (Kg/m), a range of 19-30 Kg/m was considered as normal value.

To assess ovarian reserve in such women, a clomiphene citrate challenge test was performed in the following manner :

A cycle day 3 serum FSH level was estimated using Enzyme Linked Florescent Assay (ELFA), then the patients given 100mg of clomiphene

citrate on day 5 to 9 of the cycle and again serum FSH level was measured on day 10, a cycle day 21 progesterone was assessed and a level of (10-30) nmol/l was considered as normal, (the 2.5th centile, WHO), this value is arbitrary, the higher the value, the higher probability that ovulation will be normal (12). Women who didn't complete the CCC test were rolled out from the study

After the initial assessment was done, a follow up visit was conducted, in which the clinical, laboratory and ultrasound findings were appraised and patients were assigned on the pertinent treatment. The length of the follow-up was ranged from one week to one year and was recorded for each patient accordingly .

According to the results of the investigations, the participant women were allocated into two sets: abnormal and normal CCC test.

A normal CCC test refers to day 3 and day 10 FSH below 10mIU/ml. Different cut-off figures have been stated in the previous studies owing to the use of different laboratory protocols have been used which creates confusion in interpretation of data. We use ELFA in our study as mentioned above, an FSH level equal to higher than 10mIU/ml for cycle day 3 or 10 indicates an abnormal test.

The data of this study were analyzed using Chi square (χ^2) and Fisher exact test as indicated statistically.

Results

This study includes one hundred eight patient of 22 to 50 years old who enrolled in appraisal of ovarian reserve in the course of their subfertility management from 1st of October 2004 till 1st of October 2005 .Their follow-up period ranged from one week to a year. Patients with abnormal CCC test had an average duration of follow-up of 18 weeks compared to 25 weeks in patients with an abnormal CCC test. Thirty six women had an abnormal CCC tests versus seventy two patients had a normal CCC tests. Out of 36 patients, 11 showed an abnormal CCC test, depending on day 10 FSH alone. Patients who were not finish the test were ruled out .

Table (1) shows the age distribution of women with a normal CCC versus abnormal CCC test. Patients with normal test were younger than patients with an abnormal test, in addition (94.4 %) of patients of abnormal CCC test were above the age 35 year compared to (41.7 %) those with normal CCC. (p value <0.01).

Comparison of the mean of mother's age of menopause between the two groups yields a statistically significant difference. That is to say, patients with abnormal CCC test tend

to possess a family history of earlier menopause (66.7%) as shown in table (2). Also, 66.7% women with abnormal CCC test, have mother's age of menopause 50 years.

Patients of a normal CCC test had longer cycle interval than women of an abnormal test, p value < 0.01 as shown in table (3).

We notice a statistically insignificant difference in the occurrence rate of grey hair in the two sets of women. This is true for the duration of time that the grey hair existence (p value > 0.05) as shown in table (4). Also, there was no significant difference in regard to smoking habit between the two groups (p value > 0.05) as shown in table (5).

Table (6) shows that: - The mean ages of 38.3 year in abnormal group compared to a mean age of 33.3 year in those with a normal group

-Normal CCC candidates show a higher body mass index (BMI) than those with an abnormal test, p value < 0.01. - No statistically significant difference in the age of menarche when comparing the two groups, p value > 0.05.

-There was statistically significant difference in the proportion of women with recurrent pregnancy loss (RPL) between normal CCC candidates compared to abnormal CCC group, p value < 0.01. - Abnormal CCC candidates had a lower conception rate

(5.5%) compared to normal CCC patients (25 %)

Lastly, table (7) shows that normal CCC test candidates had higher day 21 progesterone than women in the abnormal CCC group. Yet no significant difference for cycle day 21 progesterone in the range of 10 and 20 ng/ml. Higher number of normal CCC

test candidates had serum progesterone above 20 ng /ml.

Chi squared test and Fisher exact test were used to analyze data as appropriate , p value of < 0.05 and < 0.01 was considered to be significant and highly significant respectively .

Table 1: Age distribution between women with a normal and an abnormal CCC test

Age (year)	Normal CCC test		Abnormal CCC test		p value
	No	%	No	(%)	
20--35	42	(58.3%)	2	(5.6%)	<0.01
36- 49	30	(41.7%)	33	(91.6%)	<0.01
≥ 50	0		1	(2.8%)	
Total	72		36		

$x^2 = 27.7$ $df = 1$ $p \text{ value} < 0.01$

Table- 2 Comparison between mothers age at menopause in women with a normal CCC test and those with an abnormal CCC test.

Mother age (year)	Normal CCC test		Abnormal CCC test		p value
	No	(%)	No	(%)	
< 50 years	12	(16.7%)	24	(66.7%)	<0.01
≥50 years	60	(38.3%)	12	(33.3%)	<0.01
Total	72		36		

Table- 3 comparison of mean menstrual cycle length for women with a normal and those with an abnormal CCC test.

	< 26 days cycle		26 days cycle		total
	No	(%)	No	(%)	
Normal CCC test	12	(16.7%)	60	(83.3%)	72
Abnormal CCC test	23	(63.9%)	13	(36.1%)	36
p value	< 0.01		<0.01		

Table-4 The effect of grey hair on patients with a normal CCC test compared to an abnormal CCC test.

Groups	Presence of gray hair		Absence of gray hair		Total
	No	(%)	No	(%)	
Normal CCC test	44	(61.1%)	28	(38.9%)	72
Abnormal CCC test	24	(66.7%)	12	(33.3%)	36
Total	68		40		108

$\chi^2 = 0.12$ $df = 1$

p value Not significant (NS)

Table-5 Effects of smoking between patients with a normal CCC test compared to an abnormal CCC test

Groups	Smoker		Non smoker		Total
	No	(%)	No	(%)	
Normal test	8	(11.11%)	64	(88.9%)	72
Abnormal test	5(13.9%)		31	(86.11%)	36
Total	13		95		108

Fisher Exact test

p- value not significant (NS)

Table 6. Comparison of clinical characteristics between patients with anormal CCC test and those with an abnormal CCC test.

Clinical feature	Normal CCC test	Abnormal CCC test	Normal CCC test	Abnormalccc test	<i>p</i> value
	No ± SD	No ±SD	No %	No %	
Age (year)	33.1±5.30	37.5±(2.79%)			<0.01
BMI	25.9 ±2.4	22.6±2.03			0.01
Age of menarche (year)	12.86±0.53	12.7±0.61			NS
Cycle Interval (ays)	28.03±0.78	26.33±1.74			<i>p</i> <0.01
Mothers age at menopause	49.20±1.99	48.08±3.01			NS
Grey hair			44 (61.1%)	24 (66.7%)	NS
Pregnancy rate			18 25%	2 5.5%	<i>p</i> <0.05
smoking			8 (11.11%)	5 (13.9%)	NS
No of women with RPL ∞			3 (4.16%)	9 (25.0%)	<i>p</i> <0.01
Total number			72	36	

Data are presented as mean ± SD or No. (%), NS: not significant, ∞ Recurrent pregnancy loss.

Table-7 Comparison of serum progesterone values between patients with a normal CCC test and those with an abnormal test.

Laboratory values	Normal CCC test		Abnormal CCC test		p value
	No. (%)		No.	(%)	
Progesterone<10ng/ml	12	(16.7%)	21	(58.3%)	p<0.01
Progesterone 10-20ng/ml	16	(22.2%)	9	25%	>0.01
Progesterone levels >20ng/ml	44	(61.1%)	6	(16.7%)	<0.01
total	72		36		

Chi – square test : $\chi^2=23.95$ d. f = 2 $p<0.01$

Discussion:

In This study, women were tested for their ovarian reserve using CCC test. Prior studies have depended on day 3 FSH for assessment ovarian reserve. In a large study of 1,478 IVF cycles, Toner et al found that ongoing pregnancy rate were higher in patients with FSH level less than 15IU/l and decrease to about 5% in those with basal FSH more than 25 IU/L.(13,14) In our study, CCC test was abnormal in 36 patients, among those 36 patient, eleven have an abnormal CCC test depending on day 10 FSH only. Thus by relying on day 3 FSH only, 30.6 % of the women with reduced ovarian reserve were missed. Current observations agreed with prior studies in that the CCC test has higher sensitivity compared to day 3 FSH level only.

Our results agree with other studies (15) in that abnormal CCC test is associated with increased age. We have found that 94.4 % of patients with abnormal test were above 35 years .

This study also showed that patients with normal CCC test have an average BMI higher than abnormal candidates; this corresponded to a previous study which showed such association between BMI and earlier onset menopause (15). While other studies failed to show such a correlation (16,17) , this is might be related to the fact that obese patients have reduced FSH level due to excessive production of peripheral estrogen.

An abnormal CCC test was significantly correlated with shorter cycle durations, probably due to

shorter follicular phase, and this finding was similar to previous studies. Lenton (1984) showed that normal variability of the duration of follicular phase with increased age. This is clinically significant to identify young patients who require DOR assessment to avoid over testing of all women above 35 years by CCC test (which is costly and defers treatment), and to select subfertile women below 35 years with high risk of diminished ovarian reserve, thus saving time and money. (15,16,18)

The early diagnosis of diminished ovarian reserve helps to start more aggressive therapeutic protocols like controlled ovarian stimulation and ICSI thereby avoiding unnecessary delay and the loss of invaluable time.

We found that a careful history of menstrual cycle is important for rapid diagnosis and referral of patients to a reproductive endocrinology for aggressive treatment.

The mothers' age of menopause is a key clinical attribute. We notice abnormal CCC test candidates have family history of early menopause. This is confirmed by previous studies. Giovanna et al, revealed that genetic inheritance plays a more important role than environmental factors and that inherited factors might be the crucial determinant of menopause onset, the initial oocytes number, the inception of premature ovulatory

failure and physiological menopause (17,19). In our study (66.7%) of women with an abnormal CCC test had their mothers' age at menopause before 50 years compared to (16.7%) in normal group.

Women were interviewed about the presence or absence and onset of grey hair appearance. A positive response was scored if a woman has at least two grey hairs. (20) The difference in the incidence of grey hair between the two groups was statistically insignificant, This finding agreed with Fady et al (2003) (19,21).

The incidence of recurrent pregnancy loss significantly differs when the two groups was compared, in agree with a previous study. (19) This observation might be attributed to the higher occurrence of inherited defects in fetuses aborted spontaneously. in women with diminished ovarian reserve; however, other studies didn't confirm such a difference. (20,21)

Day 21 progesterone was higher in normal CCC test candidates than in abnormal CCC candidates. These observations were confirmed by other studies. (19) These low progesterone values may indicate impaired folliculogenesis or that the CCC test recruits fewer follicles causing inadequate release of progesterone. (19)

The effects of smoking was studied and we didn't find significant

differences in the rate of smoking between the two groups while previous studies found that smoker women reach menopause earlier than non-smoker by 1-4 years. The number of smoked cigarettes was previously found to correlate directly with early menopause.(19,21) We didn't find such a difference, this is may be because smoking is an unusual habit among women in our community.

It has been found that an abnormal CCC test is associated with a low conception rate irrespective of age. (5,22,23) We also found a low pregnancy rate in abnormal CCC test (5.5%) versus 25% in normal test candidates (p value < 0.01). The follow up duration was 18 weeks in abnormal group versus 25 weeks in normal group. Consequently, the reported low pregnancy rate may reflect the rather short period of follow up in our study.

Conclusions and Recommendations

According to the findings of current observations, patients with certain laboratory and clinical attributes have to be assessed for ovarian reserve those women including:

Women age more than 36 years.

Women with interval between menstrual cycles less than 26 days especially those older than 35 years.

Unexplained infertility.

Patients with endometriosis.

Previous ovarian surgery.

-6Patients with abnormal CCC test should be referred to expert gynecologist and reproductive endocrinologist for controlled ovarian hyperstimulation or IVF.

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