Clomiphene citrate challenge test: cycle to cycle variability of cycle day 10 follicle stimulating hormone level

A. Hannoun, A. Abu Musa, J. Awwad, H. Kaspar, A. Khalil

Department of Obstetrics and Gynecology, American University of Beirut, Medical Centre, Beirut (Lebanon)

Summary

The clomiphene citrate challenge test (CCCT), a means of assessing ovarian reserve, was shown by several studies to have an excellent predictive value for achieving conception in natural cycles, during ovulation induction and in-vitro fertilization cycles. Accordingly we elected to study the cycle to cycle variability of CCCT so as to determine the reliability of a single CCCT result. Two groups of patients were studied, the first (n = 40) were those patients who were performing the test because it was indicated, and the second (n = 24) were those who were receiving clomiphene citrate for ovulation induction. In both groups CCCT intercycle variability was significant in 75% of the cases, but this variability altered the prognostic values of the test in only 40% of the first group. In conclusion, our study showed a high percentage of intercycle variability of CCCT but further studies are needed to evaluate the influence of this variability on potential conception.

Key words: Clomiphene citrate; Follicle stimulating hormone.

Introduction

The clomiphene citrate challenge test (CCCT) was first described in 1987 by Navot *et al.* [1] as a means of assessing ovarian reserve in women ≥ 35 years of age. It consists of measuring baseline serum follicle stimulating hormone (FSH) levels on cycle day 3 and then on cycle day 10 after the administration of 100 mg of clomiphene citrate on cycle days 5 through 9. An elevated cycle day 10 FSH concentration, which may vary from one laboratory to another and from one study to another (range: 10 U/L-26U/L), would indicate an abnormal test.

Several studies [1-6] showed that an abnormal CCCT had excellent predictive value for diminished ovarian reserve and poor long-term pregnancy rates in natural cycles, during ovulation induction and in in-vitro fertilization (IVF) cycles. Therefore, a patient with abnormal values is usually counselled, as it is done in our IVF program, that her chance for conception is very poor and that she may want to consider other options like egg donation or adoption. However, many of these patients with abnormal tests would request a repeat test to be sure of the reported result, or they would question its predictive value and ask for IVF trial with controlled ovarian hyperstimulation.

Accordingly, we elected to study the cycle to cycle variability of CCCT, namely cycle day 10 FSH value, in the same patient in order to determine the reliability of a single CCCT result.

Materials and Methods

Starting January 1997, two groups of patients attending the infertility and IVF center at the American University Hospital in Beirut were approached for inclusion in the study. The first group were those patients in whom the CCCT was being done to check their ovarian reserve (group I, n = 40), and the second group comprised those patients in whom ovulation was being induced by the administration of 100 mg of clomiphene citrate starting cycle day 5 for 5 days (group II, n = 24). Those patients in group II who conceived from the first cycle of ovulation induction were excluded from the study. Blood was withdrawn by regular venipuncture, centrifuged after its clotting and the serum obtained was kept at 20 °C till the time of hormone assay. The sera obtained from the same patient from the two consecutive cycles were run in the same hormone assay in order to eliminate the interassay variability of the test itself.

The test used in our hormone laboratory is the time-resolved fluoroimmunoassay kit A017-201 (DELFIA hFSH kit, Wallac, Oy, USA). It is a solid phase, two site fluoroimmunometric assay in which two monoclonal antibodies (derived from mice) are directed against two separate antigenic determinants on the hFSH molecule. The reported intraassay coefficient of variation of the DELFIA test by the manufacturer is 2.8%.

In order to study the actual intraassay percentage change when the test is done in our laboratory, we performed the test on 15 samples in duplicate in the same assay. The percentage change (PC) was calculated as follows: (1st FSH value - 2nd FSH value / 1st FSH value) x 100. Concerning the issue of ovarian reserve, in our laboratory, a cycle day 10 value of FSH > 20 is considered abnormal.

Data obtained from the hormone assay were analysed as follows: cycle day 10 FSH values of the 2 consecutive CCCTs for each patient were compared to determine two objectives. First, the presence of a significant difference between the two FSH values, and second, whether this difference had shifted the patient from one prognostic category to another as related to the issue of ovarian reserve.

Results

The actual intraassay percentage change (PC) or variability of the DELFIA test, computed from the values of the 15 samples that were run in duplicate, according to the formula mentioned above, ranged from 0.2% to 9.4%

Received June 25, 1998
revised manuscript accepted for publication July 20, 1998

with a mean of 3.2%. That is why we elected to consider any PC > 10% as a significant difference between the 2 compared values when applied to the study group, namely when comparing the cycle day 10 FSH levels in the 2 consecutive cycles of CCCTs in the same patient.

Thirty (75%) out of 40 patients (mean age: 40.1 years) in group I had a significant (>10%) PC or cycle to cycle variability of the CCCT. Out of these 30 patients only 12 (40%) had a real shift in their prognostic category namely a change from a normal CCCT (cycle day 10 FSH < 20 U/L) to an abnormal test (FSH < 20 U/L) or vice versa. Similarly out of the 24 patients (mean age: 30.3 years) in group II, 18 (75%) had a significant PC but none of these 18 had a real shift in her prognostic category.

Discussion

The concept of ovarian reserve is a well clinically established entity [7] that can be assessed by different tools. Among these screening tools, the CCCT has proven to be, up till now, the best characterised and most sensitive test in providing accurate prognostic information. An abnormal CCCT has excellent predictive values for diminished ovarian reserve and poor long-term pregnancy outcome in natural or ovulation induced cycles, as well as in assisted reproductive cycles [1, 3, 5].

Although most infertility centers, including ours, do not use an abnormal CCCT as an exclusion criterion, these centers make use of the information it provides in counselling infertile patients regarding their chances for achieving pregnancy by certain therapeutic regimens. However, many of these patients inquire about the reliability and reproducibility of this test and even request a repeat test, when the result is abnormal, to be sure of the outcome.

In this study, we determined the cycle to cycle variability of cycle day 10 FSH level of CCCT and its effect on the test interpretation namely normal versus abnormal. The intercycle variability of basal cycle day 3 FSH level was assessed previously by Scott *et al.* [8]. In our study, we found that a real intercycle variability occurred in 75% of the CCCTs performed. However this variability was significant, in terms of the test prognostic value, only in the elderly group (group I in which CCCT was performed to assess the ovarian reserve) and not in the younger group (group II in which CCCT was done only as part of this study). In group I, 40% of those who had real intercycle variability had a real change in their prognostic category in comparison to none in group II.

This study lacks the correlation between the presence of the CCCT intercycle variability and/or the shift in the prognostic category and the potential for achieving pregnancy. Further studies should address this issue. In conclusion, this study showed a high percentage of intercycle variability in the cycle day 10 FSH level of the CCCT. This variability, with a wide range, could affect the test interpretation (normal versus abnormal) and hence the counselling of concerned patients in regard to their potential fertility especially in elderly patients in whom ovarian reserve is being assessed. Whether to recommend performing the CCCT more than once awaits further studies to check whether this intercycle variability has any prognostic bearing on potential conception.

References

[1] Navot D., Rosenwaks Z., Margalioth E. J.: "Prognostic assessment of female fecundity". *Lancet*, 1989, *2*, 645.

[2] Loumaye E., Billion J.-M., Mine J.-M., Psalit I., Pensis M., Thomas K.: "Prediction of individual response to controlled ovarian hyperstimulation by means of a clomiphene citrate challenge test". *Fert. Steril.*, 1990, *53*, 295.

[3] Tanbo T., Dale P. O., Abyhom T., Stokke K. T.: "Follicle-stimulating hormone as a prognostic indicator in clomiphene citrate/human menopausal gonadotrophin-stimulated cycles for in vitro fertilization". *Hum. Reprod.*, 1989, *6*, 647.

[4] Tanbo T., Dale P. O., Ludne O., Norman N., Abyholm T.: "Prediction of response to controlled ovarian hyperstimulation: a comparison of basal and clomiphene citrate stimulated follicle stimulating hormone levels". *Fertil. Steril.*, 1990, *53*, 295.

[5] Scott R. T., Leonardi M. R., Hofmann G. E., Illions E. H., Neal G. S., Navot D.: "A prospective evaluation of clomiphene citrate challenge test screening in the general infertility population". *Obstet. Gynecol.*, 1993, *82*, 539.

[6] Scott R. T. Jr., Illions E. H., Koster E. R., Dellinger C. L., Hofmann G. E., Navot D.: "Evaluation of the significance of a poor estradiol response during the clomiphene citrate challenge test". *Fertil. Steril.*, 1993, *60*, 242.

[7] Scott R. T. Jr., Hofmann G. E.: "Prognostic assessment of ovarian reserve". *Fertil. Steril.*, 1995, *63*, 1.

[8] Scott R. T. Jr., Hofmann G. E., Oehninger S., Muasher S. J.: "Intercycle variability of day 3 follicle-stimulating hormone levels and its effect on stimulation quality in vitro fertilization". *Fertil. Steril.*, 1990, *54*, 297.

Address reprint requests to:
ALI KHALIL, M.D.
Department of Obstetrics and Gynecology
American University of Beirut
Medical Centre
P.O. Box: 113-6044/C8
Beirut (Lebanon)