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Clomiphene citrate changes metabolite content of follicular fluid of PCOS women

A.A. ERSAHIN[1], E. ÇALIŞKAN[2]

[1]Department of Obstetrics and Gynecology, Bahcesehir University School of Medicine, Istanbul, Turkey
[2]Department of Obstetrics and Gynecology, Bahcesehir University School of Medicine, Kocaeli, Turkey

Abstract. – OBJECTIVE: To determine whether clomiphene citrate (CC) treatment affects the metabolite contents of a dominant follicle in polycystic ovary syndrome (PCOS).

PATIENTS AND METHODS: Twenty non-obese primary infertile anovulatory PCOS women undergoing CC treatment and ten fertile women were enrolled. 6 out of 20 patients had impaired glucose tolerance test (IGT). CC was given at a dose of 150 mg on days 3-7 of cycles in the first group. 10 fertile women with a regular menstrual cycle and normal ovaries during ultrasound examination were accepted as control group. They were not given any drug for ovarian stimulation. Transvaginal sonography and follicular tracking were done to each group of participant. Both groups of subjects underwent magnetic resonance spectroscopy when the dominant follicle with a mean diameter of at least 16-18 mm was detected. Lactate (Lac), N-acetylaspartate (NAA), creatine 1 (Cr1), creatine 2 (Cr2) and choline (Cho) signal of dominant follicles were measured. Peak of each metabolite was measured in units. Voxels were placed in the center of dominant follicle.

RESULTS: Compared to control group significantly decreased Cho signal was found in follicular fluid of PCOS subjects taking clomiphene. Almost three-fold decline in Cho signal was detected in PCOS group compared to Cho signal of control group (0.64±1.01 *vs.* 2.01 ±1.13). On the other hand, significantly increased Lac signal was detected in the dominant follicle of PCOS subjects taking clomiphene compared to control group. Almost 2 fold increase in Lac signal was noted after clomiphene treatment (1.90±0.32 *vs.* 0.93±2.21). The results of spectroscopy signals obtained from PCOS subjects without IGT and PCOS subjects with IGT were similar.

CONCLUSIONS: Unbalanced production of Cho signal in the follicular fluid may have occurred secondary to membrane damage of cumulus-oocyte-complexes due to CC therapy.

Key Words:
Clomiphene citrate, PCOS, MR spectroscopy.

Introduction

Clomiphene is a selective estrogen receptor modulator having both estrogen agonist and antagonist properties[1]. In women with WHO Type II anovulation, clomiphene has been reported to induce ovulation in almost 70% of patients and achieve a pregnancy rate of 15-50% per woman[2]. Reasons for low pregnancy rates despite high ovulation in PCOS cases using CC are not clear. While some studies claim that supplementation with exogenous estrogen is beneficial in CC cycles, other studies assert there is no positive effect[3].

Follicular fluid metabolites may be responsible for unsuccessful pregnancy rates in PCOS cases. We, therefore, thought that deterioration of membrane integrity in the cumulus-oocyte-complexes secondary to CC treatment may be a possible cause of failed pregnancy in PCOS subjects. To test our idea we used MR spectroscopy. MRS, a non-invasive technique, may provide both qualitative and quantitative information about the pathological and physiological process. Each metabolite has different function within cell. N-acetylaspartate (NAA) is a marker for neuronal health; Choline (Cho) is a marker of membrane integrity; Lactate (Lac) is a sign of defect in cell cycle[4-6]. The feasibility of MRS in reproductive tissues was previously shown by Celik et al[4]. They demonstrated the efficiency of MRS in identifying endometrial and ovarian signals in benign and malignant gynecological disorders[4-6]. They also showed that the MRS technique was successfully applicable in the endometrial cancer, ovarian cancer, endometrioma, leiomyoma as well as in the normal endometrium[4-7]. Based on above considerations, this study was planned to assess the possible effects of clomiphene citrate on development of dominant follicle in anovulatory women with PCOS using several well-known spectroscopy markers.

Corresponding Author: Aynur Adeviye Erşahin, MD; e-mail: aynur.ersahin@hotmail.com

Patients and Methods

The study was conducted in the Bahcesehir University Obstetrics and Gynecology Clinic. PCOS was diagnosed when existence of at least two of the following three features: 1. amenorrhea or oligomenorrhea with chronic anovulation, 2. clinical and/or biochemical evidence of hyperandrogenism, and 3. ultrasonographic appearance of PCOS (2). A total of 20 primary infertile anovulatory women with PCOS was enrolled in this study. Six out of 20 PCOS patients had impaired glucose tolerance test (IGT). Subjects with pelvic diseases, history of pelvic surgery, and tubal or male factor infertility, were excluded. Obese PCOS subjects having a body mass index higher than 30, and/or current or previous history of taking hormonal drugs and taking alcoholic beverages, were also excluded. All PCOS subjects were treated with clomiphene citrate (Serophene, Serono, Rome, Italy) at dosage of 150 mg/day for 5 days beginning on the 3rd day of progesterone-induced withdrawal bleeding. Transvaginal sonography and follicular tracking were done. Women underwent spectroscopy analysis as soon as the detection of a follicle with a mean diameter of at least 16-18 mm at USG examination. Age and body mass index (BMI) matched ten fertile women with regular menstrual cycles, that were enrolled as controls. Similar to PCOS subjects, control patients were sent to spectroscopy analysis when the dominant follicle with a mean diameter of at least 16-18 mm was detected. The study was performed according to the guidelines of the Helsinki Declaration on human experimentation and was approved by the Local Ethics Committee.

MR spectroscopy

Metabolite signals were obtained by way of 1.5 T MRI. In both axial and coronal plane T1-weighted images (time repetition [TR]/time echo [TE], 10/4.6) and T2-WI (1600/100) with 5 mm thick sections, were achieved. Single voxel MR spectroscopy procedure was applied via point-resolved spectroscopy with a short (31 ms) and long (136 ms) TE. Finally, the voxel was placed at the center of dominant follicle to prevent signal from neighboring structures. The metabolite ratios of acquired signals were determined using Magnetic Resonance User Interface software. N-acetylaspartate (NAA), lactate (Lac), creatine (Cr), and choline (Cho) peaks of each group was detected.

Statistical Analysis

The Statistical Package for Social Sciences, version 21.0 (SPSS Armonk, NY, USA) was used for statistical analysis. Individual group parameters were assessed with one-sample Kolmogorov-Smirnov Z test and were found to be abnormally distributed. Hence, statistical comparisons between groups were performed by nonparametric Mann-Whitney U and Wilcoxon tests. Data are presented as mean±standard deviation (SD). For all comparisons, statistical significance was defined by $p < 0.05$.

Results

BMI of CC and control group were similar. However, HOMA-IR, serum insulin and androgen levels in the PCOS women were significantly higher from those of the control group. 3/20 PCOS patients were excluded for the following reasons: inadequate development of the growing follicle at transvaginal sonography (one patient), fear of indoor space in MRI (one patient), and bad spectral image (one patient). All ten subjects in control group had good spectral image except one who ovulated before spectroscopy. Compared to control group significantly decreased Cho signal was found in follicular fluid of PCOS subjects taking clomiphene. Almost three-fold decline in Cho signal was detected in PCOS group compared to Cho signal of control group (0.64±1.01 *vs.* 2.01 ±1.13; $p<0.01$). On the other hand, significantly increased Lac signal was detected in the dominant follicle of PCOS subjects taking clomiphene compared to control group (Figure 1). Almost 2 fold increase in Lac signal was noted after clomiphene treatment (1.90±0.32 *vs.* 0.93±2.21; $p<0.03$). There was no significant difference in terms of Cr and NAA signals between two groups.The results of spectroscopy signals obtained from PCOS subjects without IGT and PCOS subjects with IGT were similar.

Discussion

This study assessed dominant follicle metabolite contents in PCOS women taking clomiphene for ovarian stimulation. A total of 20 women with PCOS given clomiphene were selected and followed-up. Before the study began, there were not significant differences in basic demographic variables among the treatment and control groups.

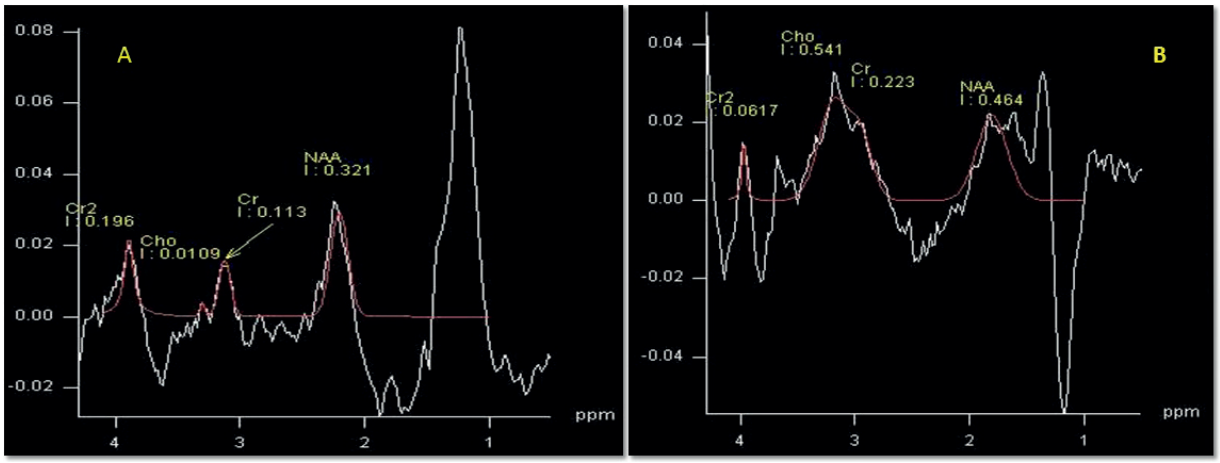

Figure 1. Single voxel MRS depicts low Cho signal in clomiphene group ***(A)*** where as high Cho signal in natural cycles ***(B).***

As expected, most PCOS cases had high serum insulin and androgen levels. Likewise, HOMA-IR of PCOS subjects was higher than those in the controls. On account of insulin resistance has an important implication in the pathogenesis of PCOS control cases with high insulin resistance, they were excluded.

The results of the present study show that clomiphene does cause a significant reduction in Cho metabolite. On the other hand Lac signal of women taking clomiphene was found to significantly increased. We have not tested the effect of the selected dose of CC (150 mg/d) on the spectra. The higher metabolites measured in the control group have led to the idea that CC use in some way negatively affects fertilization or implantation in infertile PCOS cases. This side effects may either be due to anti-estrogenic effects of clomiphene or it may be secondary to the secretion of high amounts of endogenous FSH. Clomiphene exerts anti-estrogenic effects on the endometrium that lead to thin endometrial development. This anti-estrogenic impact of clomiphene on the endometrium is likely to be one of the major causes of decreased pregnancy rates in spite of high ovulation rates[3,8-10]. Several studies that have reported that adverse effects of clomiphene on endometrium thickness might be prevented by administering exogenous estrogen or rFSH after clomiphene[3,11]. Conversely, some studies did not show that supplementation with estrogens in clomiphene cycles did not show improvement in endometrium receptivity. Collectively, high Lac and low Cho signals can be attributed to anti-estrogenic effects of clomiphene.

Conclusions

We have demonstrated for the first time that CC treatment, unlike naturally growing dominant follicle, caused failed secretion of metabolites that requiring for normal follicle development. We also noted that an increase in the signal intensity of lactate with a decrease in Cho peak intensity in the spectra obtained from PCOS cases was the specific evidence of detrimental effects of clomiphene on growing follicle. Finally, in addition to insulin resistance, defects in cell membrane integrity of PCOS patients using CC may explain low pregnancy rates. The data we have obtained with this study are not strong enough to suggest the abandonment of CC usage in infertile PCOS. It is useful to be cautious about the use of CC for ovaraian stimulation until comprehensive studies comparing CC, letrazole, and rFSH preparations on follicle development will be done.

Conflict of Interest

The Authors declare that they have no conflict of interest.

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