The effect of low-dose combined oral contraceptive containing 100 ug levonorgestrel on plasma plasminogen activator inhibitor-1 concentrations

M. Küçük[1], S.D. Sezer[2], A.R. Odabaşi[2], Z. Güner[2], H. Yuksel[2], M. Serter[3]

[1]Department of Obstetrics and Gynecology, Cine State Hospital, Aydin
[2]Department of Obstetrics and Gynecology, Adnan Menderes University, Aydin
[3]Department of Biochemistry, Adnan Menderes University, Aydin (Turkey)

Summary

Objective: The objective of the current study was to investigate the effect of a combined oral contraceptive (COC) containing 20 ug ethinyl estradiol (EE) and 100 ug levonorgestrel (LNG) which is currently used on plasma plasminogen activator inhibitor-1 (PAI-1) concentrations. *Material and Methods:* Twenty-five women who had not used any COC for at least three months before the initiation of the study were enrolled in the control group. Twenty women who had been using COC containing 20 ug EE and 100 ug levonorgestrel LNG for at least three months prior to the study were enrolled in the LNG/EE group. Serum samples for PAI-1 and other biochemical parameters were obtained at the early follicular phase (cycle day 2-5). *Results:* No significant difference was observed in PAI-1 concentrations between the LNG/EE and control group (group LNG/EE: 62.4 ± 30.2 ng/ml; control group: 58.7 ± 26.0 ng/ml). *Conclusion:* Although we observed similar PAI-1 concentrations in both groups, there is need for further interventions to evaluate the clinical relevance of our findings.

Key words: PAI-1; Low-dose oral contraceptives; Levonorgestrel

Introduction

For many years, combined oral contraceptives (COCs) have been used as a safe method of birth control [1]. Since the 1960s, when they were first introduced, decreasing the hormone dosage in COCs in an attempt to reduce COC-related complications has been a goal. It has been for this reason that drugs called "low-dose COCs" containing 20 ug ethinyl estradiol (EE) together with component progesterones such as desogestrel or levonorgestrel (LNG) have been on the market since the beginning of the 1990s [2, 3].

To date, there has been a sizeable volume of data obtained on the effectiveness of low-dose oral contraceptives and their capacity to secure adequate cycle control [4, 5]. Knowledge about the effects of COCs on hemostatic and fibrinolytic variables, however, is limited [6, 7].

Tissue plasminogen activator (tPA) is a molecule involved in the conversion of plasminogen to plasmin, facilitating an essential process in clotting. Plasminogen activator inhibitor-1 (PAI-1) is the basic molecule that determines the level of active tPA in the blood. Although the basic cells involved in PAI synthesis are unknown, PAI synthesis has been identified in hepatocytes, adipocytes and endothelial cells [8-10].

The purpose of the present study was to examine the effect of low-dose COCs containing 20 ug EE and 100 ug LNG on plasma plasminogen activator inhibitor-1 (PAI-1) concentrations.

Revised manuscript accepted for publication March 25, 2010

Materials and Method

The study was carried out at the Adnan Menderes University Medical School Gynecology and Obstetrics Clinic. The required permission was obtained for the study from the university board of ethics and an informed consent form was acquired from each of the participants in the study.

Participants who met the inclusion criteria for the study and had not been using any kind of COC for the past three months were enrolled in the control group. Women who met the inclusion criteria and had been taking a low-dose COC containing 20 ug EE and 100 ug LNG for at least three months prior to the study constituted the study group.

Inclusion criteria for the study were determined as follows: Being a non-smoker between the ages of 18-35; having no contraindication for taking COCs; having no thyroid, hepatorenal or cardiovascular disease; having no known malignant disease or history of thromboembolic disease. Apart from these criteria, patients diagnosed with polycystic ovary syndrome (PCOS) were excluded from the study.

Blood specimens were collected on the third day of the cycle after a night of fasting. Serum was separated and preserved at -80°C to facilitate analysis of PAI-1 levels. PAI-1 levels were analyzed with a high-sensitivity ELISA Kit (American Diagnostica, CT, USA).

Statistical analysis

Statistical analysis for the study was carried out using the SPSS program (Statistical Package for Social Sciences), Version 12.0. Data was expressed as mean ± standard deviation.

$p < 0.05$ was considered statistically significant. Continuous variables were analyzed using the Mann-Whitney U test. Differences between categoric variables were analyzed with the chi-square and Fisher's exact tests.

Results

The control group in the study consisted of 25 women while the LNG/EE group comprised 20 women. Demographic characteristics and PAI-1 levels of the participants are shown in Table 1.

Table 1. — *Patient characteristics and biochemical parameters*.

	LNG/EE group (n = 20)	Control group (n = 25)	*p* value
Age (years)	26.8 ± 4.3	27.7 ± 4.1	ns
BMI (kg/m²)	22.2 ± 2.6	21.9 ± 2.5	ns
Systolic Blood Pressure (mmHg)	113.1 ± 9.4	110.3 ± 10.1	ns
Diastolic Blood Pressure (mmHg)	77 ± 6.9	78 ± 6.1	ns
Waist to hip ratio	0.78 ± 0.05	0.77 ± 0.08	ns
PAI (ng/ml)	62.4 ± 30.2	58.7 ± 26.0	ns

Results in mean ± SD. NS: not significant. LNG/EE: 20 ug ethinyl estradiol/100 ug levonorgestrel group.

No difference was seen between the study and control groups in terms of systolic and diastolic blood pressure values. Similarly, no difference was observed between the groups in terms of age and body mass index.

Again, no difference was seen between the study and control groups in terms of plasma PAI-1 levels (group LNG/EE: 62.4 ± 30.2 ng/ml; control group: 58.7 ± 26.0 ng/ml).

Discussion

Over the years, the use of COCs has been associated with rare but potentially dangerous side-effects. Because of their infrequency, these side-effects have not been fully examined in clinical studies. It is for this reason that there have been efforts to define secondary parameters in the hope that some knowledge can be gathered in the context studied [11].

Knowledge about the effects of COCs on hemostatic and fibrinolytic variables is limited [6, 7]. There is also limited information on the effect on plasma PAI-1 concentrations of low-dose COCs containing 20 ug EE and 100 ug LNG. PAI-1 levels increase in pregnancy, returning to normal at the fifth week postpartum [12]. There are conflicting results in studies regarding the effect of low-dose COCs on PAI-1 levels. Some research has indicated that COCs have a reducing effect on PAI-1 concentrations. Most such studies, however, have been carried out on COCs containing 30 ug or more of EE. In their study, Prasad *et al*. [13] found a significant decrease in PAI-1 levels with COCs containing 30 ug EE and 150 ug LNG. Similarly, Meijers *et al*. [14] in their research also observed a decrease in PAI-1 levels in women taking COCs containing 30 ug EE and 150 ug LNG. On the other hand, studies on this subject have generally not been conducted using preparations that contain 20 ug EE and 150 ug LNG. Conversely, Ferreira *et al*. [15] report no significant difference in PAI-1 levels with low-dose COCs. In a study by Winkler *et al*. [16] on progesterone-only oral contraceptives containing 30 ug LNG, it was noted that PAI-1 levels did not change. In keeping with this finding, another study on the effect of low-dose COCs on human endothelial cell cultures has shown that these low-dose preparations do not increase or decrease PAI synthesis [17]. Endrikat *et al*. compared 30 ug EE and 150 ug LNG containing COC with a combination of 20 ug EE and 100 ug containing COC to find that the group taking 20 ug EE and 100 ug LNG COC had higher levels of PAI-1 compared to the group taking the higher dose.

In the present study, no change was observed in PAI-1 levels with the use of low-dose COCs containing 20 ug EE and 100 ug LNG. These findings are in line with the results of the study of Fereira *et al*. [15] .

One of the strengths of the present study was that patients with PCOS were excluded from the sampling. In a study by Agren *et al*. [18], low PAI levels were associated with insulin resistance and increased BMI. Patients with PCOS exhibit obesity and increased insulin resistance [19].

The present study has various limitations. The fact that the research was cross-sectional prevented an examination of the effect of the combined use of 20 ug EE and 100 ug LNG on plasma PAI-1 levels over time. There is a need for more longitudinal studies with larger samplings and different populations.

Another limitation of the study was the method of self-reporting that was used to obtain information from patients about their individual use of contraceptives and the length of time they were taking them. A recent study however has reported that there is a high probability that women will remember how long an oral contraceptive has been used, supporting the opinion that the information provided in this way should be readily accepted as reliable data [20].

To conclude, although similar plasma PAI-1 concentrations were observed in both the LNG/EE and control groups in this study, it is suggested that more advanced studies be carried out to better determine the clinical significance of the findings noted here.

References

[1] Blackburn R.D., Cunkelman A., Zlidar V.M.: "Oral contraceptives-an update". *Popul*. *Rep A*., 2000, *28*, 25.

[2] Endrikat J., Klipping C., Cronin M., Gerlinger C., Ruebig A., Schmidt W., Düsterberg B.: "An open label, comparative study of the effects of a dose-reduced oral contraceptive containing 20 microg ethinyl estradiol and 100 microg levonorgestrel on hemostatic, lipids, and carbohydrate metabolism variables". *Contraception,* 2002, *65,* 215.

[3] Düsterberg B., Ellman H., Müller U., Rowe E., Mühe B.: "A three-year clinical investigation into efficacy, cycle control and tolerability of a new low-dose monophasic oral contraceptive containing gestodene". *Gynecol*. *Endocrinol*., 1996, *10,* 33.

[4] Bannemerschult R., Hanker J.P., Wünsch C., Fox P., Albring M., Brill K.: "A multicenter, uncontrolled clinical investigation of the contraceptive efficacy, cycle control, and safety of a new low dose oral contraceptive containing 20 micrograms ethinyl estradiol and 100 micrograms levonorgestrel over six treatment cycles". *Contraception*, 1997, *56*, 285.

[5] Hite R.C., Bannemerschult R., Fox-Kuchenbecker P., Turck R., Brill K.: "Large observational trial of a new low-dose oral contraceptive containing 20 micrograms ethinylestradiol and 100 micrograms levonorgestrel (Miranova) in Germany". *Eur. J. Contracept*. *Reprod*. *Health Care*, 1999, *4*, 7.

[6] Kluft C., de Maat M.P., Heinemann L.A., Spannagl M., Schramm W.: "Importance of levonorgestrel dose in oral contraceptives for effects on coagulation". *Lancet,* 1999, *354*, 832.
[7] Koh K.K., Yoon B.K.: "Controversies regarding hormone therapy: Insights from inflammation and hemostasis". *Cardiovasc Res.,* 2006, *70,* 22.
[8] Kooistra T., Schrauwen Y., Arts J., Emeis J.J.: "Regulation of endothelial cell t-PA synthesis and release". *Int. J. Hematol.,* 1994; *59,* 233.
[9] Chandler W.L., Levy W.C., Stratton J.R.: "The circulatory regulation of TPA and UPA secretion, clearance, and inhibition during exercise and during the infusion of isoproterenol and phenylephrine". *Circulation,* 1995, *92,* 2984.
[10] Wiman B.: "Plasminogen activator inhibitor 1 (PAI-1) in plasma: its role in thrombotic disease". *Thromb. Haemost.,* 1995, *74,* 71.
[11] Hu P., Greendale G.A., Palla S.L., Reboussin B.A., Herrington D.M., Barrett-Connor E., Reuben D.B.: "The effects of hormone therapy on the markers of inflammation and endothelial function and plasma matrix metalloproteinase-9 level in postmenopausal women: the postmenopausal estrogen progestin intervention (PEPI) trial". *Atherosclerosis*., 2006, *185,* 347.
[12] Bremme K., Ostlund E., Almqvist I., Heinonen K., Blombäck M.: "Enhanced thrombin generation and fibrinolytic activity in normal pregnancy and the puerperium". *Obstet. Gynecol.,* 1992*, 80*, 132.
[13] Prasad R.N., Koh S.C., Viegas O.A., Ratnam S.S.: "Effects on hemostasis after two-year use of low dose combined oral contraceptives with gestodene or levonorgestrel". *Clin. Appl. Thromb. Hemost*., 1999, *5,* 60.
[14] Meijers J.C., Middeldorp S., Tekelenburg W., van den Ende A.E., Tans G., Prins M.H. *et al*.: "Increased fibrinolytic activity during use of oral contraceptives is counteracted by an enhanced factor XI-independent down regulation of fibrinolysis: a randomized cross-over study of two low-dose oral contraceptives". *Thromb. Haemost*., 2000, *84*, 9.
[15] Ferreira A.C., Montes M.B., Franceschini S.A., Toloi M.R.: "Effects of two oral contraceptives, containing 30 or 20 microg of ethinyl estradiol in combination with gestodene, on blood coagulation and fibrinolysis in Brazilian women". *Int. J. Fertil. Women's Med.,* 2001, *46,* 265.
[16] Winkler U.H., Howie H., Bühler K., Korver T., Geurts T.B., Coelingh Bennink H.J.: "A randomized controlled double-blind study of the effects on hemostasis of two progestogen-only pills containing 75 microgram desogestrel or 30 microgram levonorgestrel". *Contraception,* 1998, *57*, 385.
[17] Quehenberger P., Kapiotis S., Pärtan C., Schneider B., Wenzel R., Gaiger A., Speiser W.: "Studies on oral contraceptive-induced changes in blood coagulation and fibrinolysis and the estrogen effect on endothelial cells". *Ann. Hematol.,* 1993, *67,* 33.
[18] Agren A., Wiman B., Schulman S.: "Low PAI-1 activity in relation to inflammatory parameters, insulin profile and body mass index". *J. Intern. Med.,* 2008, *264,* 586.
[19] Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group: "Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome". *Fertil. Steril.,* 2004, *81*, 19.
[20] Coulter A., Vessey M., McPherson K., Crossley B.: "The ability of women to recall their oral contraceptive histories". *Contraception,* 1986, *33,* 127.

Address reprint requests to:
A.R. ODABAŞI, M.D.
Department of Obstetrics and Gynecology
Adnan Menderes University
Aydin (Turkey)
e-mail: arodabasi@gmail.com