Influence of Oral Contraceptive Pills on Homocysteine and Nitric Oxide Levels: As Risk Factors for Cardiovascular Disease

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Purpose: Elevation of homocysteine levels have been involved as a remarkable risk factor for cardiovascular disease. Decreased bioavailability of nitric oxide (NO) may result in abnormal reactions between the vessel wall and platelets and is thus involved in the initiation and progression of atherosclerosis. We aimed to assess the effect of a low dose oral contraceptive pills on homocysteine and NO levels which may influence the individual cardiovascular risk by regulation of endothelial function and development of atherosclerosis. **Methods:** The study was conducted in 50 healthy women with normal menstrual cycles as control group and 50 healthy women receiving oral contraceptive pill for at least three menstrual cycles.

Key words: homocysteine; nitric oxide; contraceptive pill; atherosclerosis

Homocysteine concentration was assayed by an enzyme immunoassay method and a colorimetric assay was used for determination of NO levels. **Results:** After 3 months of treatment, homocysteine levels were significantly increased ($P = 0.027$), and there was a significant and considerable decrease ($P = 0.048$) in NO concentration of oral contraceptive pill (OCP) consumers. **Conclusions:** Our data indicates that OCPs increase the homocysteine (HCY) and reduce the NO levels. These findings warrant a possible implication of OCP in change of risk of development of coronary heart disease. J. Clin. Lab. Anal. 26:120–123, 2012. © 2012 Wiley Periodicals, Inc.

INTRODUCTION

There is controversial evidence of the effects of combined oral contraceptive pills (OCPs) on the cardiovascular risk factors and the body's response to these effects. For example, it has recently been shown that the free radicals produced following the metabolic activation of low-dose OCPs can alter the activity of antioxidant enzymes glutathione peroxidase and superoxide dismutase in healthy subjects (1).

There is a growing awareness of possibility of alterations of some of cardiovascular risk biomarkers in women receiving combined OCPs. Recent data shows that the use of these pills could be associated with an increased

risk of myocardial infarction, especially in women with cardiovascular risk factors such as smoking or hypertension (2). The studies performed on women receiving hormone replacement therapy (HRT) indicate that the adverse cardiovascular effects may depend on the type,

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dosage, form, and route of administration of hormones (3). These hormones can adversely affect different cardiovascular risk biomarkers, of which, homocysteine (HCY) (4) and nitric oxide (NO) (5) are of particular importance.

NO leads to vasodilatation and inhibits platelet aggregation and migration (7,8). Reduced bioavailability of NO has been implicated to be involved in the initiation and progression of atherosclerosis (2). There is evidence for endothelial dysfunction and specific defects in NO-mediated vasodilatation in patients with hypertension, smokers, and individuals with elevated concentration of HCY (9). Several studies have defined that female sex steroids may affect NO and HCY (2). The results of the studies by Ridker et al. (11) conducted on healthy postmenopausal women indicate that cardiovascular risk is increased more than twice in women with moderately increased HCY concentration, irrespective of the conventional risk factors. HCY level is higher in postmenopausal women in comparison with those in the premenopausal period (12).

The aim of this study is to establish the normal range of homocysteine and NO concentration in healthy subjects living in Tehran, and to estimate the NO and HCY alteration as a result of OCP consumption and their possible consequences.

MATERIALS AND METHODS

Study Subjects

The effects of estrogen–progesterone LD (low dose) OCPs on the HCY and NO were determined. Sample size was determined for a power of more than 80% using data from our pilot study and G*Power 3.1.0 software package. The study consisted of 50 healthy married women aged 20–35 years not receiving any contraception with normal menstrual cycles who served as controls and 50 married women aged 20–35 years who were referred to the Valfajr Clinic in western Tehran who were consuming LD estrogen–progesterone OCPs nightly (levonorgestrel 0.15 mg, ethinyl–estradiol 0.03 mg, Darupakhsh Company, Tehran, Iran) for a minimum of three cycles. Each cycle consisted of 21 days of active treatment, followed by 7 days without treatment. This study was approved by the University Clinic Ethics Committee and written informed consent was obtained from all OCP users and control subjects. Information about age, smoking history, consumption of alcohol, medications used (including oral contraceptives) recent history of acute or chronic illness, stage of menstrual cycle and the duration of their use of OCPs were noted (not shown). In our study, individuals from the control and OCP receiving groups were matched regarding sex, age, weight, and blood pressure. Subjects with history of diabetes mellitus, liver and renal diseases, and who work with insecticides were excluded from the

study. It should be noted that cigarette smokers, alcohol drinkers, and those receiving other medications such as folic acid and vitamin B₁₂ were also excluded from the study. The diet excluded the use of the following food-stuffs which are known to influence the HCY and NO levels: cheese, meat, salad, spinach, cabbage, canned food, and tea. Blood was collected by venipuncture of the antecubital vein into sterile tubes for determination of HCY and NO levels. The blood samples (12 hr) were obtained between 9:00 a.m. and 11 a.m. to minimize possible diurnal variations.

HCY Assay

Axis homocysteine enzyme immunoassay (EIA) was intended for the quantitative determination of total L-homocysteine in serum or plasma (Axis-Shield Diagnostics, Ltd. Kit, Dundee DD2 1XA, United Kingdom). Precision of assay was 9–10%. As synthesis of HCY continues in red blood cells after drawing, it was very important to prepare specimens as follows: serum samples should be allowed to clot for no more 30 min before centrifugation and separation of serum. Serum samples should be kept on ice prior to separation.

NO Assay

Nitrate/Nitrite Colorimetric Assay (Nitrate/Nitrite Colorimetric Assay Kit, Cayman Chemical Company, ALEXIS Biochemicals Industriestrasse 17, CH-4415 Lausen, Switzerland) provided an accurate and convenient method for measurement of total nitrate/nitrite concentration as stable metabolites of NO. Analytic sensitivity of the assay was 2 μ M. Inter-assay and intra-assay coefficient of variations are 3.4% and 2.7%, respectively.

Statistical Analysis

Data are given as mean $\pm$ SD and analyzed with Student's *t*-test using SPSS version 16. A *P*-value ≤ 0.5 was considered significant.

RESULTS

As shown in Table 1, the data obtained from OCP users for the serum HCY concentration (13.268 ± 3.475 μ mol/L) show a significant increase (+1.82 fold, +45%, *P* = 0.027) as compared with control group (7.288 ± 2.621 μ mol/L). The same comparison for NO levels between control group (28.266 ± 6.506 μ M) and women receiving OCPs (14.720 ± 4.549 μ M) shows a significant decrease (–47.9%, –0.52-fold, *P* = 0.048).

DISCUSSION

In this study, we have shown that OCP can significantly affect the NO and HCY levels in healthy women. The

TABLE 1. Homocysteine and Nitric Oxide Levels in Women Receiving Oral Contraceptive Pill and Control Subjects

Duration of drug use (months)	Treatment groups (n = 50)	Homocysteine $\mu\text{mol/L}$	NO μM
0	50	7.288 $\pm$ 2.621	28.266 $\pm$ 6.506
3–36	50	13.268 $\pm$ 3.475	14.720 $\pm$ 4.549
Change, % (fold)		+47 (1.6) $P = 0.027$	–47.9 (0.52) $P = 0.048$

Values are mean $\pm$ SD.

Comparison between HCY ($P = 0.027$) and NO ($P = 0.048$) levels: control subjects versus OCP consumers for 3–36 months.

alterations include elevation of the level of HCY and reduction of concentration of NO, which have been implicated as risk biomarkers for coronary heart diseases (14). As it shown in Table 1, after at least 3 months of OCP uptake, HCY concentration was significantly higher than that observed in control group. This might be due to the effect of OCP on decreasing the availability of group B vitamins for HCY metabolic pathways. HCY metabolic pathways strongly depend on folate, vitamin B₁₂, and vitamin B₆ (16) and deficiency of one or more of these vitamins has been implicated as the most common cause of moderately elevated HCY levels among adults (16). The results of the reports on the effects of OCPs on HCY levels are not consistent. For example, in a study by Merki-Feld et al. (2) it has been showed that long-term (3) cycles) treatment with two OCPs of EE/LKG (30 μg ethinylestradiol/150 μg levonorgestrel) and EE/GSD (30 μg ethinylestradiol/75 μg gestodene) increased the HCY levels. But, Hanna et al. (3) reported that in three groups of women in the early postmenopausal stage with normal initial concentration of HCY, the three forms of HRT used had no influence on the HCY levels. These different observations may be due to the effects of various endogenous sex hormones, which have been reported to affect the HCY levels. For example, it was shown that HCY level is lower during pregnancy (16) and higher in patients with polycystic ovary syndrome (10). The effects of sex hormones on NO production have been the subject of different studies. For example, it has been shown that 17- α -estradiol elevates endothelial NO production in cultured cell and decreases inducible nitric oxide synthase (NOS) in smooth muscle cells of rat aorta (2). This is in accordance with present study showing that a decrease in NO level is observed during consumption of a combination of ethinylestradiol with levonorgestrel. Interestingly, some studies showed that NO level could be a function of HCY concentration. For example, it has been demonstrated that at high levels, HCY may decrease endothelial production of NO through oxidative mechanisms (19). In another study Boger et al. (21) found that in monkeys with hyperhomocysteinemia, the level

of Asymmetric dimethylarginine (ADMA), which is an endogenous inhibitor of NOS, increases. On the other hand, it was shown that treatment with HCY decreases the glutathione peroxidase (GPX) activity, which leads to a high susceptibility of NO to oxidative inactivation (16, 20). The changes of HCY and NO levels observed in our study are in accordance with many of previous reports. In conclusion, our results suggest that intake of OCP for three cycles result in a significant increase in HCY levels and a remarkable decrease in NO concentration. As alterations in these biomarkers have been reported to be associated with increased risk of coronary heart disease, these findings warrant a possible implication of OCP in change of risk of development of coronary heart disease.

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