Effect of oral contraceptive therapy on homocysteine and C-reactive protein levels in women: an observational study

Kadınlarda oral kontraseptif tedavisinin homosistein ve C-reaktif protein düzeyleri üzerine etkisi: Gözlemsel bir çalışma

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ABSTRACT

Objective: Increased levels of homocysteine and C-reactive protein (CRP) are considered as independent risk factors for atherosclerosis. As the level of these factors is affected by sex hormones, a population-based assessment of their changes following oral contraceptive therapy is needed to avoid the side effects that might arise of these variations. To this aim, the present study was to investigate the effect of combined oral contraceptive (OCP) on CRP and homocysteine levels among young healthy women.
Methods: We conducted an observational cross-sectional analysis of 90 healthy, non-obese women (mean age 25 years and body-mass index 22 kg/m^2). Forty-five healthy women on OCP and 45 healthy controls were studied for CRP and homocysteine levels by enzyme-linked immunosorbent assay (ELISA). Unpaired t test and Chi-square test were used for comparison of variables between oral contraceptive users and non-oral contraceptive users.
Results: The results showed that the homocysteine (13.268±3.475 vs. 7.288±2.621 μmol/L) and CRP (5863.0±1349.5 vs. 1138.3±691.12 ng/ml) levels were significantly higher in women receiving OCP in comparison with the control group (p=0.027 and p<0.001, respectively).
Conclusion: The alteration in homocysteine and CRP levels could be attributed to the OCP suggesting that use of these pills should be reviewed in women with increased risk of atherosclerosis and other cardiovascular risk factors. *(Anadolu Kardiyol Derg 2011; 11: 698-702)*
Key words: Atherosclerosis, C-reactive protein, homocysteine, oral contraceptive pill

ÖZET

Amaç: Artmış homosistein ve C-reaktif protein (CRP) düzeyleri ateroskleroz için bağımsız risk faktörü olarak kabul edilmektedir. Bu faktörlerin düzeyi, cinsiyet hormonları, oral kontraseptif tedavisi bu varyasyonlar ortaya çıkabilecek yan etkileri önlemek için gerekli değişiklikleri nüfus tabanlı bir değerlendirme de etkilenir. Bu çalışmanın amacı, genç sağlıklı kadınlarda kombine oral kontraseptif ilaçların (OCP) CRP ve homosistein üzerine etkilerini araştırmaktır.
Yöntemler: Obez olmayan, 90 sağlıklı kadında (Ortalama yaşları 25 ve vücut kitle indeksi 22 kg/m^2) gözlemsel kesitsel analiz yapıldı. Kırk beş OCP kullanan sağlıklı ve kontrol grubundaki kadınlarda ELIZA tekniği ile CRP ve homosistein düzeyleri incelenmiştir. Eşleştirilmemiş t-testi ve Ki-kare testi, oral kontraseptif kullanıcıları ve non-oral kontraseptif kullanıcıların arasındaki değişkenlerin karşılaştırılması için kullanıldı.
Bulgular: Bu sonuçlar, homosistein (13.268±3.475'e karşı 7.288±2.621 μmol/L) ve CRP (5863.0±1349.5'e karşı 1138.3±691.12 ng/ml) düzeyleri kontrol grubu ile karşılaştırıldığında (p=0.027 ve p<0.001, sırasıyla) OCP alan kadınlarda anlamlı olarak daha yüksek olduğu saptandı.
Sonuç: Homosistein ve CRP seviyelerindeki değişiklik OCP'ye atfedilebilir. Bu hapların kullanımı ateroskleroz ve diğer kardiyovasküler risk faktörleri artmış kadınlarda dikkatle gözden geçirilmelidir. *(Anadolu Kardiyol Derg 2011; 11: 698-702)*
Anahtar kelimeler: Ateroskleroz, C-reaktif protein, homosistein, oral kontraseptif tablet

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Introduction

The pathogenesis of coronary heart disease is complicated and includes abnormalities in lipids, endothelium, and thrombosis; increasingly inflammation has been recognized as an important component in the coronary artery disease pathway (1).

Increased C-reactive protein (CRP), as a marker of this inflammation, has prognostic importance for the development of vascular disease that is comparable to that of cholesterol abnormalities (1). CRP production is stimulated via an inflammatory pathway that begins with local injury and reaction at cellular level with activation of macrophages, monocytes, leucocytes, fibroblasts and endothelial cells in the immediate area surrounding the disturbance (2). Recent studies demonstrated that use of contraceptives is associated with elevated serum CRP in women, which may partially explain increased risk of future cardiovascular events (3, 4).

High levels of homocysteine are related to increased risk of venous thrombosis, cardiovascular diseases, (CVD), thrombotic, neurodegenerative, pregnancy-associated diseases and disorders of the central nervous system (3). Homocysteine is an amino acid that derives from demethylation of methionine. The total serum homocysteine level varies in the range of 5-15 µmol/L in the normal population (5). Either minor genetic abnormalities or nutritional deficiencies of B vitamins such as folic acid involved in metabolism of methionine could lead to increased total homocysteine concentration. Homocysteine metabolism may be influenced by dietary habits and lifestyle factors. In particular, homocysteine is inversely related to folate and causes oxidative stress, vascular inflammation, damages endothelial cell, inhibits endothelium-dependent relaxation, and enhances thrombogenecity (5-8).

So far, few data exist on high-sensitivity CRP and homocysteine blood levels in young, healthy, normal-weight women (9, 10). Potentially, detailed knowledge of serum concentrations of these biomarkers in specific groups of women may allow the development of effective early primary preventive strategies for cardiovascular disease.

To this aim, the present study was to investigate the effect combined oral contraceptive (COC) on CRP and homocysteine levels among young healthy women.

Methods

Study design and population

This cross-sectional observational study included 90 healthy white Iranian women recruited in Tehran from June 2009 to June 2010. Consecutive enrollment was done through announcements at the University of Tehran Campus.

The study groups consisted of 45 healthy married women aged 20-35 years who were not COC users with normal menstrual cycles who served as controls and 45 married women aged 20-35 years who were users of the second generation COC (levonorgestrel 0.15 mg, ethinyl -estradiol 0.03 mg) for a minimum of three cycles. All participants completed a self-administered questionnaire assessing demographic factors (age, ethnic group, and education), medical history (pregnancies, past or recurrent illness), contraceptive histories, health behaviors including alimentary habits, tea and alcohol drinking, use of diet supplementation and smoking (11).

Each cycle consisted of 21 days of active treatment, followed by 7 days without treatment. Subjects with a history of diabetes mellitus, liver and renal disease, and those who were in contact with insecticides were excluded from the study.

This study was approved by the University Clinical Ethics Committee and written informed consent was obtained from the subjects enrolled in the study.

Laboratory analyses

The blood samples (12 hours fasting state), were obtained between 9:00 a.m. and 11 a.m. to minimize possible diurnal variations. Blood was collected by venipuncture of the antecubital vein into evacuated tubes (Vacutainer Tubes, Becton-Dickinson, Franklin Lakes, NJ, USA) with ethylenediamine tetraacetic acid as anticoagulant, or without anticoagulant but containing beads for better serum separation.

Samples were collected at the Valfajr Hospital, University of Tehran. Personnel executing the collection and measurement of samples were blinded to clinical, demographic, and habit data.

Full blood counts and hemoglobin measurements were immediately performed on an automatic cell counter, Cell-Dyn Sapphire analyzer (Abbott Laboratories, Chicago, IL, USA). Ethylenediamine tetraacetic acid blood samples were immediately protected from light and centrifuged at 3.000 g for 15 minutes at 10°C, and the plasma was aliquoted and stored at -80°C. Serum was separated less than 30 minutes after blood coagulation by centrifugation at 2.200 g for 5 minutes at 15°C. Aliquots of serum samples were frozen at -80°C for further analysis.

Axis homocysteine Enzyme immunoassay (EIA) was intended for the quantitative determination of total homocysteine in serum (Axis-Shield Diagnostics Lts. Kit, Dundee DD2 1XA, United Kingdom). The peroxidase activity was measured spectrophotometrically (Stat Fax 303 plus Model, American) after addition of substrate, and the absorbance was inversely related to the concentration of homocysteine in sample (12, 13).

Enzyme immunoassay for the quantitative determination of CRP (LDN, Labor Diagnostika Nord GmbH & Co. KG, Am Eichenhain 1, 48531 Norderhorn) in human serum was used.

Statistical analysis

Statistical analysis was performed using the Statistical Package for Social Sciences (SPSS Inc., Chicago, IL, USA). The Kolmogorov-Smirnov test was used to assess the normality of data distribution. Normally distributed variables were presented as mean and standard deviation (±SD). The t test for independent samples was used for comparison of continuous variables,

as appropriate. The difference of proportions between COC users and non-COC users was assessed by χ^2 test. A p value ≤0.05 was considered significant.

Results

Demographic and behavioral data gathered at the start of the study on the 90 participants, according to COC use, are shown in Table 1. As can be seen from the Table, there were no significant differences in the main demographic and lifestyle characteristics between 45 COC users and 45 non-COC users. Nearly 18% of the study women used multivitamin and mineral supplements, with no difference according to COC use.

As shown in Table 2, the data obtained from subjects on OCP for the serum homocysteine concentration (13.268±3.475 µmol/L) show a significant increase (+45%, p=0.027) as compared with control group (7.288±2.621 µmol/L). The same comparison for CRP levels between control group (1138.3±691.12 ng/ml) and women receiving OCP (5863±1349.5 ng/ml) shows a significant increase (+84%, p<0.001) as well.

Table 1. Demographic and behavioral characteristics of studied groups

Variables	OCP users (n=45)	Non-OCP users (n=45)	p*
Age, years	25.20±2.34	25.40±3.21	NS
Height, cm	1.65±0.04	1.65±0.06	NS
Weight, kg	61.20±5.53	63.50±7.45	NS
BMI, kg/m^2	22.10±1.22	22.10±2.12	NS
Cigarettes/day in smokers, n	2.70±3.21	3.40±5.56	NS
Cups of tea/day in drinkers, n	1.50±0.43	1.70±0.55	NS
Nutritional supplement use, n (%)	9 (20)	8 (17.80)	NS

Data are presented as mean±standard deviation or number (percentage)
*Unpaired t test, Chi-square test
BMI - body mass index, NS - non significant, OCP - oral contraceptive pill

Table 2. Homocysteine and C-reactive protein levels in women receiving oral contraceptive pill and control subjects

Treatment groups	Homocysteine, µmol/L	C-reactive protein, ng/ml
Control (n=45) No OCP use	7.288±2.621	1138.30±691.12
OCP users (n=45) Duration - 3-36 month	13.268±3.475	5863.0±1349.5
Difference % (fold)	+45 (5)	+84 (5)
p*	0.027	<0.001

Data are presented as mean±standard deviation
*Unpaired t test
OCP- oral contraceptive pill

Discussion

In this study, we found that there were no significant group differences in baseline characteristics and low dose contraceptive pills have a major effect on CRP and homocysteine levels. Our results suggest that 3 months use of OCP caused a significant increase in serum CRP concentration and homocysteine levels.

Previous studies have shown that OCP can increase the risk of cardiovascular diseases. In a study by Hak et al. (14) the mean values of homocysteine were observed in postmenopausal women; they were higher than in premenopausal. The results of studies by Ridker et al. (15) conducted on healthy postmenopausal American women without coronary disease and cancer showed that cardiovascular risk was increased more than twice in women with moderately elevated homocysteine levels (14.1 vs 12.4 µ mol), irrespective of the conventional risk factors.

Previous reports of the Women's Health Initiative (1), demonstrated that progestin, with or without estrogens, increases CRP levels (1). They identified that progestin in combination with conjugated equine estrogen increases the interleukin 6 (IL-6, an inflammatory-mediated stimulation) of CRP, and showed that when the subjects were treated only with the equine estrogen, IL-6 change was negatively related to CRP change (1). It was suggested that the presence of a progestin causes the IL-6 mediated stimulation of CRP, whereas other mechanisms are likely responsible for CRP production in women receiving only conjugated equine estrogen (1). They demonstrated that the combined regimen of conjugated equine estrogen plus low dose medroxyprogestrone acetate (MPA) administrated continuously may have a distinct role in conferring this increased coronary artery disease risk (1).

The biological pathways underlying progestin-enhanced IL-6 stimulation of CRP production are not clear but could include effects on IL-6 receptors, particularly glycoprotein 130 (gp 130) or direct augmentation of intracellular CRP production (1). European studies by Doring et al. (16) demonstrated that mean CRP concentration in third-generation OCP (containing desogestrel orgestodene) consumers were 3.4 fold higher than in non users. Another study (17) showed that 31 hormonal contraceptive users had 2.7 fold higher average concentration of CRP than 27 non users. A recent study (10) found a frequency of CRP more than 3 mg/l of 35.3% in 295 OCP users and 10.3% in 751 nonusers.

It is proposed that the whole body homocysteine and CRP metabolism could be impaired due to OCPs conversion to reactive species. Our results suggest that OCP treatment may induce the formation of free radicals, which could stimulate CRP and homocysteine synthesis. Probably, OCP has a direct effect on hepatic CRP and homocysteine synthesis. Previous studies have shown that OCPs may directly modulate hepatic synthesis of several factors at the transcriptional level and may also have various immunomodulatory effects, therefore predispose to thromboembolic events by stimulating inflammatory mechanisms (2).

The CRP and homocysteine levels seem to be sensitive to hormonal changes in oral contraceptive users. It is suggested that even a small amount of steroid compounds in OCP could be converted to peroxide and is sufficient to induce synthesis a new molecules of homocysteine and CRP. Homocysteine can be degraded by two mechanisms, the remethylation and transsulfu-

ration pathways, which depend on folate and vitamin B_{12} respectively (18). It is postulated that OCP consumption might decrease the bioavailability of these vitamins and the possible deficiency of one or more of these vitamins might be cause for elevated homocysteine in OCP users. Deficiency of one or more of these B-vitamins is the most common cause for mild to moderately increased homocysteine levels among adults (19). These evidences suggest that lowering total homocysteine concentration such as folate and B_{12} supplementation could reduce the risk of cardiovascular events. Guelpen et al. (20) study in patients with stroke, suggested a protective role of folic acid for hemorrhagic stroke, possibly in addition to its effects on homocysteine status, when used in patients with hyperhomocysteinemia.

All these results suggest that it is possible that women on OCPs with high total homocysteine levels could benefit from lowering total homocysteine as secondary prevention. As it is shown in results, OCPs affect the serum homocysteine levels, which is in agreement with some (21) but not all (22) studies. On the other hand, the alteration of homocysteine levels in OCP users is probably due to reduced renal function or disturbed renal degradation of homocysteine. Youssef et al. (23) study demonstrated that high total homocysteine plasma concentration was associated with increased CRP levels in cerebrovascular diseases in an association analysis of a small sample. Also, Jiangtao et al. (5) demonstrated this linear association between plasma total homocysteine and CRP levels in a larger sample study on 291 Chinese patients with stroke. In a 5-year follow up study, they showed that increased total homocysteine levels are linearly associated with CRP elevation and they found that higher total homocysteine level combined with higher CRP has much more predictive value for the relative risk factor of death or new vascular events in first-onset stroke patients during long-term follow-up period. They also identified that the relative risk factor of death or new vascular events was 4.67 in patients with high homocysteine and CRP levels compared with those with low total homocysteine and CRP concentrations (5).

It should be noted that if we could determine the homocysteine and CRP levels before initiation of OCPs, we would be able to compare the levels of homocysteine and CRP before and during oral contraceptive use to understand their effects on the aforementioned biomarkers, which would improve the conclusion drawn from this study.

Study limitations

There were several limitations for our study. Firstly, this was a small study, and these observations must be confirmed in a larger sample of patients with more analysis works. Nevertheless, a large-scale prospective study should be launched to confirm the associative effect of homocysteine, CRP with coronary artery disease. Secondly, we analyzed only two markers in relation to CAD, it will be interesting also to see if other markers of inflammation have an effect on the risk of coronary artery disease. The other point to be considered in interpretation of these results is the role of genetic variations in homocysteine concentration, which we have not studied in the current project and might have affected our results. Our study is also limited by the non-representative nature of our study sample, because the general female population has greater proportions of obese, low-social status, and older premenopausal women.

Conclusion

In conclusion, this study showed increased homocysteine levels and elevated plasma C-reactive protein concentration in OCP users. The homocysteine level may have implications for development of cardiovascular diseases and venous or inflammatory diseases. Further studies are required to elucidate mechanism underpinning increased cardiovascular and inflammatory risks associated with OCP consumption.

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Conflict of interest: None declared.

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