The Effects of Raloxifene on Serum Lipid Profiles, C-Reactive Protein and Homocysteine Levels in Postmenopausal Women

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Abstract

Objective: To investigate the effects of raloxifene on serum lipid profiles and C-reactive protein and homocysteine levels in postmenopausal women.

Materials and Methods: A single center, longitudinal, open-labeled, uncontrolled study was performed with 64 postmenopausal women with the mean age 56.53±6.92 years. Patients received daily 60 mg of raloxifene for 6 months. Serum levels of total cholesterol, LDL cholesterol, triglycerides, HDL cholesterol, lipoprotein (a), C-reactive protein and homocysteine were measured at basal and 6th month of therapy.

Results: Raloxifene significantly lowered serum levels of total cholesterol (-3.4%), LDL cholesterol (-11.2%) and lipoprotein (a) (-3.4%) levels. HDL cholesterol levels increased significantly by 11.1%. However, there was no significant change on triglycerides. Serum inflammation marker homocysteine significantly decreased by 21% with raloxifene, but no change in the C-reactive protein levels was observed.

Discussion: Raloxifene treatment for 6 months favorably alters cardiac risk factor levels by decreasing total cholesterol, LDL cholesterol, lipoprotein (a), and homocysteine and by increasing HDL cholesterol without changing triglyceride and C-reactive protein levels significantly.

Key words: postmenopausal women, raloxifene, lipoproteins, C-reactive protein, homocysteine

Özet

Postmenopozal Kadınlarda Raloksifen Tedavisinin Serum Lipidleri, C-Reaktif Protein ve Homosistein Düzeyleri Üzerine Etkisi

Amaç: Postmenopozal kadınlarda raloksifen tedavisinin serum lipidleri, C-reaktif protein ve homosistein düzeyi üzerine etkilerini araştırmak.

Materyal ve Metot: Tek merkezli, longitudinal, açık uçlu ve kontrolsüz olan bu çalışma, ortalama yaşları 56.53±6.92 olan 64 postmenopozal kadın üzerinde yapıldı. Çalışmaya dahil edilen hastalar, 6 ay süresince günlük 60 mg dozunda raloksifen kullandılar. Tedavi öncesinde ve altıncı ayın sonunda serum total kolesterol, LDL kolesterol, trigliserid, HDL kolesterol, lipoprotein (a), C-reaktif protein ve homosistein düzeyleri ölçüldü ve bazal değerler ile tedavi sonrası değerler karşılaştırıldı.

Sonuç: Raloksifen tedavisi total kolesterol (-%3.4), LDL kolesterol (-%11.2) ve lipoprotein (a) (-%3.4) serum düzeylerini anlamlı olarak düşürdü. Serum HDL kolesterol düzeyleri ise anlamlı olarak %11.1 arttı. Ancak, serum trigliserid düzeylerinde anlamlı bir değişim saptanmadı. Serum enflamasyon belirteci homosistein, raloksifen tedavisi ile anlamlı olarak %21 azaldı, ancak serum C-reaktif protein düzeyinde anlamlı bir değişim saptanmadı.

Tartışma: Raloksifen tedavisi total kolesterol, LDL lipoprotein (a) ve homosistein düzeylerini azaltarak ve HDL kolesterol düzeylerini artırarak kardiyak risk faktörlerini olumlu yönde değiştirir. Ancak, trigliserid ve C-reaktif protein düzeyleri üzerine anlamlı bir etki oluşturmaz.

Anahtar sözcükler: postmenopozal kadınlar, raloksifen, lipoproteinler, C-reaktif protein, homosistein

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Introduction

Cardiovascular disease (CVD) is the leading cause of death among women in industrialized countries and the incidence increases in postmenopausal women dramatically compared to premenopausal years (1). Menopause is associated with unfavorable lipid modifications, such as an increase in total cholesterol, low-density lipoprotein cholesterol (LDL-C) and triglycerides that can increase the risk of CVD. This increase in total cholesterol results from the increases in the levels of LDL-C, very-low-density lipoprotein and lipoprotein (a) [Lp(a)]. The oxidation of LDL-C which is the most atherogenic particle is also enhanced (2). Additionally, high-density lipoprotein cholesterol (HDL-C) levels may decrease over time, and this change is a more powerful predictor of CVD in postmenopausal women (3). Lp(a) is another independent risk factor for coronary heart disease, and elevated levels are reduced with postmenopausal hormone therapy (4). Lp(a) contains a low-density lipoprotein (LDL)-like moiety, which is covalently linked to apolipoprotein (a). Apolipoprotein (a) is similar to the fibrinolytic proenzyme plasminogen. Lp(a) is isolated in the arterial wall at sites of atherosclerosis and it may serve as a link between the pathogenic process of atherosclerosis and thrombosis (5).

C-reactive protein (CRP) is an inflammation marker synthesized mainly in liver. Studies indicate that CRP is an independent marker of cardiovascular risk in men and women (6). CRP predicts an increased risk of cardiovascular events in individuals who have normal lipid levels. Therefore, it is argued that both CRP and lipid profiles should be used for screening purposes (7). Most studies report that oral estrogen with or without progestins increases CRP level, indicating that estrogen is primarily responsible for this action (8,9).

Elevated homocysteine levels are also associated with an increase in CVD. It is an independent risk factor for atherosclerosis and thromboembolic disease. Homocysteine levels increase after menopause and the levels are significantly lowered by hormone replacement therapy (HRT) (10).

Hormone replacement therapy effectively reverses these unfavorable lipid profiles in postmenopausal women. Observational studies indicated that HRT was associated with 50% reduction in the incidence of myocardial infarction and other CVD (11,12). However, more recent prospective randomized trials showed no benefit of HRT for cardiovascular protection (8,13), and the concerns about the increased risk of breast and endometrium cancer has replaced this therapy by alternative drugs. Raloxifene is a selective estrogen receptor modulator that binds to estrogen receptor. It has antagonistic effects in breast tissue and endometrium, while it has estrogenic effects on bone and cardiovascular system (14).

There are several clinical studies performed with raloxifene demonstrating beneficial effects on lipid metabolism and the vascular wall (15-17). Several animal studies have shown favorable changes in the lipid profile, including inhibition of LDL oxidation, increased HDL-C, improved endothelial function, and the release of nitric oxide (18,19).

The aim of the current clinical study was to evaluate the effects of raloxifene on serum lipid profiles of postmenopausal women. CRP and homocysteine levels were also measured in order to investigate how raloxifene treatment modifies these cardiovascular risk markers.

Materials and Methods

Sixty-four postmenopausal women who were admitted to the outpatient clinic of Dokuz Eylül University, Department of Obstetrics and Gynecology and who had either osteoporosis (*t* score, <–2.5 at lumber spine or total hip) or osteopenia (*t* score, –1 to –2.5) with any risk factor for osteoporotic fracture were recruited for the study. Ethics Committee approval from the Institutional Review Board of our university and written informed consents from the patients were obtained. None of the patients had vasomotor symptoms and were using or had used HRT or any other medication that might alter serum lipid profiles. Patients with diabetes mellitus, history of smoking, thyroid disease, and bone disease other than osteoporosis, coronary artery disease, thromboembolic disease, and chronic liver or kidney disease were excluded. Patients with unexplained uterine bleeding and any kind of malignancy were also not included.

At the initial visit, a complete history was taken, and a physical examination was performed for each woman. Height and body weights were measured while patients were wearing light clothes and no shoes. The body mass index (BMI) was calculated as body weight divided by height-squared (kg/m^2). All patients received 60 mg of raloxifene (Evista; Lilly Pharmaceutical Company, Istanbul, Turkey) each day for 6 months with additional calcium carbonate (1500 mg/d) and vitamin D (400 IU/d) as a supplement. Compliance was assessed by interview at the end of treatment. No patients dropped out of the study.

At baseline and after 6 months total serum cholesterol, triglycerides, HDL-C, LDL-C, lp(a), CRP and homocysteine levels were measured. For the serum analysis, blood samples were obtained by venopuncture after overnight fasting of 12 hours. Samples were centrifuged within 45 minutes of collection at 3000 x g for 10 minutes and stored at –70°C until assayed. Total cholesterol, HDL-C and triglycerides were measured with enzymatic reagents in Hitachi modular DP autoanalyser. LDL-C was calculated using the Friedewald equation: LDL-C = (total cholesterol-HDL-C) – (triglycerides x 0.20). Lp(a) was quantified by turbidometric method with Integra 400 plus machine (Roche, Switzerland). Homocysteine was measured in plasma with fluorescence polarization immunoassay method. CRP was measured in serum with immunoturbidometric method in the Integra 400 plus machine.

Statistical analysis was performed with the SPSS (Statistical Package for Social Sciences) statistical software (version 11; SPSS Inc, Chicago, Ill). Data were presented as mean values ± SD. The Mann Whitney *U* test was used to compare the

mean values between the obese and non-obese groups; paired sample *t* test was used to compare the mean values before and after treatment. *p* value of <0.05 was considered to be statistically significant.

Results

The mean age of the study group was 56.53±6.92 (range 42-77). Thirty-eight (59.4%) patients were considered obese (BMI>25) and the remaining twenty-six (40.6%) were normal weighted. The mean duration of menopause was 8.6±4.3 years. Majority of the patients (87.5%) were natural menopause patients.

The mean serum lipid profiles and homocysteine levels were not significantly different among obese and non-obese groups at baseline (Table 1). However, the basal mean CRP level of the obese group was significantly higher than the non-obese group (p=0.021).

Mean serum triglyceride levels increased 11.9% with raloxifene treatment at 6th month, but the difference was not significant (p>0.05). However, at 6 months of therapy total cholesterol, LDL-C and lp(a) levels decreased significantly by 3.4%, 11.2% and 3.5%, respectively. Additionally, there was a 11.2% increase in HDL-C levels, which was also statistically significant.

The mean CRP levels increased by 24.9%, but the difference did not reach a statistical significance (p>0.05). Serum homocysteine concentrations decreased by 21.0% and the difference was significant. Serum lipid concentrations and the cardiac risk factors at basal and 6th month of raloxifene treatment and the mean percent changes are given in Table 2 and Figure 1, respectively.

Table 1. Basal concentrations of serum lipids and inflammation markers of obese and non-obese patients

	Obese (n=38)	Non-obese (n=26)	*p*
Triglycerides (mg/dl)	122.50±53.50	120.81±58.85	NS
Total Cholesterol (mg/dl)	215.05±35.60	215.12±35.14	NS
HDL Cholesterol (mg/dl)	57.65±11.20	57.84±14.43	NS
LDL Cholesterol (mg/dl)	131.97±32.29	133.15±30.78	NS
Lipoprotein-a (g/L)	0.30±0.30	0.29±0.25	NS
C-Reactive Protein (mg/L)	3.48±2.04	2.26±1.45	0.021
Homocysteine (µmol/L)	12.39±4.52	12.88±2.73	NS

NS: not significant

Table 2. Serum concentrations of lipids and inflammation markers at basal and after 6 months treatment with raloxifene

	Basal (n=64)	6th Month (n=64)	*p*
Triglycerides (mg/dl)	121.81±55.28	136.34±76.57	NS
Total Cholesterol (mg/dl)	215.08±35.13	207.78±36.12	0.036
HDL Cholesterol (mg/dl)	57.73±12.50	64.15±17.29	<0.001
LDL Cholesterol (mg/dl)	132.45±31.44	117.63±29.84	<0.001
Lipoprotein-a (g/L)	0.30±0.28	0.28±0.28	<0.001
C-Reactive Protein (mg/L)	3.01±1.85	3.76±4.56	NS
Homocysteine (µmol/L)	12.47±4.07	9.85±3.23	<0.001

NS: not significant

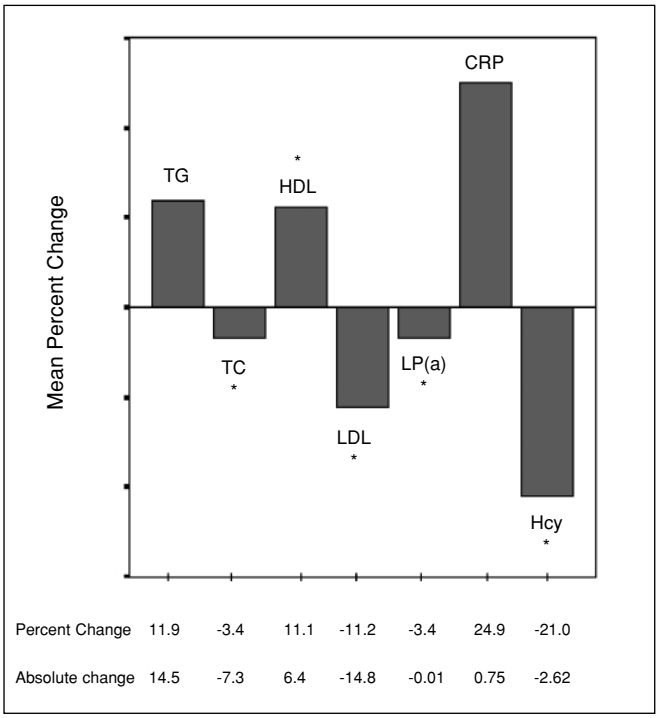

Figure 1. Mean changes in serum lipids and cardiac risk factors after raloxifene treatment
*Statistically significant; TG: triglyceride; T-C: total cholesterol; HDL: HDL cholesterol; LDL: LDL cholesterol; Lp(a): lipoprotein (a); Hcy: homocysteine

Discussion

Dyslipidemia plays a major role in atherosclerosis. Atherosclerotic process starts with an insult to the vascular endothelium, and mediators like oxidized LDL-C, lp(a), homocysteine, CRP and other inflammatory markers are involved. Modifying these substances in a favorable way with drug therapy may decrease CVD events in postmenopausal women.

The results of our study showed that 6 months treatment with raloxifene altered lipid profile of healthy postmenopausal women in a favorable way. Additionally, although it did not change serum CRP levels, raloxifene significantly decreased one of the major cardiac risk factor homocysteine. These results mostly confirm the previous reports.

In clinical trials, raloxifene has been shown to lower total cholesterol and LDL-C levels by 3.9% to 12% (14,16,17). This effect is evident after 3 months of therapy and is sustained over 4 years of treatment. Our results are consistent with the literature in that total and LDL cholesterol levels are lowered 3.4% and 11.2%, respectively. In addition to reducing LDL-C levels, raloxifene has been demonstrated to inhibit the oxidation of LDL-C *in vitro*, which appears to be more potent than estrogen in this regard (20).

Raloxifene does not seem to raise serum levels of HDL-C (21). The results of MORE (Multiple Outcomes of Raloxifene Evaluation) study (14) showed that after 24 months of treatment with raloxifene, HDL-C levels did not change significantly. Walsh et al. (16) also reported that at the 6th month of therapy there was no change in the HDL-C level. However, HDL_2-C, the most cardioprotective subfraction, was significantly raised. Contrary to these results, we observed that HDL-C levels significantly raised by 11.1% after 6 months of treatment. Some animal studies also showed an increase in HDL-C concentrations with raloxifene (22). A recent study with a small group of patients also reported a significant increase in HDL-C levels with raloxifene (23). These dissimilar effects of raloxifene on HDL-C may be due to different individual responses to the drug and deserves further investigation.

Elevated levels of lp(a) have been identified as an independent risk factor for CVD. In a 6 month study (16) raloxifene decreased lp(a) levels by 7% in 390 healthy postmenopausal patients which was less than the 19% decrease with estrogen plus progestin therapy. Our results also showed a 3.4% significant reduction in lp(a) levels.

C-reactive protein is an independent marker for the risk of CVD in postmenopusal women without clinically evident coronary heart disease (24). Moreover, it adds to the predictive value of total cholesterol and HDL-C in determining risk of first myocardial infarction (25). Even moderate increases in CRP levels within the range generally considered normal are associated with significant increases in future cardiovascular events. It is reported that, contrary to estrogen plus progestin treatment, raloxifene has a neutral effect on CRP levels. Walsh et al. (26) reported a 5.6% reduction in CRP levels which was not statistically significant. In our study, serum CRP levels increased by 24.9% after 6 months of treatment, but the difference did not reach a statistical significance. There are a number of pathophysiologic mechanisms by which CRP may increase the risk of CVD have been proposed. CRP stimulates the release of inflammatory cytokines from human macrophages and may promote coagulation by stimulating the tissue factor from the endothelium (27). Furthermore, CRP activates the complement cascade, which has been implicated in early stages of atherosclerosis. CRP has been found to be colocalized with complement components in atherosclerotic lesions of human coronary arteries (28).

Increased levels of circulating homocysteine have damaging effect on the vascular endothelium (29) and high plasma levels have been suggested as predictive of an increased risk of CVD events (6). It also increases the levels of circulating adhesion molecules and coagulation markers in humans by a prooxidant effect on the vascular endothelium (30). Previous studies indicate a significant reduction in plasma homocysteine levels in postmenopausal women treated with raloxifene, a similar effect also observed with estrogen progestin therapy. De Leo et al (21). reported a 19.5% reduction after 6 months of treatment with raloxifene, which is in agreement with our results (21% reduction). In another study with limited number of patients plasma homocysteine concentrations were decreased significantly with raloxifene 150 mg/day but not with raloxifene 60 mg/day (31). Walsh et al. (26) also concluded that raloxifene lowered homocysteine levels by 8%, similar to the 7% reduction obtained with estrogen progestin treatment.

In conclusion, six months treatment with raloxifene changes serum lipid levels of postmenopausal women in a favorable way. This effect was also observed in homocysteine concentrations. Therefore, because of its beneficial effects on biochemical markers of cardiovascular risk, it can be speculated that raloxifene might substantially reduce the risk of CVD in postmenopausal women. Clinical trials with cardiovascular events as the definitive end point are required for raloxifene treatment.

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