Hormonal Modulation of the Proinflammatory Cytokines, Leptin and Plasminogen Activator Inhibitor-1 in Healthy Postmenopausal Women

Sağlıklı Postmenopozal Kadınlarda HRT Kullanımı ile Sitokinler, Leptin ve Plazminojen Aktivatör İnhibitör-1 Düzeylerinin Araştırılması

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SUMMARY

In this study we aimed to determine the levels of TNF-α, IL-6, IL-1β, PAI-1, leptin in healthy premenopausal and postmenopausal subjects who were evaluated before and after hormone replacement therapy (HRT). Twenty premenoposal woman with regular cycles and 45 postmenopausal women were included in the study. Twenty-five of the postmenopausal women received conjugated estrogen (CCE) + medroxyprogesterone acetate (MPA) and the other 20 received 17-β estradiol + norethisterone acetate (NETA). The levels of the above identified cytokines in these two treatment groups were compared with that of the healthy pemenopausal group as well as with each other before and after 6 months of HRT. IL-1 levels were significantly higher in the postmenopausal group before HRT when compared to the premenopausal group. Levels of IL-1 were elevated even more after 6 months of HRT. IL-6 was significantly higher in postmenopausal group before HRT compared to the premenopausal group. After 6 months of HRT there was no significant decrease in levels of IL-6. TNF-α was significantly higher in postmenopausal group before HRT compared to the premenopausal group. No significant decrease was seen after HRT. PAI-1 was significantly higher in the postmenaposal group before HRT compared to the premenopausal group. After 6 months of HRT, there was a significant decrease in levels of PAI-1. Leptin levels were not different between the premenopausal and the postmenopausal groups (p=0.97). But leptin levels were higher in the postmenoposal group after receiving HRT for 6 months. Menopausal status and HRT may be the causative factor of biochemical proinflammatory response, leptin, fibrinolytic process and coagulation in healthy postmenopausal women.

Key Words: *Menopause, proinflammatory cytokines, leptin, plasminojen activator inhibitor-1*

Devamı sayfa 92'de

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ÖZET

Bu çalışmanın amacı, sağlıklı pre- ve postmenopozal kadınlarda HRT kullanımı öncesi ve sonrasında TNF-α, IL-6, IL-1β, PAI-1 ve leptin düzeylerini belirlemektir. Sağlıklı premenopozal 30 ve sağlıklı postmenopozal 45 kadın çalışmaya dahil edildi. Postmenopozal kadınların 25'i konjüge östrojen (CCE) + medroksiprogesteron asetatı (MPA), 20'si ise 17-β östradiol + noretisteron asetatı (NETA) altı ay süreyle kullanmıştı. IL-1 düzeyleri; postmenopozal grupta, HRT öncesi premenopozal grupla karşılaştırıldığında anlamlı olarak yüksekti. HRT kullanımı sonucu IL-1 düzeyleri yüksekliklerini korudu. IL-6 düzeyleri benzer oranlarda postmenopozal grupta HRT öncesi yüksek iken, HRT kullanımı sonucu anlamlı olmamakla beraber bu düzeylerde düşme saptandı (p>0.05). TNF-α düzeyleri postmenopozal grupta HRT öncesi yüksek iken, HRT kullanımı sonucu bir değişiklik saptanmadı. PAI-1, postmenopozal grupta tedavi öncesi, premenopozal gruba göre yüksek düzeylerde iken, HRT kullanımı sonrası anlamlı oranda düşüş gösterdi. Leptin düzeyleri pre- ve post menopozal gruplar arasında fark göstermez iken (p=0.97) , altı aylık HRT kullanımı sonrası yüksekti. Menopozal durum ve HRT kullanımı; biyokimyasal proenflamatuvar yanıt, leptin, fibrinolitik süreç ve koagülasyon mekanizmalarında rol oynayan önemli unsurlardır. Bu konudaki çalışmaların sayı ve niteliğinin artırılması, bu mekanizmaların anlaşılmasında bizlere ışık tutacaktır.

Anahtar Kelimeler: *Menopoz, proenflamatuvar sitokinler, leptin, plazminojen aktivatör inhibitör-1*

Introduction

Hormone replacement therapy (HRT) aims at preventing the vasomotor symptoms in short term, osteoporosis and cardiovascular disease in long term. Venous thromboembolism, coronary artery diseases, endometrium and breast cancer are the possible pathological outcomes. Coronary events increase substantially in postmenopausal period.[1] Deep venous thrombosis and cardiovascular event risks were found to be increased in the first randomized placebo-controlled trial HERS (Heart and Estrogen/Progesterone Replacement Study) using the conjugated estrogen (CCE) and MPA.[2] The results of the Woman's Health Initiative (WHI) study have supported this data.[3] HRT's procoagulant and proinflammatory effects may be the causative factor for these outcomes. Increased risks may be associated with the proinflammatory cytokines which may be proatherogenic.[4]

Cytokines are the important modulators of inflammatory reaction. They are polypeptide in nature and are secreted by T-lymphocytes and monocytes that bind to their own membrane receptor with paracrine, autocrine, and endocrine functions.[4] Tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), interleukin-1β (IL-1β) may increase the expression of adhesion molecules in vascular endothelium and promote the endothelial-monocyte interaction, thereby being an important part of proatherogenic process.[4]

Leptin is another molecule that is secreted from the adipose tissue and has a role in regulating the satiety in hypotalamus and thermoregulation. It also processes immune regulatory function. Body mass index (BMI) is the most important determinant of leptin levels in the serum. There is controversy about the leptin levels estimated in different studies in postmenapousal women.[5-8]

Plasminogen activator inhibitor-1 (PAI-1) is one of the most important determinants of the fibrinolytic process together with the tissue plasminogen activator and tissue plasminogen activator antigen-1. PAI-1 levels are elevated in patients with a history of atherosclerosis and this may be an indicator of future cardiovascular morbidity.[9] PAI-1 is also related to deep venous thrombosis which is increased 2-3 times in patients receiving HRT.[10] Conflicting data are present about the effects of the HRT on the coagulative and the fibrinolytic processes.[9,11,12]

In this study we aimed to investigate the levels of TNF-α, IL-6, IL-1, PAI-1, leptin in healthy premenopausal and postmenopausal subjects. Postmenopausal women receiving either CCE + MPE or 17-β estradiol + norethisterone acetate (NETA) were also evaluated after 6 months of HRT.

Material and Methods

This study was performed in the Endocrinology and the Menopause outpatient clinics of Obstetrics and Gynecology Department of I.U. Cerrahpasa Hospital Medical School. All postmenopausal women had FSH >30 mIU/ml. Premenopausal women were selected from the general gynecology outpatient clinic seeking care for unrelated complaints. Thirty premenopausal women with regular cycles and 45 postmenopausal women were included in the study. None of the patients had a history of coronary heart disese, diabetes, chronic or acute systemic illness such as SLE or rheumatoid arthritis. Both groups were similar in terms of BMI. None were presc-

ribed drugs other than HRT. Twenty-five of the 45 postmenopausal subjects were given 0.625 mg CCE + 2.5 mg MPA, and 20 were given 17-β estradiol + NETA for 6 months. The study was approved by the Ethics Commitee of Cerrahpasa Medical School and every patient gave an informed consent.

Sample Collection

Serum levels of the markers were checked as follows: Blood samples were drawn after 12-14 hours of fasting in the morning (before and six months after HRT in the postmenopausal group). Serum was obtained after at least 30 minutes clotting by centrifugation at 2500 g for 15 minutes. For PAI-1 assays blood samples (9 ml) were drawn into chilled 0.109 M trisodium citrate anticoagulant (1 ml) tubes, centrifuged as previously described to obtain platelet free plasma. Plasma and serum were stored at –80°C for determination of TNF-α, IL-6, IL-1-β, leptin, PAI-1 levels until assayed.

Assay of PAI-1

Supernatant PAI-1 antigen was assayed by using Asserachrom®, PAI-1 (Diagnostica Stago, France) as previously described.[13]

Assay of TNF-α, IL-6, IL-1-β and Leptin

Serum TNF-α, IL-6, IL-1-β and leptin levels were measured by enzyme-immunometric assays according to the manufacturers instructions (Quantikine High Sensitivity TNF-α, Quantikine IL-6, Quantikine IL-β and Quantikine leptin, R&D Systems, Minneapolis, USA). All samples were tested in duplicate on serum samples, and those above the standard curve were retested with appropriate dilutions. All measurements were done blinded.

Statistical Analysis

The data were given as means ±SD (standard deviation). The groups were compared with Anova Tukey HSD. Tukey test was employed to determine the p-values. The level of statistical significance was taken as $p<0.05$.

Results

IL-1 was significantly higher in postmenopausal group before HRT, compared to the premenopausal group ($p<0.001$, *Table 1*). The levels of IL-1 were elevated further after receiving HRT for 6 months when compared to the levels before treatment ($p<0.001$, *Table 2*). Although the increase in levels of IL-1 were higher in the 17-β estradiol + NETA group, there was no statistically significant difference between CCE + MPA and 17-β estradiol + NETA groups ($p=0.12$, *Table 3*).

IL-6 was significantly higher in postmenopausal group before HRT compared to the premenopausal group ($p<0.001$, *Table 1*). After 6 months of HRT there was an non-significant decrease in IL-6 levels ($p>0.05$, *Table 2*). Although the the decrease in IL-6 levels was higher

Table 1. The parameters of the pre- and postmenopausal women (mean ±SD)

	Premenopause	Postmenopause	p
PAI-1 (IU/ml)	5.23±1.27	7.84±2.01	<0.001
Leptin (ng/ml)	7.14±0.48	9.25±0.33	0.97
IL-1 (pg/ml)	10.56±1.8	17.8±3.6	<0.001
IL-6 (pg/ml)	13.7±2.3	29.30±4.4	<0.001
TNF (pg/ml)	14.2±3.2	21.08±1.03	<0.001

Table 2. Parameters of postmenopausal women before and after HRT (mean ±SD)

	Premenopause	Postmenopause + HRT	p
PAI-1 (IU/ml)	3.01±1.5	6.23±1.1	0.03
Leptin (ng/ml)	16.1±4.2	11.7±3.5	0.04
IL-1 (pg/ml)	25.1±3.7	28.9±2.1	0.12
IL-6 (pg/ml)	23.8±1.9	22.1±4.4	0.939
TNF (pg/ml)	24.01±1.9	20.54±1.6	0.24

Table 3. Comparing postmenopausal treatment groups (mean ±SD)

	CCE+MPA	17-β Estradiol + NETA	p
PAI-1 (IU/ml)	3.01±1.5	6.23±1.1	0.03
Leptin (ng/ml)	16.1±4.2	11.7±3.5	0.04
IL-1 (pg/ml)	25.1±3.7	28.9±2.1	0.12
IL-6 (pg/ml)	23.8±1.9	22.1±4.4	0.939
TNF (pg/ml)	24.01±1.9	20.54±1.6	0.24

in the 17-β estradiol + NETA group, there was no statistically significant difference between the CCE + MPA and the 17-β estradiol + NETA groups (p=0.939, *Table 3*).

TNF-α was significantly higher in postmenopausal group before HRT compared to premenopausal group (p<0.001, *Table 1*). No significant decrease was seen after HRT (p=0.98, *Table 2*). There was no statistically significant difference between the CCE + MPA and the 17-β estradiol + NETA groups (p=0.24, *Table 3*).

PAI-1 was significantly higher in the postmenopausal group before receiving HRT compared to premenopausal group (p<0.001, *Table 1*). After 6 months of HRT treatment, there was a significant decrease in PAI-1 levels (p=0.02, *Table 2*). The decrease was more prominent in the 17-β estradiol + NETA group than in the CCE + MPA group (p=0.03, *Table 3*). However, leptin levels were not different between the premenopausal and the postmenopausal groups (p=0.97, *Table 1*). But leptin levels were higher in postmenopausal group receiving HRT for 6 months (p<0.001, *Table 2*). This increase in leptin was more significant in the CCE + MPA group compared to that in the 17-β estradiol + NETA group (p=0.04, *Table 3*).

Discussion

The relationship between cytokines and cardiovascular events and the value of the cytokines in predicting the cardiovascular events has been the subject of scientific research.

These molecules have been reported to be proatherogenic.[14] These cytokines may increase the expression of various adhesion molecules contributing to the monocyte –endothelial cell interaction. So they may be an important inflamatory component of atherogenesis.[4] The protective role of HRT (if there is any) in terms of cardioprotection may be that of supression of those cytokines and may be predictive of future events. In terms of cardioprotection, the cytokine that has proven proinflammatory role is the CRP. Although disputed, the role of TNF-α, IL-6, IL-1β in cardiovascular events were investigated and conflicting results were obtained with HRT; i.e., decreased, increased or unchanged. Walsh et al.,[15] in their study comparing the IL-1, CRP, TNF-α in patient groups receiving CCE + MPA, raloxifene 60 mg, raloxifene 120 mg, and placebo were not able to show the proinflammatory response in other groups that was evident by CRP in the CCE + MPA group, but showed a decrease in TNF-α levels in all groups. Although IL-6 is the most important stimulator of CRP from liver, no significant change of IL-6 was shown in CCE + MPA group, and they could not explain the proinflammatory response that was evident by the rise in CRP. The increased IL-1β levels and unchanged TNF-α levels in our study challenge the literature. In most studies the decrease in the levels of IL-1 and TNF-α with HRT are linked with loss of inhibition of osteoclastic activity and suppressed inflammatory responses.[16] Although IL-6 was decreased with HRT, this decrease was not significant statistically, also no significant change was noted betwen HRT groups. Additionally HRT was associated with biochemical proinflammatory response. This response may be responsible at least partially for the inflammatory component of atherosclerosis that may be seen with HRT. Menopause is not related only to weight gain, but also to the centralisation of the fat distribution. HRT helps to decrease the weight gain in menopause and also reverses the fat distributon mimicing the premenopausal period.[17,18] The positive effects of the HRT on fat metabolism may be based on the increased metabolism, increased activity of lipoprotein lipase and increased physical activity, but also with changing levels of leptin.[19] Literature is quite confusing about the levels of leptin among patients receiving HRT. This is probably a result of the inconsistancy of the studies in terms of age, menapouse age, HRT indication, adipose tissue composition and BMI of the patient populations. Leptin levels were reported to be increased, decreased and unchanged with HRT.[5-8] Lambrinoudaki et al.[19] in their investigations with 88 patients reported that oral ERT and HRT did not change the levels of leptin and the most important determinant of leptin was the BMI. Haffner et al.[5] and Hadji et al.[7] reported similar findings with Lambrinoudaki et al.[19] Konukoğlu et al.[20] reported increased leptin levels with oral HRT especially in the obese group. Brooks-Asplund et al.[8] reported unchanged levels with HRT but showed a positive correlation of IL-1 with leptin that may be important in thermoregulation. Our results are consistent with that of Konukoğlu et al. showing increased levels of leptin. Konukoğlu et al.[20] reported an increase of leptin in both the obese and non obese groups although it was more prominent in the obese group. But in the current study the groups were similar in terms of BMI. All the same, the metabolic, endocrine, cardiovascular consequences of leptin levels changing with HRT are unknown.

The effects of HRT on coagulation and fibrinolysis are debatable. While some have reported increased fibrinolysis, others have reported no change.[2,21,22] The predictive value of PAI-1 as a marker of fibrinolysis is known for cardiovascular

events.[23] PAI-I is related to central obesity and its serum levels increase with the expression of insulin resistance in the adipose tissue.[9] The study of Salobir et al.[24] is the largest in literature focusing on the relation between 17-β estradiol + NETA and hemostasis. They have shown a decline of PAI-1 with 6 months of HRT. They also concluded that HRT triggered the fibrinolytic process and had no effect on coagulation. Teede et al.[12] in their study with 17-β estradiol + NETA for 6 weeks showed an increase in both the fibrinolytic process and coagulation. Salobir reported that his study had missed this early procoagulant activity. By the way, Scarabin and Sporrong could not detect any change in fibrinolysis and coagulation with HRT.[21,22] Douketis demonstrated the activation of fibrinolysis in 3 months of HRT with CCE + MPA, resulting from the decline of levels of PAI-1, and reported that the cardiovascular protection may result from this effect.[25] As far as we know, there is no data in literature directly comparing the effects of 17-β estradiol + NETA and CCE + MPA in terms of PAI-1 levels. The rise of PAI-1 levels in the postmenopausal period and the decline by HRT in this study is consistent with literature on the HRT –fibrinolysis relationship. Also the decrease was more significant in the 17-β estradiol + NETA group. This may be a clue to the relatively less risk of cardiovascular events in this patient group, although a single parameter is not reliable for definite conclusions. Prospective studies are needed for comparison of the effects of treatment with 17-β estradiol + NETA and CCE + MPA in terms of coagulation and fibrinolysis parameters.

In conclusion, we can say that we detected a partial biochemical proinflammatory response although we could not find out any significant difference between the HRT groups. Detection of proinflammatory reaction despite the progestagen component of HRT is an interesting finding. The decrease in PAI-1 serum levels is important for cardioprotection. Significance of leptin increase with the HRT is currently unknown.

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