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EFFECTS OF HORMONE THERAPY ON SERUM HOMOCYSTEINE LEVELS IN PERI- AND POSTMENOPAUSAL WOMEN

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SUMMARY

Objective: *To assess the differences in serum Hcy levels induced by different HT regimens in peri- and postmenopausal women.*
Desing: *Prospective clinical study.*
Setting: *Academic medical center.*
Patients: *Eighty-four healthy non-hysterectomized peri-and postmenopausal women.*
Intervention: *Blood samples were collected between 8:00 and 10:00 a.m. after at least 12 h fasting from a peripheral vein at study entry and after 3 months of therapy.*
Main Outcome Measures: *Serum total Hcy concentrations.*
Results: *There were no significant changes in the values of homocysteine after the tibolone therapy compared with baseline values (P=0.06). Neither continuous nor sequential conjugated equine estrogens plus medroxyprogesterone acetate regimens causes significant effects in serum homocysteine levels after 3 months of therapy (P=0.56 and P=0.84, respectively).*
Conclusions: *Our data suggest that tibolone and continuous or sequential conjugated equine estrogens plus medroxyprogesterone acetate therapies did not have significant changes on serum homocysteine levels. Further research is needed to better understand the effect of hormone therapy on homocysteine metabolism.*

Key words: *estrogen plus progestin, homocysteine, menopause, tibolone*

ÖZET

Peri ve Postmenopozal Dönemde Farklı HT Rejimlerinin Serum Homosistein Düzeyleri Üzerine Etkileri

Objektif: *Peri- ve postmenopozal dönem kadınlarda farklı HT rejimlerinin serum Hcy düzeyleri üzerine etkilerini değerlendirmek. Planlama: Prospectif klinik çalışma.*
Ortam: *Akademik tıp merkezi.*
Hastalar: *Seksendört sağlıklı nonhisterektomize peri- ve postmenopozal kadın.*
Girişim: *Çalışma başlangıcında ve 3 ay sonra en az 12 saat açlık sonrası sabah 8:00- 10:00 arasında periferik ven serum örnekleri toplandı.*
Değerlendirme Parameteleri: *Serum total Hcy konsantrasyonları.*
Sonuç: *Tibolon tedavisinden sonra, bazal değerlerle karşılaştırıldığında homosistein düzeylerinde anlamlı değişiklik gözlenmedıı (P=0.06). Kontinü ve aralıklı konjuge östrojen+ medroksiprogesteron asetat tedavisinden sonra da 3. ay serum homosistein düzeylerinde anlamlı değişiklik olmadı (sırasıyla P=0.56 ve P=0.84).*
Yorum: *Bulgularımıza göre hem tibolon, hem de kontinü veya aralıklı konjuge östrojen+ medroksiprogesteron asetat tedavileri*

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serum homosistein düzeylerinde anlamlı değişikliğe yol açmamıştır. Hormonal tedavinin homosistein metabolizması üzerine etkilerini daha iyi anlamak için yeni çalışmalara ihtiyaç vardır.

Anahtar kelimeler: *homosistein, menopoz, östrojen + progestin, tibolon*

INTRODUCTION

Elevated serum levels of the sulphur amino-acid homocysteine (Hcy) represent an independent risk factor for atherosclerotic vascular disease[1,2]. Hcy may promote atherosclerosis by injuring the vascular endothelium[3] and has been associated with cardiocerebrovascular disease and thromboembolism [4,5]. Substantial evidence links elevated Hcy levels to an increased risk of cardiovascular disease (CVD)[6] and mortality[7]. The incidence of cardiovascular morbidity and mortality increases substantially in women after menopause[8]. Elevated levels of Hcy may in part contribute to the increased risk of developing CVD in postmenopausal women[9].

Several epidemiological data have suggested that hormone therapy (HT) is cardioprotective[10,11]. But, recently, data from Women Health Study[12]. and from Heart and Estrogen/progestin Replacement Study[13]. have shown that HT does not have cardioprotective effect in postmenopausal women.

Serum Hcy levels are partly genetically determined [14], but nongenetic states, such as folic acid, vitamin B6, or vitamin B12 deficiency, or renal or liver failure may increase these levels[15]. There are indications that Hcy levels are related to estrogen status. Males have higher Hcy levels compared to age-matched females[16]. Although several investigators have reported increased Hcy levels in postmenopausal women when compared to premenopausal women[9], there is no concensus on whether they increase after menopause[17].

HT is suggested to decrease levels of Hcy[18-21], but cardioprotective effects of Hcy lowering therapy have not been known yet. The objective of our study was to assess the differences in serum Hcy levels induced by different HT regimens in peri- and postmenopausal women.

MATERIAL and METHODS

A total of 84 healthy non-hysterectomized peri- and postmenopausal women with climacteric symptoms (hot flushes and/or outbreaks of sweating) were enrolled in the study. Eighty-four postmenopausal (defined as amenorrhoeic for >6 months) women, aged 42 to 62 years, and 20 perimenopausal women with irregular cycles (generally oligomenorrhoeic), aged 36 to 47 years. They had diastolic blood pressures less than 105 mmHg. Postmenopausal status was confirmed by serum FSH concentrations of > 30 mIU/L. None of the women received HT for at least 3 months before entering the study and none took vitamin B supplements, such as folic acid, B6 or B12. Exclusion criteria included a history of cardiovascular, cerebrovascular, thrombo-embolic, metabolic, hepatic or renal disease. The work was approved by a medical ethics committee and written informed consent was obtained from each subject. Eighty-four healthy peri-and postmenopausal women were enrolled in the study. A total of 64 postmenopausal women were randomly assigned to receive tibolone 2.5mg/day (Livial 2.5 mg, Organon) (n=32) or continuous conjugated equine estrogens 0.625mg/day plus medroxyprogesterone acetate 2.5mg/day (CEE + MPA) (Premelle, 2.5 mg, Wyeth) (n=32). Remaining 20 perimenopausal women with irregular cycles received sequential CEE + MPA regimen (Premelle cycle, 5 mg, Wyeth) (0.625mg of CEE on days 1-14, 0.625mg of CEE plus 5mg of MPA on days 15-28). Serum Hcy levels were measured at baseline and after 3 months of therapy. Blood samples were collected between 8:00 and 10:00 a.m. after at least 12 h fasting from a peripheral vein at study entry and after 3 months of therapy. All specimens were collected in Vacutainer (Becton-Dickinson, Franklin Lakes, NJ) blood-collecting tubes according to standard hospital guidelines for venipuncture and sample collection. Hcy specimens were placed on ice and all specimens were transported to the laboratory within 30 minutes of collection. Serum was obtained after centrifugation at 2,000 x g for 10 minutes, frozen, and stored at –20 ºC until analysis. Serum total Hcy concentrations were measured by using an IMX (Abbott Diagn. USA) Hcy assay. Assay is based on the fluorescence polarization immunoassay (FPIA) technology.

Statistical Analysis: All variables were expressed as mean ± standard deviation. For all measured parameters, statistical analyses of between-group differences were performed by using Kruskal-Wallis variance analysis.

Mann-Whitney U test was used for comparing basal values of menopause duration of both postmenopausal groups. For three groups, serum Hcy levels measured at baseline and after 3 months of treatment were compared by using Wilcoxon Signed Ranks test. P values < 0.05 were considered statistically significant. A power analysis was not performed before study initiation. All data were entered into and processed by SPSS 9.05 for Windows statistical package.

RESULTS

During treatment, 12 women were excluded from the analysis. Three women receiving tibolone therapy withdrew due to vaginal spotting and headache. Two women receiving continuous combined CEE-MPA therapy complained of vaginal spotting and mastalgia. 7 women (four women with tibolone therapy, two women with continuous CEE + MPA therapy and one woman with sequential CEE + MPA therapy) left the study for reasons not related to the treatment. The remaining 72 patients completed the study. Baseline characteristics of the three assignment groups differed only in age values, which were lower in the sequential CEE + MPA group (p= 0.000). Other clinical and laboratory parameters were similar in the groups (p > 0.05) (Table I). There were no statistically significant differences in the baseline levels of Hcy between the study groups. (Table II).

Table 1: *Descriptive characteristics at baseline*

Variable	Tibolone	continuous CEE + MPA	sequential CEE + MPA
Age (y)	50.7 ± 3.5	51 ± 5	43.3 ± 3.3
Menopause duration (months)	42.2 ± 54.1	43.7 ± 36.7	-
Body mass index (kg/m2)	28.9 ± 4.8	31.8 ± 4.9	29.2 ± 3.5
Blood pressure (mmHg)			
Systolic	119.7 ± 23.1	133.1 ± 28.2	119.3 ± 21.7
Diastolic	76.5 ± 11.7	85.6 ± 14.1	76.8 ± 16.6
FSH (mIU/mL)	74.3 ± 32.1	72.2 ± 31.6	67.9 ± 35.9
Total Cholesterol (mg/dl)	223.1 ± 42.2	220.8 ± 40.6	200.4 ± 46.2
Triglyceride (mg/dl)	132.5 ± 48.1	129.1 ± 54.4	154.8 ± 108.7
LDL (mg/dl)	138.9 ± 41.8	140.4 ± 37.4	116 ± 36.7
HDL (mg/dl)	57.4 ± 14.1	52.4 ± 15	54.4 ± 13.3
Vitamin B12 (pg/ml)	241.6 ± 110.5	273.5 ± 127.8	254.6 ± 93.9
Folate (ng/ml)	5.5 ± 1.9	4.8 ± 1.3	4.6 ± 1.1

Note: Values are expressed as mean ± SD.
CEE, conjugated equine estrogens; MPA, medroxyprogesterone acetate; FSH, follicle stimulating hormone; LDL, low-density lipoprotein; HDL, high-density lipoprotein.

Table II: *Serum homocysteine levels at baseline and after 3 months*

Group	Hey (μmol/L)		
	Baseline Mean ± SD	After 3 months Mean ± SD	P value
Tibolone (n=25)	15.3 ± 7.6	16.3 ± 7.8	0.06
Continuous CEE + MPA (n=28)	12.9 ± 3.5	12.9 ± 4.6	0.56
Sequential CEE + MPA (n=19)	12.4 ± 2.1	12.7 ± 1.9	0.84

Values are given as mean ± SD (standard deviation).
CEE, conjugated equine estrogens; MPA, medroxyprogesterone acetate; Hcy, homocysteine.

There were no significant changes in the values of Hcy after the tibolone therapy compared with baseline values (P= 0.06). Neither continuous nor sequential CEE + MPA regimens causes significant effects in serum Hcy levels after 3 months of therapy (P= 0.56 and P= 0.84, respectively) (Table 2).

DISCUSSION

Several studies have reported that estrogen therapy significantly decreases Hcy levels[18,19], but, such an effect may not be observed when combined with progestin in postmenopausal women. A decrease of greater magnitude has been related to estrogen[19], while the presence of progestin has been demonstrated to either attenuate[18] or augment[20] the effect. Christodoulakos et al reported that continuous CEE + MPA administration resulted in a decrease of a lesser magnitude (2.6 %) compared with CEE[19]. Smolders et al demonstrated that treatment with oral 17_-E2 decreases fasting plasma concentration of Hcy and that addition of the progestogen gestodene attenuates the reduction induced by oral E2 therapy[18]. On the other hand, Yildirir et al reported that the mean Hcy levels decreased by 17.8 % in the CEE group and by 24.2 % in the CEE + MPA regimen group[20].

Although combined HT is reported to decrease serum levels of Hcy[19,21]., this observation was not confirmed by others[22-24]. In the only study evaluating the effect of HT on Hcy levels after a methionine load, the association of estrogens with cyclic MPA induced an elevation of post-load Hcy levels[23]. Park et al reported that the mean values of Hcy levels did not change significantly after oral CEE (0.625 mg) combined with MPA (2.5 mg) therapy for 3 months[24]. Similarly, in

our study, neither continuous nor sequential CEE + MPA regimens causes significant effects in serum Hcy levels after 3 months of therapy.

Tibolone is a synthetic steroid with mixed estrogenic, progestogenic, and androgenic activity used for postmenopausal HT. We did not find any significant change in serum Hcy with tibolone after 3 months of treatment. Our results are in agreement with other studies investigating the effect of tibolone on Hcy levels[25,26]. Celik et al found that Hcy levels did not change significantly after 6 months of treatment with tibolone[25]. In the other study, tibolone had no effect on serum Hcy levels at least during the first 18 months of therapy[26].

Estrogenic potency of tibolone is about 1/50 that of ethinyl-estradiol, the progestogenic potency is 1/8 that of norethisterone (an androgen derivative), and the androgenic potency is about 1/3 that of norethisterone [27]. Although tibolone is reported to have no effect on Hcy levels[25,26], the effect of combined estrogen-androgen derivative is controversial. Eviö et al detected that oral or transdermal combination of sequential estradiol and norethisterone acetate did not cause significant changes on Hcy levels in postmenopausal women[22]. On the other hand, Ventura et al demonstrated that continuous combined oral HT with 17_-E2 plus norethisterone acetate reduced post-methionine load Hcy levels in postmenopausal women (28). In the other study, Hak et al reported a decrease in serum Hcy levels with sequential combined regimen of 17_-E2 and desogestrel or with combination of CEE and norgestrel in perimenopausal women after 6 months of therapy[29].

In conclusion, our data suggest that tibolone and continuous or sequential CEE + MPA therapies do not lead significant changes on serum Hcy levels during 3 months of observation. Further research is needed to better understand the effect of HT on Hcy metabolism.

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