EFFECTS OF SHORT-TERM HORMONE REPLACEMENT ON ATHEROGENIC INDICES IN INDIAN POSTMENOPAUSAL WOMEN

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ABSTRACT

Indian ethnicity by itself is a strong risk factor for development of CAD in Indian postmenopausal women due to lower HDL levels as compared to Whites and women of oriental origin. We evaluated and compared the short-term effects of menopause, estrogen replacement therapy and combined estrogen and progestin replacement therapy on various atherogenic indices. 40 postmenopausal women, both surgical and natural (20 each) were selected. 10 surgical postmenopausal women were given 0.625 mg conjugated estrogens daily for 6 months and 10 natural postmenopausal women were given 0.625 mg conjugated estrogen with 2.5 mg medroxyprogesterone acetate daily. 20 women were included in the control group and given placebo. Fasting venous blood samples were analyzed for extended lipid profile and calculated atherogenic indices before starting the therapy and after 1, 3 and 6 months. LDL and Apolipoprotein B increased ($p < 0.05$) and those of Apolipoprotein A1 and HDL decreased in the control groups. In both the study groups levels of serum cholesterol and LDL decreased ($p < 0.05$) and those of HDL and Apolipoprotein A1 increased ($p < 0.01$). LDL/HDL, Apo B/ ApoA1, Total Cholesterol/ HDL decreased significantly ($p < 0.05$) in both the study groups compared to the control groups. The effect of estrogen alone was more significant as compared to combination therapy. Log Triglycerides (TG)/HDL ratio showed a decrease in women on estrogen alone but the difference was not significant. Our study confirms that short term HRT has a favorable effect on atherogenic indices in Indian postmenopausal women.

KEY WORDS

LDL/ HDL Ratio, Total cholesterol/ HDL, Apo B / Apo A1, Conjugated estrogens, Medroxyprogesterone acetate.

INTRODUCTION

Menopause is an estrogen-deficient state resulting from the loss of ovarian activity (1). Estrogen secretion plays a major qualitative role in guaranteeing a women's psychophysical equilibrium during fertile period (2). The endocrine changes resulting from the menopause lead to cessation of reproduction and accompanying vasomotor and genito-urinary symptoms in women like hot flashes, mood swings, depression, nervousness, irritability, recurrent vaginitis, dysuria, etc. along with long term health effects like coronary artery disease and osteoporosis (3). Due to the increased awareness and longevity in

21st century, most women seek help of the clinician. Women are about 10 years older than men at first manifestation of Coronary Artery Disease (CAD), although they have a similar plaque burden (4). Among Indian women, the presence of hypertension, obesity, diabetes and abnormal lipid profile are correlated with CAD (5).

Plasma lipid and apolipoprotein ratios that include both an atherogenic and an anti-atherogenic lipid component (e.g, total cholesterol/ high density lipoprotein (HDL) cholesterol ratio, Low Density Lipoprotein (LDL)/ HDL ratio and Apolipoprotein (apoB/ apo A1) ratio have been found to be strong predictors of coronary artery disease (6). One study reports that these ratios have strong and independent predictive values, with a 1-unit increase in the TC/HDL and LDL/HDL associated with 49% and 75% increases in risk of Myocardial Infarction (MI), respectively (7).

From a biologic point of view, estrogen has multiple effects that would be expected to be cardio protective, including favorable changes in lipids, endothelial

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function, vascular reactivity and blood-flow (8). Hormone replacement therapy is in fact considered a physiological approach to the management of menopause related problems rather than a pharmacological approach (9).

Epidemiological, angiographic and postmortem data suggest that 25-50% of protective effect of estrogen replacement in reduction of cardiovascular disease is due to improvements in lipid status, however progestin may partly attenuate the benefits of estrogen on cardiovascular disease (10).

The objective of this study was to compare the short-term effects of estrogen [conjugated estrogens (CE)] and combination of estrogen (CE) and progestin [medroxyprogesterone acetate (MPA)] on serum lipid and lipoprotein levels and other atherogenic indices in healthy Indian postmenopausal women belonging to lower middle-income group.

MATERIALS AND METHODS

The experimental design was that of clinically controlled, single blinded phase III trial. Forty postmenopausal women, presenting with vasomotor or genito-urinary complaints were included in the study from the wards and out patient department of Lady Hardinge Medical College and Smt Sucheta Kriplani Hospital, New Delhi with informed consent. The clinical study was started in February 2000, and the last patient finished the clinical phase in March 2001. Sixty-four women were initially enrolled for the study but in the end only forty postmenopausal women completed the 6-month trial phase. Patients were randomly assigned to the study and control groups in each category.

Inclusion criteria

Age 40 -60 years, either surgically or naturally menopausal with FSH levels ≥ 30 mIU/ml. Surgical menopausal women were those who had undergone hysterectomy with bilateral salpingo- oophorectomy for some benign disease of uterus and adenexa. They were included in the study after their histopathology report was negative for malignancy.

The natural menopausal group consisted of those women who had their last menstrual period at least 1 year earlier but not more than 10 years back.

Exclusion criteria

Subjects with history of major medical illnesses like myocardial infarction, stroke, congestive heart failure, hepatitis or a history of malignant disease of breast or known or suspected estrogen dependent tumors, myomas or endometrial carcinoma.

All women with high triglyceride levels ≥ 500 mg/dl and those who were receiving anti-hyperlipidemic drugs

were excluded from study.

Women participating in this study were assigned to four groups;

Study Group I- Ten surgical menopausal women who were given only Conjugated Estrogen (CE) 0.625 mg daily.

Study Group II- Ten natural menopausal women who were given continuous combined CE 0.625 mg with medroxyprogesterone acetate (MPA) 2.5 mg daily.

Control Groups I and II -Twenty post-menopausal women (10 surgical and 10 natural menopause) were given placebo.

Women in all the groups were matched for age, parity, hemoglobin levels, body mass index (BMI) and mean blood pressure.

Detailed history of the patient was taken for any major illness, drug intake and family history of breast or genito-urinary malignancy. On each follow up visit, women underwent routine clinical examination including examination of breast and mammography, chest, cardiovascular system and per abdominal examination and PAPS smear.

Urine midstream sample was tested for presence of albumin and sugar. Women with intact uterus were subjected to ultra-sonogram lower abdomen and PAPS smear at the beginning and end of 6 months of therapy to rule out any premalignant/ malignant changes in uterus and adenexa. All the women were followed up at 1, 3 and 6 months of therapy. 6.0 ml of venous blood sample was taken after an overnight fast and analyzed for blood glucose, renal function test, liver function tests, calcium, phosphorus, lipid profile, Apolipoprotein A1 and B (immunoturbidimetric) on Synchron CX5 Clinical Chemistry Autoanalyser (Beckman) using standard reagents and kits from Randox (U.K.) / Beckman (USA). Because of the skewed nature of the raw TG distribution, we calculated log of TG/HDL (logTG/ HDL).

All results were analyzed on the spss statistical package. The data are presented as the mean $\pm$ Standard error (SE). The differences in mean values in three groups were analyzed by one-way Analysis of variance (ANOVA). Paired t-test was carried out to compare the pretreatment and 1, 3 and 6 months post-treatment values of both treatment regimens and placebo. p-values less than 0.05 were considered significant and those less than 0.01 were highly significant.

RESULTS

Analysis of variance did not show significant intergroup differences in age, parity, Body Mass Index, systolic and diastolic blood pressure, blood glucose levels in

the postmenopausal women at the beginning of the therapy. (Table 1).

Symptoms

Nearly 70% of the women taking HRT in both the study groups reported a subjective improvement in the genito-urinary and psychosomatic symptoms as early as first follow-up.

By the end of 3 months 95 % women did not have any episode of hot flashes or night sweats. This symptomatic improvement persisted till the end of 6 months. Only 3 out of 10 women taking the combination therapy complained of intermittent vaginal bleeding.

However in the control group the subjects reported a slight improvement in their psychosomatic symptoms but little/ no improvement in genitourinary complaints like dysuria and vaginitis even with alternative non-estrogenic creams.

Changes in LDL/HDL Ratio

The baseline LDL /HDL ratio in all the four groups is shown in Table 2. The difference in mean in the pretreatment period was not significant by ANOVA ($p=0.522$).

LDL/HDL ratio decreased in both the study groups but this decrease was highly significant and was observed earlier (at 3 months) in women taking only estrogen as compared to women on combination therapy (after 6 months), indicating attenuation of positive effects of estrogen on HDL and LDL by progesterone.

In contrast, LDL/HDL ratio increased in the control groups, though the increase was not significant.

Changes in Total Cholesterol/ HDL ratio (TC/ HDL)

The difference in mean in the pretreatment period was not significant ($p=0.207$) (Table 2).

TC/HDL ratio decreased in both the study groups significantly by the end of 3 months and this beneficial effect persisted even till the end of 6 months, indicating that both estrogen and the combination therapy are beneficial in reducing the total cholesterol levels and increasing the HDL levels.

In contrast, TC/HDL ratio increased in the control groups, though the increase was not significant.

Changes in Apolipoprotein B/ Apolipoprotein A1 ratio

The pretreatment ratio of Apo B/ ApoA1 was not significant ($p=0.996$) as shown in Table 2.

Apo B/ Apo A1 ratio decreased significantly in both the study groups but this decrease was observed earlier (at 1 month) in women taking only estrogen as compared to women on combination therapy, indicating possible attenuation of positive effects of estrogen by Progesterone.

In contrast, Apo B/ Apo A1 ratio increased in the control groups. Although the increase was not significant in natural menopause group but in the surgical menopause group the increase was significant at 6 months

Table 1. Subject characteristics and baseline pretreatment values

	Study Group-I (n=10)	Study Group-II (n=10)	Control Group-I (n=10)	Control Group-II (n=10)	p-value
Age (Years)	46.8±1.53	49.2±1.2	47.7±1.74	49.3± 1.76	NS
Parity	4.1±0.67	4.4±0.65	4.0±0.49	4.3±0.93	NS
BMI (Kg/m ²)	20.62± 1.34	24.06±1.09	22.76±1.98	23.40±1.40	NS
SBP (mm Hg)	120.6±6.12	127.0±5.19	117.8±4.82	125.4±3.94	NS
DBP (mm Hg)	77.8±3.4	82.0±1.89	78.0± 3.08	78.6± 2.89	NS
Hemoglobin (g/dl)	10.29±0.49	10.29±0.25	10.6±0.25	10.8±0.49	NS
Fasting Serum Glucose (mg/dl)	94.68±4.97	98.81±6.03	96.47± 5.06	99.72±6.88	NS
Abbreviations: SBP- Systolic Blood pressure; DBP- Diastolic Blood Pressure; BMI - Body Mass Index All data are expressed as mean ± Standard Error. One -way analysis of variance (ANOVA) was performed between the four groups. NS = not significant ($p>0.05$).					

Table 2. Effect of hormone replacement on various atherogenic indices

Indices		Pretreatment	1 month	3 months	6 months
LDL/ HDL Ratio	Study Group I	1.94±0.12	1.73±0.17	1.51±0.15*	1.40±0.11*
	Study Group II	2.58±0.48	2.16±0.32	1.82±0.21	1.72±0.15\$
	Control Gp I	2.36±0.17	2.74±0.26	2.81±0.18	2.83±0.27
	Control Gp II	2.18±0.33	2.21±0.26	2.57±0.34	2.58±0.26
TC/ HDL Ratio	Study Group I	4.16±0.54	3.63±0.24	3.05±0.25\$	2.86±0.23\$
	Study Group II	4.46±0.67	3.81±0.39	3.28±0.25\$	3.36±0.33\$
	Control Gp I	4.95±0.62	5.07±0.84	5.17±0.95	5.12±0.97
	Control Gp II	3.78±0.50	3.89±0.40	4.31±0.44	4.34±0.29
Apo B/ Apo A1 Ratio	Study Group I	0.722±0.10	0.54±0.06\$	0.58±0.15\$	0.53±0.05\$
	Study Group II	0.725±0.087	0.669±0.065	0.592±0.059	0.525±0.031\$
	Control Gp I	0.727±0.100	0.81±0.17	0.84±0.17	0.99±0.21\$
	Control Gp II	0.75±0.067	0.63±0.06	0.74±0.07	0.74±0.07
Log TG/ HDL Ratio	Study Group I	0.118±0.06	0.125±0.08	0.128±0.07	0.111±0.05
	Study Group II	0.132±0.11	0.125±0.06	0.104±0.06	0.099±0.07
	Control Gp I	0.184±0.08	0.181±0.09	0.184±0.08	0.197±0.08
	Control Gp II	0.148±0.08	0.163±0.06	0.174±0.07	0.172±0.07
<p>Abbreviations: LDL - Low Density Lipoprotein; HDL- High Density Lipoprotein; TC- Total Cholesterol; Apo B- Apolipoprotein B; Apo-A1- Apolipoprotein A1; TG- triglycerides.</p> <p>All data are expressed as mean ± Standard Error. * p < 0.01 ; \$ p < 0.05 .</p> <p>Study Group I -Postmenopausal women on CE 0.625 mg daily.</p> <p>Study Group II- Postmenopausal women on combination therapy.</p> <p>Control groups I & II- No hormone therapy given.</p>					

Changes in log Triglycerides/ HDL (log TG /HDL)

The difference in the baseline values of log-transformed ratio of serum Triglycerides/ HDL (molar concentration) in all the four groups was not significant (p=0.558) (Table 2).

Log TG/ HDL ratio increased initially in women taking unopposed estrogen followed by a decrease by the end of 6 months. However in women on combination therapy, a decreasing trend was seen throughout the follow-up.

In contrast, log Triglyceride/HDL ratio increased in the control groups, though this increase was not significant till the end of 6 months.

DISCUSSIONS

Currently, postmenopausal women account for more than 30% of the female population at risk for CAD (11). The total cholesterol (or LDL cholesterol) to HDL cholesterol ratio represents a simple, efficient way to help estimate coronary disease risk. Indian women have a high TC/HDL ratio by virtue of low HDL, even

when TC levels are not elevated. The HDL level among Indian women (45 mg/ dl) is about 10 mg/dl lower than in Whites (55 mg/dl) and 20 mg/dl lower than in Blacks and oriental races (65 mg/dl) and these levels of HDL are parallel to their risk of CAD. The optimum TC/HDL ratio is 3 and TC/HDL ratio >5 appears to be a strong predictor of CAD, and is observed in 25% of industrial and 32% of urban female populations in India (5).

In our study when hormone replacement therapy was given for 6 months, a significant decrease was observed in the levels of LDL/HDL ratio, Total cholesterol/ HDL ratio and Apolipoprotein B/ Apolipoprotein A1 ratio. In contrast, the women who were not taking hormones were seen to progress slowly towards more atherogenic lipid profile though the observed changes were not statistically significant for the duration of the study.

These changes could be attributed to an observed decrease in total cholesterol levels and LDL levels and an increase in HDL levels with hormone intake. The observed increase in serum HDL concentration with

unopposed estrogen therapy was higher (29%) than combined estrogen-progestin therapy (16%) but nonetheless the increase was found significant in both groups as compared to control group where 7.6% decrease was observed over 6 months. Thus it appears that though progesterone appears to negate the positive effects of estrogen on HDL-cholesterol but still the combination therapy affords greater protection against CAD risk as compared to non-users in Indian women. PEPI Trial in 1995 reported 9% increase in HDL after 36 months of unopposed estrogen therapy (12) whereas Menopause Study Group (13) reported 14% increase after one year of therapy. The increase in HDL-C levels in response to HRT is caused by increased production of apolipoprotein A-I, the major apolipoprotein of HDL-C, and by decreased hepatic lipase activity. Hepatic lipase is known to enhance the uptake of HDL-C and the catabolism of HDL₂, the HDL sub particle considered the most active in reverse cholesterol transport (14).

Serum LDL-cholesterol concentration was found to increase in the control group by 16.6%. In subjects taking unopposed estrogen, 15.8% decrease was seen in LDL levels ($p < 0.01$) which is similar to those of PEPI Trial (12) findings that reported 10% decrease in 36 months, Menopause Study Group (13) reported 7.5% decrease in 1 year and Lafferty *et al* (15) reported 21% decrease after 3 months. In subjects on combination therapy an overall 10.9% decrease was observed over 6 months. Our findings were similar to those of Kim *et al* (16) which reported 13.7% decrease in 3 months, PEPI Trial (12) that reported 12% decrease in 36 months, Menopause Study Group (13) that reported 9% decrease in 12 months. Thus both unopposed estrogens as well as combination therapy showed beneficial effects on serum LDL-cholesterol levels. Metabolic studies suggest that the reduction in LDL-C levels with HRT is a result of enhanced hepatic lipoprotein uptake, accelerated conversion of hepatic cholesterol to bile acids, and increased expression of LDL-C receptors on hepatocytes (17). The result is augmented clearance of LDL-C particles from plasma.

The observed decrease in Apolipoprotein B/Apolipoprotein A1 ratio was mainly due to an increase in Apolipoprotein A1 levels with Hormone therapy. In the subjects taking unopposed estrogen daily for 6 months, 26.7% increase was seen whereas with combined therapy 24% increase was seen in levels of serum Apolipoprotein A1 at the end of 6 months. Our findings in Indian postmenopausal women were similar to those of vander Mooren MJ *et al* (Netherlands) (18) where 23 % increase was reported after 1 year and Miller *et al*. (9-18% increase reported after 3 months in American women) (19). Thus unopposed estrogens have highly beneficial effects on serum Apolipoprotein A1 and these beneficial effects may be attenuated to some degree by the addition of progestin as seen by

the delayed appearance of significantly raised Apolipoprotein A1 levels in the combined therapy group but nonetheless it too has a favorable impact on the risk of coronary artery disease.

Our study did not show significant changes in serum Apolipoprotein B during the 6 months trial but it may be possible that on long-term basis estrogen replacement as well as combined therapy may have a beneficial effect on levels of serum Apo B compared to non-users. A 35.6% increase was seen in Apo B/Apo A1 ratio in Control group I (surgical menopause patients) ($p < 0.05$). In control Group II, the changes were in a biphasic manner similar to that observed by Pancini *et al*. (20).

Log TG/HDL ratio may be an important, albeit crude, marker of abnormal TG metabolism (7). The values of log Triglycerides / HDL did not show any significant changes in all the groups. (Table 2). The initial increase in the log TG/HDL ratio with estrogen monotherapy in present study could be attributed to an increase in levels of triglyceride-rich lipoproteins like VLDL and chylomicrons. As per studies by Sanada *et al*. (21) estrogen appears to increase the hepatic synthesis of very low-density lipoproteins, particularly large VLDL that is directly catabolized by liver rather than being delipidated to small VLDL and LDL and therefore may be less harmful.

In contrast, the recently published WHI (Women's Health Initiative trial) report (22) refutes the use of hormone replacement therapy in postmenopausal women because it has shown that the overall health risks (breast cancer, stroke, Pulmonary embolism, CHD) exceeded benefits (probable decrease in incidence of colorectal cancer and osteoporosis) from the long term use of combined estrogen plus progestin for an average 5.2 year follow-up among healthy postmenopausal US women. This has lead to a controversy regarding the use of combination HRT in women with intact uterus. However, they have also reported a 12.7% reduction in LDL-cholesterol and an increase of 7.3% in HDL cholesterol with combination therapy. Though the study has been discontinued but the estrogen arm of this study is scheduled to continue till March 2005.

The Heart and Estrogen-progestin replacement study (HERS) has added critical data about cardio-protective effects of Hormone Replacement therapy in elderly women with proven coronary artery disease (23).

Indian postmenopausal women are at great risk of CAD due to genetically low HDL levels (5). The benefits of HRT may be greater in Indian women, who have substantially higher rates of CAD (3-fold) and lower rates of breast cancer (one-half) compared with Whites (5).

Though Hormone Replacement Therapy has fallen into disrepute among Western countries with reported risks outweighing the benefits, short term HRT is still recommended in Indian scenario for alleviation of acute menopausal symptoms. Also, the average age group of women being prescribed HRT in India (45-55 years) is much less than age group considered in WHI and HERS. A woman is enrolled for Hormone replacement therapy only after thoroughly ruling out any history of uterine fibroids, deep vein thrombosis in the woman or a family history of breast cancer. Also a clinical examination is done including PAPS smear, mammography and ultra sonogram abdomen in women with intact uterus. The women taking Hormone replacement are routinely followed-up with lipid profile, mammography, and PAPS smear. However the clinician always has a choice to balance risks against the benefits and then take a decision for or against use of hormones in that woman.

Thus, authors conclude that short-term HRT can be safely used for the management of acute vasomotor and urogenital symptoms in Indian postmenopausal women. In addition this therapy will have beneficial effects on serum cholesterol, LDL-Cholesterol, HDL-Cholesterol and Apolipoprotein -A1 levels, thus decreasing the risk for development of CAD in future.

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