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Research Article

BIOCHEMICAL CHANGES AND HORMONE REPLACEMENT THERAPY IN POSTMENOPAUSAL WOMEN

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A R T I C L E I N F O

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A B S T R A C T

Objective- Present study was conducted to ascertain the effect of hormone replacement therapy (HRT) in post-menopausal women on degree of oxidative stress by measuring levels of serum malondialdehyde (MDA), an end product of lipid per-oxidation and serum glutathione (GSH), a component of antioxidant defense system and other parameters like fasting blood Sugar (FBS), Alkaline Phosphatase (ALP), Triglycerides(TG), High density lipoprotein (HDL), Low density lipoprotein (LDL) , Total Cholesterol (TC).

Design- The present study was carried out on 40 post-menopausal OPD patients, whose menses have ceased for one year, in the range of 35-60 years, the same subjects were then put on HRT for four months in form of oral conjugated equine estrogens, Premarin 0.625mg/day alone or in combination with Medroxy progesterone acetate 2.5 mg, to counteract the negative effect of estrogen.

Result- Estrogen resulted in alleviation of oxidative stress as indicated by decreased level of MDA and increased level of GSH.

Conclusion- It's reasonable to suggest that estrogen is acting as antioxidant. Moreover, postmenopausal women who are having a high-risk cardiovascular disease due to oxidative stress and osteoporosis get protected by HRT.

INTRODUCTION

Oxidative stress plays a key role in pathogenesis of several age associated diseases. The antioxidant properties of estradiol reduce oxidative stress related complications[1]. Menopause is typified by drop in endogenous estradiol (E2). Before menopause main circulating estrogen compound is 17β estradiol (E2) produced by ovary. During menopause main estrogen is estrone, derived peripherally. Estrogenscan reduce oxidative stress at two levels by preventing ROS generation and by scavenging free radicals. Estrogens can up-regulate the endogenous anti-oxidative defense by indirect action on expression and activity of enzymes, as SOD (Super-oxide Dismutase), GPX1 and GPX4and has synergistic interaction with glutathione[2].

Estrogen is an important athero-protective molecule with marked effects on vasculature that are mediated at least in part by increased availability of signalingnitric oxide, one third of clinical advantages of estrogens can be attributed to effects on lipid balance[3].Estrogen replacement therapy also causes elevation in plasma NO and NO metabolites. The effects of HRT provide insight into NOS regulation by estrogen and endothelium dependent vasodilatation of brachial and coronary arteries in post-menopausal women[4].

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The key to use of estrogen replacement therapy is to prevent CVD before atherosclerosis is evident and to identify women who have genetic susceptibility to adverse outcome to therapy. Recent studies have shown potential benefits of HRT even in setting of established atherosclerosis. Atherosclerotic plaques in post-menopausal women receiving HRT contained fewer inflammatory leukocytes, proteolytic pathways, lower levels of TNF-α, activated NFkB (nuclear factor), matrix metalloproteinase (MMP-9). Moreover, more collagen and elastin degradation, reduced vascular smooth muscle cells, inhibition of NF-kB-β, increased ROS production, induction of MMPs and TGF-β expression as compared to postmenopausal women who never took therapy[5]. Inflammation induced aging vasculature is related to excessive accumulation and impaired dynamic O-GlcNAcylation of critical protein in IkBα/NFkB signaling pathway leading to loss of estrogen inducedvaso-protection[6].

Post menopausal women with osteoporosis have increased level of TNF-α as compared to without osteoporosis. Activation of TNF-α, NF-k B and PI3K/Akt signaling contributes to osteoporosis. TNF-α contributes to postmenopausal osteoporosis by synergistically promoting RANKL. IL-7 through T cell stimulation by estrogen regulates hematopoietic and immune functions that are critical for bone homeostasis. T cells produce proinflammatory cytokines like receptor activator of NF-k B ligand (RANKL), TNF-α (Tumor

necrosis factor-α) and interlukin-17(IL-17) all of which augment osteoclastogenicity[7].

T cells produce proinflammatory cytokines including receptor activated of NF-k B ligand (RANKL), TNF-α (Tumor necrosis factor) and IL-17 (Interlukin-17) all of which augment osteoclastogenicity. Anti TNF-α, anti-RANKL in estrogen deficient mice had no effects on CD4 + T-cell proliferation and B-lymphopoiesis, anti-IL-17 suppressed both events with reversal of CD-28 loss. Anti-IL-17 produced proinflammatory cytokine production and induced Tregs. All three antibodies restored trabecular micro architecture with comparable efficacy, however cortical bone parameters, bone biomechanical properties histomorphometry were best preserved by anti-IL17 antibodies. Likely attributable to its inhibitory effect on osteoblast apoptosis and increased member of bone lining cells and Wnt 10b expression[8].

MATERIAL AND METHODOLOGY

TYPE- Cohort study

Subjects- Forty post-menopausal women with menopause more than one year were put on hormone replacement therapy for at least four months either estrogen or estrogen and progesterone if they have their uterus intact. Detailed report regarding socio-economic status, family history of diabetes mellitus, coronary heart disease, obesity, hypertension, menstrual history, obstetrical history, mode of onset of menopause, per vaginal examination and clinical investigation were recorded as per Performa. A written consent from the patient was taken.

Exclusion Criteria- Patients having renal, neuromuscular, pulmonary, hepatic diseases and patients on steroids were excluded from the study.

Assays- All patients were subjected to routine investigations like hemoglobin, blood sugar, blood urea, serumcreatinine, serum glutamate-pyruvate transaminase, serum glutamate-oxaloacetate transaminase, total cholesterol, high density lipoprotein cholesterol(HDL-C),low density lipoprotein cholesterol (LDL-C), triglyceride, urine complete examination after twelve hours of fasting on fully automated analyzer by kit-methods and ECG(electro-cardiogram).

Blood glutathione by Beutler *et.al*. and malondialdehyde by Ohkawa *et.al*. and estradiol by ELISA kit provided by Syntron bioresearch inc. were also estimated. Ten mililitre of venous blood was taken under all aseptic conditions using disposable syringe and needle of this 0.2 ml of 3.8% sodium citrate solution and immediately used for blood glutathione estimation, rest of blood allowed to clot and retract; centrifuged at 3000 rpm for ten minutes and used for estimating MDA and routine tests. These patients were put on conjugated equine estrogen, Premarin 0.625 mg daily orally for 4 months. Patients with intact uterus were put on additional progesterone, dydrogesterone. Ten mg daily orally at least 10-12 days per month. All tests were repeated after four months, after twelve hours of fasting.

Statistical Analysis- This was done by mean +/-standard error of mean (SEM) using paired student t-test. The level of significance or p-value was taken <0.05.

RESULTS

The results of this study are summarized as below:-
The majority of cases belong to 46-50 age group. The MDA level in postmenopausal women after giving HRT were found to be lowered ($p<0.001$) as compared to those before giving HRT. In the present study MDA level is comparable to that conducted by Leal *et.al*.(2002).The disparity in values of serum MDA is probably due to different methodologies used and the time period of application of HRT. Leal *et.al*.(2002) gave HRT FOR 3.5+/-1.3 months and in present study women were given HRT for four months. In our present study there is increase in GSH value which is consistent with Leal *et.al*. study and glutathione level is significantly higher after HRT ($p<0.001$) as compared to before HRT.

Sugar shows significant decrease after giving HRT which is in agreement with Rajan (1994) $p<0.01$. Alkaline phosphatase is significantly increased ($P<0.001$) after HRT. This is in agreement with Holzer G, Einhorn TA and Majeska RJ.

Triglyceride, cholesterol and LDL-C levels are decreased significantly ($p<0.001$) after HRT and HDL-C is increased significantly ($p<0.001$). These effects are comparable to previous study of Kim HY and Eow K (2002).

Table Comparison of Routine Investigations in post menopausal women on Hormone replacement therapy

Parameters	Hormone Replacement Therapy				t	p	s
	Mean+/-SD		Range				
	Before HRT	After HRT	Before HRT	After HRT			
Haemoglobin (gm%)	10.93+/-0.73	11.03+/-0.63	10-12	10-12	1.28	>0.05	NS
Serum creatinine (mg%)	1.082+/-0.139	1.077+/-0.097	0.8-1.3	0.7-1.3	0.42	>0.05	NS
Blood urea (mg%)	27.32+/-4.64	27.0+/-4.94	20-36	20-40	0.96	>0.05	NS
SGOT(IU/l)	26.24+/-8.95	25.30+/-7.50	13-48	10-40	1.73	>0.05	NS
SGPT(IU/l)	25.08+/-10.87	26.32+/-10.72	7-50	10-44	0.85	>0.05	NS
Parameters		Before HRT		After HRT			
Urine		NAD		NAD			

The table shows that routine investigations were within normal ranges before and after HRT.

Effect of HRT on Various Parameters

Parameters	MEAN+/- SEM(t)		p	s
	Before HRT	After HRT		
FBS(mg%)	72.17+/- 1.37	70.15+/-1.36	<0.01	HS
Alkaline phosphatase KA units	7.33+/- 0.23	8.05+/- 0.27	<0.001	HS
TG(mg%)	157.68+/-7.29	154.35+/-7.62	<0.001	HS
HDL-C(mg%)	41.48+/- 1.79	44.79+/- 1.65	<0.001	HS
LDL-C(mg%)	114.2+/- 4.93	99.75+/- 4.62	<0.001	HS
Cholesterol(mg%)	196.98+/-5.81	178.05+/-5.77	<0.001	HS
Malondialdehyde (µmol/l)	3.13+/-0.13	2.93+/-0.12	<0.001	HS
Glutathione(mg%)	53.36+/-1.32	64.16+/-1.61	<0.001	HS
Estradiol(pmol/l)	42.8+/-2.32	212+/-4.43	<0.001	HS

t is test of significance of difference in mean, p-values/level of significance. HS-Highly significant change; mg%=milligram percent; µmol/l=micromole per liter; pmol/l=picomole per liter.

The table shows routine investigations like FBS, shows highly significant change ($p<0.01$) after giving HRT. The other routine investigations like ALP, TG, HDL-C, LDL-C, Total cholesterol and special investigations like malondialdehyde, glutathione and estradiol show highly significant changes ($p<0.001$) after HRT.

Comparison of Mean Change From Base In FBS (mg%) in estrogen, estrogen and progesterone group after four months of HRT.

Group	Mean change from base	Difference (mg%)	t	p	s
Estrogen	3.77+/-5.80				
Estrogen and Progesterone	0.59+/-1.09	3.18	2.52	<0.05	S

Comparison of Mean Change From Base in Triglyceride (mg%) in estrogen, estrogen and progesterone group after four months of HRT.

Group	Mean change from base	Difference (mg%)	t	p	s
Estrogen	14.44+/-14.2				
Estrogen and Progesterone	5.31+/-13.91	9.13	2.05	<0.05	S

DISCUSSION

Risk factor according to Freidewald's equation total cholesterol/HDL-C comes out to be 3.91; this is less than 7 showing low risk for cardiovascular disease. This also shows HRT protects postmenopausal women from cardiovascular disease. There is high reactivity of free radicals which damages multiple biological substrates including lipids, lipoproteins and carbohydrates. As high concentration of lipoperoxides, total cholesterol, triglycerides are seen in postmenopausal women these decreases after hormone replacement therapy. Oxidation and increased cellular resistance to cytotoxic effects of oxidized LDL-C is seen. Estrogen demonstrates antioxidant properties by inhibiting LDL-C as is also seen in present study estrogen thus prevent cardiovascular disease. This is also confirmed by present study. Estrogen deficiency leads to coronary heart disease and osteoporosis. Infact estradiol produces better antioxidant status after hormone replacement therapy.

In post-menopausal women after HRT (hormone replacement therapy) i.e. estrogen or combined therapy (estrogen and progesterone) of four months malondialdehyde decreases and glutathione increases after treatment with HRT.

CONCLUSION

Short term use of HRT relieves symptoms of post-menopause. The long-term use of HRT remains controversial. The potential benefits for cardiovascular system must be weighed against the potential risk of breast cancer and endometrial cancer. For post-menopausal females treatment has to be individualized.

Oxidative stress plays a key role in pathogenesis of several age associated diseases. The antioxidant properties of estradiol reduce oxidative stress related complications. Menopause is typified by dropin endogenous estradiol that might subsequently affect women well-being.

Younger women 50-59 years or within 10 years of menopause have decreased coronary disease and did not have the perceived risks including breast cancer after HRT. Inflammation induced injury in aging vasculature is related to excessive accumulation and impaired dynamic O-GlcNAcylation of critical proteins

Many women who abruptly stop HRT have more risk, including more osteoporotic fractures and benefits are there if predominantly focused on estrogen component.

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