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SERUM HOMOCYSTEINE LEVELS AND HORMONE REPLACEMENT THERAPY

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ABSTRACT

Objectives: This study was designed to compare the level of homocysteine in the premenopausal and postmenopausal women and to evaluate the effects of Hormone Replacement Therapy on Homocysteine and total cholesterol level in postmenopausal women.

Material and Methods: Total Homocysteine was estimated by Florescent Polarization Immuno Assay and total Cholesterol by the enzymatic endpoint method.

Results: A significant difference in both total Homocysteine and total cholesterol levels was observed after six months of Hormone Replacement Therapy supplements.

Conclusion: Hormone replacement therapy reduces homocystein and cholesterol levels in postmenopausal women which may reduce the risk of cardiovascular disease.

Keywords: Postmenopausal women, Homocysteine, Hormone Replacement Therapy.

INTRODUCTION

Homocysteine (Hcy) is an intermediate product during the catabolism of sulfur containing essential amino acid, methionine[1,2,3]. It is found either as free homocysteine, cysteine-homosysteine mixed disulfide or protein bound homocysteine. The one that is bound with protein in plasma reflects total plasma homocysteine (tHcy)[4].

Endothelial dysfunction contributes to vascular disorder linked to Hyperhomocysteinemia[5,6]. Hyperhomocysteinemia is an independent risk factor for atherosclerotic diseases, including ischaemic heart disease, stroke and peripheral vascular disease[5,7-11].

Gender, age and circulating levels of folate and B12 affect plasma tHcy level[12] and also by estrogen status[7]. Hcy concentration rises progressively with age in men and women, likely causes include clinical or subclinical folate and B12 deficiencies[1,2]. Reiss et al.[13] reported basal homocysteinemia significantly higher in men than women which increased significantly after menopause in women, approaching those in men. This is most probably due to estrogen deficiency because in young women where estrogen production is high, serum lipids and Hcy levels are normal[14,15]. But after menopause abnormal lipid profile and hyperhomocysteinemia and increased incidence of coronary heart disease show a possible relationship among estrogen, normal lipid profile, normal tHcy levels and relative immunity to coronary heart disease[14,16].

Hormone replacement therapy (HRT) in postmenopausal women was the focus of medical research for the last so many decades so the present study was designed to compare the level of homocysteine in the premenopausal and postmenopausal women and to evaluate the effects of Hormone replacement therapy on tHcy and total cholesterol level in postmenopausal women.

MATERIAL AND METHODS

Thirty premenopausal and thirty postmenopausal women were randomly selected amongst patients attending different units of Abbottabad Teaching Hospital Complex, Ayub and District Head Quarter Hospital, Abbottabad. A detailed medical history and informed consent was taken from the subjects. Premenopausal women served as control. Subjects on Hormone replacement therapy, multivitamins, lipid lowering drugs, and those having coronary heart disease, thyroid disease, diabetes mellitus, renal disease were not included in the study.

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10ml of venous blood was collected from each subject after an overnight fast of 12-14 hours. Serum was separated and stored at -20°C until analyzed. After blood sample collection, the postmenopausal women were given 0.625mg conjugated equine estrogen daily for a period of six months. The subjects were called to the Out-Patient Department every month for routine check-up and to enquire and ensure about the adherence with the estrogen supplementation plan. At the end of supplementation period blood samples were again collected for analysis of tHcy and cholesterol.

Serum Hcy was determined quantitatively by Flourescence Polarization Immunoassay (FPIA) method using IMx homocysteine Kit Cat. No. B3D390, 33-0781/R5 obtained from Abbott, USA on the IMx Analyser. Total serum cholesterol was measured by quantitative enzymatic endpoint method using cholesterol kit Cat. No. CH207 obtained from Randox, USA.

Mean and Standard error mean (SEM) were calculated. Results from different group of subjects were compared using student "t" test and the level of significance was set at $p < 0.05$.

RESULTS

In total 60 women (30 premenopausal and 30 postmenopausal) participated in the study. The age range of premenopausal women was 29-31 years whereas that of postmenopausal women was 56-58 years. The level of total homocysteine and total cholesterol were found significantly different in both pre- and postmenopausal groups (Table 1).

The postmenopausal group was given hormone replacement therapy for a period of six months. Blood samples before and after the hormone replacement therapy were taken and analyzed for total homocysteine and total cholesterol. A significant ($P<0.001$) reduction in both these parameters was observed after the six month hormone replacement therapy period (Table 2).

Table 1: Age, Total Homocysteine and Total Cholesterol in Premenopausal and Postmenopausal Women

	Pre-menopausal (n=30)	Post-menopausal (n=30)
Age (years)	30.1±1.03	57.46±0.79*
Total Homocysteine (μmol/L)	8.65 ± 0.29	18.90 ± 0.35*
Total Cholesterol(mg/dL)	170.80±2.86	220.46±2.94*

*=$p < 0.001$ as compared to Pre-menopausal women.

Table 2: Total Homocysteine and Total Cholesterol before and after six months of Hormone Replacement Therapy (HRT)

	Before HRT (n=30)	After HRT (n=30)
Age (years)	29-31	56-58
Total Homocysteine (μmol/L)	8.65 ± 0.29	18.90 ± 0.35*
Total Cholesterol (mg/dL)	220.46±2.94	200.53 ±1.96*

*$p < 0.001$ as compared to Before HRT.

DISCUSSION

Coronary Heart Disease is a major cause of morbidity and mortality in the modern society[17]. High serum homocysteine concentration is recognised as risk factor for atherosclerosis and other vascular diseases[18] with consequent higher risk of cardiovascular disease[6,15,19] approaching as strong for women as for men[20]. After menopause the incidence of coronary heart disease rises in women to approach that for men of similar age[21]. In the postmenopausal period, cardiovascular diseases are a frequent chronic condition leading to high risk of myocardial infarction and death. Recently hyperhomocysteinemia and even mildly elevated plasma concentrations of homocysteine have been recognized as independent risk factors for vascular damage predisposing to arteriosclerosis[22]. The adverse effects of homocysteine involve oxidative damage to vascular endothelial cells, increased proliferation of smooth muscle cells, and oxidative modification of low density lipoprotein, all leading to atherosclerosis[17]. Our study showed a significant improvement in serum total cholesterol and tHcy level after six months of HRT with estrogen which is in conformity with several other studies[9,23-26].

Man et al[23] concluded that long term HRT is associated with improvement in plasma lipoprotein profile and a reduction in homocysteine level in postmenopausal women which may attribute to the beneficial effect of HRT on cardiovascular risk. Exact mechanism of estrogen in lowering Hcy is not so clear, but proposed mechanisms relate to an increase in kidney methionine synthetase activity or may be related to the transamination of methionine[25].

Estrogens are thought to increase high density Lipoprotein cholesterol (HDL-c) concentration by reducing hepatic triglyceride lipase enzyme activity that catabolizes HDL[27,28]. A reduction of hepatic triglyceride lipase activity might also reduce delivery of HDL-c from circulation to liver, an effect closely associated with enhanced concentration of HDL-c in plasma. The effect of estrogens in increasing HDL-c

level may also be related in part to an enhanced turnover of very low density lipoprotein cholesterol (VLDL-c) and transfer of surface remnants to HDL-c[29]. Estrogens lower low density lipoprotein cholesterol (LDL-c) level by an estrogen stimulated increase in LDL-c receptors in liver[30]. There is an increase in receptor synthesis related to increased levels of LDL-c receptors messenger RNA inside the cell[29]. According to Merola and Arnold[31], estrogen inhibits cholesterol biosynthesis at the mevalonic acid stage. Demirovic et al[32] and Tikkanen et al[30] have suggested that postmenopausal rise in serum cholesterol is primarily through an increase in LDL-c concentration because in estrogen presence it is more likely that reduced HDL-c delivery to liver is compensated by increased LDL-c delivery to liver, via the upregulated LDL-c receptors[29] so estrogen effects circulating lipoproteins level favourably[33,34]. In addition to this favour estrogen also influences the complex metabolism of lipoproteins in arterial wall, helping to impede the formation of atherosclerotic plaque[35].

CONCLUSION

Hormone replacement therapy in postmenopausal women is beneficial in reducing the blood levels of homocysteine. This in turn may help prevent atherosclerosis with consequent reduction in the incidence of coronary heart disease in postmenopausal women.

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