

## EFFECT OF LOW-DOSE FOLIC ACID SUPPLEMENTATION VERSUS HRT AND COMBINATION OF FOLIC ACID AND HRT ON PLASMA LEVEL OF HOMOCYSTEINE IN POSTMENOPAUSAL WOMEN

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### ABSTRACT

**Objective:** This study was designed to evaluate the effect of hormone replacement therapy (HRT) and folic acid supplementation on Homocysteine (Hcy) level in postmenopausal women.

**Methodology:** This is a randomized clinical trial conducted on 66 postmenopausal women (menopause at least for 2 years) attending Hamedan outpatient Fatemeh teaching hospital. Since March 2006 till April 2007. The patients were randomly divided into four groups. Before studying plasma level of Albumin, total protein, folate, fasting level of plasma Hcy was measured. The patients of first group received folic acid 5mg daily. In the second group of patients took oral conjugated estrogen 0/625mg and medroxy progesterone acetate 2/5 mg daily. In third group the patients received combination of folic acid and HRT and placebo in fourth group. Fasting plasma level of Hcy was measured four weeks later.

**Results:** Plasma level of Hcy was significantly decreased in group 1, 2 and 3 ( $P<0.05$ ) after treatment. There was significant difference between group 1, 3 ( $p=0.026$ ) and 1, 4 ( $P=0.005$ ) for plasma level of Hcy.

**Conclusion:** HRT and folic acid therapy can reduce plasma Hcy. Combination of both HRT and folic acid supplementation is more effective than HRT or folic acid alone in reduction of hyperhomocysteinemia.

**KEYWORDS:** Homocysteine, Folic acid supplementation, Menopause women.

Pak J Med Sci April - June 2010 Vol. 26 No. 2 310-313

### How to cite this article:

Nasrolahi S, Radnia N, Neghab N, Shafie A. Effect of Low-dose Folic acid Supplementation versus HRT and Combination of folic acid and HRT on Plasma Level of Homocysteine in Postmenopausal Women. Pak J Med Sci 2010;26(2):310-313.

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\* Received for Publication: July 28, 2009

\* Accepted: February 2, 2010

### INTRODUCTION

Hyperhomocysteinemia is an independent risk factor for atherosclerotic diseases including ischemic heart disease, stroke and peripheral vascular disease. Hcy is an intermediate formed during the catabolism of essential sulphur containing amino acid methionine. Increased Hcy is associated with endothelial dysfunctions in healthy human. Plasma Hcy is significantly lower in premenopausal women than young men but after menopause basal homocysteinemia increases significantly in

women approaching those in men. It seems likely that altered hormonal status and age related low folate intake are responsible for this. Cardio vascular disease (CVD) is number one killer of both men and women in western societies and is attributed primary to age and lifestyle. For women CVD is largely a disease of post menopause. A woman's risk of heart disease is far lower than man's risk until after menopause. This change in incidence of heart disease may be related to advancing age, changes in hormonal milieu, or other unknown factors.<sup>1</sup>

Elevated Hcy levels are associated with an increase in coronary heart disease, endothelial dysfunctions, atherosclerosis and thrombosis. Increasing folic acid intake and HRT reduce the circulating levels of Hcy.<sup>2</sup>

Gender, age and circulating levels of folate and B12 effect plasma Hcy level and also by estrogen status. After menopause CVD protection is lost most probably due to estrogen deficiency. Simply folic acid supplementation can be beneficial to postmenopausal women in protecting from CVD.<sup>3</sup>

The present study was conducted to investigate the effect of HRT and folic acid on Hcy.

## METHODOLOGY

This study was a randomized clinical trial, the subjects included in this study were selected from women attending outpatient department of Hamedan Fatemeh Teaching Hospital since March 2006 till April 2007. Informed written consent was obtained on voluntary basis from all subjects included in the study. Sixty six postmenopausal women (at least for two years)

enrolled randomly in four groups. Plasma levels of Albumin, total protein and folate measured with ELISA method. Women with normal lab test were included in this study.

Blood samples were obtained from antecubital veins after an over night fasting in beginning of study and after 4 weeks. Sera were stored at -50°C until analyzed. Group 1 consist of 16 women received folic acid 5mg daily for 4 weeks. Eighteen women in group two received HRT oral conjugated estrogen 0/625mg combined with 2/5mg medroxy progesterone acetate daily for four weeks. Eighteen women in group 3 received both folic acid 5mg daily and HRT for 4 weeks. Seven women in group 4 did not take any drug as control. All cases had normal diet and physical activity and were not on B6 or B12 supplementation.

Inclusion criteria were menopause women without any CVD, GI or renal disease. Exclusion criteria were smoking, abnormal plasma level of albumin, total protein, folate or taking B6 or B12 supplementation. Result from different group of subjects were compared using paired t-test and ANOVA the level of significance was set at p<0/05.

## RESULTS

The results of this study are summarized in Table-I,II&III. The mean age, duration of menopause, plasma albumin, total protein, folate and Hcy level in the beginning of study in all groups had no statistical difference. Plasma Hcy was lowered in treatment groups (p<0/05). Combination therapy with HRT and folic acid supplementation can lower plasma Hcy than HRT or folic acid supplementation alone.

Table-I: Mean age, duration of menopause, plasma albumin, total protein and primary plasma Hcy in study groups

	Group1(n=16)	Group2(n=18)	Group3(n=15)	Group4(n=17)	P Value
Mean age(years)	53.31±6.04	54.61±7.01	51.67±4.67	56.18±3.3	0.13
Duration of menopause(yr)	6.94±3.75	7.33±4.92	5.20±2.91	8±3.24	0.21
Plasma albumin (g/dl)	4.13±1.01	4.39±0.83	3.73±1.01	4.24±0.90	0.48
Plasma total protein (g/dl)	6.39±0.43	6.45±0.62	6.30±0.37	6.26±0.80	0.15
Plasma folate (mg/ml)	6.13±2.5	5.83±2.55	8.35±3.14	7.47±4.47	0.08
Primary HcY (micromol/L)	17.65±6.07	19.50±3.80	19.06±4.41	17.82±5.17	0.63

Table-II: Plasma Hcy level before and after study in four groups

	<i>Plasma Hcy (micromol/L) level before treatment</i>	<i>Plasma Hcy (micromol/L) level after treatment</i>	<i>Pvalue</i>
Group 1	17.65±6.07	11.12±4.25	P=0.002 t=3.70
Group 2	19.5±3.80	10.22±3.22	P=0.000 t=7.58
Group 3	19.06±4.15	7.73±4.21	P=0.000 t=9.43
Group 4	17.82±5.17	18.11±4.85	P=0.603 t=0.531

## DISCUSSION

In the present study both HRT and folic acid supplementation alone or in combination could decrease plasma Hcy in postmenopausal women. Hak et al, in 2000 concluded that plasma Hcy is affected by menopause. After menopause high Hcy level seems to be sum of altered hormonal status and low folate level.<sup>3</sup> Data regarding the impact of various formulas and method of administrating HRT on Hcy plasma level are not fully evaluated. Many studies were designed to evaluate the effect of folic acid on Hcy level.<sup>4</sup> Folic acid supplementation (500-5000 microgr) can reduce plasma level of Hcy in post menopausal women.<sup>5,6</sup>

It is proved that folic acid supplementation either in combination with HRT or alone has beneficial effect in lowering Hcy level in low estrogen status subsequent to ovariectomy.<sup>3,4</sup>

Moderate hyperhomocysteinemia, defined as total Hcy concentration between 12 to 13 micro

mol/L, represent as independent risk factor for heart disease, vascular brain disease, phlebothrombosis and thromboembolic complication.<sup>3</sup> Hyperhomocysteinemia is metabolic syndrome based on interaction between genetic factors diseases and demographic factors (smoking, aging, hormonal and nutritional factors).<sup>3,6</sup>

Treatment of hyperhomocysteinemia is based on administration of pharmacological doses of folic acid, which can decrease total homocysteine concentration by 25 to 30%. Such decrease, which is average 3 micromol/L, results in the decrease of relative risk of ischemic heart disease by 11 to 16%, phlebothrombose by 25% and vascular brain diseases by 19 to 24%.<sup>3</sup> In recent years great attention has been focused on the role of folates on public health.

Villa P et al in Italy designed a clinical trial on 20 menopause women without any history hypoglycemic agent or anti hypertension drug consumption, low dose of folic acid can decrease

Table-III: Plasma Hcy level before and after treatment in each group and other groups.

<i>Groups</i>	<i>Other groups</i>	<i>Mean of Hcy (micromol/L)</i>	<i>P value</i>	<i>95% confidence interval</i>	
				<i>Upper bound</i>	<i>Lower bound</i>
Group1	Group2	3.18±1.79	0.297	1.56	7.92
	Group3	5.43±1.87	0.026	0.47	10.40
	Group4	6.38±1.82	0.005	11.19	1.57
Group2	Group1	3.18±1.79	0.297	7.92	1.56
	Group3	2.25±1.82	0.608	2.57	7.08
	Group4	9.57±1.76	0.000	14.24	4.90
Group3	Group1	5.43±1.87	0.026	10.40	0.47
	Group2	2.25±1.82	0.608	7.08	2.57
	Group4	11.82±1.85	0.000	16.71	6.93
Group4	Group1	6.83±1.82	0.005	1.57	11.19
	Group2	9.57±1.76	0.000	4.90	14.24
	Group3	11.82±1.85	0.000	6.93	16.71

plasma level of Hcy, insulin and better lipid profile.<sup>6</sup>

Animal study was undertaken to examine whether lowering Hcy with HRT or folic acid in ovariectomized rats could attenuate cardiovascular complication.<sup>7</sup> De Leo et al found that low dose folic acid supplementation for four weeks reduced plasma level of Hcy in 18 post menopause women. This reduction is seen especially in women with high level of Hcy. This result is compatible with our study.<sup>5</sup>

In this study combination of HRT and folic acid therapy has statistically significant decrease in Hcy in comparison to folic acid alone, but there is no significant difference to HRT alone. Hcy level was significant decreased before and after treatment. Mean Hcy level in folic acid supplementation group (group1) was 13.9 microgr/L before treatment and 9.3 microgr/L after treatment. It was 30% reduction in Hcy. Although HRT in combination with folic acid supplementation has greater effect in lowering plasma Hcy, in postmenopausal women with contraindication for HRT folic acid supplementation prevents CVD with lowering Hcy. Even though postmenopausal HRT is not used as means of cardiovascular protection, it is a treatment available for vasomotor symptoms and prevention of urogenital atrophy during menopause. Until the relationship between menopause, HRT, homocysteine, folate are clearly elucidated with more comprehensive studies,

including all the details leading to plasma Hcy increment in Hcy metabolism, we recommend that menopausal women should be provided with accurate information and risk benefit analysis on HRT treatment and the decision should be made by the patient.

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