**COMPARISON OF LIPOPROTEINS AS CORONARY RISK FACTORS IN POSTMENOPAUSAL WOMEN WITH AND WITHOUT HORMONAL REPLACEMENT THERAPY IN RAWALPINDI/ISLAMABAD AREA**

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**ABSTRACT**

**Objective:** To compare serum concentration of Total Cholesterol, Triglyceride, LDL-cholesterol, HDL-cholesterol and lipoprotein (a) between post menopausal women on HRT and without HRT.

**Study Design:** Comparative, cross-sectional study.

**Place and Duration of Study:** The study was conducted in the department of Chemical Pathology and Endocrinology, Armed Forces Institute of Pathology, Rawalpindi from 2006 to 2007.

**Materials and methods:** Postmenopausal women with no co-morbidities and on any type of HRT for at least one year were selected as cases and healthy postmenopausal women not taking any type of HRT were taken as controls. All these cases were selected randomly. After clinical history and physical examination, blood sampling was carried out for lipid profile including Lp(a) in fasting state.

**Results:** A total of 50 postmenopausal women on HRT and their age matched healthy controls (postmenopausal women without HRT) were studied. The mean age of HRT treated group was 56.7 ± 5.0 (mean ± SD) years. The mean age of menopause of study group was 50.4 ± 1.6 years and the mean years since menopause were 8.4 ± 4.6 years. There was no statistically significant difference in age, age at menopause and years since menopause between study and control groups. The frequencies for the type of HRT were calculated. Thirty two of the women were using combination therapy i.e.; estrogen plus progesterone while 18 of them were using estrogen therapy alone. None of them were using other types of HRT like SERMs or tibolone. The mean values of TC, LDL-C and Lp (a) were lower than the control group. The mean value of HDL-C and TG was higher in study group as compared to control group.

On comparative analysis of the lipid profile of the two groups employing unpaired t-test, statistically significant difference was observed in the levels of LDL-C, HDL-C and Lp(a). However TC and TG levels were not significantly different in women belonging to either group.

**Conclusions:** HRT has beneficial effects on lipoprotein levels in postmenopausal women.

**Keywords:** Postmenopausal, Hormone replacement therapy, lipoprotein coronary risk factors

**INTRODUCTION**

Hyperlipidemia is an important modifiable risk factor for the development and progression of CHD. The relationship between elevated total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), decreased high-density lipoprotein cholesterol (HDL-C) and CHD is now firmly established. Lipoprotein (a) (Lp (a)) is an independent risk factor for CHD and this has also been demonstrated in Pakistani population with CHD. The incidence of CHD is much lower in younger women than in age-matched men and it is believed that estrogen may be responsible for these protective effects in younger women. However, this difference tends to disappear after menopause and the risk rises significantly in postmenopausal women.

After menopause exogenous administration of estrogen and progesterone preparation is known as hormone replacement therapy (HRT). As incidence of CHD is high in postmenopausal women, it is assumed that HRT has favorable effects on the lipoprotein profile. It increases the HDL-C and decreases the LDL-C, and Lp (a). On the other hand some recent studies have shown that HRT does not provide cardiac protection and may increase the risk of CHD among healthy postmenopausal women. Therefore, the risk of
treatment overweighs the benefits and it should not be used for the prevention of CHD alone.\(^8\)

Percentage of postmenopausal women taking HRT\(^9\) is very low in our country, while incidence of CHD is on rise. Considering unique ethnicity, socio-economic status and health-education status of our female population, there is a dire need to evaluate the effects of HRT on various biochemical coronary risk factors e.g. lipoprotein factors, insulin sensitivity, C-reactive protein (CRP) and homocysteine etc\(^10\). Therefore, a study was planned to evaluate effects of HRT on the levels of lipoprotein coronary risk factors. Such a study would be helpful in formulating recommendations for use of HRT or otherwise in postmenopausal women.

**MATERIALS AND METHODS**

The study was conducted in the Department of Chemical Pathology and Endocrinology, Armed Forces Institute of Pathology (AFIP), Rawalpindi. It was a comparative cross sectional study.

Fifty postmenopausal women taking any kind of HRT for at least last one year were selected as cases and fifty postmenopausal women with duration of menopause for at least one year but not taking any kind of HRT were taken as control group after taking consent from the subjects. Women with ischemic heart disease, hypertension and diabetes mellitus and on lipid lowering drugs were excluded from both groups for the study. All these women were selected randomly.

All women who were selected for the study were then given appointment for interview, clinical evaluation and specimen collection. The patients were explained the necessary requirements of medical fasting and were requested to bring along their relevant documents. They were advised to report for evaluation in the morning on the day of appointment.

The subjects were thoroughly examined. Salient features of general physical examination like pulse, blood pressure, height and weight were recorded. Other relevant information like duration since menopause, type of HRT and duration of HRT were recorded.

**Biochemical Analysis:** The patients were seated comfortably for about 15 minutes before sampling for lipid profile. Five ml of blood was collected in fasting state for serum lipid profile in plain tube. Lipid profile analysis included TC, TG, HDL-C, LDL-C and Lp (a). Serum was separated by centrifugation at a relative centrifugal force of 2500-3500 g for about 15 minutes. Each serum sample was aliquoted into two test tubes; from one aliquot, TC, TG and HDL-C were analyzed, whereas LDL-C was calculated by using Friedewald formula. The second serum aliquot was stored at -20 ºC, for analysis of Lp (a) to be done in batches.

Serum TC was measured on Saturno 300, random access chemistry autoanalyzer utilizing the cholesterol oxidase-phenyl ampyrone (CHOD-PAP) enzymatic colorimetric method (Linear Chemicals, S.L).

Serum TGs were also analyzed on Saturno 300, random access chemistry autoanalyzer utilizing the glycerol phosphate-phenylamypyrone (GPO-PAP) enzymatic colorimetric method (Human, Wiesbaden, Germany). The TG liquicolour commercial assay utilizes enzymatic colorimetric test with Lipid Clearing Factor (LCF). The LCF clears up totally a turbidity caused by lipaemic specimens.

The analysis of HDL-C was done using an indirect method by precipitation with phosphotungstic acid, and was carried out on Microlab-200, semiautomated chemistry analyzer.

LDL-C can be calculated or estimated directly. In this study the LDL-C was calculated indirectly by using the Friedewald equation/formula. This formula is based on the assumption that TC is predominantly composed of cholesterol found in VLDL, LDL and HDL. It is important to note that the Friedewald formula doesn’t hold true at high TG levels (>4.0 mmol/L), in which case it would under estimate LDL-C. However, none of the patients in this study had this level of hypertriglyceridaemia. Lp (a) samples were allowed to thaw at room temperature. The samples were then analyzed in batches, on
Hitachi 911 (clinical chemistry autoanalyzer by Roche), utilizing immunoturbidimetric assay technique by reagent kits manufactured by Roche.

**STATISTICAL ANALYSIS**

All the data including demographical and clinical details and biochemical parameters was stored and compiled for statistical analysis using Statistical Package for Social Sciences Programme (Version 11.0, SPSS). Descriptive statistics were carried out to summarize the data. Frequency and percentage were calculated for type of HRT. Mean and SD were calculated for numerical data including actual age, age at menopause, number of years since menopause and lipid profile (TC, TG, HDL-C, LDL-C and Lp (a)). Unpaired t-test was applied to determine the significance of results at a level of 0.05 in two tails.

**RESULTS**

A total of 50 postmenopausal women on HRT and their age matched healthy controls (postmenopausal women without HRT) were studied. The mean age of HRT treated group was 56.7 ± 5.0 (mean ± SD) years. The mean age of menopause of study group was 50.4 ± 1.6 years and the mean years since menopause were 8.4 ± 4.6 years. There was no statistically significant difference in age, age at menopause and years since menopause between study and control groups (Table I).

The frequencies for the type of HRT were calculated. Thirty two of the women were using combination therapy i.e.; estrogen plus progesterone while 18 of them were using estrogen therapy alone. None of them were using other types of HRT like SERMs or tibolone (Figure).

The main outcome measure in this group was the lipid profile analysis, which included serum TC, TG, HDL-C, LDL-C and Lp(a). Concentrations of the lipid profile have been expressed as mean ± SD (Table I).

The mean values of TC, LDL-C and Lp (a) were lower than the control group. The mean value of HDL-C and TG was higher in study group as compared to control group.

On comparative analysis of the lipid profile of the two groups employing unpaired t-test, statistically significant difference was observed in the levels of LDL-C, HDL-C and Lp(a) (Table II). However TC and TG levels were not significantly different in women belonging to either group.

**Table-1: Demographical characteristics and mean serum lipid concentrations of study group (n=50) and controls (n=50)**

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Study Group (mean±SD)</th>
<th>Controls (mean±SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age(years)</td>
<td>56.7±5.0</td>
<td>57.9±7.0</td>
</tr>
<tr>
<td>Age at menopause</td>
<td>50.3±1.6</td>
<td>50.6±1.5</td>
</tr>
<tr>
<td>TC (mmol/L)</td>
<td>4.60±0.62</td>
<td>4.84±0.69</td>
</tr>
<tr>
<td>TG (mmol/L)</td>
<td>2.32±0.7</td>
<td>2.24±0.8</td>
</tr>
<tr>
<td>HDL-C (mmol/L)</td>
<td>1.0±0.09</td>
<td>0.9±0.08</td>
</tr>
<tr>
<td>LDL-C (mmol/L)</td>
<td>2.44±0.62</td>
<td>2.80±0.75</td>
</tr>
<tr>
<td>LP(a) (mg/dl)</td>
<td>35.2±23.0</td>
<td>50.5±38.7</td>
</tr>
</tbody>
</table>

**Table-2: Comparison of mean serum lipid concentrations of study group (n=50) and controls (n=50)**

<table>
<thead>
<tr>
<th>Lipid parameter</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>TC (mmol/L)</td>
<td>0.057</td>
</tr>
<tr>
<td>TG (mmol/L)</td>
<td>0.59</td>
</tr>
<tr>
<td>HDL-C (mmol/L)</td>
<td>0.006</td>
</tr>
<tr>
<td>LDL-C (mmol/L)</td>
<td>0.01</td>
</tr>
<tr>
<td>LP(a) (mg/dl)</td>
<td>0.01</td>
</tr>
</tbody>
</table>

**DISCUSSION**

Women are at lower risk of fatal coronary heart disease (CHD) compared with men. After menopause this risk increases and becomes equal to men. Several types of epidemiological evidence suggest that women’s universal protection against CHD is explained...
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by estrogen\textsuperscript{11}. Lipoproteins are important coronary risk factors and their association with CHD is well established. In this study effects of HRT on lipoproteins were compared with healthy postmenopausal women without HRT. In other studies conducted on effects of HRT, the mean ages were comparatively higher\textsuperscript{12} than the mean age of our study group. However, the mean age of menopause in the study group was comparable with studies conducted in other parts of the world\textsuperscript{13}.

About two third of the women were using estrogen therapy alone. None of them were using any other type of HRT like SERMs or tibolone. The reason for not using these kinds of HRT could be financial constraint. Keeping in view the socioeconomic setup of our population, the prescription of these regimens does not appear to be cost effective.

The mean values for TC, HDL-C, LDL-C and Lp(a) were within normal range (as determined by NCEP cutoffs) which was in accordance to the results of Women Health initiative group (WHI)\textsuperscript{14}. Although mean values for TC and LDL-C in control group were also within normal limits, but these values for some subjects were much higher as compared to the study group. The mean value of HDL-C was significantly higher in study group as compared to controls. Where as, mean values for Lp(a) were low in the study population but the same were higher than the cut off value in the subjects of control group.

While comparing the mean values of HDL-C between the two groups the difference was statistically significant. This was the most significant finding of the study because the plasma concentration of HDL-C is a significant independent predictor of risk for CHD. Increased plasma concentrations of HDL-C have a cardio protective effect, mostly because it acts as a mediator of reverse cholesterol transport. HDL also exerts anti-inflammatory and antioxidant effects and promotes fibrinolysis\textsuperscript{15}.

Targeting and lowering the LDL-C levels in at risk groups has received greater attention in the NCEP revised guidelines\textsuperscript{16}. Estrogen lowers LDL-C by up regulating LDL receptors in the liver and enhancing LDL catabolism\textsuperscript{17}. The lowering of LDL-C after HRT use has also been observed in other international\textsuperscript{18} as well as local studies\textsuperscript{19,15}. Information is sparse regarding LDL subclasses and atherogenic modifications such as a shift towards smaller LDL. Similarly, in the present study, LDL has been quantified in terms of its cholesterol content only and LDL size variation has not been taken into account.

Elevated triglyceride levels in women are independent predictors of CHD mortality and angiographic progression of coronary atherosclerosis\textsuperscript{20}. Unopposed estrogen usually but not invariably increases triglycerides levels\textsuperscript{21} as in this study the mean value of TG in HRT treated group was higher than the NCEP recommended cut-off. However, the difference between the two groups was statistically not significant. These findings are in agreement with WHI study where TG levels in the study group were elevated compared to placebo group.

The mean value of TC was lower in study group as compared to control group but this difference was not statistically significant (p>0.05). This finding is, however, in accordance with many studies in which raised HDL-C and reduced LDL-C was recorded in HRT treated postmenopausal women but no effect on total cholesterol\textsuperscript{22}.

Lp(a) is associated with CHD risk probably due to its structural similarity to LDL-C, thereby binding to LDL-C receptors and blocking the degradation of LDL-C. Its long plasma half life and sequence homology with plasminogen have also been implicated in its pathogenesis. Though not measured routinely as a part of lipid profile, a special effort was made to assess the levels of Lp(a) in the study group. At present, the reference methods or standardization procedures have not been established, hence the lack of definition for precise cut-off levels for Lp(a).

In the present study we have utilized a cut off 40 mg/dl for Lp(a) to assess the number of HRT treated women at significant risk of CHD. Lp(a) analysis gave highly variable results and
varied from almost un-detectable levels to 135 mg/dl. There was statistically significant difference in mean values between two groups (P=0.01). The same fact was also established in Framingham Heart Study\textsuperscript{23}. They observed a 17.6% difference in the mean Lp(a) between women receiving HRT when compared with women of the same age not receiving estrogen. The effect of estrogen on Lp(a) is further supported by the work of Kim et al\textsuperscript{24}, who reported 23% reduction in Lp(a) mass in postmenopausal women placed on HRT; when HRT was discontinued, the concentration of Lp(a) mass returned to normal\textsuperscript{25}. The structural heterogeneity of Lp(a) as a consequence of its apolipoprotein size heterogeneity has important implications in the accurate measurement of Lp(a) in human plasma, and hence the variable results seen in various studies.

Lastly, certain important aspects like the association of HRT with thrombosis, hyperhomocysteinaemia and C-reactive protein levels which were obviously beyond the scope of this study have not been addressed and need further population based studies.

CONCLUSION

The use of HRT has favorable effects on lipid parameters. This effect is more pronounced in parameters strongly linked with coronary artery disease. Use of HRT should be individualized, the risks and benefits of HRT for each woman being taken into consideration.

REFERENCES