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Original Article

Biochemistry Section

Prevalence of Elevated Serum Homocysteine and Serum Lipoprotein 'a' in Women

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ABSTRACT

Background: Recent studies indicate that the risk of coronary artery disease (CAD) in women is no less than that in men and menopausal women are equally vulnerable as men. Studies of recent risk factors like hyperhomocysteinemia and elevation in lipoprotein (a) reveal controversial role of the same. This study hence is an attempt to study the prevalence of these factors in women and their correlation with lipid profile.

Materials and Methods: Two hundred women were enrolled in the study- 100 premenopausal women (21-45y) and 100 menopausal (50-55y). All the subjects were screened for homocysteine by ELISA and lipoprotein (a) and lipid profile by automation.

Results: Prevalence of hyperhomocysteinemia was 52% and 62% in premenopausal and menopausal women respectively. A significant positive correlation was seen for total cholesterol and triacylglycerol with serum Homocysteine in premenopausal women while pronounced positive correlation for serum cholesterol with serum Homocysteine in menopausal women. The prevalence of elevated lipoprotein (a) was 42% and 45% in premenopausal and menopausal women respectively. There was no correlation between lipoprotein (a) and lipid profile in both groups.

Conclusion: The findings of the study conclude that premenopausal and menopausal women constitute a subpopulation where recent risk factors like hyperhomocysteinemia and elevated lipoprotein(a) could be assessed along with lipid profile as screening tests to identify the risk of CAD. This would help in proper counselling of the concerned women and minimize the risk.

Keywords: Cardiovascular risk factors, Homocysteine, Lipoprotein (A), Menopausal women, Premenopausal women

INTRODUCTION

Coronary artery disease (CAD) is a leading cause of death globally [1]. It was in early 1990s that more attention was focused on women with CAD [2]. Although most risk factors contribute to CAD in both men and women [3] the impact of individual risk factors may be different for example, diabetes mellitus confers greater risk [4] and high density lipoprotein (HDL) greater protection in women [5]. The prevalence of modifiable risk factors generally increases with age [6]. The need for effective prevention measures is particularly acute during and after menopause [7]. Women are less likely to be referred for diagnostic and therapeutic procedures [2]. The influence of menopause on both cardiovascular risk factors and CAD is unique for women.

Considering recently identified risk factors the available research data reveals a controversial role of homocysteine (Hcy) and lipoprotein 'a' (Lp(a)) as individual risk factors for CAD. Lp(a) levels are independent of other lipid parameters [8] Levels greater than 30mg/dl are associated with a 2 fold greater risk of CAD [9]. Lp(a) is considered as a dual pathogen, atherogenic due to its Low Density Lipoprotein (LDL) like properties and thrombogenic due to its ability to interfere with fibrinolysis [10]. Hcy is synthesized as an intermediate of amino acid methionine metabolism. Worldwide studies have elucidated the reference range of Hcy as 5- 15µmol/L [11]. Its level rises with age, and fasting Hcy levels appear to be generally lower in women than in men [12]. Hcy itself is an atherogenic substance as it oxidizes and modifies LDL, produces superoxides thus resulting in vascular injury [11].

Though much progress has been made in assessment of CAD in women, fewer attempts have been made to study the prevalence and prevention of CAD in women as distinct subpopulation. This study hence is an attempt to compare the prevalence of serum Hcy and Lp(a) in premenopausal and menopausal women and study their correlation with lipid profile.

MATERIALS AND METHODS

Study design

1. Sample size: Two hundred healthy women enrolled in the study 100 premenopausal women (age- 21-45 y) and 100 menopausal women (age- 50-55 y). The sample size was decided by power calculation in consultation with the statistician. The female subjects were identified from faculty and other non teaching staff of Dr DYPMC and Research Centre Pimpri, Pune, India.

The subjects were screened for the following biochemical parameters-

a) Serum Hcy

b) Serum Lp(a)

c) Serum lipid profile-

i. total cholesterol (TC)

ii. LDL-c

iii. HDL-c

iv. Triacylglycerol

2. Inclusion criteria: Two hundred healthy women not on any medication or supplements like HRT, vitamins and minerals were included in the study.

3. Exclusion criteria: Women having H/O any major illness like diabetes mellitus, hypertension, malignancy and pregnant women were excluded from the study.

The five year period in menopausal women was used as wash out period to ensure the menopausal status.

4. Collection of sample: 10-12 ml of fasting venous blood sample was collected in sterile plain bulb for estimation of biochemical parameters with aseptic measures after obtaining informed consent.

5. Methods of assay

1. Serum Hcy – ELISA [13].

Biochemical Parameter (Measuring unit)	Premenopausal Women(n=100)	Menopausal Women(n=100)	p Value t-test
Homocysteine (µmol/L) (Hcy)	20.73 ± 29.0	21.65 ± 14.5	0.77
Lipoprotein(a) (mg/dl) (Lp(a))	32.20 ± 27.6	33.51 ± 23.0	0.71
Total Cholesterol (mg/dl) (TC)	159.73 ± 28.7	168.08 ± 28.3*	0.04
LDL Cholesterol (mg/dl) (LDL-c)	105.9 ± 17.0	106.91 ± 19.3	0.7
HDL Cholesterol (mg/dl) (HDL-c)	48.86 ± 7.8	48.9 ± 7.9	0.97
Triglyceride (mg/dl) (TG)	97.84 ± 25.1	108.83 ± 26.5*	0.004

[Table/Fig-1]: Ranges of Means of Homocysteine, Lipoprotein (a) and Lipid profile, ** p value < 0.05*

Biochemical Parameter (Measuring unit)	Premenopausal Women(n=100)	Menopausal Women(n=100)	p Value t-test
Homocysteine (µmol/L)	55%	68%	0.296
Lipoprotein(a) (mg/dl)	41%	46%	0.325

[Table/Fig-2]: Prevalence of raised homocysteine and Lipoprotein (a) levels in the study participants

Lipid Profile	Premenopausal Women		Menopausal Women	
	Homocysteine	Lipoprotein (a)	Homocysteine	Lipoprotein (a)
Total Cholesterol mg/dl	0.43	0.07	0.49	0.3
LDL Cholesterol mg/dl	0.14	0.13	0.12	0.2
HDL Cholesterol mg/dl	-0.21	-0.35	-0.19	-0.1
Triglycerides mg/dl	0.59	0.31	0.26	0.27

[Table/Fig-3]: Correlation of Homocysteine & Lipoprotein (a) with Lipid profile

2. Serum Lp(a) – Spectrophotometric [14]
3. Serum Lipid profile-i. Total cholesterol - Spectrophotometric [15]
   ii. LDL - Spectrophotometric [16]
   iii. HDL -- Spectrophotometric [17]
   iv. Triacylglycerol - Spectrophotometric [18].

6. Statistical analysis: Prevalence of hyperhomocysteinemia and elevated Lp(a) was calculated using WINPEPI statistical software version 11.28 in consultation with the statistician from Department of Community Medicine, DYPMC Pimpri Pune, India.

7. Ethics Statement: This study confirms the declaration of Helsinki. The research proposal was granted approval by University review board with reference no. DYPU/209(F)/08 dated 1.3.08.

RESULTS

A total of 200 women were enrolled in the study. Out of 200, 100 were premenopausal subjects & 100 were menopausal. The results of the study are described in the following paragraphs & tables.

Referring to [Table/Fig-1] no significant change was seen in serum Hcy and Lp(a) in both the groups.

Considering the lipid profile parameters, significant increase was seen in serum TC and triacylglycerol in the menopausal group. No change was seen in LDL and HDL cholesterol in both the groups.

As far as prevalence of raised Hcy and Lp(a) is concerned, no significant difference was seen between the two groups [Table/Fig-2].

Referring to [Table/Fig-3] a positive correlation was seen in total cholesterol and Hcy in both the groups. L (a) showed no correlation in premenopausal group but it was positive in menopausal group.

No correlation was seen between LDL-C and both homocysteine and lipoprotein (a) in the two groups. A negative correlation was observed between HDL-C and lipoprotein (a) in premenopausal group.

Serum TG showed a positive correlation with serum Hcy and Lp (a) in premenopausal group while no correlation is seen in the menopausal group.

The linear relationship depicted by correlation coefficient is not causal can be by chance occurrence & it is proposed to study the causal association between the parameters in the above table in a different epidemiological study.

DISCUSSION

This study revealed high prevalence of hyperhomocysteinemia both in premenopausal and menopausal women with insignificant rise in the later group. This is in concurrence with other studies [19,20]. Hcy levels rise with age, and fasting Hcy levels appear to be generally lower in women than in men. The males have relatively higher values as compared to age matched females. Higher Hcy levels in males are attributed to muscle mass [19]. Menopausal women have higher mean values as compared to premenopausal women. This increase is attributed to estrogen deficiency. Estrogen is reported to have Hcy lowering effect. Hyperhomocystenemia around menopause hence may increase the risk of CAD or may worsen the condition in existing CAD status [21]. At menopause hyperhomocysteinemia many times may coexist with other traditional risk factors like raised TC and LDL-C concentration. Hyperhomocysteinemia recently is established as an independent risk factor for CAD in 10% of the total risk factors. Elevated Hcy levels adversely influence the endothelial surface, vascular smooth muscle, connective tissue interaction with plasma lipoproteins, clotting factors and platelets which may induce vascular injury [22].

Besides hyperhomocysteinemia, this study also reveals a positive correlation between serum Hcy and TC in both premenopausal and menopausal women. This would be an additional factor to consider control of serum Hcy especially in premenopausal group where a positive correlation of serum Hcy with TG is seen as well.

The study also demonstrates a high prevalence of elevated Lp(a) in premenopausal and menopausal women with insignificant rise in the later. Circulating Lp(a) levels increase with menopause as with other lipids TC, LDL-C, and TG [23]. Lp(a) and LDL are two independent and important risk factors for CAD, each playing an important role in development of atherosclerosis through the effects on thrombolysis, the endothelium and the platelets [10]. In recent studies it has been documented that in male patients with CAD who had elevated LDL-C and Lp (a), Lp (a) seems to lose its atherogenic potency once LDL-C was aggressively lowered. In another study it was seen that Lp(a) was not an independent risk factor for CAD but seemed to increase the deleterious effects of mildly elevated LDL-C. Elevated LDL-C levels in addition to elevated Lp (a) are known to increase the risk of CAD exponentially [24]. This study also shows that Lp(a) levels are not significantly correlated with TC, TG, LDL-C or HDL-C levels in menopausal group though there is a fairly negative linear relationship between HDL-C and Lp (a) in premenopausal women. Hence the current evidence does not justify routine screening of Lp(a). However, the Lp (a) levels in special populations i.e. patients with premature CAD, those with strong family history of CAD, those who have undergone CABG and those with documented CAD in absence of traditional factors should be measured. In these patients aggressive lowering of elevated level of LDL-C with decrease in Lp(a) should be suggested [25]. This could be applicable to premenopausal and menopausal women with elevated levels of LDL-C and Lp(a) to prevent or minimise risk of CAD.

CONCLUSION

This study concludes that premenopausal and menopausal women constitute a subpopulation where the recent risk factors like serum Hcy and Lp (a) could be assessed along with lipid profile as screening test to identify the risk of CAD.

RECOMMENDATIONS

Women could be educated regarding modification of life style especially importance of diet and exercise for maintaining normal lipid profile. Supplementation with folate, B6 and B12 may help in maintaining serum Hcy levels. In premenopausal women with elevated Lp(a) nicotinic acid may be supplemented and HRT may be advised in menopausal women. Further research is required regarding the effect of supplementation.

LIMITATIONS

1. Larger sample size could have accentuated the findings.
2. Serum oestriol levels could be measured and correlated.

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