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Original Article

Biochemistry Section

Impact of Diet on Serum Lipids, Atherogenic Index of Plasma and Non HDL-c in Pre and Postmenopausal Women

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ABSTRACT

Introduction: Menopause is an inevitable phase of a woman's natural ageing process, marked by cessation of ovarian function. Hormonal changes during the phase causes derangement of lipid metabolism and thereby increasing cardiovascular risk in postmenopausal women. Diet plays a major role in influencing serum lipids.

Aim: To determine and compare lipid profile, Atherogenic Index of Plasma (AIP) and non High-Density Lipoprotein-cholesterol (HDL-c) in pre and postmenopausal women based on vegetarian and non vegetarian diet.

Materials and Methods: This cross-sectional study was comprised of 92 women (46 were premenopausal and 46 were postmenopausal) carried out at AJ Institute of Medical Sciences and Research Centre, Mangaluru, Karnataka, India between December 2019-May 2020. The groups were further divided into vegetarian and non vegetarians. Fasting lipid profile was determined by enzymatic methods. AIP and non HDL levels were calculated. Comparison of means between two groups was done using student t-test. Association between categorical variables was analysed using Chi-square test. Statistical significance was considered at $p<0.05$.

Results: Serum Total Cholesterol (TC), Triglycerides (TG), Low-Density Lipoprotein-cholesterol (LDL-c), Very Low-Density Lipoprotein-cholesterol (VLDL-c), AIP and non HDL-c levels were LDL-c and HDL-c was high (184.09±17.49, 131.96±9.49, 106.00±20.92, 26.46±1.96, 0.05±0.07, 132.45±22.39 and 51.64±5.88, respectively) in vegetarians compared to non vegetarians in premenopausal women. In postmenopausal women, similar pattern was observed with regards to serum TC, TG, LDL-c, VLDL-c, AIP, non HDL-c and HDL-c in vegetarians and non vegetarians ($p<0.05$). An alarming proportion of non vegetarian postmenopausal women showed "very high" TC (91.3%), "low" HDL-c (56.5%), "very high" LDL-c (69.6%) and "high-risk" AIP (91.3%).

Conclusion: The findings of this study indicated that all lipid parameters, AIP and non HDL-c were higher in non vegetarians except HDL-c in pre and postmenopausal women. Relevant dietary recommendations can be given to premenopausal women to promote positive health outcomes and alleviate cardiovascular risk.

Keywords: Atherosclerosis, Cardiovascular, Dyslipidemias, Menopause

INTRODUCTION

Menopause is a physiological state in a woman's life, marked by a decrease in endogenous estrogen level and cessation of menstruation [1]. Following menopause, significant changes in lipid metabolism are known to occur which result in a deranged lipid profile [2]. Dyslipidemia is the major cause of Cardiovascular Disease (CVD) among women, after the onset of menopause. Postmenopausal women are four to eight times likely to succumb due to CVD compared to other diseases [3].

Dyslipidemia is characterised by an increase in LDL-c, often associated with a decrease in HDL-c and an increase in serum TG in the form of chylomicrons and VLDL-c. Among these lipid parameters, an increase in LDL-c levels is considered a risk factor for CVD. Therefore, the primary therapeutic aim is to lower the LDL-c concentration in order to treat or prevent CVD [4]. Blood lipids are influenced by various factors, one of them being diet. Vegetarian diet prevents and protects individuals from various diseases including CVD, as opposed to a non vegetarian diet. Plant-based food that include fruits, vegetables, legumes, whole grains, nuts and seeds are rich in unsaturated fatty acids, dietary fibres, plant proteins, vitamins, minerals and phytonutrients. Unsaturated fatty acids are known to lower LDL-c which in turn reduces the risk of developing CVD or Coronary Heart Disease (CHD). Dietary fibres also play a major role in reducing the intestinal absorption of cholesterol and re-absorption of bile acids, thus contributing to reduction in LDL-c levels. The replacement of animal proteins with plant proteins in diet has been shown to lower LDL-c levels further [4]. Although, the impact of diet on serum lipids in pre-and postmenopausal women have been explored earlier [5,6], not much is known about the effect on atherogenic parameters such as AIP and non HDL among pre-and postmenopausal women. AIP and non HDL are known predictors of CVD risk [7,8].

This study aims to determine and compare serum lipid profile and atherogenic parameters such as AIP and non HDL, among premenopausal and postmenopausal women based on the type of diet.

MATERIALS AND METHODS

A cross-sectional study was carried out at AJ Institute of Medical Sciences and Research Centre, Mangaluru, Karnataka, India between December 2019-May 2020. Ethical clearance (AJEC/REV/54/2019) was obtained and informed written consent was taken from all participants at the beginning of the study.

Inclusion criteria: Healthy premenopausal women aged between 20-40 years with regular menstruation and healthy postmenopausal women aged between 40-65 years with cessation of menstruation for more than one-year duration.

Exclusion criteria: Pregnant or lactating women and women with a history of diabetes mellitus, obesity, ovarian pathologies, renal disease, Coronary Artery Disease (CAD), liver disease, smoking,

consuming alcohol and those on anti-hyperlipidemic drugs or hormone replacement therapy were excluded from the study.

Sample size calculation: A minimum sample size of 82 was calculated based on a study conducted by Shenoy R and Vernekar P where serum TC levels observed in premenopausal and postmenopausal women were 201.60±48.50 mg/dL and 234.77±58.13 mg/dL, respectively [9]. The confidence interval and power of the study were considered as 95% and 80%, respectively. Assuming a 10% non response rate, the final sample size was calculated as 90.

Study Procedure

Ninety-two women were recruited for the study, of which, 46 were premenopausal (aged between 20 and 40 years) and 46 were postmenopausal (aged between 45-65 years). The two groups were divided further based on their diet into vegetarians and non vegetarians, comprising of 23 participants in each sub-group. A detailed history was taken from participants in the form of a pre-fixed questionnaire. Participants who consumed plant-based products such as fruits, vegetables, legumes, whole grains, nuts and seeds; with or without consumption of milk or eggs or their processed products for more than one year were considered as vegetarians [10]; while others were considered as non vegetarians. Non vegetarians were those who consumed meat, poultry and/or fish regularly, in addition to vegetables, milk and/or eggs, for more than one year [11].

Fasting venous blood specimens were collected in plain (red-topped) vacutainers containing clot activator, under strict aseptic precautions and subjected to centrifugation to obtain sera. The sera were analysed for TC, TG and HDL-c by cholesterol oxidase-peroxidase [12], glycerol phosphate oxidase-peroxidase [13], cholesterol oxidase using accelerator selective detergent methods [14], respectively in Abbott Architect *ci*4100 automated analyser, using reagent kits from the same company. LDL-c was calculated using Friedewald formula {LDL-c (in mg/dL)=TC-HDL-c-(TG/5)}. VLDL-c was calculated as TG (in mg/dL) divided by 5 [15].

The AIP was calculated as log {TG/HDL-c}, where TG and HDL-c were expressed in molar concentration [16]. Non HDL-c was computed as the difference between TC and HDL-c [17]. The study participants were further evaluated on the basis of serum TC, HDL-c, LDL-c and AIP levels. Serum TC levels of <200, 200-239 and ≥240 mg/dL were considered as "desirable", "borderline high" and "high" respectively. Serum HDL-c levels of <40 and ≥60 mg/dL were categorised as "low" and "high" respectively. Serum LDL-c levels of <100, 100-129, 130-159, 160-189 and ≥190 mg/dL were considered as "optimal, near optimal/above optimal, borderline high, high and very high" respectively [18]. AIP levels of -0.3 to 0.1, 0.1 to 0.24 and >0.24 were categorised as "low risk, intermediate risk and high-risk" for development of CVD [19].

STATISTICAL ANALYSIS

It was done using SPSS version 20. Categorical variables were represented as frequency and percentage while continuous variables were represented as mean±SD. Comparison of means between two groups was done using student t-test. Association between categorical variables was analysed using Chi-square test. Statistical significance was considered at p<0.05.

RESULTS

The mean ages of premenopausal women and postmenopausal women were 31.05±6.52 and 56.39±5.38 years, respectively. The lipid profile, AIP and non HDL-c levels were compared between vegetarians and non vegetarians among premenopausal women and postmenopausal women as shown in [Table/Fig-1,2]. Significant increase in TC was noticed in non vegetarian pre and postmenopausal woman (p<0.05). HDL cholestrol was significantly lower in non vegetarians women with premenopausal status and non vegetarian postmenopausal women. (p<0.001). Non HDL cholesterol was significantly higher in non vegetarian women with postmenopausal status (p<0.001).

The dietary influence among participants was further assessed based on categories of TC, HDL-c, LDL-c and AIP levels as shown in [Tables/Fig-3,4]. A 21.7% of premenopausal women were at high-risk TC. Very high LDL-c was noticed in 13% premenopausal non vegetarian women. A proportion of non vegetarian postmenopausal women showed "very high" TC (91.3%), "low" HDL-c (56.5%), "very high" LDL-c (69.6%) and "high-risk" AIP (91.3%).

Parameters	Premenopausal women		Postmenopausal women	
	Vegetarian (n=23)	Non vegetarian (n=23)	Vegetarian (n=23)	Non vegetarian (n=23)
Total Cholesterol (TC) (mg/dL)	184.09±17.49	215.36±39.86*	212.96±19.48	280.55±39.69*
Triglycerides (TG) (mg/dL)	131.96±9.49	146.36±30.55*	142.55±18.14	172.32±10.52**
HDL cholesterol (mg/dL)	51.64±5.88	44.14±6.71**	45.46±5.29	38.46±4.97**
LDL cholesterol (mg/dL)	106.00±20.92	146.50±38.25**	138.91±20.72	207.64±37.79**
VLDL cholesterol (mg/dL)	26.46±1.96	29.27±6.04*	28.59±3.62	34.46±2.18**
Atherogenic index of plasma (AIP)	0.05±0.07	0.15±0.12*	0.14±0.09	0.29±0.06**
Non HDL-cholesterol (mg/dL)	132.45±22.39	171.23±39.59**	167.50±23.77	242.09±39.56**

[Table/Fig-1]: Comparison of various parameters between vegetarians and non vegetarians based on menopause status.
Data are mean±SD; HDL: High density lipoprotein; LDL: Low density lipoprotein; VLDL: Very low density lipoprotein
Comparison of biochemical parameters between vegetarian and non vegetarian groups was done using student t-test. *p<0.05; **p<0.001

Parameters	Vegetarians		Non vegetarians	
	Premenopausal (n=23)	Postmenopausal (n=23)	Premenopausal (n=23)	Postmenopausal (n=23)
Total Cholesterol (TC) (mg/dL)	184.09±17.49	212.96±19.48**	215.36±39.86	280.55±39.69**
Triglycerides (TG) (mg/dL)	131.96±9.49	142.55±18.14*	146.36±30.55	172.32±10.52*
HDL cholesterol (mg/dL)	51.64±5.88	45.46 ±5.29*	44.14±6.71	38.46±4.97*
LDL cholesterol (mg/dL)	106.00±20.92	138.91±20.72**	146.50±38.25	207.64±37.79**
VLDL cholesterol (mg/dL)	26.46±1.96	28.59±3.62*	29.27±6.04	34.46±2.18**
Atherogenic Index of Plasma (AIP)	0.05±0.07	0.14±0.09*	0.15±0.12	0.29±0.06**
Non HDL-cholesterol (mg/dL)	132.45±22.39	167.50±23.77**	171.23±39.59	242.09±39.56**

[Table/Fig-2]: Comparison of various parameters between premenopausal and postmenopausal women based on diet.
Data are mean±SD; HDL: High density lipoprotein; LDL: Low density lipoprotein; VLDL: Very low density lipoprotein
Comparison of biochemical parameters between vegetarian and non vegetarian groups was done using Student t test. *p<0.05; **p<0.001

Based on diet and TC categories				
	TC categories			
Diet	Desirable (TC <200 mg/dL)	Borderline (TC 200-239 mg/dL)	High-risk (TC >240 mg/dL)	Chi-square
Vegetarian	18 (78.3%)	5 (21.7%)	0 (0.0%)	χ^2 (2)=7.978, p=0.019
Non vegetarian	10 (43.5%)	8 (34.8%)	5 (21.7%)	
Based on diet and HDL-c categories				
	HDL-c categories			
Diet	Low (HDL-c <40 mg/dL)	Normal (HDL-c 40-59 mg/dL)	High (HDL-c ≥60 mg/dL)	Chi-square
Vegetarian	0 (0.0%)	21 (91.3%)	2 (8.7%)	χ^2 (2)=7.231, p=0.027
Non vegetarian	5 (21.7%)	18 (78.3%)	0 (0.0%)	
Based on diet and LDL-c categories				
	LDL-c categories			
Diet	Optimal (LDL-c <100 mg/dL)	Near optimal (LDL-c 100-129 mg/dL)	Borderline high (LDL-c 130-159 mg/dL)	High (LDL-c 160-189 mg/dL)	Very high (LDL-c ≥190 mg/dL)	Chi-square
Vegetarian	10 (43.5%)	9 (39.1%)	4 (17.4%)	0 (0.0%)	0 (0.0%)	χ^2 (4)=16.923, p=0.002
Non vegetarian	0 (0.0%)	9 (39.1%)	9 (39.1%)	2 (8.8%)	3 (13.0%)	
Based on diet and AIP categories				
	AIP categories			
Diet	Low risk (AIP-0.3-0.1)	Intermediate risk (AIP 0.1-0.24)	Increased risk (AIP >0.24)	Chi-square
Vegetarian	17 (73.9%)	6 (26.1%)	0 (0.0%)	χ^2 (2)=14.547, p=0.001
Non vegetarian	6 (26.1%)	8 (34.8%)	9 (39.1%)	

[Table/Fig-3]: Distribution of subjects based on diet and categories of TC, LDL-c, HDL-c and AIP in premenopausal women.

Based on diet and TC categories				
	TC categories			
Diet	Desirable (TC <200 mg/dL)	Borderline (TC 200-239 mg/dL)	High-risk (TC >240 mg/dL)	Chi-square
Vegetarian	9 (39.1%)	10 (43.5%)	4 (17.4%)	χ^2 (2)=25.893, p<0.001
Non vegetarian	0 (0.0%)	2 (8.7%)	21 (91.3%)	
Based on diet and HDL-c categories				
	HDL-c categories			
Diet	Low (HDL-c <40 mg/dL)	Normal (HDL-c 40-59 mg/dL)	High (HDL-c ≥60 mg/dL)	Chi-square
Vegetarian	0 (0.0%)	23 (100.0%)	0 (0.0%)	χ^2 (1)=18.121, p<0.001
Non vegetarian	13 (56.5%)	10 (43.5%)	0 (0.0%)	
Based on diet and LDL-c categories				
	LDL-c categories			
Diet	Optimal (LDL-c <100 mg/dL)	Near optimal (LDL-c 100-129 mg/dL)	Borderline high (LDL-c 130-159 mg/dL)	High (LDL-c 160-189 mg/dL)	Very high (LDL-c ≥190 mg/dL)	Chi-square
Vegetarian	0 (0.0%)	10 (43.5%)	8 (34.8%)	5 (21.7%)	0 (0.0%)	χ^2 (3)=29.600, p<0.001
Non vegetarian	0 (0.0%)	0 (0.0%)	2 (8.7%)	5 (21.7%)	16 (69.6%)	
Based on diet and AIP categories				
	AIP categories			
Diet	Low risk (AIP-0.3-0.1)	Intermediate risk (AIP 0.1-0.24)	Increased risk (AIP >0.24)	Chi-square
Vegetarian	8 (34.8%)	10 (43.5%)	5 (21.7%)	χ^2 (2)=23.179, p<0.001
Non vegetarian	0 (0.0%)	2 (8.7%)	21 (91.3%)	

[Table/Fig-4]: Distribution of subjects based on diet and categories of TC, LDL-c, HDL-c and AIP in postmenopausal women.

DISCUSSION

Postmenopausal women have higher TC, LDL-cholesterol and TG levels, accompanied with lower HDL-cholesterol levels [20]. Decline in ovarian hormone production also leads to variations in size and density of lipoproteins [21]. In addition to hormonal influence, factors such as central obesity, increased blood pressure and insulin resistance can put postmenopausal women, especially those who had an early menopause onset, at risk of developing CVD [19]. Dietary influence on lipid profile in pre-and postmenopausal women has not been studied extensively. Based on the limited available literature, varying results have been obtained while considering the impact of both, diet and menopause, on lipid parameters. This may be due to differences in geographical distribution, socioeconomic status, cultural practices and lifestyle.

In the current study, serum TC, TG, LDL-c and VLDL-c levels were low and HDL-c was high in vegetarians compared to non vegetarians, irrespective of the menopause status (p<0.05). Huang Y et al., reported significantly high TG and low HDL-C in vegetarian premenopausal women while TC was significantly high in vegetarian postmenopausal women, compared to omnivores in either group [5]. Chaudhary A et al., studied the impact of diet on lipid profile and autonomic functions in postmenopausal women and reported significant increase in serum TC, TG, LDL-c and TC/HDL-c in non vegetarians compared to vegetarians. There was no significant difference in HDL-c between non vegetarians and vegetarians [22].

Another study carried out on tribal women in North India, showed no significant difference in lipid profile parameters between vegetarians and non vegetarians, among pre-and postmenopausal women [6].

This study observed that a significant proportion of non vegetarian postmenopausal women showed "very high" TC (91.3%), "low" HDL-c (56.5%) and "very high" LDL-c (69.6%). These findings indicate the importance of screening postmenopausal women by taking a good diet history and assessing the lipid profile. Atherogenic parameters such as AIP and non HDL-c were significantly higher among non vegetarians compared to vegetarians, in both pre-and postmenopausal women. As per recent literature search, there are no studies available which compares AIP and non HDL-c in vegetarian and non vegetarian pre-and postmenopausal women. Further evaluation of AIP revealed, among premenopausal women, the percentage of non vegetarians with high-risk of CVD was 39.1% while none of the vegetarians were at high-risk of developing CVD. Although, 21.7% and 43.5% of the vegetarian postmenopausal women showed high and intermediate risks respectively, a significant 91.3% of the non vegetarian postmenopausal women were at high-risk of developing CVD with a mean AIP level of 0.29±0.06, while remaining 8.7% showed intermediate risk.

AIP is considered a good predictor of atherosclerosis and CAD compared to conventional lipid profile parameters [23]. AIP has been proven to be an independent predictive indicator of CAD, particularly in postmenopausal women [19]. According to a cross-sectional study carried out by Barua L et al., on 265 postmenopausal Bangladeshi women, AIP showed significant association with CVD risk factors such as duration of menopause, waist-hip ratio, postprandial glucose, TC and LDL-c. A 35.5% of the study participants, not considering diet, exhibited high-risk AIP level with mean AIP of 0.16±0.25 [7]. Non HDL-c is yet another promising marker of CVD risk. The calculated parameter encompasses the levels of chylomicrons, VLDL-c and their remnants, Intermediate Density Lipoprotein (IDL), LDL and lipoprotein (a) [8]. In a recent study, Iannuzzi A et al., reported that the highest tertile of non HDL-c among 220 postmenopausal women with subclinical atherosclerosis was significantly associated with the presence of coronary plaques (OR=2.38, 95% CI, p=0.038) while the highest tertile of HDL-c provided protection against development of carotid plaques (OR=0.36, 95% CI, p=0.017), indicating its relevance in detecting atherosclerosis in postmenopausal women [24].

The World Health Organisation (WHO) has estimated that 80% of CAD can be prevented among individuals with dietary and lifestyle modifications. Relevant dietary recommendations can be given to perimenopausal women to promote positive health outcomes [25]. The American Heart Association (AHA) has also laid down recommendations to improve cardiovascular health which include intake of ≥4.5 cups of fruits/day, ≥200 g of shellfish/week, ≤1500 mg of sodium/day, ≤36 fl oz of sweetened beverages, 3 or more 1-oz-equivalent servings of whole grains/day and ≥4 servings of nuts, seeds and legumes/week [26]. Higher consumption of fruits and vegetables are associated with lower CVD related mortality, as reported in a meta-analysis of cohort studies, which further supports the dietary recommendations [27].

The mechanism by which diet influences cardiovascular health mainly depends on the food group consumed. Consumption of whole grains and legumes improve serum TC due to the high content of soluble fibers. Nutrients like vitamin C, folic acid, potassium, magnesium, flavonoids and carotenoids present in fruits, green leafy vegetables and legumes are known to have beneficial effect on endothelial function and may cause vasodilation, thereby lowering blood pressure. Increased consumption of unsaturated fatty acids and decreased intake of saturated fats can reduce the risk of developing CVD [28]. [Table/Fig-5] is showing the comparison of present study with similar studies [5,6 22].

S. No.	Author's name and year	Place of study	Sample size	Parameters compared	Conclusion
1.	Huang YW et al., 2014 [5]	China	2397 premenopausal women and 1154 postmenopausal women, who were divided into vegans, ovo-lacto-vegetarians and omnivores.	Lipid profile parameters, apo-A1, apo-B, TC/HDL-c, TG/HDL-c and LDL-c/HDL-c ratios.	Vegans and ovo-lacto-vegetarian diets showed significantly low HDL-c levels in both pre-and postmenopausal women. The authors concluded that ovo-lacto-vegetarian diet would be beneficial for premenopausal women.
2.	Chaudhari A et al., 2013 [22]	India	120 postmenopausal women, divided into 60 vegetarians and 60 non vegetarians	Lipid profile parameters, TC/HDL-c ratio	Serum TC, TG, LDL-c and TC/HDL-c ratio were significantly high among non vegetarians compared to vegetarians. They concluded that diet influences serum lipids.
3.	Kumari P et al., 2019 [6]	India	98 premenopausal women, divided into 40 vegetarians and 58 non vegetarians and 63 perimenopausal/ postmenopausal women, divided into 14 vegetarians and 49 non vegetarians	Lipid profile parameters and TC/HDL-c ratio	There was no significant difference in serum TC, TG, VLDL, LDL, HDL and TC/HDL-c ratio between vegetarians and non vegetarians in premenopausal and perimenopausal/postmenopausal groups. As per the study, diet had no influence on serum lipids.
4.	Present study	Karnataka, India	46 premenopausal and 46 postmenopausal women	Serum lipid profile, Atherogenic Index of Plasma (AIP) and non HDL-c	Serum TC, TG, LDL-c and VLDL-c levels were low and HDL-c was high in vegetarians compared to non vegetarians, irrespective of the menopause status. AIP and non HDL-c levels were high in non vegetarians compared to vegetarians, in pre- and postmenopausal women. The authors concluded that vegetarian diet is beneficial to both pre- and postmenopausal women.

[Table/Fig-5]: Comparison of findings from similar studies and present study [5,6,22].

Limitation(s)

The main limitation of the study was the small sample size. Furthermore, vegetarian diet could have been further classified into pure vegan, ovo-vegetarian and lacto-vegetarian. Such classifications were not applied in the current study as further increase in sample size would have been warranted.

CONCLUSION(S)

Diet does play a role in influencing serum lipids. Dyslipidemia with elevated AIP and non HDL-c levels were observed among non vegetarian women irrespective of menopausal status. The proportion of non vegetarian postmenopausal with high-risk AIP, in the current study, is quite alarming and warrants necessary dietary interventions. Early screening of postmenopausal for lipid parameters and indices along with dietary assessment can be a boon in alleviating CVD risk.

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