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Original Article

Clinical Biochemistry Section

Lipid Profile Patterns in a Rural Area of Ramanagara District

MR KUSUMA[1], Y SAHANA[2], HD SHILPA[3]

ABSTRACT

Introduction: Dyslipidemia is one of the risk factors contributing to atherosclerosis leading to Cardiovascular Disease (CVD), which in-turn is a major contributor to the mortality and morbidity globally.

Aim: To examine the burden of lipid profile pattern in a rural area.

Materials and Methods: The present study was a cross-sectional study done in the rural area of Harohalli, Ramanagara district, Karnataka, India. A total of 560 patients who came for routine blood check-up were enrolled for the study. The study population was further divided into subgroups as <20 years, 21-40 years, 41-60 years, >60 years. Percentage analysis and association of lipid profile parameters and age as well as gender was established using appropriate statistical methods like percentage analysis, Chi-square test, Venn-diagram and trend chart.

Results: Less than half of the population were found to have high serum levels of Triglyceride (TG), Low Density Lipoprotein (LDL) and TG/HDL ratio. High-Density Lipoprotein cholesterol (HDL-c) was found to be significantly associated with gender (p value <0.01). HDL-c was found to increase progressively after 50 years of age in females compared to males of same age. Also, an increasing trend of TG/HDL-c ratio was noted in both genders with a marked elevation being noted in post-menopausal females.

Conclusion: Abnormal lipid profile was noted even in young population of the study group; thus, an early screening and follow-up in both males and females is required keeping in view their future progression to CVDs.

Keywords: Cardiovascular disease, Dysfunctional lipoproteins, Dyslipidemia, Post-menopausal

INTRODUCTION

Dyslipidemia is one of the major risk factors which contribute to CVD apart from other risk factors such as hypertension, diabetes mellitus, obesity, cigarette smoking and sedentary lifestyle. In turn CVD is the leading contributor to mortality and morbidity globally [1]. The World Health Organisation (WHO) estimated dyslipidemia to be associated with more than 50% of Ischemic Heart Disease (IHD) cases in the world also accounts for more than four million deaths every year [2]. In India an alarming increase in prevalence of CVD is noted over past few decades. CVD is said to cause a considerable contribution to the mortality among adults belonging to the age group of 25-69 years [3]. Dyslipidemia also leads as atherosclerosis [4,5]. Many population-based studies have demonstrated an inverse association between HDL level and the risk of Coronary Artery Disease (CAD) [6,7]. Dyslipidemia is defined by the presence of one or more abnormalities in the levels of various lipids in the circulation. Lipid profile refers to and depicts the pattern of lipids in the blood. A lipid profile usually includes the estimation of Total Cholesterol (TC), TG, LDL and HDL-c fractions in the serum.

It is evident through various studies that hypercholesterolemia is one of the major risk factors for CVD and stroke [8]. It accounts for about one third of the IHD burden worldwide [9,10]. The role of LDL cholesterol in atherosclerosis is very well established. Hypercholesterolemia persisting for a longer period favors deposits in the subintimal region of arteries following endothelial dysfunction. Catabolism of LDL-c occurs through apo-B LDL receptor and a fraction of LDL-c particles is degraded by macrophages (non-specific uptake). Foam cells are formed when these macrophages are overloaded with cholesterol. This is the hallmark of atherosclerotic plaques. It is a well-known fact that atherosclerosis is the primary cause of CVDs [11]. Other than levels of lipids per se, lipoprotein indexes too have found a role in risk estimation such as TG/HDL ratio. High TG/HDL ratio is found to be associated with cardiovascular risk in patients with familial hypercholesterolemia [12]. Several population-based studies conducted over the years have demonstrated an atypical pattern of dyslipidemia in Indians. These studies revealed a lower HDL-c, borderline high LDL-C and increased TG levels (atherogenic dyslipidemia) in the Indian population [13].

The rural area (Harohalli) of Ramanagara in which the current study is based, has no prior studies examining the pattern of lipid profile in the resident population. Hence, this study was undertaken to examine the burden of lipid profile pattern.

MATERIALS AND METHODS

This was a cross-sectional study conducted in the Department of Biochemistry, CDSIMER, Harohalli, a rural area of Ramanagara district, Karnataka, India. After obtaining approval from the Institutional Ethical Committee, 560 patients who came for routine check-up were included in the current study. Study was conducted for the period of six months i.e. from November 17th, 2019 to May 30th, 2020. Written informed consent was obtained from all the enrolled study subjects. Relevant information was collected and fasting (8-10 hours) blood samples were collected for lipid profile estimation. Samples obtained were processed within two hours of collection.

Inclusion criteria: Apparently healthy subjects aged between 18-85 years, who came for routine blood check-up were enrolled for the study.

Exclusion criteria: Smokers and alcoholics, study subjects who were on lipid lowering drugs, known cases of CVD, hypertension, diabetes were excluded from the study.

The study population was divided into different age groups {i.e., <20 (n=2), 21-40 (n=99), 41-60 (n=341), >60 (n=118)} and gender {i.e., males (n=310), females (n=250)}.

- Serum lipid profile was analysed in a semi-auto analyser.
- TC was estimated using CHOD-PAP end point method

Principle: This method is based on Trinders methodology [14].

Cholesterol ester in the presence of cholesterol esterase is cleaved to form cholesterol and fatty acid. The obtained cholesterol is converted to cholest-4-en-3-one and H_2O_2 (hydrogen peroxide). H_2O_2 then reacts with 4-aminoantipyrine and phenol in the presence of peroxidase to form quinonimine dye, a chromogen which is read at 505 nm against a blank.

- TG was estimated using GPO Trinder end point method

Principle: TG's in the presence of lipoprotein lipase is cleaved to form glycerol and free fatty acids. Glycerol is then phosphorylated to form glycerol-3-phosphate in the presence of glycerol kinase and a co-factor Mg^{2+}. Glycerol-3-phosphate is then oxidised by glycerol phosphate oxidase to form H_2O_2. H_2O_2 condenses with 4-aminoantipyrine in the presence of peroxidase to yield quinonimine dye, a chromogen whose intensity is directly proportional to the concentration of TG in the given serum sample at 546 nm.

- HDLc was estimated using phosphotungstic acid, end point method

Principle: Chylomicrons, LDL and VLDL and other non-HDL cholesterol are precipitated from serum by phosphotungstic acid in the presence of Mg^{2+}. The solution is then centrifuged to obtain the supernatant which includes HDL-c which is estimated using CHOD-PAP end point method.

- LDLc was calculated used Friedwald equation:
- TG/HDL ratio was calculated:

(LDL for those study subjects with TG value >400 mg/dL were not calculated and were excluded from statistical analysis).

$$LDL\ cholesterol = Total\ cholesterol - HDL\ cholesterol - \frac{Triglyceride}{5}$$

Values were considered as dyslipidemic as per National Cholesterol Education Program (NCEP) and Adult Treatment Panel III (ATP III) guidelines [15]. As per the guidelines, dyslipidemia is defined by the presence of one or more abnormal serum lipid concentration:

- TC >200 mg/dL (hypercholesterolemia)
- TG >150 mg/dL (hypertriglyceridemia)
- HDLc <40 mg/dL
- LDLc >100 mg/dL

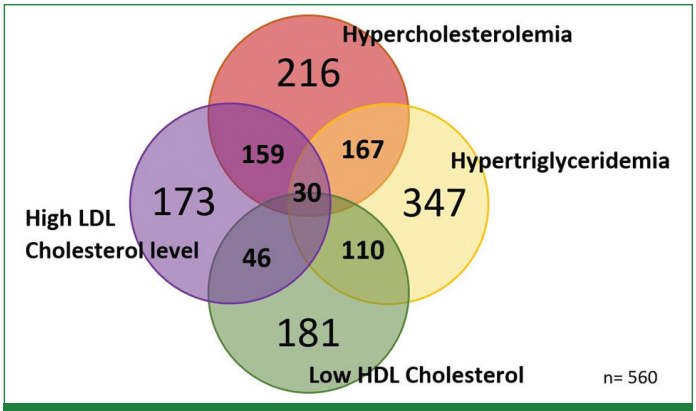
STATISTICAL ANALYSIS

Percentage distribution of the lipid profile parameters was calculated in the different age groups and in the gender subgroups. Percentage distribution of TG/HDL ratio in different age groups as well as gender was calculated. To study the association between the lipid profile and age and gender by statistical analysis, normality of the data was first calculated using Shapiro-wilk test. A Chi-square test was done using Statistical Package for the Social Sciences (SPSS) version 19, to obtain the association of lipid profile parameters with age as well as gender. A p-value <0.01 was considered to be significant.

RESULTS

The total study population comprised of 560 patients which included 310 males and 250 females. The study population was further divided into four groups based on age i.e., <20, 21-40, 41-60, >60. Overlapping of the individual parameters of lipid profile is shown in the form of Venn diagram [Table/Fig-1].

Gender specific and age group specific percentage distribution of normal and abnormal lipid profile patterns (as defined by NCEP, ATP III) was calculated and is shown in [Table/Fig-2,3].



[Table/Fig-1]: Venn diagram showing the overlap of lipid profile among the study population.

	Male (n=310)	Female (n=250)
TC (mg/dL)		
<200 (Normal)	64.8	59.2
200-239 (Borderline)	15.2	22.4
≥240 (High)	20	18.4
TG (mg/dL)		
<150	35.8	41.6
>150	64.2	58.4
HDL (mg/dL)		
<40 (Low)	34.6	24
40-60	60.7	72
≥60 (High)	4.7	4
LDL (mg/dL)		
<100 (Normal)	72	66.6
130-159 (Borderline high)	13.3	16.8
160-189 (High)	5.8	8.0
≥190 (Very high)	9.2	8.6

[Table/Fig-2]: Gender specific percentage distribution of different levels of lipid profile.

	Age <20 (n=2)	Age 21-40 (n=99)	Age 41-60 (n=341)	Age >60 (n=118)
TC (mg/dL)				
<200 (Normal)	100%	67.7%	59.7%%	64.7%
200-239 (Borderline)	0%	13.1%	20.3%	17.7%
≥240 (High)	0%	19.2%	20%	17.6%
TG (mg/dL)				
<150	50%	41.4%	38.5%	37%
>150	50%	58.6%	61.5%	63%
HDL (mg/dL)				
<40 (Low)	50%	21.7%	31.5%	32%
40-60	50%	75.3%	62.6%	65.5%
≥60 (High)	0%	3%	5.9%	2.5%
LDL (mg/dL)				
<100 (Normal)	50%	75%	68.6%	69%
130-159 (Borderline high)	50%	12%	17.1%	12%
160-189 (High)	0%	2.0%	6.6%	10%
≥190 (Very high)	0%	11.0%	7.7%	9%

[Table/Fig-3]: Percentage distribution of different levels of lipid profile in different age group.

Age specific percentage distribution of TG/HDL ratio is shown in [Table/Fig-4]. A remarkably higher percentage of subjects in all the age groups were shown to have a TG/HDL ratio >3.

To evaluate the significance of the difference noted and strength of association in lipid profile parameters between the gender and the various age groups, Chi-square test was done as shown in

Age group (years)	Percentage of TG/HDL ratio <3	Percentage of TG/HDL ratio >3
<20	0%	100%
21-40	9%	90.9%
41-60	10.3%	89.7%
>60	6.7%	93.3%

[Table/Fig-4]: Percentage distribution of TG/HDL ratio in different age groups.

[Table/Fig-5,6]. Analysis showed a significant association between HDL-c and gender (p-value 0.01), where in higher HDL-c values were noted in the male population [Table/Fig-5]. However, the differences in the lipid parameter values among the different age groups were found to be insignificant [Table/Fig-6].

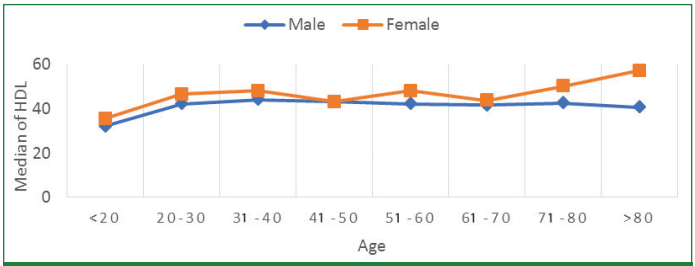
Parameters	Male	Female	Total	Chi square value (d.f.= 1)	p-value
TC <200	202	148	350	1.794	0.18NS
TC >200	108	101	210		
Total	310	250	560		
TG <150	111	104	216	1.932	0.16 NS
TG >150	199	146	344		
Total	310	250	560		
HDL <40	114	69	183	5.876	0.01*
HDL>40	196	181	377		
Total	310	250	560		
LDL<130	222	165	387	1.696	0.19 NS
LDL>130	88	85	173		
Total	310	250	560		

[Table/Fig-5]: Chi-square test showing association of lipid profile parameters with gender.
NS: Not significant; *Significant; p-value <0.01 is significant

Parameters (mg/dL)	<30 years	31-60 years	>60 years	Total	Chi square value (d.f=1)	p-value
TC <200	24	247	78	349	2.26	0.32NS
TC >200	10	161	40	211		
Total	34	408	118	560		
TG <150	15	156	45	216	0.4703	0.790NS
TG >150	19	252	73	344		
Total	34	408	118	560		
HDL <40	13	128	42	183	1.249	0.54NS
HDL >40	21	280	76	377		
Total	34	408	118	560		
LDL<130	23	277	85	385	0.7513	0.68NS
LDL>130	11	131	33	175		
Total	34	408	118	560		

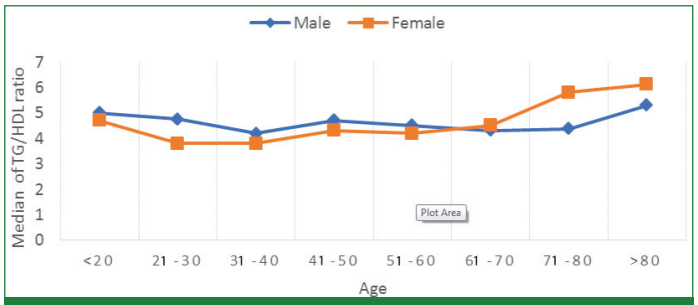
[Table/Fig-6]: Chi-square test showing association of lipid profile parameters with age.
NS: Not significant

HDL-c value was noted to be slightly higher in females in all the age groups. Also, HDL-c value show a progressive increase after the age of 50 in case of females compared to age matched males [Table/Fig-7].



[Table/Fig-7]: Graph showing trend in HDL value in different age groups and gender.

Trend analysis of the prevalence of higher TG/HDL ratio among different age groups and gender initially showed lower levels in females up to the age group of 60. However, a trend reversal was noted in the age groups >60 year with females having higher values. In addition to having higher values, a progressive increase in the TG/HDL ratio was also noted in females in the age group >60 years [Table/Fig-8].



[Table/Fig-8]: Graph showing trend in TG/HDL ratio in different age groups and gender.

DISCUSSION

In the current study dyslipidemia pattern was noted in less than half of the study population in Harohalli, a rural area of Ramanagara, Karnataka, India. Percentage analysis of different parameters of lipid profile with different age groups, it was noted that most of the study population had normal lipid profile levels with the exception of TG levels. It is interesting to note that more than half of the population has normal HDL-c levels despite hypertriglyceridemia. Increased HDL-c fractions were seen in post-menopausal women in this study population as against the norm; which may be attributed probably due to their dietary and lifestyle practices. The diet of this rural population, predominantly consisting of farming community is said to be more balanced including larger proportions of leafy vegetables, pulses, dietary fibres and folate rich food [16]. However, further studies evaluating the dietary patterns of the local population and its association with lipid profile patterns are required to establish the nature of their influence on the lipid profile.

However, despite the high HDL-c levels in post-menopausal females in this study, which can imply a better protection against the risk of CVD, the TG/HDL-c ratio was higher in them which in turn portends a higher risk of developing atherosclerosis and its sequelae. TG/HDL-c ratio is considered to be one of the powerful and better predictors of development of CAD [17] as well as CHD and CVD [18]. In a study done by Gaziano JM et al., TG/HDL ratio predicted a 16-fold increase in myocardial infarction in patients with no previous history of CAD [19]. It has been established that hypertriglyceridemia and low HDL-c are key metabolic abnormalities associated with Insulin Resistance (IR) status [20]. Hence, TG/HDL ratio depicts Insulin Resistance (IR) which paves the way for progression to Diabetes Mellitus and CVD [21]. IR causes increased circulatory free fatty acid level as adipose tissue retains less fatty acid. Elevated free fatty acid levels in circulation causes increased uptake by the liver, to synthesise more TGs and TG containing Very Low-Density Lipoprotein (VLDL). Resulting increased blood TG concentration causes exchange of TG of VLDL and Cholesteryl ester of HDL. TG rich HDL which is formed as a result of this exchange is easily catabolised. Also, decreased oestrogen levels in post-menopausal females, contributes to abnormal lipid profile pattern including hypertriglyceridemia [21,22]. This mechanism can explain the high TG levels, high TG/HDL ratio observed in the above-mentioned, analysis, implying risk of CVD [23]. Although TG levels progressively elevate with age in both genders, a few studies have stated that women are at high risk of coronary events [24].

This study has shown some surprising results in terms of outcome, in-spite of increased of HDL-c it is noted that TG/HDL ratio is more in post-menopausal females this apparent paradox can be explained probably by increased catabolism of TG rich HDL and also presence

of circulating dysfunctional HDL-c levels. The current study also highlights the fact that although there is no significant change between the lipid profile parameters with age upto 60 years, a considerably large proportion of the population in these age groups demonstrates a higher TG/HDL-c ratio which can be explained by the above-mentioned mechanism of the presence of dysfunctional lipoproteins. More studies on HDL-c associated enzymes, dysfunctional HDL-c and detailed analysis of the modifications in the lipoprotein subfractions would add some knowledge regarding the same [25]. The alarming aspect noted is the prevalence even in younger age group as against the general notion of presence of dyslipidemia in the older population. This point towards the need for considering the possible factors that has caused the rise of new trend.

Prospective studies on a large scale in this population to monitor the outcomes with respect to CVD and CAD are required to strengthen the association of the above parameters with increased mortality and morbidity outcomes.

Limitation(s)

Limitation of the present study was limited sample size, cross-sectional study and anthropometric measurements were not considered.

CONCLUSION(S)

It is disturbing to observe dyslipidemia even among young adults in various studies, including present study. As it is a known fact that effective treatment of dyslipidemia can reduce mortality and morbidity rate, screening from a younger age may aid in combating the disease burden or to slow down the process of atherosclerosis leading to CVD. This study also reinforces the prior evidence provided, that post-menopausal women are vulnerable to cardiac events. The current study is a step towards the treating clinicians with regards to the trends in this demographic area.

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PARTICULARS OF CONTRIBUTORS:
1. Tutor, Department of Biochemistry, Dr. Chandramma Dayananda Sagar Institute of Medical Education and Research, Ramanagara, Karnataka, India.
2. Assistant Professor, Department of Biochemistry, Dr. Chandramma Dayananda Sagar Institute of Medical Education and Research, Ramanagara, Karnataka, India.
3. Professor, Department of Biochemistry, Dr. Chandramma Dayananda Sagar Institute of Medical Education and Research, Ramanagara, Karnataka, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:
Dr. Y Sahana,
Assistant Professor, Department of Biochemistry, Dr. Chandramma Dayananda Sagar Institue of Medical Education and Research, Harohalli, Kanakapura Taluk, Ramanagara District-562121, Karnataka, India.
E-mail: sanu.brains@gmail.com

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