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Original Research Paper

Cardiology

A CROSS-SECTIONAL STUDY OF THE ASSOCIATION BETWEEN HOMOCYSTEINE AND LIPID PROFILES, 5-YEAR STUDY IN AN INDIAN REFERENCE LABORATORY

Dr Moumita Misra Pathologist, Metropolis Healthcare Limited, Mumbai, Maharashtra

Dr Shailesh Desai* Pathologist, Metropolis Healthcare Limited, Mumbai, Maharashtra, *Corresponding Author

Raj Jatale Biostatistician, Metropolis Healthcare Limited, Mumbai, Maharashtra

Dr Kirti Chadha Chief Scientific Officer, Metropolis Healthcare Limited, Mumbai, Maharashtra

ABSTRACT

AIMS AND OBJECTIVES: The study investigated the correlation between homocysteine and lipid profiles in Indian urban based population in reference laboratory. **METHOD:** The participants were composed of 540339 Indian people aged 18 - 92 years old, who were recruited from urban population of Mumbai in India over 5years retrospectively. Non-parametric test and logistic regression were used to examine the distribution of homocysteine and lipid profiles (triglyceride [TG], total cholesterol [TC], low-density lipoprotein cholesterol [LDLC], high-density lipoprotein cholesterol [HDL-C]) and the relationship between them. **RESULTS:** The median age of subjects at risk was 40 years old or more, and 55.67% were male with female being 44.33%.In multivariate logistic regression analyses, high homocysteine was associated with increasing risk of low HDL-C (HDL-C < 40mg/dl; adjusted odds ratio [OR] =1.1293, 95% confidence interval [CI]: 1.0923- 1.1675, P < 0.0001), hypertriglyceridemia (TG ≥ 500mg/dl; adjusted OR = 1.33, 95% CI: 1.0607–1.6676, P < 0.0001), high CHOL/HDL (CHOL/HDL ≥ 5; adjusted OR = 1.2235, 95% CI: 1.1752–1.2737, P < 0.0001), high LDL/HDL (LDL/HDL ≥ 3.5; adjusted OR = 1.2378, 95% CI: 1.1737–1.3055, P < 0.0001), high LDL-C (LDL-C >150mg/dl; adjusted odds ratio [OR] =1.8763, 95% confidence interval [CI]: 1.744 – 2.0321, P < 0.0001) and high non HDL-C (non HDL-C >160mg/dl; adjusted odds ratio [OR] =1.09, 95% confidence interval [CI]: 1.0113 – 1.1714, P < 0.0001) after adjusting the confounders. However, there were no significant associations between homocysteine and ApoA1, Lipoprotein (a). The regression analysis results would provide a basis for the treatment and prevention of abnormal lipid metabolism among Indian population. **CONCLUSION:** The present study showed that hyperhomocysteine was independently associated with high LDL-Cholesterol, hypertriglyceridemia and low levels of HDL-C. Our results also suggest that the homocysteine levels might involved in HDL-C and TG metabolism.

KEYWORDS : High-density lipoprotein (HDL-C), Homocysteine (Hcy), Low-density lipoprotein cholesterol (LDL-C), Total cholesterol (TC), Triglyceride (TG).

INTRODUCTION:

Hyperhomocysteinemia (HHcy) is an independent risk factor for cardiovascular disease (CVD) through various mechanisms which includes endothelium damage, stimulation of smooth muscle cell proliferation, enhanced peroxidation of low-density lipoprotein cholesterol (LDL-C) and activation of thrombosis as per Antoniades C et al [26]. As per Geoff H. Werstuck et al [5]; homocysteine-induced endoplasmic reticulum (ER) stress activates both the unfolded protein response and the sterol regulatory element–binding proteins (SREBPs) in cultured human hepatocytes as well as vascular endothelial and aortic smooth muscle cells. Activation of the SREBPs is associated with increased expression of genes responsible for cholesterol/triglyceride biosynthesis and uptake and with intracellular accumulation of cholesterol. This explained the development and progression of atherosclerotic lesions observed in hyperhomocysteinemia.

This study aims to investigate the association of plasma homocysteine level with lipid profiles in Indian community based population without lipid-lowering treatment.

STUDY DESIGN:

This retrospective study was conducted on 540339 lipid profile test results obtained over a period of 5 years, from 2017-2021 in a referral laboratory based in the metropolitan city of Mumbai, India.

The population studied belonged to the city of Mumbai (Urban population).

Investigations:

1. Homocysteine
2. Apolipoprotein profile including Apolipoprotein A1,Apolipoprotein B, Apolipoprotein B/A1 ratio
3. Lipoprotein(a)
4. Lipid profile including Triglycerides level, HDL Cholesterol, Non HDL Cholesterol, VLDL Cholesterol, LDL Cholesterol, Cholesterol-Total, LDL/HDL ratio, CHOL/HDL ratio.

We studied the incidence and prevalence of lipid profiles and homocysteine in different age groups and gender.

We also studied the correlation between lipid profiles with plasma homocysteine for the diagnostic sensitivity of each in view of cardiovascular risk.

METHODS:

After an overnight fast of at least 12 h, a venous blood sample was obtained from the forearm of each individual. Plasma samples were separated within 30 min of collection and were stored in refrigerator.

Analysis of plasma homocysteine:

For this study, all reports results were obtained on usage of Roche analyser.

Principle of Roche analyzer for plasma homocysteine evaluation:

Homocysteine (Hcy) enzymatic assay is based on a novel enzyme cycling assay principle that assesses the co-substrate conversion product instead of assessing co-substrate or Hcy conversion products of Hcy. In this assay, oxidized Hcy is first reduced to free Hcy which then reacts with a co-substrate, S-adenosylmethionine (SAM), to form methionine (Met) and S-adenosylhomocysteine (SAH), catalysed by Hcy S-methyltransferase.

SAH is assessed by coupled enzyme reactions where SAH is

hydrolysed into adenosine (Ado) and Hcy by SAH hydrolase, and Hcy is cycled into the Hcy conversion reaction to form a reaction cycle that amplifies the detection signal. The formed Ado is immediately hydrolyzed into inosine and ammonia. In the last step, the enzyme glutamate dehydrogenase (GLDH) catalyzes the reaction of ammonia with 2-oxoglutarate and NADH to form NAD+. The concentration of Hcy in the sample is directly proportional to the amount of NADH converted to NAD+ (A340 nm).

Reference Range of plasma homocysteine: 5.46 to 16.2micromol/L

Analysis of lipid profile:
Analysis of reports of lipid profile which includes triglycerides, total cholesterol, HDL cholesterol was done using enzymatic colorimetric test method on Roche analyzer.VLDL cholesterol, CHOL/HDL ratio, LDL/HDL ratio, LDL Cholesterol, serum Non HDL Cholesterol are calculated parameters.

Reference ranges for lipid profile were defined as per **The National Cholesterol Education Programmer (NCEP) Adult Treatment Panel III Reporting guidelines:**

Parameters	Interpretation (in mg/dl)
LDL Cholesterol	Optimal: < 100 Near Optimal / Above Optimal: 100-129 Borderline High: 130-159 High: 160-189 Very High: >= 189
Triglycerides level	Normal: < 150 Borderline High: 150-199 High: 200-499 Very High: >= 500
Cholesterol-Total	Desirable - Upto 200 Borderline high - 200-240 High - Above 240
HDL Cholesterol	Major risk factor for heart disease: < 40 Negative risk factor for heart disease: >= 60
Non HDL Cholesterol	Optimal: < 130 Desirable: 130-159 Borderline high: 159-189 High: 189-220 Very High: >= 220
LDL Cholesterol	Optimal: < 100 Near Optimal: 100-129 Borderline high: 130-159 High: 160-189 Very High: >= 190
VLDL Cholesterol	6-38
LDL/HDL RATIO	2.5-3.5
CHOL/HDL RATIO	3.5-5

Analysis of Serum Apolipoprotein profile:
Serum Apolipoprotein profile includes Apolipoprotein A1, Apolipoprotein B and Apolipoprotein B/A1 Ratio was done using Immunoturbidimetry method on Roche module analyzer.

Reference Range of Serum Apolipoprotein profile:
Apolipoproteins A1: 104-202 mg/dl
Apolipoproteins B: 66-133 mg/dl
Apolipoproteins B/A1 Ratio: 0.35-1.0 mg/dl

Analysis of Serum Lipoprotein (a):
Serum Lipoprotein test was done using particle enhanced immunoturbidimetry method on Roche module analyzer.

Reference Range of Lipoprotein (a):
0 - 30mg/dl

Statistical Analysis Methods:
Data was analysed using "R Software Version 3.5.2". Result of Quantitative variable are expressed as Mean + SD, Median (Interquartile Range {IQR}) and Range. Result of Qualitative variable are expressed as Frequency and Percentage. Shapiro-Wilks Test was used to determine whether data sets differed from a normal distribution. For categorized variables chi square test or Fisher's exact test is been used. To determine the independent effects of variables associated with the abnormal Homocysteine a multiple binary logistic regression analysis was then performed including variables with a P value of < 0.1 from univariate analysis. Result was considered significant at p value < 0.05.

RESULT:
Total 540339 cases were included in the study over period of 5 years. Out of 540339 cases, 239543 were females (44.33%) and 300796 were males (55.67%). Maximum patients belonged to age group of above 41 and majority of them above 60 years of age. (Table No. 1)

Table No.1:

	Frequency	Percentage
Gender		
Female	239543	44.33
Male	300796	55.67
Age Group		
18–30	47963	8.88
31–40	98799	18.28
41–50	117363	21.72
51–60	117028	21.66
>60	159186	29.46

From among all the cases, 41.83% had abnormal homocysteine. Apolipoproteins A1, Apolipoproteins B and Apolipoproteins B/A1 were observed to be abnormal in 10.57%, 24.27% and 2.05% cases respectively.

In lipid profiles (Table No.2), total cholesterol were seen high in 22.49% cases and low in 69.40%.

Total Cholesterol was high in 8.11% and borderline high in 22.49%. HDL Cholesterol was major risk factor for heart disease in 32.74% cases and it was negative risk for heart disease in 11.44% cases. CHOL/HDL ratio was high in 22.09% cases and was low in 34.89% cases.

LDL Cholesterol was optimal and near optimal in 46.27% and 28.74% cases respectively and it was abnormal in 24.98% cases i.e.(Borderline high: 17.55%, High: 5.76% and Very high: 1.67%)

LDL/HDL ratio was high in 13% cases and was low in 58.09% cases. Non HDL Cholesterol level was optimal in 48.25% cases, desirable in 25.93% cases, borderline high in 16.28% cases, high in 6.63% and very high in 2.91%,

Triglyceride level was normal in 64.34% cases, borderline high in 18.66% cases, high in 16.07% cases and was very high in 0.94% cases. VLDL Cholesterol was high in 15.49% cases and normal in 84.51% cases.

Lipoprotein (a) was abnormal in 37.34% and was normal in 62.66% cases.

Table No.2:

	Frequency	Percentage
Homocysteine		
High	68570	40.18
Low	2823	1.65
Normal	99271	58.17
ApolipoproteinsA1		
High	834	1.05

Low	7531	9.52
Normal	70717	89.42
Apolipoproteins B		
High	7267	9.19
Low	11929	15.08
Normal	59886	75.73
Apolipoproteins B/A1		
High	11424	1.71
Low	2244	0.34
Normal	655414	97.96
Cholesterol Total		
Bordline High	89430	22.48
Normal	276014	69.4
Low	32246	8.11%
HDL Cholesterol		
Major Risk	130204	32.74
Borderline risk	222005	55.82
Negative Risk	45499	11.44
CHOL/HDL Ratio		
High	87806	22.09
Low	138714	34.89
Normal	171042	43.02
LDL Cholesterol		
Optimal	175571	46.27
Near optimal	109060	28.74
Borderline High	66588	17.55
High	21869	5.76
Very High	6332	1.67
LDL/HDL Ratio		
High	49322	13
Low	220371	58.09
Normal	109700	28.91
Lipoprotein(a)		
Abnormal	29526	37.34
Normal	49553	62.66
Non HDL Cholesterol		
Optimal	191977	48.25
Desirable	103157	25.93
Borderline High	64785	16.28
High	26376	6.63
Very High	11573	2.91
Triglycerides Level		
Normal	256027	64.34
Borderline High	74240	18.66
High	63955	16.07
Very High	3726	0.94
VLDL Cholesterol		
Normal	320576	84.51
High	58778	15.49

Based on gender differentiation, correlation of various parameters and risk factors for cardiovascular risk was found to be significant statistically with p value < 0.05. Males were more significantly at risk with higher levels of homocysteine (46.75%), Total Cholesterol (71.80%), Apo B/Apo A (18.19%), Chol/HDL (27.94%), HDL Cholesterol (43.98%), Non- HDL Cholesterol (26.42%), Triglyceride (39.59%), LDL/HDL Cholesterol (16.57%) and VLDL (17.64%) as compared to females. On contrary, had higher levels of ApoA1 (1.87%), Apo B (9.4%), LDL Cholesterol (25.86%), Lipoprotein A (40.88%) was found to be significantly higher in females. (Table No. 3)

Table No.3

	Gender				
	Female		Male		P Value
	Frequency	Percentage	Frequency	Percentage	
Homocysteine					
High	10272	22.35	58298	46.75	<0.0001
Low	2100	4.57	723	0.58	
Normal	33597	73.09	65674	52.67	
Apolipoproteins A1					
High	704	1.87	130	0.31	<0.0001
Low	1854	4.91	5677	13.73	
Normal	35173	93.22	35544	85.96	
Apolipoproteins B					
High	3548	9.4	3719	8.99	<0.0001
Low	5150	13.65	6779	16.39	
Normal	29033	76.95	30853	74.61	
Apolipoproteins B/Apolipoproteins A1 ratio					
High	3902	10.34	7522	18.19	<0.0001
Low	1388	3.68	856	2.07	
Normal	32441	85.98	32973	79.74	
Cholesterol Total					
Desirable	127482	66.8	148532	71.8	<0.0001
Borderline high	46016	24.11	43414	20.99	
High	17336	9.08	14910	7.21	
HDL Cholesterol					
Major Risk	39226	20.55	90978	43.98	<0.0001
Borderline Risk	117129	61.38	104876	50.7	
Negative Risk	34486	18.07	11013	5.32	
Chol/HDL ratio					
High	30022	15.74	57784	27.94	<0.0001
Low	78666	41.23	60048	29.04	
Normal	82088	43.03	88954	43.02	
LDL Cholesterol					
Optimal	80964	43.83	94607	48.59	<0.0001
Near optimal	55987	30.31	53073	27.26	
Borderline High	33391	18.08	33197	17.05	
High	10988	5.95	10881	5.59	
Very High	3373	1.83	2959	1.52	
LDL/HDL Ratio					
High	17067	9.24	32255	16.57	<0.0001
Low	118197	64	102174	52.48	
Normal	49424	26.76	60276	30.96	
Lipoprotein(a)					
Abnormal	15424	40.88	14102	34.1	<0.0001
Normal	22305	59.12	27248	65.9	
Non HDL Cholesterol					
Optimal	91683	48.03	100294	48.46	0.00001
Desirable	51159	26.8	51998	25.12	
Borderline High	30397	15.92	34388	16.61	
High	12082	6.33	14294	6.91	
Very High	5564	2.91	6009	2.9	
Triglycerides Level					
Normal	130956	68.6	125071	60.41	<0.0001
Borderline High	33550	17.57	40690	19.65	
High	25328	13.27	38627	18.66	
Very High	1076	0.56	2650	1.28	
VLDL Cholesterol					
High	24430	13.23	34348	17.64	<0.0001
Normal	160217	86.77	160359	82.36	

As per Table no.4 high triglyceride, non-HDL cholesterol, LDL cholesterol, was significantly associated with elderly age group. High homocysteine also significant association with age, although was not specific to any age group.Table No.4

TableNo.4

	Age Group										
	18-30		31-40		41-50		51-60		>60		P value
	N	%	n	%	N	%	n	%	n	%	
Homocysteine											
High	7556	40.24	14259	41.93	15308	40.34	13108	38.73	18339	39.79	<0.0001
Low	698	3.72	843	2.48	524	1.38	382	1.13	376	0.82	
Normal	10525	56.05	18903	55.59	22117	58.28	20352	60.14	27374	59.39	

Apolipoproteins A1											
High	33	0.7	97	0.79	119	0.74	153	0.86	432	1.55	<0.0001
Low	701	14.87	1568	12.69	1577	9.75	1446	8.09	2239	8.01	
Normal	3979	84.43	10689	86.52	14484	89.52	16278	91.06	25287	90.45	
Apolipoproteins B											
High	316	6.7	1441	11.66	1832	11.32	1817	10.16	1861	6.66	<0.0001
Low	516	10.95	1001	8.1	1499	9.26	2404	13.45	6509	23.28	
Normal	3881	82.35	9912	80.23	12849	79.41	13656	76.39	19588	70.06	
Apolipoproteins B/Apolipoproteins A1Ratio											
High	804	17.06	2766	22.39	3023	18.68	2480	13.87	2351	8.41	<0.0001
Low	59	1.25	110	0.89	190	1.17	428	2.39	1457	5.21	
Normal	3850	81.69	9478	76.72	12967	80.14	14969	83.73	24159	86.38	
Cholesterol Total											
Desirable	23629	77.57	46111	66.07	54780	63.45	58311	65.75	93183	76.12	<0.0001
Borderlin High	5442	17.87	17673	25.32	23346	27.04	21665	24.43	21304	17.4	
High	1390	4.56	6009	8.61	8209	9.51	8706	9.82	7932	6.48	
HDL Cholesterol											
Major Risk	9647	31.67	26409	37.84	30583	35.42	27969	31.54	35596	29.02	<0.0001
Borderlie Risk	17350	56.95	37613	53.9	47947	55.53	50432	56.86	68883	56.16	
Negative Risk	3467	11.38	5767	8.26	7809	9.04	10287	11.6	18169	14.81	
Chol/HDL Ratio											
High	5883	19.32	20890	29.95	23922	27.72	19629	22.14	17482	14.28	<0.0001
Low	11710	38.45	17199	24.66	22293	25.83	28952	32.66	58560	47.85	
Normal	12859	42.23	31669	45.4	40088	46.45	40077	45.2	46349	37.87	
LDL Cholesterol											
Optimal	13923	47.21	23563	36.12	30089	37.17	37247	43.96	70749	59.45	<0.0001
Near Optimal	9799	33.22	23143	35.47	26427	32.65	23560	27.81	26131	21.96	
Borderlie High	4339	14.71	13307	20.4	17103	21.13	16502	19.48	15337	12.89	
High	1072	3.63	4069	6.24	5837	7.21	5734	6.77	5157	4.33	
Very High	360	1.22	1159	1.78	1495	1.85	1687	1.99	1631	1.37	
LDL/HDL Ratio											
High	3616	12.26	11595	17.77	13117	16.2	11157	13.2	9837	8.27	<0.0001
Low	17505	59.36	30761	47.15	39928	49.33	48103	56.91	84074	70.66	
Normal	8369	28.38	22884	35.08	27903	34.47	25265	29.89	25079	21.08	
Lipoprotein(a)											
Abnormal	1442	30.6	4113	33.3	5944	36.74	6903	38.61	11124	39.79	<0.0001
Normal	3271	69.4	8240	66.7	10235	63.26	10974	61.39	16833	60.21	

Non HDL Cholesterol											
Optimal	16662	54.67	27152	38.88	33629	38.93	39899	44.97	74635	60.95	<0.0001
Desirable	8014	26.3	21484	30.77	25334	29.33	23401	26.38	24924	20.35	
Borderine High	3940	12.93	13350	19.12	17199	19.91	15750	17.75	14546	11.88	
High	1276	4.19	5457	7.81	7241	8.38	6697	7.55	5705	4.66	
Very High	583	1.91	2387	3.42	2981	3.45	2975	3.35	2647	2.16	
Triglycerides Level											
Normal	23134	75.9	42393	60.69	51425	59.52	55044	62.03	84031	68.61	<0.0001
Borderlin High	3868	12.69	12870	18.42	17290	20.01	17999	20.28	22213	18.14	
High	3263	10.71	13484	19.3	16478	19.07	14965	16.86	15765	12.87	
Very High	215	0.71	1107	1.58	1211	1.4	731	0.82	462	0.38	
VLDL Cholesterol											
Normal	26501	89.95	53471	81.97	66358	81.98	70711	83.46	103535	87.01	<0.0001
High	2962	10.05	11764	18.03	14583	18.02	14013	16.54	15456	12.99	

Association of homocysteine with other parameters in the table no.5 was statistically significant for Apo B, Apo B/A1, Total cholesterol, LDL Cholesterol, Chol/HDL ratio, LDL/HDL ratio, non-HDL cholesterol, Triglycerides level, VLDL Cholesterol, HDL Cholesterol and association of homocysteine with ApoA1, Lipoprotein (a) was statistically insignificant. (Table No. 5)

Table No.5

	Homocysteine						
	High		Low		Normal		P value
	n	%	N	%	N	%	
ApolipoproteinsA1							
High	7	1.1	1	3.45	26	1.47	0.2195
Low	73	11.46	2	6.9	153	8.67	
Normal	557	87.44	26	89.66	1586	89.86	
Apolipoproteins B							
High	70	10.99	1	3.45	126	7.14	0.0018
Low	74	11.62	5	17.24	293	16.6	
Normal	493	77.39	23	79.31	1346	76.26	
Apolipoproteins B/ Apolipoproteins A1							
High	111	17.43	0	0	208	11.78	0.0007
Low	12	1.88	1	3.45	54	3.06	
Normal	514	80.69	28	96.55	1503	85.16	
Cholesterol Total							
Desirable	13252	70.54	214	78.97	20174	72.39	<0.0001
Borderline High	4179	22.25	43	15.87	5778	20.73	
High	1355	7.21	14	5.17	1918	6.88	
HDL Cholesterol							
Major Risk	6991	37.21	96	35.42	8667	31.1	<0.0001
Borderline Risk	10356	55.11	142	52.4	16294	58.47	
Negative Risk	1443	7.68	33	12.18	2907	10.43	
Chol/HDL Ratio							
High	4774	25.41	44	16.24	5373	19.28	<0.0001
Low	5655	30.1	116	42.8	10937	39.25	
Normal	8357	44.49	111	40.96	11554	41.47	
LDL Cholesterol							
Optimal	8094	44.94	137	53.1	13505	50.3	<0.0001

Near Optimal	5346	29.68	85	32.95	7331	27.31	
Borderline High	3307	18.36	30	11.63	4270	15.9	
High	1032	5.73	5	1.94	1368	5.1	
Very High	233	1.29	1	0.39	374	1.39	
LDL/HDL Ratio							
High	2692	14.95	19	7.36	2954	11	<0.0001
Low	9447	52.45	173	67.05	16655	62.03	
Normal	5871	32.6	66	25.58	7239	26.96	
Lipoprotein(α)							
Abnormal	230	36.11	11	37.93	684	38.75	0.4988
Normal	407	63.89	18	62.07	1081	61.25	
Non HDL Cholesterol							
Optimal	8791	46.78	148	54.61	14688	52.69	<0.0001
Desirable	5049	26.86	81	29.89	6735	24.16	
Borderline High	3214	17.1	28	10.33	4149	14.88	
High	1279	6.81	10	3.69	1664	5.97	
Very High	461	2.45	4	1.48	641	2.3	
Triglycerides level							
Normal	11831	62.96	177	65.31	18253	65.47	<0.0001
Borderline High	3739	19.9	44	16.24	5290	18.97	
High	3075	16.37	48	17.71	4182	15	
Very High	145	0.77	2	0.74	154	0.55	
VLDL Cholesterol							
Normal	15081	83.72	211	82.42	22875	85.24	<0.0001
High	2932	16.28	45	17.58	3962	14.76	

Correlation of homocysteine with other parameters in the table below was statistically significant. Homocysteine showed positive correlation with Apo B, Apo B/A1, Total cholesterol, LDL Cholesterol, Chol/HDL ratio, LDL/HDL ratio, non-HDL cholesterol, Triglycerides level, VLDL Cholesterol. Homocysteine showed negative correlation with ApoA1, HDL Cholesterol, Lipoprotein (α). (Table No. 6)

Table No.6

Variables	n	Correlatio coefficient (r)	95% CI of r	P value
Apolipoproteins A1	2431	-0.1187	-0.1578 to -0.0793	<0.0001
Apolipoproteins B	2431	0.1001	0.06055 to 0.1393	<0.0001
Apolipoproteins B/A1	2431	0.1574	0.1184 to 0.1959	<0.0001
Cholesterol Total	46927	0.0473	0.0382 to 0.0563	<0.0001
HDL Cholesterol	46929	-0.1027	-0.1116 to -0.09370	<0.0001
Chol/HDL Ratio	46921	0.1142	0.1052 to 0.1231	<0.0001
LDL Cholesterol	45118	0.07361	0.06443 to 0.08278	<0.0001
LDL/HDL Ratio	45116	0.1353	0.1263 to 0.1444	<0.0001
Lipoprotein(α)	2431	-0.05563	-0.09518 to -0.01591	0.0062
Non HDL Cholesterol	46942	0.07507	0.06607 to 0.08406	<0.0001
Triglycerides level	46940	0.03199	0.02295 to 0.04103	<0.0001
VLDL Cholesterol	45106	0.03324	0.02402 to 0.04246	<0.0001

Association of lipoprotein(α) with other parameters in the table no.7 was statistically significant for Apo B, Apo B/A1, Total cholesterol, LDL Cholesterol, Chol/HDL ratio, non-HDL cholesterol, Triglycerides level, VLDL Cholesterol, HDL Cholesterol and association of lipoprotein(α) with LDL/HDL ratio was statistically insignificant. (Table No. 7)

Table No.7

	Lipoprotein(α)				
	Abnormal		Normal		P Value
	Frequency	Percentage	Frequency	Percentage	
Apolipoproteins A1					
High	359	1.22	475	0.96	<0.0001
Low	2302	7.8	5229	10.55	
Normal	26864	90.99	43849	88.49	
Apolipoproteins B					
High	3123	10.58	4143	8.36	<0.0001
Low	3878	13.13	8051	16.25	
Normal	22524	76.29	37359	75.39	
Apolipoproteins B/ Apolipoproteins A1 ratio					
High	4270	14.46	7153	14.44	<0.0001
Low	661	2.24	1583	3.19	
Normal	24594	83.3	40817	82.37	
Cholesterol Total					
Desirable	15439	67.5	27670	71.57	<0.0001
Borderline high	5116	22.37	8093	20.93	
High	2317	10.13	2901	7.5	
HDL Cholesterol					
Major Risk	7054	30.84	14838	38.38	<0.0001
Borderline Risk	13028	56.96	20164	52.15	
Negative Risk	2791	12.2	3662	9.47	
Chol/HDL Ratio					
High	4892	21.39	9302	24.06	<0.0001
Low	8456	36.97	13286	34.37	
Normal	9522	41.64	16069	41.57	
LDL Cholesterol					
Optimal	10339	46.52	18561	50.56	<0.0001
Near optimal	5588	25.14	9676	26.36	
Borderline High	4052	18.23	5927	16.14	
High	1636	7.36	1945	5.3	
Very High	611	2.75	603	1.64	
LDL/HDL Ratio					
High	3274	14.73	5153	14.04	0.0059
Low	12942	58.23	21255	57.9	
Normal	6008	27.03	10303	28.07	
Non HDL Cholesterol					
Optimal	11316	49.46	19562	50.56	<0.0001
Desirable	5268	23.02	9529	24.63	
Borderline High	3671	16.04	5950	15.38	
High	1737	7.59	2507	6.48	
Very High	889	3.89	1142	2.95	
Triglycerides Level					
Normal	15658	68.42	23713	61.27	<0.0001
Borderline High	4189	18.3	7731	19.98	
High	2952	12.9	6859	17.72	
Very High	86	0.38	398	1.03	
VLDL Cholesterol					
Normal	19334	86.99	30415	82.87	<0.0001
High	2891	13.01	6286	17.13	

Correlation of lipoprotein (α) with other parameters in the Table No.8 below was statistically significant except Apolipoprotein B/A1. Lipoprotein (α) showed positive correlation with ApoA1, Apo B, Apo B /A1, Total cholesterol, HDL Cholesterol, LDL Cholesterol, Non-HDL cholesterol. Lipoprotein (α) showed negative correlation with Chol/HDL

ratio, LDL/HDL ratio, Triglycerides level, VLDL Cholesterol.

Table No.8

Variables	N	Correlation Coefficient (r)	95% CI of r	P value
Apolipoproteins A1	79078	0.09289	0.0859 to 0.0998	<0.0001
Apolipoproteins B	79078	0.05995	0.05300 to 0.0668	<0.0001
Apolipoproteins B/A1	79078	0.0006	-0.0062 to 0.0076	0.8467
Cholesterol Total	61536	0.05391	0.04603 to 0.06178	<0.0001
HDL Cholesterol	61537	0.1099	0.1021 to 0.1177	<0.0001
Chol/HDL Ratio	61527	-0.05233	-0.0602 to -0.0444	<0.0001
LDL Cholesterol	58938	0.06093	0.0528 to 0.06897	<0.0001
LDL/HDL Ratio	58935	-0.01052	-0.01860 to -0.00245	0.0106
Non HDL Cholesterol	61571	0.02599	0.01809 to 0.03388	<0.0001
Triglycerides level	61556	-0.07525	-0.08310 to -0.06739	<0.0001
VLDL Cholesterol	58926	-0.05832	-0.06636 to -0.05027	<0.0001

In multivariate logistic regression analyses (Table No. 9), HHcy was associated with risk age above 41years statistically significant (p value <0.0001) and males were at increased risk adjusted odds ratio [OR] = 2.4405 (p value <0.0001). HHcy was associated with increasing risk of low HDL-C (HDL-C < 40mg/dl; adjusted odds ratio [OR] =1.1293, 95% confidence interval [CI]: 1.0923- 1.1675, P < 0.0001), hypertriglyceridemia (TG ≥ 500mg/dl; adjusted OR = 1.33, 95% CI: 1.0607–1.6676, P < 0.0001), high CHOL/HDL (CHOL/HDL ≥ 5; adjusted OR = 1.2235, 95% CI: 1.1752–1.2737, P < 0.0001), high LDL/HDL (LDL/HDL ≥ 3.5; adjusted OR = 1.2378, 95% CI: 1.1737–1.3055, P < 0.0001), high LDL-C (LDL-C > 150mg/dl; adjusted odds ratio [OR] =1.8763, 95% confidence interval [CI]: 1.744 – 2.0321, P < 0.0001) and high non HDL-C (non HDL-C > 160mg/dl; adjusted odds ratio [OR] =1.09, 95% confidence interval [CI]: 1.0113 – 1.1714, P < 0.0001) after adjusting the confounders. However, there were no significant associations between Hcy and ApoA, ApoB, Apo B/A1, TC or Lipoprotein (a).

Table No.9

Parameters	Univariate analysis				Multivariate analysis		
	Variable	P value	OR	95% CI OR	P value	OR	95% CI OR
Age Group		<0.0001					
	18 - 30	Ref			Ref		
	31 - 40	0.3107			<0.0001	0.918	0.88 to 0.95
	41 - 50	<0.0001	0.9128	0.88 to 0.94	<0.0001	0.7985	0.7 to 0.83
	51 - 60	<0.0001	0.8452	0.81 to 0.87	<0.0001	0.746	0.71 to 0.77
	>60	<0.0001	0.8718	0.84 to 0.90	<0.0001	0.7791	0.75 to 0.81
Gender		<0.0001					
	Female	Ref					
	Male	<0.0001	2.4405	2.38 to 2.49	<0.0001	2.5899	2.53 to 2.65
Apolipoproteins A1		0.0017					
	Normal	Ref					
	High	0.0356	0.4276	0.19 to 0.94			
	Low	0.0065	0.6812	0.52 to 0.89			
Apolipoproteins B		<0.0001					
	Normal	Ref					
	High	0.0075					
	Low	<0.0001	0.3741	0.29 to 0.48	<0.0001	0.5456	0.42 to 0.71
Apolipoproteins B/A1		<0.0001					
	Normal	Ref					
	High	0.011	0.7414	0.59 to 0.93			
	Low	0.0004	0.3344	0.18 to 0.61			
Cholesterol Total		0.7083					
	Normal	Ref					
	High	0.4222					
	Low	0.8515					
HDL Cholesterol		<0.0001					
	Negative	Ref					
	Borderline	<0.0001	0.8898	0.87 to 0.91			
	Majo Risk	<0.0001	1.1293	1.09 to 1.17			
Chol/HDL ratio		<0.0001					
	Normal	Ref					
	High	<0.0001	1.2235	1.17 to 1.27	0.0002	1.1459	1.07 to 1.23
	Low	<0.0001	0.7199	0.69 to 0.7	<0.0001	0.7837	0.74 to 0.83
LDL Cholesterol		0.0002					
	Optimal	Ref					
	Near Optimal	0.0483	1.0375	1.01 to 1.08			
	Borderlinhigh	0.0001	1.0945	1.04 to 1.15			
	High	0.01495	1.0617	0.98 to 1.15	0.00663	1.011	0.81 to 1.11
	Very High	0.01138	1.8763	1.74 to 2.03	<0.0001	1.6794	1.57 to 1.81
LDL/HDL Ratio		<0.0001					
	Normal	Ref					

	High	<0.0001	1.2378	1.17 to 1.31	0.0256	1.1022	1.01 to 1.20
	Low	<0.0001	0.7791	0.79 to 0.80	<0.0001	0.8444	0.80 to 0.88
Lipoprotein (a)		<0.0001					
	Normal	Ref					
	Abnormal	<0.0001	0.4882	0.42 to 0.56	<0.0001	0.6352	0.55 to 0.74
Non HDL Cholesterol		<0.0001					
	Optimal	Ref					
	Desirable	0.0004	1.0702	1.03 to 1.11			
	Borderlinhigh	0.0001	1.0979	1.05 to 1.15	0.0007	0.9076	0.85 to 0.96
	High	0.0238	1.0884	1.01 to 1.17	0.0257	0.8874	0.79 to 0.98
	Very High	0.0075	1.019	0.90 to 1.15	0.0001		
Triglyceride level		0.0328					
	Normal	Ref					
	Borderlinhgh	0.8686					
	High	0.1012			0.0001	0.8742	0.8 to 0.93
	Very High	0.0135	1.33	1.06 to 1.67	<0.0001	0.908	0.86 to 0.98
VLDL Cholesterol		0.0654					
	Normal	Ref					

DISCUSSION:

In our cross-sectional study, we found that homocysteine, TG, TC, LDL, Lipoprotein (a), non-HDL, VLDL and HDL-C levels were different between sexes. Homocysteine, TG, LDL, non-HDL, VLDL levels among females were lower than males, while HDL-C, TC, lipoprotein (a) in females was higher than males. Differences in homocysteine and lipid profiles among sexes may further lead to difference in the relationship between homocysteine and lipid profiles, along with the strength of the relationship.

Carlson et al has reported that the lipid profiles and lipoproteins between adult males and adult females differ from birth, and HDL-C of females is more than that of males at birth and at all ages after adulthood [7, 8]. Rosano et al observed that the level of homocysteine was low in the majority females during pregnancy, although it does not significantly differ from that of males of the same age in postmenopausal females which suggested that estrogen can reduce homocysteine level in premenopausal females [9, 10].

Thus, homocysteine and lipid profiles among females differ from males due to estrogen, which is a fat-soluble steroid hormone and is the main female sex hormone. Estrogen is involved in the development of the female reproductive system and maintains menstruation. It also plays a vital role in menstrual cycle and pregnancy, and has other physiological functions such as anti-platelet and regulating lipid metabolism as per Dimitrova et al [11].

The difference in relationship between homocysteine and lipid profiles among different sexes can mainly be attributed to the differences in the physiological structure and estrogen of different sexes.

Qujeq et al. analysed 126 patients with myocardial infarction and found that homocysteine was significantly negatively correlated with HDL-C ($r = -0.93$, $p < 0.05$), and positively correlated with LDL-C ($r = 0.98$, $p < 0.05$) [12]. In a study done by Qin et al [14] at the First Affilia ted Hospital of Guangxi Medical University from 2015 to 2016, blood was collected from 8043 patients undergoing physical examinations, Hcy was found to be positively correlated with TG ($r = 0.084$, $p = 0.000$), TC ($r = 0.045$, $p = 0.000$), and LDL-C ($r = 0.059$, $p = 0.000$), and negatively correlated with HDL-C ($r = -0.189$, $p = 0.000$). Kiseljakovic et al [13] reported that there was negative association between serum hyper homocysteinemia and lipids in atherosclerotic vascular disease (ASVD) patients. Xiaona Niu et al study found that homocysteine was associated with high levels of TG, TC, LDL-C, and low level of HDL-C among Chinese population of Hunan, which was consistent with the above research results [2].

However, Yadav et al. study was conducted on 60 patients with ischemic heart disease had no significant correlation between homocysteine and TG, TC, and HDL-C [3]. In a study by de Luis et al conducted in 2005 on 155 diabetes patients, there was no significant correlation between homocysteine and lipid profiles [15]. Very few previous studies have studied the relationship between homocysteine and lipid profiles between different sexes [1,2]. The results of our study on relationship between homocysteine and lipid profiles between different sexes are in concurrence to the findings of Niu et al and Momin et al [1,2]. This study suggested that there may be significant difference about the relationship between homocysteine and lipid profiles in different sexes.

Many studies have shown that elevated homocysteine is an independent risk factor for cardiovascular disease and closely associated with the development of atherosclerotic vascular disease (ASVD), coronary heart disease, diabetes, dementia, and other diseases [16, 17, 18,19]. Moreover, clinical and epidemiological studies have demonstrated that abnormal lipid metabolism is associated with atherosclerosis [20, 21, 22].Abnormal lipid metabolism is also an important risk factor for atherosclerosis, and hyperhomocysteine can induce hyperlipidemia thereby damaging of blood vessels through various mechanisms [5].

Most studies suggesting mechanisms through which homocysteine affects lipid metabolism are mentioned below. As per Rubin et al, apolipoprotein A (Apo A–I) is the major protein component of HDL-C and overexpression of human Apo A-I can increase high-density lipoprotein level [23].

On contrary raised hyperhomocysteine may decrease expression of Apo A-I which may further reduce HDL- C synthesis, leading to the occurrence of cardiovascular disease as per Mikael et al [24]. Yideng et al. suggested that DNA hypomethylation could be a mechanism of homocysteine associated to abnormal lipid and atherosclerosis in vascular smooth muscle cells [25]. Werstuck et al suggested that homocysteine may enhance the expression of sterol regulatory element binding proteins (SREBP) and increase the intracellular accumulation of TC and TG in cells [5].

This study showed that hyperhomocysteine was negatively correlated with HDL-C and positively correlated with LDL-C, total cholesterol, triglycerides among Indian population, which was in concurrence with the previous experimental studies. However, only few studies done on the relationship between homocysteine and lipid profiles between different sexes. Although this study explores large sample of population data to establish the difference in the relationship between the between homocysteine and lipid profiles

between different sexes.

To summarize, studies involving the association between homocysteine and lipid profiles have had been inconclusive. As per Xiaona Niu et al, the discrepancy is due to either the selected subjects have different types of diseases or they are from different ethnic groups [2]. So, confounding factors are not controlled, or included confounding factors are different in regression analysis, which could also lead to different results.

Our study has random selection of subjects from urban population, which could be used to assess the correlation between homocysteine and lipid profiles providing enough statistical significance. Also, we used univariate analysis to control confounding factors which could have possibly affected homocysteine levels. Our study analyzed the relationship between homocysteine and lipid profiles among urban based population and gender based, however, being very few studies aimed at establishing the association. However, our study has limitations such as we cannot eliminate unknown confounding factors such as dietary habits of subjects or subjects consuming some drugs, which may interfere with homocysteine and lipid profiles. Also, our study was cross-sectional study and could not establish a causal association between the homocysteine and lipid profiles. Due to the unavailability of such data, detailed evaluation cannot be performed.

The regression analysis showed that hyperhomocysteine was also associated with hypertriglyceridemia, hypercholesterolemia, high level of LDL-C and low level of HDL in males and females from Indian community based population, which would help to establish treatment of abnormal lipid metabolism and prevention of cardiovascular risk.

CONCLUSION:

The present study has analysed the relationship between homocysteine and lipid profiles among Indian community-based population. We have limited confounding factors by usage of univariate analysis among randomly selected subjects. Hyperhomocysteine was independently associated with an increasing risk of low HDL-C among males. The regression analysis showed that hyperhomocysteine was also associated with hypertriglyceridemia, hypercholesterolemia, and high level of LDL-C in males more than females from Indian community based population, which would form the basis of management and prevention of abnormal lipid metabolism.

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