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A study to evaluate the correlation between increased homocysteine level and hyperlipidaemia: An observational study

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ABSTRACT

Introduction and Aims: Numerous studies have suggested a possible relation between increased homocysteinemia level and cardiovascular diseases. This relationship is clinically important in the management of cardiac diseases specially in elderly population. However, there is limited data which suggests association between hyperhomocysteinemia and hyperlipidaemia. The aim of this study is to find a relation between hyperlipidaemia and hyperhomocysteinemia in patients who are not on lipid-lowering treatment.

Materials and Methods: A total of 300 hyperlipidemic patients were enrolled who attended medicine OPD. After obtaining informed consent, data were collected by a trained technician according to a standard operating protocol of the laboratory. After an overnight fast of minimum 12 hours, 5 ml of venous blood was taken under aseptic conditions in EDTA tube. The blood was late centrifuged, plasma samples were then separated within half hour of sample collection and were stored at −80 °C in the laboratory. Total cholesterol (TC), Low Density Lipid-Cholesterol (LDL-C), High Density Lipid-Cholesterol (HDL-C) and Triglycerides (TG) were measured on Bene Sphera autoanalyzer. Homocysteine was measured on Robonic ELISA reader.

Result: Homocysteine level in blood was directly proportional to increased level of TC, TG, LDL-C and age of the patient. In the same group homocysteine level was inversely proportional to high density lipoproteins.

Conclusion: There is strong correlation of increased homocysteine level with hyperlipidaemia.

1. Introduction

Homocysteine (Hcy) is considered to be associated with hyperlipidaemia. An understanding of its metabolism and factors that effect its regulation will help in the development of therapeutic strategies which may eventually lower the risk of atherosclerosis in humans. Possible mechanisms of atherosclerosis due to hyperhomocysteinemia (HHcy) include damage to inner vascular membrane, augmentation of smooth muscle cell proliferation, increase low–density lipoprotein cholesterol peroxidation and activation of thrombos formation.[1,2] Association between HHcy, dyslipidemia and atherosclerosis has already been studied extensively.[3] An inverse association between HHcy and HDL-C has been found in humans in various studies.[4] Recent studies have strongly showed the importance of metabolic balance between homocysteine metabolism, hypolipoproteinemia, liver function and cardiovascular disease.[5,6] It has been suggested that Hcy disturbs HDL-C metabolism via inhibiting ApoA-I protein synthesis.[7,8] We conducted this study with the aim to evaluate the correlation between the level of plasma Hcy with hyperlipidaemia in Indian perspective in patients who were not on lipid lowering medications.

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2. Materials and Methods

This prospective, randomized study was conducted at a tertiary referral hospital for a duration of one and half year after obtaining approval from the hospital ethical committee. A total of 300 hyperlipidemic patients were enrolled who attended medicine OPD. After obtaining informed consent, samples were collected by a trained technician according to a standard operating protocol of the laboratory. After an overnight fast of minimum 12 hours, 5 ml of venous blood was taken under aseptic conditions in EDTA tube. The blood was late centrifuged, plasma samples were then separated within half hour of sample collection and were stored at −80 °C in the laboratory. Total cholesterol (TC), Low Density Lipid-Cholesterol (LDL-C), High Density Lipid-Cholesterol (HDL-C) and Triglycerides (TG) were measured on BeneSphera autoanalyzer**.** Homocysteine was measured on Robonic ELISA reader.

2.1. Data collection

Each participant was asked a standardized questionnaire designed specifically for the present study that collected information related to medical history, past and current medication use and personal habits such as exercise, cigarette and alcohol consumption.

After taking informed consent, baseline data were collected by trained research staff according to a standard operating procedure. After an overnight fast of at least 12 hours, 5 ml of venous blood was taken under aseptic conditions in EDTA tube. The blood was centrifuged, plasma samples were separated within 30 min of collection and were stored at −80 °C. Plasma total cholesterol (TC), LDL-C, HDL-C, triglycerides (TG) were measured on BeneSphera autoanalyzer**.** Homocysteine was measured on Robonic ELISA reader.

2.2. Sample size

The sample size was calculated for estimating the mean homocysteine levels in the study population. The expected standard deviation of the homocysteine was taken as 13.1,[9] margin of error was assumed as 1.5, alpha error as 5%, and beta error as 20%. Using these parameters, the sample size was calculated as 296 which was rounded off to 300 for the study

2.3. Statistical analysis

Categorical variables were presented as percentages with 95% CI, and continuous variables were presented as mean with standard deviation (SD). The study population was divided into quartiles on the basis of their homocysteine levels. Various study parameters were compared across these 4 groups. For categorical variables across groups, chi-square test was used and for the comparison of continuous variables across 4 groups, one-way ANOVA was used. To identify the predictors of homocysteine levels, linear regression was done. Age, total cholesterol, triglyceride, HDL, and LDL levels were used as independent variables in the regression model. In first model, bivariate analysis was done without adjustment for other variables. In the second model, adjustment was done for age, TC, TG, HDL, and LDL. Data with p-value <0.05 was considered as significant. Statistical analysis was performed using Statistical Package for Social Sciences (IBM SPSS Statistics for Windows, Version 23.0).

3. Result

300 patients were evaluated in the study. They were distributed in 4 groups (Quartiles) according to the value of homocysteine level obtained. Similarly mean values of Age, Total cholesterol, triglyceride and HDL were evaluated in the four quartiles (Table 1).

There was a positive correlation between Age and Hcy levels in all the four quartiles.

Similarly, there was also a positive correlation between TC and TG and homocysteine levels in all the four quartiles.

Linear regression for age and lipid profile on homocysteine levels was evaluated (Table 2) in the form of 2 models.

Model 1 denotes Bivariate analysis and b coefficient was statistically significant between age, total cholesterol, triglyceride, HDL & LDL level. The b coefficient was found to be negative between HDL level and homocysteine level.

Model 2 denotes Multivariate analysis after adjusting for age, total cholesterol, triglyceride, HDL, and LDL and b coefficient was statistically significant between age, Total cholesterol, HDL & LDL level. The b coefficient was found to be negative between HDL level and homocysteine level in the model 2. b coefficient was statistically insignificant for triglyceride level.

4. Discussion

Cholesterol is a major component of cellular membranes. Hypercholesterolemia is the presence of high amount of lipids in the blood.[10] It has been seen that there is a positive correlation between homocysteine level and cardiovascular diseases.[11,12]

We evaluated 300 patients and the mean value of Hcy was found to be 10.9 (3.2) mcmol/L. All the patients were further evaluated for their Hcy level and their correlation with other factors by grouping them into 4 quartiles of 75 patients each. The values of Hcy in the quartile 1,2,3 and 4 was 6.4 (0.9), 10.0 (0.9), 12.3 (0.6), 14.8 (0.9) mcmol/L respectively.

The mean age of the patients was 58.8 (12.3), and the mean age in the quartile 1,2,3 and 4 was 46.6 (2.7), 54.0 (9.1), 63.9 (8.7), and 71.0 (9.6) respectively. We found a

Table 1: Comparison of lipids across quartiles of homocysteine levels

Mean (SD)	Total	Quartile 1	Quartile 2	Quartile 3	Quartile 4	
N	300	75	75	75	75	**p-value**
Homocysteine (mcmol/L)	10.9 (3.2)	6.4 (0.9)	10.0 (0.9)	12.3 (0.6)	14.8 (0.9)	
Age	58.8 (12.3)	46.6 (2.7)	54.0 (9.1)	63.9 (8.7)	71.0 (9.6)	<0.001
Total cholesterol(mg%)	221.5 (35.3)	205.2 (18.9)	212.6 (27.5)	219.6 (31.8)	248.6 (42.7)	<0.001
Triglyceride(mg%)	158.3 (18.8)	146.2 (13.4)	149.4 (11.6)	160.7 (14.9)	176.8 (17.7)	<0.001
HDL(mg%)	47.1 (6.7)	53.8 (2.8)	49.0 (5.2)	45.6 (5.2)	40.0 (4.5)	<0.001
LDL(mg%)	119.1 (19.6)	99.4 (5.5)	112.2 (9.9)	126.2 (14.1)	138.6 (18.5)	<0.001

Table 2: Linear regression for effects of age and lipid profile on homocysteine levels

	Model 1		**Model 2**	
	b (SE)	p-value	b (SE)	p-value
Age	0.211 (0.009)	<0.001	0.111 (0.009)	<0.001
Total cholesterol	0.041 (0.005)	<0.001	0.006 (0.003)	0.018
Triglyceride	0.100 (0.008)	<0.001	0.006 (0.005)	0.230
HDL	-0.362 (0.018)	<0.001	-0.160 (0.015)	<0.001
LDL	0.127 (0.006)	<0.001	0.040 (0.006)	<0.001

Model 1: Bivariate analysis

positive correlation which is statistically significant between increasing age and Hcy level.

The total cholesterol level was evaluated and the mean value was 221.5 (35.3) mg% and the mean value in the quartile 1,2,3 and 4 was 205.2 (18.9), 212.6 (27.5), 219.6 (31.8) and 248.6 (42.7) mg% respectively. It increased with the increasing value of Hcy level and the it was statistically significant.

The total Triglyceride level was studied and the mean value was found to be 158.3 (18.8) mg% and the mean value in the quartile 1,2,3 and 4 was 146.2 (13.4), 149.4 (11.6), 160.7 (14.9) and 176.8 (17.7) mg% respectively. It increased with the increasing value of Hcy level and the it was statistically significant.

The total HDL level was examined and the mean value was 47.1 (6.7) mg% and the mean value in the quartile 1,2,3 and 4 was 53.8 (2.8), 49.0 (5.2), 45.6 (5.2) and 40.0 (4.5) mg% respectively. We found that HDL level decreased with the increasing level of Hcy(inverse relationship) which was statistically significant.

The total LDL level was examined and the mean value was 119.1 (19.6) mg% and the mean value in the quartile 1,2,3 and 4 was 99.4 (5.5), 112.2 (9.9), 126.2 (14.1) and 138.6 (18.5) mg% respectively. The level of LDL cholesterol increased with the increasing homocysteine level and the increase was statistically significant.

We found that increased Hcy level is independently associated with lower HDL-C and increased level of triglycerides and total cholesterol. We also found that HHcy was also associated with the increased level of LDL. The interaction between hyperlipidaemia and Hcy metabolism has been extensively studied.[7,8] It has been found that methionine can alter cholesterol metabolism and there is a weak positive correlation between circulating homocysteine and plasma cholesterol.[13]

Linear regression regarding effects of age and lipid profile on homocysteine levels was evaluated in our study. On doing bivariate analysis we found that there exists a positive correlation between Age and homocysteine levels.

Similarly, there also exists a positive correlation between total cholesterol and TG and homocysteine levels in all the four quartiles of our result. The b coefficient came out to be negative between HDL level and homocysteine level. The b coefficient was statistically significant between all the lipid profiles and Hcy levels. Positive correlation shows that as age progresses the level of Hcy level in the body also increases. Similarly, the increase in total cholesterol and triglyceride level leads to gradual increase in Hcy level. Negative b coefficient shows inverse relationship between HDL and Hcy level in the human body.

Similarly, when we performed multivariate analysis, we found positive correlation and a statistically significant data (b coefficient) between Hcy level and age, TC, TG and LDL. b coefficient was statistically insignificant between Hcy level and triglyceride. The b coefficient was negative and statistically significant between HDL level and homocysteine level which supports various studies that have shown beneficial effects of HDL in preventing cardiovascular diseases.[14–16]

We found that Plasma Hcy levels increased sharply in persons above the age 50 years as compared to age below 50 years. This may be due to altered metabolism of homocysteine after the age of 50 which causes increased level of homocysteine. It may be a possible explanation for

the progressive increase in ischemic strokes especially in elderly population.

However, the relationship between HHcy and hyperlipidaemia have not been conclusively proved uptil now.[17] It has been postulated that the low ratio between phosphatidylcholine (PC) and phosphatidylethanolamine (PE) caused by HHcy is a major factor for triglycerides accumulation. Hcy enhances the augments the expression of sterol regulatory element-binding proteins which leads to increased intracellular accumulation of TC and TG.[18] Hcy also leads to protein misfolding in the endoplasmic reticulum which affects lipoprotein particle production in the cell.[19] DNA hypomethylation has been postulated to be the mechanism which suggests that Hcy leads to lipid disorders and atherosclerosis in blood vessels.[20]

No significant relation between plasma Hcy and TC, HDL-C, and TG in diabetic patients has been found.[21]

Therefore, though studies conducted uptil now have suggested mixed results for the relation between Hcy and hyperlipidaemia, the most consistent findings indicate that higher Hcy is associated with decreased serum HDL-C and increased TG levels, which are consistent with the results of our present study.

The clinical and epidemiological data regarding the relations between Hcy and TC, LDL are very limited and more clinical trials need to be conducted for ascertain the real facts.

5. Conclusion

Based on the outcome of the present study we conclude that Hyperhomocysteinemia status is independently associated with hypertriglyceridemia, high total cholesterol level, high LDL level and low HDL levels in the blood.

6. Sources of Funding

Nil

7. Conflict of Interest

Nil.

References

1. Antoniades C, Antonopoulos AS, Tousoulis D, Marinou K, Stefanadis C. Homocysteine and coronary atherosclerosis: from folate fortification to the recent clinical trials. *Eur Heart J*. 2008;30(1):6–15.
2. Wierzbicki AS. Homocysteine and cardiovascular disease: a review of the evidence. *Diab Vasc Dis Res*. 2007;p. 143.
3. Linton MRF, Yancey PG, Davies SS. The role of lipids and lipoproteins in atherosclerosis. In: Feingold KR, Anawalt B, Boyce A, editors. Endotext. Dartmouth (MA): MDText.com, Inc; 2000. Available from: https://www.ncbi.nlm.nih.gov/books/NBK343489.
4. Obeid R, Herrmann W. Homocysteine and lipids: S-Adenosyl methionine as a key intermediate. *FEBS Lett*. 2009;583(8):1215–25.
5. Barter PJ, Rye KA. Homocysteine and Cardiovascular Disease. *Circ Res*. 2006;99(6):565–6.
6. Liao D, Tan H, Hui R. Hyperhomocysteinemia decreases circulating high-density lipoprotein by inhibiting apolipoprotein A-I Protein synthesis and enhancing HDL cholesterol clearance. *Circ Res*. 2006;99(6):598–606.
7. Watanabe M, Osada J, Aratani Y, Kluckman K, Reddick R, Malinow MR, et al. Mice deficient in cystathionine beta-synthase: animal models for mild and severe homocyst(e)inemia. *Proc Natl Acad Sci*. 1995;92(5):1585–9.
8. Frauscher G, Karnaukhova E, Muehl A, Hoeger H, Lubec B. Oral administration of homocysteine leads to increased plasma triglycerides and homocysteic acid — additional mechanisms in homocysteine induced endothelial damage? *Life Sci*. 1995;57(8):813–7.
9. Refsum H, Yajnik CS, Gadkari M, Schneede J, Vollset SE, Örning L, et al. Hyperhomocysteinemia and elevated methylmalonic acid indicate a high prevalence of cobalamin deficiency in Asian Indians. *Am J Clin Nutr*. 2001;74(2):233–41.
10. Durrington P. Dyslipidaemia. *Lancet*. 2003;362(9385):717–31.
11. Ubbink JB, Vermaak WJH, Bennett JM, Becker PJ, Staden DA, Bissbort S. The prevalence of homocysteinemia and hypercholesterolemia in angiographically defined coronary heart disease. *Klin Wochenschr*. 1991;69(12):527–34.
12. Genest JJ, McNamara JR, Salem DN, Wilson PWF, Schaefer EJ, Malinow MR. Plasma homocyst(e)ine levels in men with premature coronary artery disease. *J Am Coll Cardiol* . 1990;16(5):1114–9.
13. Mahalle N, Kulkarni MV, Garg MK, Naik SS. Vitamin B12 deficiency and hyperhomocysteinemia as correlates of cardiovascular risk factors in Indian subjects with coronary artery disease. *J Cardiol*. 2013;61(4):289–94.
14. Hirche F, Schröder A, Knoth B, Stangl GI, Eder K. Methionine-Induced Elevation of Plasma Homocysteine Concentration Is Associated with an Increase of Plasma Cholesterol in Adult Rats. *Ann Nutr Metab*. 2006;50(2):139–46.
15. Bardagjy AS, Steinberg FM. Relationship Between HDL Functional Characteristics and Cardiovascular Health and Potential Impact of Dietary Patterns: A Narrative Review. *Nutr*. 2019;11(6):1231.
16. Kosmas CE, Martinez I, Sourlas A, Bouza KV, Campos FN, Torres V, et al. High-density lipoprotein (HDL) functionality and its relevance to atherosclerotic cardiovascular disease. *Drugs Context*. 2018;7. doi:10.7573/dic.212525.
17. Ali KM, Wonnerth A, Huber K, Wojta J. Cardiovascular disease risk reduction by raising HDL cholesterol - current therapies and future opportunities. *Br J Pharmacol*. 2012;167(6):1177–94.
18. Yadav AS, Bhagwat VR, Rathod IM. Relationship of plasma homocysteine with lipid profile parameters in ischemic heart disease. *Indian J Clin Biochem*. 2006;21(1):106–10.
19. Momin M, Jia J, Fan F, Li J, Dou J, Chen D, et al. Relationship between plasma homocysteine level and lipid profiles in a community-based Chinese population. *Lipids Health Dis*. 2017;16(1):54.
20. Ji C, Kaplowitz N. Hyperhomocysteinemia, endoplasmic reticulum stress, and alcoholic liver injury. *World J Gastroenterol*. 2004;10(12):1699–1708.
21. Tabaei S, Tabaee SS. DNA methylation abnormalities in atherosclerosis. *Artif Cells Nanomed Biotechnol*. 2019;47(1):2031–41.

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