J Cardiovasc Disease Res., 2017; 8(2): 46-49
A Multifaceted Peer Reviewed Journal in the field of Cardiology
www.jcdronline.org | www.journalonweb.com/jcdr

Original Article

Homocysteine-Is there any role in Coronary Heart Disease?

Ghosh Shanoli[1], Roy Sanchita[2], Kumar Soumitra[3], Pal Pritha[1], Dutta Atreyee[1], Halder Ajanta[1]*

[1]Department of Genetics, Vivekananda Institute of Medical Sciences, Ramakrishna Mission Seva Pratishthan, 99 Sarat Bose Road, Kolkata, West Bengal, INDIA.
[2]Department of Anatomy, Institute of Post-Graduate Medical Education and Research, 244 A.J.C. Bose Road, Kolkata, West Bengal, INDIA.
[3]Department of Medicine, Vivekananda Institute of Medical Sciences, Ramakrishna Mission Seva Pratishthan, 99 Sarat Bose Road, West Bengal, INDIA.

ABSTRACT

Purpose: To test the hypothesis that the coronary heart disease is associated with high serum homocysteine and low vitamin B-12, and folic acid concentrations. **Methods:** In this population-based case control study, 116 coronary artery diseases with appropriate ECG and/or Echo findings and 96 controls without abnormal ECG and/or Echo findings were selected as the study population. **Results:** The mean value of serum homocysteine levels of the cases (16.88 µ mol/L ± 10.84) was not significantly higher than the values of the control group (14.18 ± 4.19 µ mol/L) (p=0.02). The mean value of folic acid levels was lower both in male and female cases than that in controls but this difference was also not statistically significant (p= 0.51& 0.05).The mean B12 level also showing higher level in male and female cases but not significantly higher than those of their control group (p=0.48 & 0.62). Serum lipid profile [Total Cholesterol TCHL, Low density lipoproteins (LDL), High density lipoproteins (HDL), Triglyceride (TG)] was also found to be inadequate as a potential predictive risk factor in this particular population. **Conclusion:** These results do not support the hypothesis that coronary heart disease is related to high serum homocysteine concentration. The results are not conclusive due to inability to adequately control for potential confounders as well as inadequate sample size.
Key words: Coronary heart disease, Homocysteine, Vitamin B12, Folic acid, Lipid profile.

Correspondence
Dr. Ajanta Halder,

Associate Professor, Department of Genetics, Vivekananda Institute of Medical Sciences, Ramakrishna Mission Seva Pratishthan, 99 Sarat Bose Road, West Bengal, INDIA.
Mobile No: +91 9830196735
E-mail address: ajantahaldar@yahoo.com

Submission Date: 19-08-2016;
Revision Date: 25-01-2017;
Accepted Date: 28-02-2017.
DOI : 10.5530/jcdr.2017.2.11

INTRODUCTION

Cardiovascular disease (CVD) is an important cause of mortality and morbity in India and is reported to have a high prevalence of premature CVD. Even different surveys indicate substantial regional variations in CVD prevalence and mortality rates. Sometimes traditional risk factors like smoking, hypertension, dyslipidemia and non-modifiable risk factors such as age, sex, and family history fail to explain this excess occurrence of CVD. Therefore, many emerging risk factors have been investigated, and among these, elevated plasma homocysteine level (hyper-homocysteinemia) is of particular interest. Homocysteine is a Sulphur-containing non-protein α-amino acid that results as an intermediary product of the methionine degradation pathway. It is not a dietary constituent and its sole source is methionine. The normal concentration of homocysteine in plasma is 5 – 15 µ mol/L. Typically a level less than 15 µ mol/L is considered normal, between 15 – 18 µ mol/L is considered as mildly elevated, between 19 – 60 µ mol/L as moderately elevated and > 60 µ mol/L as severely elevated.[1] Folate and vitamin B_{12} are essential components for the metabolic pathways for decreasing the serum homocysteine concentrations which occurs through remethylation to methionine or trans-sulfuration to cysteine. In this metabolic pathway the enzyme methylene-tetrahydro-folate-reductase (MTHFR) is responsible for the reduction of 5,10-methylene-THF to 5-methyl-THF, where vitamin B_{12} acts as a co-factor.[2] Hyperhomocysteinemia causes increased oxidation of LDL side chains, leading to increased free radical formation which in turn may cause endothelial injury initiating the atherosclerosis.[3] Increased homocysteine level is also reported to cause vascular spasm by impairing the production of endothelial derived relaxing factor (E.D. R.F) and interfere with the vasodilatory and antithrombotic function of nitric oxide.[4] Life style, age, gender, having another chronic disease, vitamin deficiencies and some drugs may increase the levels of homocysteine concentrations. On the contrary, Vitamin B fortification and supplementation (especially vitamin B-12) may decrease the serum homocysteine levels.[5,6] Hyperhomocysteinemia can be present as a cardiovascular risk factor depending on the geographic population studied; thus, the increased risk due to hyperhomocysteinemia seems to be different in Indians as compared to Europeans.[7] Low vitamin B_{12} concentration and hyperhomocysteinemia are common in Indian, particularly in vegetarians and urban residents.[8] There are few studies about this relationship in Eastern Indian populations; therefore, this study aims to determine the relationship between Vit B_{12}, folate , homocysteine and the risk of CAD in this population. In this population-based case control study of adults aged 35-64 years we aimed; 1. To determine serum vitamin B-12, homocysteine and folic acid concentrations among individuals with ischemic findings in their ECGs and echocardiographies (i.e., cases), 2. To determine serum vitamin B-12, homocysteine and folic acid concentrations among individuals without ischemic findings in their ECGs and echocardiographies (i.e., controls), 3. To assess the relationship between the coronary heart diseases and serum vitamin B-12, homocysteine and folic acid concentrations in the study population, comparing cases and controls.

MATERIALS AND METHODS

A total of 116 patients, 94 males and 22 females, clinically diagnosed cases with coronary artery disease (CAD) were selected from the Cardiology out Patient Department (OPD), Ward and ICU of Ramakrishna Mission Seva Pratishthan. Vivekananda Institute of Medical Sciences (RKMSP, VIMS) and all of them were ECG and/ or ECHO cardiograph confirmed CAD patients, <65 yr of age. Patients out of the age range or with history of coronary heart disease resulting from the use of drugs such as carbamazepine, amphetamines, estrogens, or from cocaine consumption, or who were under current or chronic use of certain drugs that influence homocysteine levels, such as methotrexate, trimethoprim, cholestyramine and cyclosporine were excluded. Patients with chronic or acute renal insufficiency, diabetes mellitus, hypothyroidism, and other forms of atherosclerosis or chronic heart failure were also excluded. Controls were 96 (69 males and 27 females) subjects treated in the outpatient clinic of the Hospital, who had no cardiovascular disease or other chronic diseases such as renal failure. The same exclusion criteria for the cases were also applied to the control subjects .Controls were age- and sex matched healthy individuals included in study. Clinical details of cas-

es were obtained after examination in consultation with attending cardiologist. Detailed history of their clinical, lifestyle, and socioeconomic conditions were taken up in a proper questionnaire under their proper consent. Details of type of cardiac problems along with ECG, ECHO Cardiograph findings, blood pressure, lipid profile, history of smoking, hypertension etc. were also recorded in addition. The protocol of present study was approved by ethics committee of VIMS, RKMSP.

Laboratory assays: Venous blood samples from the cases and controls were obtained and plasma levels of lipid profile, homocysteine, vitamin B_{12} and folic acid were assayed by the standard procedure. Normal range of homocysteine levels was 5-15 µ mol/l. Folic acid levels were considered normal between 6 to17 ng/ml, and those of vitamin B_{12} were considered normal between 187 to 900 pg/ml.[13] Hypercholesterolemia was defined as cholesterol > 200 mg/dl.

Statistical analysis

Statistic version 6 (Tulsa, Oklahoma: Stat Soft Inc., 2001) and Graph Pad Prism version 5 (San Diego, California: Graph Pad Software Inc., 2007) were used for data entry and analysis. Analyses included frequency and percent distributions, calculations of means, standard deviations, medians, and percentiles and standard error. The mean and standard deviation (SD) and median of homocysteine, lipid profile, folic acid, and vitamin B_{12} levels of the different groups were measured. As potential confounders current smoking daily or most days, age, veg/non-veg dietary habits, hypertension were taken into consideration. All the variables were tested for normal distribution by Kolmogorov-Smirnoff goodness-of-fit test. .In our study population Lipid profile and age are normally distributed but Hct, B_{12} and Folate are not. Comparison of normally distributed numerical variables between groups were done by One-way ANOVA followed by post hoc comparison using Tukey's test . For variables with non-normal distribution the Kruskal-Wallis ANOVA was performed followed by post hoc comparison using Dunn's test .p value was considered to be significant if <.001. The Correlations between lipid profile parameters and Hct, B_{12}, Folate were performed between the groups, using spearman's test for continuous variables(r value >0.3 cosidered to be significant).

Ethical considerations

The study was revised and approved by the Institutional Ethics Committee and an informed consent was signed by the subjects who agreed to participate in the study.

RESULT

A total number of 212 subjects (116 cases, 96 controls) were enrolled for this study. The basic characteristics of the cases and control population which are relevant for our study are depicted in Table 1. No significant difference was noted between the age and sex distribution, dietary habits and prevalence of hypertension among case and control group in the study population. Prevalence of smoking is significantly high in male cases than controls, no females both in cases and controls were found to be smokers. Prevalence of diabetes was also found to be significantly (p<0.001) high in cases than controls.

The descriptive statistics of different parameters like lipid profile, homocysteine, folate and vitamin B_{12} are shown in Table 2. In our study except the HDL level all other lipid profile parameters didn't show any significant differences among cases and controls. HDL level were significantly low among male cases compared to the levels in control group (p value < 0.001), though in female we didn't find this difference among cases and controls.

The mean plasma level of homocysteine in patients (16.88 ± 10.84µmol/lit) was higher than the control group (14.18 ± 4.19 µmol/lit) but not statistically significant (p=0.02) though in 50% of cases the homocysteine level is above the normal range (>15.1 µmol/lit) whereas this is only 28.4% in cases of controls. Considering the sex factor the mean value of serum homocysteine levels of the male and female cases were also slightly but not significantly higher than those of the corresponding values of control group (p= 0.12 and 0.64 respectively). The mean value of folic acid levels was lower both in male and female cases than that in controls but this difference was also not statistically significant (p= 0.51& 0.05). The mean B_{12} level also showing higher level in male and female cases but not significantly higher than those of their control group (p=0.48 & 0.62).

Table 1: Basic characteristics of study population

Characteristics	Cases		controls	
	M	F	M	F
Age(yrs)	55.66± 6.86	56.23±5.42	54.97±6.45	57.30±4.14
Sex(%)	81.03	18.97	71.88	28.13
Smoking (%)	64.39	---	59.42	---
Diabetes (%)	50	42.6	38.5	33.3
Hypertension(%)	58	63.9	52	59.2
Diet (vegetarian)%	26.6	18.8	28.49	23.3

Correlation between plasma Hcy, FA and B_{12} were also investigated. In case of male and female CAD patient's homocysteine has strong negative correlation with FA(r value for male and female -0.32 and -0.66 respectively) and B_{12} level(r value for male and female -0.39 and -0.58 respectively).

DISCUSSION

The number of females within the CAD+ group were small due to the lesser number of women qualified for the study; in addition this may provide some evidence that females may be at lower risk of CAD compared to males in this Eastern Indian population similar to what was found in western populations. Cigarette smoking is known to increase the risk of CAD. Our data show that smokers were more prevalent in the CAD+ group. Diabetes is well-known to have strong relationship with CAD. In this study, diabetes was found to be dominant in CAD positive group. Diabetes may influence the metabolic syndrome, lipid abnormalities and atherosclerosis, all of which may lead to CAD as this was previously reported in Indian population.

Hypertension was equally distributed in both CAD+ and CAD- groups, but in lesser proportion compared to the non-hypertensive subjects within each group. This finding does not provide strong evidence to support the role of hypertension in the etiology of CAD in this population, which differs from what was found in other populations. In addition, hypertension may or may not play a major role in development of CAD in this population and could possibly be secondarily or non-causally present within the subjects with heart disease.

Many population based prospective studies has been conducted to evaluate the impact of dyslipidemia on CVD.[9-12] Several studies have reported varying prevalence and type of dyslipidemia from different regions of India.[9,13-17] These variations can be explained by differences in the study population with respect to age and sex distribution, inclusion of patients with CVD and population or hospital-based study. We have found HDL level were significantly low among male cases compared to the levels in control group (p value < 0.001)but found no significant differences in TG, TC and non-HDL level among cases and controls suggesting that these levels have less predictive power on the incidence of CHD in this Eastern Indian population which differs from the studies done in the

Table 2: Descriptive statistics of different parameters

Parameters		Group	sex	Plasma level			
			Mean	SD	median	SE	
Lipid profile	TG	cases	M	147.61	69.28	150.00	7.15
			F	145.14	21.72	152.0	4.63
		Controls	M	126.58	47.36	123.0	5.70
			F	122.70	39.32	125.0	7.57
	TC	cases	M	157.76	42.46	154.00	4.38
			F	160.05	44.81	149.0	9.55
		controls	M	148.65	40.36	145.0	4.86
			F	147.70	41.28	143	7.94
	HDL	cases	M	36.69	9.31	35	0.96
			F	45.36	10.08	43.5	2.15
		controls	M	44.58	11.01	45	1.33
			F	43.33	11.61	42	2.23
	nHDL	cases	M	112.43	35.48	108.00	3.66
			F	109.05	39.12	103.00	8.34
		controls	M	109.78	40.30	109.0	4.85
			F	105.41	36.72	100.0	7.07
Homocysteine (µmol/L)		cases	Male	17.70	11.63	15.44	1.20
			Female	13.38	5.34	11.00	1.14
		control	Male	14.33	4.08	14.2	0.41
			Female	13.80	4.51	12.36	0.87
Folic Acid (ng/ml)		patients	Male	9.95	7.46	8.4	0.77
			Female	10.26	6.45	8.55	1.37
		controls	Male	13.21	12.52	8.8	1.51
			Female	28.43	31.03	20	5.97
B_{12} level (pg/ml)		cases	Male	267.6	151.3	212.5	15.60
			Female	285.7	108.7	261	23.18
		controls	Male	262.6	157.3	200	18.93
			Female	242.3	144.7	190	27.86

past in this country. In favour of this finding, some recent research has provided more evidence suggesting that smaller LDL particles are more atherogenic and hence more closely associated with CHD, regardless of total or LDL cholesterol levels.[18] In our study it is interesting that even in CHD patients the levels of the blood lipids are lower which definitely indicates a need to extend the study on a larger population to determine the normal range in this region and implication of appropriate interventions to maintain the serum total cholesterol, triglyceride and non-HDL levels within a prescribed normal range.

Since 1950, various studies have been conducted about the risk factors of coronary heart diseases. Recently, scientists have been studying on the new independent risk factors like FA, Vit B_{12}, and homocysteine. A review of data from a cross-sectional and retrospective case control study published by Hankey GJ *et al*[19] demonstrated that the homocysteine level in CAD ranged from 11.3-16.7 µ mol/L while the control group was slightly lower 8.9-14.7 µ mol/L. The present data is concordant with other investigators; however, the present study could not prove a significant difference in means of the homocysteine level betweeen these two groups as in many previous reports. Although there is a great controversy regarding the relationship of homocyteine with coronary artery disease, as results of some researchers have shown a positive correlation[20,21] and some a negative correlation.[22-24]

Regarding Vitamin B_{12} and folate, both study groups showed comparable Vitamin B_{12} and folate level. In cases of CAD patients of this population only 14.60% and 12.93 % are having B_{12} and FA level lower than the reference range. Freqent use of vitamin supplementation by the general practitioner in this age group may be responsible for this favorable level of serum vitamin B_{12} and FA .Dietary practices of consumption of more non-vegetarian foods of this population may be contributed for normal range of Vit B_{12} level. We have found strong negative correlation between homocysteine, FA and B_{12} level which support other studies that increasing circulating folate and/or vitamin B_{12} concentrations may reverse the elevated Hct. Vitamin B_{12} and folate levels can be improved by either in-

creasing intake or treating inefficient absorption due to gastrointestinal disorders or pharmaceutical side effects.

Our study has some limitations. One of the most important limitations of this study is about the diagnosis criterion. We determined the CVS diseases according to the ECG and echocardiographic findings. However, similar ECG changes might have been due to hypertension, pericardial infections and digital usage as well as myocardial infarction. This should be taken into consideration while interpreting the results. On the other hand, in such a population-based study, ECG seems to be a cost effective method for it is a practical, economic and easy measurement tool. Absence of any ischemic changes in ECG was considered in the study as "absence of coronary disease". Yet, atherosclerotic process increases with age, even in the absence of clinical coronary heart disease.

Another limitation of our study is the smaller sample size of female participants in comparison to males. This limitation causes our inability to precisely evaluate the role of hyperhomocysteinemia in young females with CAD. Cases and controls were selected among the participants of a population based study. A prospective cohort study is a more appropriate study in this case which can be designed to prevent the bias like 'premature deaths' and 'selective survival' but they are expensive and require long term follow up.

CONCLUSSION

In conclusion, the findings of this study would suggest that the assessment of CHD risk by measurement of a traditional serum lipid profile (TC, LDL-cholesterol, HDL-cholesterol, TG) may be inadequate in this particular population. This research finding also suggests that the measurements of homocysteine are insufficient to discriminate between individuals at risk of CHD and those who are not at risk.

ACKNOWLEDGEMENT

The authors would like to thank Dr (Prof) Abhijit Hazra for his guidance in statistical analysis and Secretary of Ramkrishna Mission Seva Pratisthan,Vivekananda Institute of Medical Sciences, kolkata for his support.

CONFLICT OF INTEREST

None

ABBREVIATIONS USED

TG: triglyceride; TC: total cholesterol; HDL: high density lipoprotein; nHDL: non high density lipo-protein.

REFERENCES

1. 1. Selhub J *et al.* Association between plasma homocysteine concentration and carotid artery stenosis. N Engl J Med. 1995;332(5):286-91. https://doi.org/10.1056/NEJM199502023320502; PMid:7816063.
2. Boushey CJ, Beresford SA, Ommenn GSS, Fallahi F, Salarifar M, Davoodi G, *et al.*Tehran Heart Center, homocysteine, vitamin B_{12} and folate levels in premature coronary artery disease. BMC Cardiovasc Disord. 2006;6.
3. Thmbyarajah J and Towned JN. Homocysteine and atherosclerosis: Mechanism for injury. Europian Heart Journal. 2000;21.
4. Tsuda K, Nishio I. Serum Homocysteine and endothelial Dysfunction in Circulatory Disorders in Women. Circulation. 2004:27;110(4)e37.
5. Selhub J, Jaques PF, Wilson P, Rush D, Rosenberg IH. Vitamin status and intake as primary determinants of homocysteinemia in and eldery population. JAMA. 1993;270(22):2693-8. https://doi.org/10.1001/jama.1993.03510220049033: https://doi.org/10.1001/jama.270.22.2693; PMid:8133587.
6. Schnyder G, Roffi M, Flammer Y. Effect of homocysteine – lowering therapy with folic - acid, vitamin B(12), and Vitamin B(6) on clinical outcome after percutaneous coronary. Intervention: the Swiss Herat study a randomized controlled trial. JAMA. 2002;288.
7. Sastry BKS, Indira N, Anand B, Kedarnath, Surya PB, Soma RB. A case–control study of plasma homocysteine levels in South Indians with and without coronary artery disease. Indian Heart J. 2001;53.
8. Yajnik CS, Deshpande SS, Lubree HG, Naik SS, Bhat DS, Uradey BS, *et al.* Vitamin B_{12} deficiency and hyperhomocysteinemia in rural and urban Indians. J Assoc Physicians India. 54;2006.
9. Joshi P, Islam S, Pais P, Reddy S, Dorairaj P, Kazmi K, *et al.* Risk factors for early myocardial infarction in South Asians compared with individuals in other countries. JAMA. 2007;297(3);286-94. https://doi.org/10.1001/jama.297.3.286; PMid:17227980.
10. Enas EA, Mehta J. Malignant coronary artery disease in young Asian Indians: thoughts on pathogenesis, prevention, and therapy. Clinical cardiology. 1995;18(3):131-5. https://doi.org/10.1002/clc.4960180305.
11. Labreuche J, Touboul PJ, Amarenco P. Plasma triglyceride levels and risk of stroke and carotid atherosclerosis: A systematic review of the epidemiological studies. Atherosclerosis. 2009;203(2):331-45. https://doi.org/10.1016/j.atherosclerosis.2008.08.040; PMid:18954872.
12. Bhalodkar NC, Blum S, Rana T, Bhalodkar A, Kitchappa R, Kim KS, *et al.* Comparison of levels of large and small high-density lipoprotein cholesterol in Asian Indian men compared with Caucasian men in the Framingham offspring study. Am J Cardiol. 2004;94(12)1561-3. https://doi.org/10.1016/j.amjcard.2004.08.040; PMid:15589018.
13. Karthikeyan G, Teo KK, Islam S, McQueen MJ, Pais P, Wang X, *et al.* Lipid profile, plasma apolipoproteins, and risk of a first myocardial infarction among Asians: An analysis from the INTERHEART Study. J Am Coll Cardiol. 2009;53(3):244-53. https://doi.org/10.1016/j.jacc.2008.09.041 ; PMid:19147041.
14. Gupta R, Vasisht S, Bahl VK, Wasir HS. Correlation of lipoprotein (a) to angiographically defined coronary artery disease in Indians. Int J Cardiol. 1996;57(3):265-70. https://doi.org/10.1016/S0167-5273(96)02800-8.
15. Mohan V, Deepa R, Rani SS, Premalatha G. Chennai Urban Population Study (CUPS No. 5) Prevalence of coronary artery disease and its relationship to lipids in a selected population in South India: The Chennai urban population study (CUPS No. 5) J Am Coll Cardiol. 2001;38(3):682-7. https://doi.org/10.1016/S0735-1097(01)01415-2.
16. Arca M, Montali A, Valiante S, Campagna F, Pigna G, Paoletti V, *et al.* Usefulness of atherogenic dyslipidemia for predicting cardiovascular risk in patients with angiographically defined coronary artery disease. Am J Cardiol. 2007;100(10):1511-6. https://doi.org/10.1016/j.amjcard.2007.06.049 ; PMid:17996510.
17. Sharma SB, Garg S, Veerwal A, Dwivedi S. hs-CRP and oxidative stress in young CAD patients: A pilot study. Indian J Clin Biochem. 2008;23(4):334-6. https://doi.org/10.1007/s12291-008-0073-8 ; PMid:23105781 PMCid:PMC3453131.
18. Guazzeli R, Fatini C, Piazzini M, *et al.* Lipoprotein (a): genetic marker of precociousmyocardial infarction. Ann Ital Med Int. 1996;11.
19. Hankey GJ, Eikeboom JW. Homocysteine and vascular disease. Lancet. 1999;354(9176):407-13. https://doi.org/10.1016/S0140-6736(98)11058-9.
20. Yang Q, Lorenzo *et al.* Improvement in stroke mortality in Canada and USA. Circulation 2006;113(10):1335-43. https://doi.org/10.1161/CIRCULATIONAHA.105.570846 ; PMid:16534029.
21. Sabahat T, Lale T, Gülşen H, Banu Ç, Dilek A. A case control study assessing the relationship between coronary heart disease and serum vitamin B_{12}, homocysteine and folic acid levels. Gazi Medical journal 2006;17(2).
22. Willett W. Diet and Coronary Heart Diseases in Nutritional Epidemiology. Second Edition. New York-Oxford University Press, 1998.
23. Shojaie M, Naghshvar F, Izadi HR, Eshraghian A , Pourahmad M. Homocysteine level in Iranian patients with premature acute myocardial infarction. Clin Med J. 2009;122(16):1952-4.
24. Lee M, Hong KS, Chang SC, Saver JL. Effiacy of homocysteine lowering therapy with Folic acid in stroke prevention: a meta-analysis. Stroke. 2010;41(6):1205-12. https://doi.org/10.1161/STROKEAHA.109.573410; PMid:20413740 PMCid:PMC2909661.

Cite this article: Shanoli G, Sanchita R, Soumitra K, Pritha P, Atreyee D, Ajanta H. Homocysteine-Is there any role in Coronary Heart Disease?. Journal of Cardiovascular Disease Research. 2017;8(2):46-49.