ORIGINAL ARTICLE

CORRELATION OF SERUM HOMOCYSTEINE LEVELS AND LIPID PROFILE IN CORONARY HEART DISEASE

Ayesha Naureen Awan, Humaira Imtiaz*, Alruba Taimoor, Aamir Nazir**, Malik Mahmood Ahmed***, Sarmud Latif Awan†**

Department of Biochemistry, Anatomy, **Physiology, Ayub Medical College, Abbottabad, ***Department of Pathology, †Surgery, AJK Medical College, Muzaffarabad, Pakistan

Background: Coronary heart disease occurs when cholesterol accumulates on the artery walls, creating plaques. Reduced blood flow occurs when one or more of these arteries become partially or completely blocked. Homocysteine is an intermediate formed during the catabolism of methionine. Development of atherosclerotic change is common feature in patients with hyperhomocysteinemia. This study was conducted to access the relationship of Hcy, lipid profile and CHD in our population. **Methods:** This cross-sectional analytical study was carried out at Biochemistry Department, Ayub Medical College, Abbottabad. Eighty subjects were divided into 2 groups. Cases: 40 patients were confirmed MI patients. Controls: 40 were age and gender matching healthy individuals. Lipid profile and serum total Hcy were measured. **Results:** The mean age of the cases was 59.68±8.06 years. The controls were 58.93±6.93 years. Mean tHcy level of the cases was 17.15±4.45 µmol/l while that of controls was 12.20±2.53 µmol/l ($p<0.001$). In lipid profile TG and VLDL-C showed a statistically significant rise ($p<0.05$) in the cases compared to controls. There were statistically significant differences between HDL-C, LDL-C and TC/HDL-C ratios ($p<0.001$). Mean TG in cases was 166.01±30.03 mg/dl with ($p<0.05$). Similarly the mean VLDL-C in cases was 33.20±6.01 mg/dl and in controls it was 30.48±2.80 mg/dl ($p<0.05$). Mean TC in cases was 212.82±32.48 mg/dl and in controls it was 202.80±17.03 mg/dl ($p>0.05$) which is statistically not significant**. Conclusion:** Plasma tHcy level and deranged lipid profile are powerful predictor value of CHD but they are independent risk factors.

Keywords: Hyperhomocystienemia, lipid profile, coronary heart disease

Pak J Physiol 2019;15(2):56–60

INTRODUCTION

Homocysteine (Hcy) is an intermediate formed during the catabolism of sulfur containing essential amino acid, methionine.[1,2] Methionine can be resynthesized from Hcy by methionine synthase reaction requiring folate and B_{12}.[3] Methyl cobalamin is a coenzyme in the combined conversion of Hcy to methionine and of methyltetrahydrofolate to tetrahydrofolate. Conversion of Hcy to methionine via methioine synthase is the only reaction in human body that requires both folate and B_{12}. Without adequate amounts of folate, vitamin B_{12} and $B_{6,}$ Hcy cannot be converted into useful amino acids and thus it is built up in the blood.[4] Hcy concentration rises progressively with age in men and women, likely causes include clinical or sub clinical folate and B_{12} deficiencies.[2,5] Smoking, excessive coffee consumption and lack of exercise are associated with elevation in Hcy as well.[6]

Some genetic defects of enzymes involved in the metabolism of methionine like Cystathionine beta synthase deficiency, impaired Methionine synthase activity and Methylene tetra hydro folate reductase (MTHFR) Deficiency are also associated with high levels of Hcy.[7,8] Similarly some diseases, drugs and Chronic high alcohol consumption are notorious folic acid inhibitors.[7,9,10]

In the mid 1960s a Harvard physician, Dr Kilmer Mc Cully, suggested an association between raised Hcy levels and massive generalized atherosclerosis.[11] Recent studies have confirmed that atherosclerotic changes and thrombo-embolism are common features in patients with heritable defects of different enzymes involved in methionine catabolism.[12] Hence a positive correlation between Hcy and atherosclerosis was postulated.[1,11] Individuals with elevated levels of Hcy tend to have higher incidence of cardiovascular disease.[12] Hcy may cause vascular events which may contribute to heart disease by the epithelial cell injury, effect coagulation proteins and enhance pro-coagulant activity alter platelet function, modify LDL and may stimulate smooth muscle cell proliferation.[13,14]

Coronary heart disease (CHD) is a leading cause of morbidity and mortality in many countries worldwide including Pakistan and is estimated that it will be the single largest cause of disease burden. CHD can develop at any age.[15,16] Coronary heart disease occurs when cholesterol accumulates on the artery walls, creating plaques. Reduced blood flow occurs when one or more of these arteries become partially or completely blocked.[17] The lipid profile is a group of tests that are often done together to identify the risk of heart disease. These tests are good indicators of whether

someone is likely to have a heart attack or stroke caused by the blockage of blood vessels or hardening of the arteries. The high levels of cholesterol in blood circulation are strongly associated with progression of heart disease.[18,19] but most patients with Myocardial Infarction have normal cholesterol levels. On careful literature search, no local study about the relationship of Hcy, lipid profile and CHD in Pakistani population was traceable. There is a need to recognize Hcy as a potential risk factor in local population with the help of local evidence.

MATERIAL AND METHODS

This cross-sectional analytical study was carried out at the Department of Biochemistry, Hazara University Manshera, and Ayub Medical College, Abbottabad.

A total of 80 subjects were included in this study by Convenience (non-probability) sampling technique. They were divided into 2 groups.

- **Cases:** 40 patients were confirmed patients of MI. Patients who had confirmed Myocardial Infarction (MI) diagnosed by the cardiologist, coming for routine follow up (first visit) in the Cardiology OPD after the acute attack
- **Controls:** 40 were matching healthy individuals in age and gender.

Patients with known history of liver, kidneys or taking any of the known drugs that affect the liver functions such as isoniazide, Phenytoin, Largectil, chlorpromazine etc. were excluded from the study. The subjects taking Folic acid therapy or multivitamins were also excluded from the study.

After Informed consent the Demographic data including age, gender, dietary habits, height and weight was documented in proforma. Blood pressure was taken in sitting posture. Ten (10) mL blood was collected from each subject after an overnight fast of 10−12 hours (as advised by the cardiologist) with their due permission under antiseptic technique using a disposable syringe. Samples were centrifuged in a bench top centrifuge at 4,000 revolutions per minute for 10 minutes to get serum.

Lipid profile was performed immediately, for which serum lipid profile was estimated using different kits supplied by the Randox laboratories UK (Kit Cat. No. CH207 for serum total cholesterol, Kit Cat. No. CH204 for HDL-C and kit Cat. No. TR 210 for serum Triglycerides). LDL and VLDL were estimated according to formula proposed by Wilson.

Serum for Total Hcy was stored in Eppendorf tube at -20 ºC in a refrigerator; and was measured within a week by IMx homocysteine assay (FPIA Kit catalog No. B3D390, 33-0781/R5). Data was entered into SPSS-22 for analysis. Reference range of serum Hcy is 4–15 µmol/l.

RESULTS

A total of 80 subjects selected as per inclusion and exclusion criteria were investigated. Out of these 40 were cases that had a positive history of CHD and the rest of them were controls (40) that did not have the history of CHD. The mean age of the cases was 59.68±8.06 (30–70) years and that of the controls was 58.93±6.93 (48–76) years (Table-1). Twenty-three (57.5%) cases and 22 (55%) controls were males, and 17 (42.5%) cases and 18 (45%) controls were females.

Twenty-four (60%) cases and 25 (62.5%) of the controls were on mixed diet. Nine (22.5%) cases and 8 (20%) controls were predominantly on meat diet, while 7 (17.5%) cases and 7 (17.5%) controls were predominantly on vegetable diet. The average weight of the cases was 75.80±12.37 Kg while that of the controls was 58.5±11.73 Kg. Mean height of the cases was 162.62±6.97 Cm and of controls was 163.75±8.51 Cm.

Mean tHcy level of the cases was 17.15±4.45 µmol/l while that of controls was 12.20±2.53 µmol/l. There is a statistically significant difference between cases and controls with respect to Hcy levels (p<0.001). Hcy levels by age groups are tabulated in Table-2.

Table-3 and 4 shows the comparison of the lipid profile in cases and controls. The different parameters of lipid profile which are compared include the TG, TC, HDL-C, LDL-C levels and TC/HDL-C ratio.

Mean TG in cases was 166.01±30.03 mg/dl with (p<0.05). Similarly the mean VLDL-C in cases was 33.20±6.01 mg/dl and in controls was 30.48±2.80 mg/dl with (p<0.05). While the mean TC in cases was 212.82±32.47 mg/dl and in controls was 202.80±17.03 mg/dl with (p<0.088) which is not statistically not significant.

In contrast to the afore mentioned, the levels of HDL-C in cases were 40.58±3.74 as compared to 58.43±6.21 in control with (p<0.001). Similarly the levels of LDL-C in cases were 138.67±29.08 as compared to 114.79±19.02 in controls with (p<0.001). The TC/HDL-C ratio in cases were 5.27±0.88 as compared to 3.52±0.55 in controls with (p<0.001).

Table-5 shows no statistical significant correlation between serum tHcy levels the parameters of lipid profile with all variables of interests. The variables were distributed along x and y axis to obtain bivariate correlations.

Table-1: Study subjects by age groups (n=40)

Age Groups (in years)	Cases		Controls	
	Numbers	Percent	Numbers	Percent
30–39	1	2.5	0	0
40–49	1	2.5	3	7.5
50–59	16	40.0	21	52.5
60–69	19	47.5	13	32.5
70 & Above	3	7.5	3	7.5
Total	40	100.0	40	100.0

Table-2: Hcy levels by age groups

Age (year)	CASES			CONTROL		
	Number	Mean	SD	Number	Mean	SD
30–39	1	19.200	–	–	–	–
40–49	1	16.000	–	3	11.367	4.3822
50–59	16	16.500	2.6415	21	12.219	1.8891
60–69	19	17.074	5.3845	13	12.031	3.1420
≥70	3	20.867	6.7419	3	13.667	2.5166
Total	40	17.155	4.4471	40	12.202	2.5310

Table-3: Comparison of Total Cholesterol, Triglycerides and VLDL-C in cases and controls

Groups	TG (mg/dl)	TC (mg/dl)	VLDL-C (mg/dl)
Cases (40)	166.01±30.03	212.82±32.48	33.20±6.01
Controls 40)	152.38±13.73	202.80±17.03	30.48±2.80
P-value	<0.05	>0.05 (p=0.088)	<0.05

Table-4: Comparison of HDL-C, LDL-C and TC/HDL-C ratio in cases and controls

Groups	HDL-C (mg/dl)	LDL-C (mg/dl)	TC/HDL-C ratio(mg/dl)
Cases (40)	40.58±3.74	138.67±29.08	5.27±0.88
Controls (40)	58.43±6.21	114.79±19.02	3.52±0.55
P-value	<0.001	<0.001	<0.001

Table-5: Bivariate correlation among variables of interest in cases and controls

Case(n=40)Vs Control(n=40)	x-axis variable (levels)	y-axis variable (levels)	Pearson's *r*	*p*
Case	tHcy	TG	-0.125	0.442
Control	tHcy	TG	-0.066	0.688
Case	tHcy	TC	-0.131	0.422
Control	tHcy	TC	0.189	0.242
Case	tHcy	HDL-C	0.059	0.719
Control	tHcy	HDL-C	-0.010	0.952
Case	tHcy	LDL-C	-0.125	0.443
Control	tHcy	LDL-C	0.147	0.365
Case	tHcy	VLDL-C	-0.125	0.442
Control	tHcy	VLDL-C	-0.066	0.688
Case	tHcy	TC/HDL-C ratio	-0.151	0.352
Control	tHcy	TC/HDL-C ratio	0.139	0.392

DISCUSSION

Atherosclerotic Cardiovascular disease accounts for half of all the premature deaths in men, in the developed as well as in the developing countries.[20] Studies in the last decade have indicated that HHcy is an independent risk factor in the premature development of vascular disease.[21] The principal observation of our study was that the mean of serum tHcy levels found in the CHD cases was significantly higher (17.155±4.44 µmole/L) compared to the age and gender matched healthy controls (12.2±2.53 µmol/L). This finding is in agreement with several studies in the west as well as in Pakistan.[22,23]

In a meta-analysis for articles published from Jan 1966 to Jan 1999, relevant studies were identified by systematic research of the literature and data from 30 prospective or retrospective studies was included. It involved a total of 5,073 ischemic heart disease events and 1,113 stroke events. This meta-analysis suggested elevated Hcy is at the most a modest independent predictor of IHD and stroke risk in healthy population.[24] In another meta-analysis of 27 case-control studies Boushey *et al* found that an increase of 5 µmol/l in basal tHcy level was associated with a 60% increase in the odds of CHD among men and an 80% increase in the odds of CHD among women based on which an increase of 5 µmol/l of serum tHcy was estimated to increase the risk of CAD by as much as 20 mg/dl increase in cholesterol concentration.[25]

The present study also showed that in CHD cases Hcy levels were only moderately elevated. The range of serum tHcy was 8.8–28.6 µmol/l. This finding is also in line with recent observation. This indicates that moderately elevated Hcy level is a risk factor for CHD. None of the cases had Hcy levels more than 100 µmol/l ie severe HHcy. The concentration of Hcy at which the risk begins to increase is not clear but all results show an association between elevated Hcy levels and CHD.[26]

The strong association between deranged lipid profile and CHD has been proved in several studies while the concentrations of HDL-C are inversely associated with the risk of future cardiovascular events as it is a strong negative predictor of CHD.[27,28] The classical function of HDL is to mobilize cholesterol from extrahepatic tissues for delivery to the liver for excretion. HDL plays a key role in this pathway, known as reverse cholesterol transport (RCT).[29] HDL can also transport free cholesterol directly to the liver.[30] Drug induced extremely low levels of cholesterol rich LDL-C (<20 mg/dL) have been shown to be safe and possibly beneficial in short term follow-up.[31,32]

Although Hcy concentration rose progressively with age in men and women, likely causes include clinical and subclinical folate deficiencies.[1] No matter what may be the underlying cause, Hcy is known to play an important role in CHD.[33,34] Similarly lipid profile is also disturbed due to age changes. There are high levels of serum TC, TG, VLDL-C, LDL-C and TC/HDL-C (ratio) while low levels of HDL-C.[35] Attempt was made to establish a correlation between serum tHcy and different parameters of Lipid profile. There was significant increase in serum tHcy levels in the CHD cases and significant increase was observed for serum TC, TG, VLDL-C, LDL-C and TC/HDL-C (ratio) in the CHD cases, while HDL-C levels decline significantly in them as compared to age and gender matched healthy controls. Our study shows negative correlation between TC, TG, VLDL.[36] LDL-C and TC/HDL-C ratio with tHcy and a positive correlation between HDL-C and tHcy.[36]

Although statistically no significant correlation was found between serum tHcy and various parameters of Lipid profile this confirms that elevated plasma tHcy is an 'Independent Risk Factor'[37,38] for atherosclerotic

disease in Pakistani population like the western population as proved by many western studies in addition to other risk factors.

CONCLUSION

Lipid profile is good and affordable indicators of a likely heart attack or stroke caused by the blockage of blood vessels or hardening of the arteries. Plasma tHcy levels are independent risk factor for CHD. Routine screening for elevated Hcy concentrations is not yet recommended but it is advisable for individuals who manifest at herothrombotic disease without its traditional risk factors or who have a family history of premature CHD.

REFERENCES

1. Virdis A, Ghiadoni L, Salvetti G, Versari D, Taddei S, Salvett A. Hyperhomocysteinaemia: is the novel risk factor in hypertention. J Nephrol 2002;15:414–21.
2. Ceperkovic Z. The role of increased levels of homocysteine in the development of cardiovascular diseases. Med Pregl 2006:59(3–4):143–7.
3. Blencowe H, Cousens S, Modell B, Lawn J. Folic acid to reduce neonatal mortality from neural tube disorders. Int J Epidemiol 2010;39(Suppl 1):i110–21.
4. Selhub J, Jacques PF, Wilson PW, Rush D, Rosenberg IH. Vitamin status and intake as primary determinants of homocysteinemia in an elderly population. JAMA 1993;270:2693–8.
5. Moustapha A, Robinson K. Homocysteine an emerging age-related cardiovascular risk factor. Geriatrics 1999;54(4):41,44–6, 49–51.
6. Rozen R. Genetic modulation of homocysteinemia. Semin Thromb Hemost 2000;26:255–61.
7. Lattanzio R, Sampirtro F, Ramoni A, Fattorini A, Brancato R, D'Angelo A. Moderate hyperhcymia and early-onset central retinal vein occlusion. Retina 2006;26(1):65–70.
8. Sharma P, Senthilkumar RD, Brahmachari V, Sundaramoorthy E, Mahajan A, Sharma A, *et al.* Mining literature for a comprehensive pathway analysis: a case study for retrieval of Hcy related genes for genetic and epigenetic studies. Lipids Health Dis 2006;5:1. doi: 10.1186/1476-511X-5-1
9. Malinow MR. Homocyst(e)ine and arterial occlusive disease J Intern Med 1994;236:603–17.
10. Stehouwer CD, Gall MA, Hougaard P, Jakobs C, Parving HH. Plasma homocysteine concentration predicts mortality in non-insulin-dependent diabetic patients with and without albuminuria. Kidney Int 1999;55:308–14.
11. Fodor JG, LeGrand C. Homocysteine: A new coronary heart disease risk factor. University of Ottawa Heart Institute Prevention and Rehabilitation Center: CACR Newsletter; 1999. Retrieved on: 24-2-2003. Available at: http://www.cacr.ca/news/1999/9909fodor.htm.
12. Tofler GH, D'Agostino RB, Jacques PF, Bostom AG, Wilson PW, Lipinska I, *et al*. Association between increased Hcy levels and impaired fibrinolytic potential: Potential mechanism for cardiovascular risk. Thromb Haemost 2002;88:799–804.
13. Genser D, Prachar H, Hauer R, Halbmayer WM, Mlczoch J, Elmadfa I. Homocysteine, folate and vitamin B12 in patients with coronary heart disease. Ann Nutr Metab 2006;50:413–9.
14. Stanger O, Semmelrock HJ, Wonisch W, Bos U, Pabst E, Wascher TC. Effects of folate treatment and homocysteine lowering on resistance vessel reactivity in atherosclerotic subjects. J Pharmacol Exp Ther 2002;303:158–62.
15. Nishtar S. Prevention of coronary heart disease in South Asia. Lancet 2002;360(9338):1015–8.
16. Daphale A, Acharya S, Shukla S. Detection of asymptomatic coronary artery disease (CAD) in newly detected type 2 diabetes mellitus (DM) by exercise treadmill test. Int J Contempor Med Res 2017;4:2269–75.
17. Gupta S, Lakhani KK, Munshi H. A study of risk factors in young patients of acute coronary syndrome. Int J Contempor Med Res 2017;4:2144–7.
18. Nkomo VT, Gardin JM, Skelton TN, Gottdiener JS, Scott CG, Enriquez-Sarano M. Burden of valvular heart diseases: a population based study. Lancet 2006;368:1005–11.
19. Rahilly-Tierney C, Bowman TS, Djoussé L, Sesso HD, Gaziano JM. Change in high-density lipoprotein cholesterol and incident coronary heart disease in apparently healthy male physicians. Am J Cardiol 2008;102:1663–7.
20. Ayub M, Waseem T, Nadeem MA, Irshad H, Khalid AW, Imam SF, *et al*. Risk stratification of patients presenting with first acute myocardial infarction with serum cardiac troponin-T. Pak J Cardiol 1999;10(2–3):54–62.
21. Okada E, Oida K, Tada H, Asazuma K, Eguchi K, Tohda G, *et al*. Hyperhomocysteinemia is a risk factor for coronary arteriosclerosis in Japanese patients with type 2 diabetes. Diabetes Care 1999;22:484–90.
22. Aamir M, Sattar A, Dawood MM, Dilawar M, Ijaz A, Anwar M. Hyperhomocystenimia as a risk factor for ischemic heart disease. J Coll Physicians Surg Pak 2004;14:518–21.
23. Salah uddin M, Karira KA, Motahir AS, Samad A. Serum Hcy in patients with acute myocardial infarction. Pak J Cardiol 2000;11:93–9.
24. Homocyteine Studies Collaboration. Hcy and risk of ischemic heart disease and stroke: a meta-analysis. JAMA 2002;288:2015–22.
25. Boushey CJ, Beresford SA, Omenn GS, Motulsky AG. A quantitative assessment of plasma homocysteine as a risk factor for vascular diseases: probable benefits of increasing folic acid intakes. JAMA 1995;274:1049–57.
26. Kaul S, Zadeh AA, Shah PK. Homocysteine hypothesis for athrothrombotic cardio vascular disease. J Am Coll Cardiol 2006;48:914–23.
27. Sabatine MS, Giugliano RP, Keech AC, Honarpour N, Wiviott SD, Murphy SA, *et al*. Evolocumab and clinical outcomes in patients with cardiovascular disease. N Engl J Med 2017;376:1713–22.
28. Cannon CP, Blazing MA, Giugliano RP, McCagg A, White JA, Theroux P, *et al.* Ezetimibe added to statin therapy after acute coronary syndromes. N Engl J Med 2015;372:2387–97.
29. Phillips MC. Molecular mechanisms of cellular cholesterol efflux. J Biol Chem 2014;289(35):24020–9.
30. Tanigawa H, Billheimer JT, Tohyama J, Fuki IV, Ng DS, Rothblat GH, *et al*. Lecithin: cholesterol acyltransferase expression has minimal effects on macrophage reverse cholesterol transport in vivo. Circulation 2009;120(2):160–9.
31. Writing Committee, Lloyd-Jones DM, Morris PB, Ballantyne CM, Birtcher KK, Daly DD Jr, DePalma SM, *et al*. 2016 ACC Expert Consensus Decision Pathway on the role of non-statin therapies for LDL-cholesterol lowering in the management of atherosclerotic cardiovascular disease risk: A report of the American College of Cardiology Task Force on Clinical Expert Consensus Documents. J Am Coll Cardiol 2016;68:92–125.
32. Watts GF, Ooi EM, Chan DC. Demystifying the management of hypertriglyceridaemia. Nat Rev Cardiol 2013;10:648–61.
33. Obwegeser R, Hohlagschwandtner M, Sinzinger H. Homocysteine —a patho-physiological corner stone in obstetrical and gynaecological disorders. Hum Reprod Update 1999;5(1);64–72.
34. Stoney CM. Plasma homocysteine levels increase in women during psychological stress. Life Sci 1999;64:2359–65.
35. Cai M, Zou Z. Effect of aerobic exercise on blood lipid and glucose in obese or overweight adults: A meta-analysis of randomised controlled trials. Obes Res Clin Pract 2016;10(5):589–602.

36. Somay G, Aliskan T, Erenogla NY. Carotid artery stenosis and homocysteine in ischemic stroke; a case control study. J NeurolSci [Turkish] 2005;22:394–402.
37. American Heart Association Nutrition Committee, Lichtenstein AH, Appel LJ, Brands M, Carnethon M, Daniels S, Franch HA, *et al*. Diet and lifestyle recommendations revision 2006: a scientific statement from the American Heart Association Nutrition Committee. Circulation 2006;114:82–96.
38. Schulz RJ. Homocysteine as a biomarker for cognitive dysfunction in the elderly. Curr Opin Clin Nutr Metab Care 2007;10:718–23.

Address for Correspondence:
Dr. Ayesha Naureen Awan, Associate Professor, Department of Biochemistry, Ayub Medical College, Abbottabad, Pakistan. **Cell:** +92-333-7879702
Email: ana-khyber@gmail.com

Received: 13 Mar 2019 **Reviewed: 7 Jun 2019** **Accepted: 17 Jun 2019**