Correlation of hyper-homocysteinemia with coronary artery disease in absence of conventional risk factors among young adults

Hammad Shah [a,*], Muneeb Ullah Jan [a], Afrasyab Altaf [a], Momin Salahudin [a]

[a] Cardiology Department, Rehman Medical Institute, Peshawar, Pakistan

Objective: Coronary artery disease is major cause of mortality and morbidity. Homocysteine has long been postulated as an underlying factor for atherosclerosis leading to coronary artery disease, yet its role in young patients is uncertain. This study was aimed to analyze the correlation between plasma homocysteine and coronary artery disease among young adults in the absence of conventional risk factors.

Methods: It was a case-control study carried out at Rehman Medical Institute, Peshawar, Pakistan from October 1, 2016, to September 30, 2017. Universal sampling technique was adopted and 158 participants were included. A total of 30 participants were in the control group and 128 were in the patient group, who had moderate to severe stenosis in either single or multiple major coronary arteries on coronary angiography and aged <40 years.

Results: Cases and controls had similar characteristics but differed significantly in serum homocysteine concentration. In the control group, the mean plasma homocysteine concentration of 6.3 (þ2.05) μmol/L and in the patient group a mean plasma homocysteine concentration of 44.5 (þ14.01) μmol/L was observed. All the patients with moderate to severe stenosis in single or major coronary arteries had raised plasma homocysteine concentrations. Among 128 patients, 15 (11.7%) had moderate increase, 109 (85.2%) had intermediate increase, and four (3.1%) had severe increase in plasma homocysteine levels. Single vessel coronary artery disease was observed in 118 (92.2%) patients, whereas 10 (7.8%) had more than one major coronary artery involvement.

Conclusion: Hyper-homocysteinemia has positive correlation with coronary artery disease among young adults in the absence of conventional risk factors.

Keywords: Atherosclerosis, Cardiovascular, Coronary artery disease, Hyper-homocysteinemia, Risk factor, Thrombosis, Young adults

Disclosure: Authors have nothing to disclose with regard to commercial support.
Received 23 January 2018; revised 4 April 2018; accepted 27 April 2018.

* Corresponding author at: Cardiology Department, Rehman Medical Institute, Sector 5-B/II, Peshawar, Pakistan; Residence: House 171, Street 3, Sector E-7, Phase 7, Hayatabad, Peshawar, Pakistan.
E-mail address: drhammadshah@gmail.com (H. Shah).

P.O. Box 2925 Riyadh – 11461KSA
Tel: +966 1 2520088 ext 40151
Fax: +966 1 2520718
Email: sha@sha.org.sa
URL: www.sha.org.sa

1016-7315 © 2018 The Authors. Production and hosting by Elsevier B.V. on behalf of King Saud University. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

Peer review under responsibility of King Saud University.
URL: www.ksu.edu.sa
https://doi.org/10.1016/j.jsha.2018.04.002

Production and hosting by Elsevier

Introduction

One third of global deaths are caused by cardiovascular events [1]. In developed countries such as the United States, >600,000 deaths annually are attributed to cardiovascular diseases [2]. Ischmeic heart disease is the biggest culprit contributing to mortality from heart diseases [3]. Atherosclerosis is the hallmark of ischemic heart disease which is characterized by narrowing or blockage of coronary arteries supplying all the nutrients and oxygen to the heart [4]. Coronary angiography is the gold standard for detecting coronary artery disease [5].

Homocysteine accelerates atherosclerosis by increasing the prothrombotic factors XII and V and decreasing the antithrombotic factors along with endothelial derived nitric oxide. Hyperhomocysteinemia also inculcates direct endothelial cell damage, which leads to smooth muscle cells hyperplasia, contributing to occlusion or narrowing of the vessels. Increased plasma levels of homocysteine contribute to cardiac morbidities and it has positive association with hypertension [6]. Aspirin resistance is also increased with high levels of homocysteine in the blood [7]. Therefore, homocysteine has been thought of as an independent risk factor contributing to coronary artery disease and the Framingham Risk score has been challenged [8]. Low levels of vitamin B12 and high serum concentration of homocysteine have been associated with coronary artery disease in Asians and investigated by two different studies in the Indian population [9,10]. Homocysteine is a modifiable risk factor and folic acid supplementation improves the endothelial dysfunction caused by high serum concentration of homocysteine [11].

The vascular toxicity of hyper-homocystenemia is well established, yet we have limited evidence regarding the isolated effect of hyperhomocystenemia on coronary artery disease in the younger population in absence of cumulative and synergistic effect of conventional strong risk factors. This study aimed to analyze the correlation of serum homocysteine and coronary artery disease in young adults in the absence of conventional risk factors. As it is a modifiable risk factor, targeting it will decrease both the cardiovascular mortality and morbidity.

Materials and methods

This study was carried out at the Cardiology Department of Rehman Medical Institute (RMI), Peshawar, Pakistan. RMI is a specialized tertiary care hospital providing modern state of the art facilities to patients from across the province and neighboring countries. It was an observational case-control study carried out for a period of 12 months from October 1, 2016, to September 30, 2017. A total of 158 participants were included; 128 participants in the patient group and 30 participants in the control group.

In the patient group, a universal sampling technique was adopted and every patient who had angiographic evidence of moderate to severe stenosis in single or multiple major coronary arteries on coronary angiography aged <40 years and >25 years was included. In the control group, random sampling from the general population was done having features similar to the participants of the patient group. Patients from each sex, different ethnic backgrounds, multiple geographic locations, and different socioeconomic statuses were part of the study population in both groups.

All those participants who had mild stenosis in coronary arteries, were using antihypertensive medications including calcium channel blockers, beta blockers, diuretics, or had a blood pressure measurement of >140 mmHg systolic and >100 mmHg diastolic, were using antidiabetic medications including oral antidiabetics or insulin or had a random blood glucose level of >200 mg/dL, fasting blood glucose level of >126 mg/dL or HbA1c of >6.5%, were active smokers and smoked more than one cigarette daily, had familial hyperlipidemia or dyslipidemia, or had blood cholesterol level >250 mg/dL, taking drugs such as antidepressants (S-adenosyl methionine), methotrexate, phenytoin, carbamazepine, 6-azauridine triacetate, recently undergone general anesthesia or were exposed to nitrous oxide were excluded from the study population.

In an EDTA tube, 5 mL fasting blood sample was collected under aseptic technique, having 8 hours fasting cutoff index. Plasma homocysteine

Abbreviations

RMI	Rehman Medical Institute
FPIA	Fluorescence Polarization Immunoassay
CAD	Coronary artery disease
QCA	Quantification of coronary atherosclerosis
SVCAD	Single vessel coronary artery disease
DVCAD	Double vessel coronary artery disease
TVCAD	Triple vessel coronary artery disease
BMS	Bare metal stent
PCI	Percutaneous Cutaneous Intervention

level was assessed, using Fluorescence Polarization Immunoassay (FPIA) technique on AxSYM System (Abbott Laboratories Lake Bluff, Illinois, United States), by standard method. Highly selective conversion of homocysteine to S-adenosylhomocysteine by the enzyme S-adenosylhomocysteine hydrolase was the basic principle of this technique. FPIA is the accepted method of homocysteine analysis worldwide [12,13].

This study was approved by the research and evaluation unit of Rehman Medical Institute after scrutiny of synopsis by research evaluation committee vide letter reference No: RMC/NOC/16 dated September 20, 2015, and abided by the declaration of Helsinki. Informed written consent obtained and confidentiality of the patient was ensured.

The Shapiro-Wilk test was applied to check the distribution of data. Continuous variables determined as mean ± standard deviation test and categorical variables expressed as frequencies and percentages. Analysis of variance (ANOVA) was used for comparison of the mean. Pearson test was used to compare the severity of hyper-homocysteinemia with severity of coronary artery disease and number of coronary artery disease. A p value <0.5 was considered significant.

Operational definitions

Coronary artery disease (CAD) was defined as stenosis on coronary angiography using the quantification of coronary atherosclerosis (QCA) scoring system. Mild CAD was defined as <30% stenosis in coronary arteries. Moderate CAD was defined as 30–70% stenosis in coronary arteries. Severe CAD was defined as >70% stenosis in coronary arteries.

Patients having moderate to severe stenosis in single vessels were labelled as single vessel coronary artery disease (SVCAD). Patients having moderate to severe stenosis in two major vessels were labelled as double vessel coronary artery disease (DVCAD). Patients having moderate to severe stenosis in three major vessels were labelled as triple vessel coronary artery disease (TVCAD).

Mild hyper-homocysteinemia was defined as plasma homocysteine level of >12–14.9 μmol/L, moderate hyper-homocysteinemia was defined as plasma homocysteine level of >15–29.9 μmol/L, intermediate hyper-homocysteinemia was defined as plasma homocysteine level of >30–00 μmol/L, and severe hyper-homocysteinemia was defined as plasma homocysteine level of >100 μmol/L [14].

Results

The total number of the study population was 158 with 128 participants in the patient group and 30 participants in the control group. Case and control groups were identical in baseline characteristics and matched for each variable using the Chi-square test as shown in Table 1.

The control group had mean plasma homocysteine concentration 6.3 (±2.05) μmol/L whereas the patient group had mean plasma concentration of 44.5 (±14.01) μmol/L and there was a statistically positive correlation between mean homocysteine concentration of case and control groups with p value of 0.003 from the t test as shown in Fig. 1

The association of hyper-homocystenemia with the number of coronary arteries involved and severity of coronary artery stenosis is depicted in Table 3.

The correlation between homocysteine level and severity of coronary artery disease (moderate or severe stenosis in 1 or more major coronary arteries) was statistically significant on Pearson's test with $r = 0.85$ and $p < 0.01$. Homocysteine level also had statistically significant correlation with the number of major coronary arteries having moderate to severe stenosis with $r = 0.22$ and $p < 0.01$ on Pearson's test.

Table 1. Basic characteristics of controls and cases.

		Control	Case	p
Age		32.84 (±2.14)	33.81 (±2.74)	<0.001
Sex	Males	68.1	67.2	<0.001
	Females	31.9	32.8	<0.001
Smoking		13.5	14.3	<0.001
Diabetes mellitus		6.4	7.1	<0.001
Hypertension		9.4	10.2	<0.001
BMI		22.95 (±2.65)	23.28 (±2.08)	<0.001
Homocysteine (μmol/L)		6.3 (±2.05)	44.5 (±14.01)	0.86

Data are presented as% or mean (±SD).
BMI = body mass index.

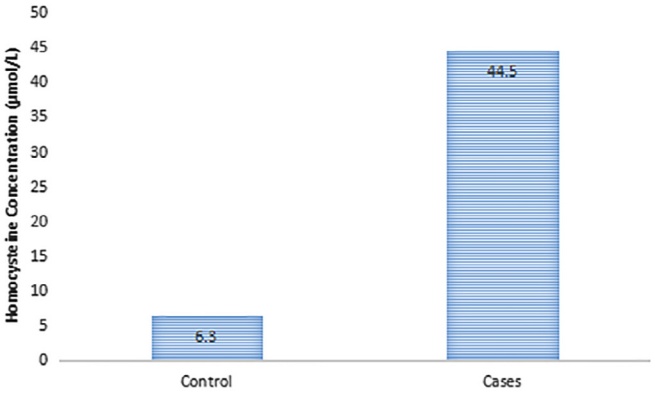

Fig. 1. Comparison of plasma homocysteine concentration between cases and controls. The intensity of increase in hyper-homocystenemia, its classification as mild, moderate, intermediate, and severe, and its relationship with sex among patients is shown in Table 2.

Table 2. Intensity of hyper-homocysteinemia and its relationship with sex.

Sex	Intensity of Hyper-homocystenemia in patients group				Total
	Mild increase (12–14.9 µmol/L)	Moderate increase (15–29.9 µmol/L)	Intermediate increase (30–100 µmol/L)	Severe increase (>100 µmol/L)	
Male	0	11 (8.6)	72 (56.3)	3 (2.3)	86 (67.2)
Female	0	4 (3.1)	37 (28.9)	1 (0.8)	42 (32.8)
Total	0	15 (11.7)	109 (85.2)	4 (3.1)	128

Data are presented as *n* (%) or range.

Table 3. Association between hyper-homocysteinemia severity, number of coronary vessels involved, and severity of CAD.

Severity of hyperhomocystenemia	No. of vessels involved			Severity of CAD	
	SVCAD	DVCAD	TVCAD	Moderate stenosis	Severe stenosis
Mild hyper-homocysteinemia (12–14.9 µmol/L)	0	0	0	0	0
Moderate hyper-homocysteinemia (15–29.9 µmol/L)	15	0	0	10	5
Intermediate hyper-homocysteinemia (30–100 µmol/L)	103	5	1	0	109
Severe hyper-homocysteinemia (>100 µmol/L)	0	3	1	0	4

CAD = coronary artery disease; DVCAD = double vessel coronary artery disease; SVCAD = single vessel coronary artery disease; TVCAD = triple vessel coronary artery disease.

Discussion

A recent study conducted in the Indian population showed that plasma homocysteine levels are highter in men compared with females which is congruent to our result in which 67.2% of the patients suffering from hyper-homocysteinemia were males compared with 32.8% of females [15]. Homocysteine has a prothrombotic effect by enhancing platelet aggregation via the hydrogen sulfide pathway, thus contributing to intravascular thrombosis as reported by different studies [16,17]. It is hence identified and reported as an independent risk factor for atherosclerosis [18,19]. Ganguly and Alam [10] also identified the detrimental effect of high homocysteine levels on a cardiovascular system mediated by catecholamine and Zang et al. [20] showed its deleterious effect in causing arterial stiffness. All these studies support our results which showed that all the patients with moderate to severe stenosis in one or more major coronary arteries had hyper-homocysteinemia. Amongst them, 92.2% had intermediate, 4.7% had moderate, and 3.1% had severe increase in plasma homocysteine levels. None of the patients who had moderate or severe stenosis in one or more major coronary arteries had a normal homocysteine level.

Different geographic locations and different ethnic backgrounds have different genetic responses and to evaluate hyper-homocysteinemia effect in our population we compared our results to similar studies from Asian populations. Mahalle et al. [9] conducted a similar study in Asia having 216 patients with coronary artery disease, among whom 95.3% had hyper-homocysteinemia in the

Indian population. Hyper-homocysteinemia was identified as a risk factor for cardiovascular thrombosis in Asian population. Mendis et al. [21] conducted a case control study to look for association between the hyper-homocysteinemia and coronary artery disease in Sir Lankan patients and established a statistically significant association between the raised level of homocysteine and coronary artery disease, strengthening results of our study.

Schaffer et al. [8] ran a large prospective study from March 2007 to October 2013 to look for the relationship between homocysteine and coronary artery disease including 3056 patients. They found a positive correlation between raised homocysteine levels and coronary artery disease and concluded that homocysteine was an independent risk factor for coronary artery disease.

The impact of homocysteine as an isolated and very important risk factor was hallmarked in a meta-analysis of observational studies published in *The Journal of the American Medical Association* correlating homocysteine levels with risk of ischemic heart disease, stroke, and found that low levels of homocysteine was associated with lower risk of ischemic heart disease and stroke [22]. We also compared our target population age group with other studies. Raised levels of homocysteine was reported as cardiovascular risk factor in young patients in another case report, supporting our result and hypothesis [23].

Yeh et al. [24] showed that plasma homocysteine level was $16.4 + 6.44$ μmol/L in patients with myocardial infarction. This study had limited participants and included all those patients who had primary Percutaneous Coronary Intervention (PCI) done with bare metal stent (BMS). BMS has a very limited usage and scope due to increased risk of acute stent thrombosis. Renal impairment patients were also excluded in that study which is one of the contributing factors in raising serum homocysteine concentration [25]. Besides this all the patients of myocardial infarction were included regardless of their age and risk factors. These results do not truly reflect cardiovascular event as a sole result of pure hyper-homocystenemia but a cumulative role of all the risk factors in which hyper-homocystenemia contribution can be negligible in the presence of conventional strong risk factors. On the contrary, results of our study show the vascular toxic effect of pure hyper-homocystenemia in absence of conventional risk factors without synergistic and cumulative effects. High levels of plasma homocysteine are required to bring the cardiovascular toxic effects in the absence of other conventional risk factors as observed in our study. Furthermore, it was carried out in underprivileged population who are prone to develop hyper-homocystenemia [26,27].

Our results showed that all the young patients with coronary artery disease had a raised homocysteine level with no young patient suffering from moderate to severe stenosis in single or multiple major coronary arteries having a normal serum homocysteine concentration. Hyper-homocystenemia is a modifiable risk factor, therefore it needs urgent attention. Targeting it will decrease both cardiovascular morbidity and mortality both in primary and secondary prevention.

Conclusion

In absence of conventional risk factors, hyper-homocysteinemia alone can be a major risk factor for cardiovascular thrombosis and it has a positive correlation with coronary artery disease in young patients. Further studies are required to elaborate such results.

Limitations of the study

Having been a case-control study, our results cannot truly depict the general population but it will provide a basis for further population based epidemiological studies and bring into the limelight the grave impact hyper-homocysteinemia is projecting as cardiovascular risk factor.

The sample size was relatively small due to low incidence of hyper-homocysteinemia in the general population and stringent inclusion–exclusion criteria.

Plasma separation time was not standardized which might have prompted technical delays in plasma separation and slightly high false levels of plasma homocysteine concentration.

References

[1] World Health Organization. Available at: http://www.who.int/news-room/fact-sheets/detail/cardiovascular-diseases-(cvds); May 17, 2017.
[2] Mangge H, Becker K, Fuchs D, Gostner JM. Antioxidants, inflammation, and cardiovascular disease. World J Cardiol 2014;6:462–77.
[3] Santulli G. Epidemiology of cardiovascular disease in the 21st century: updated numbers and updated facts. J Cardiovasc Dis 2013;1:1–2.
[4] Shenoy V, Mehendale V, Prabhu K, Shetty R, Rao P. Correlation of serum homocysteine levels with the severity of coronary artery disease. Indian J Clin Biochem 2014;29:339–44.

[5] Jiangping S, Zhe Z, Wei W, Yunhu S, Jie H, Hongyue W, et al.. Assessment of coronary artery stenosis by coronary angiography: a head-to-head comparison with pathological coronary artery anatomy. Circ Cardiovasc Interv 2013;6:262–8.
[6] Baszczuk A, Kopczyński Z, Thielemann A. Endothelial dysfunction in patients with primary hypertension and hyperhomocysteinemia. Postepy higieny i medycyny doswiadczalnej 2013;68:91–100 (Polish).
[7] Karolczak K, Kamysz W, Karafova A, Drzewoski J, Watala C. Homocysteine is a novel risk factor for suboptimal response of blood platelets to acetylsalicylic acid in coronary artery disease: a randomized multicenter study. Pharmacol Res 2013;74:7–22.
[8] Schaffer A, Verdoia M, Cassetti E, Marino P, Suryapranata H, De Luca G, et al.. Relationship between homocysteine and coronary artery disease: results from a large prospective cohort study. Thromb Res 2014;134:288–93.
[9] 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;61:289–94.
[10] Ganguly P, Alam SF. Role of homocysteine in the development of cardiovascular disease. Nutr J 2015;14:6. https://doi.org/10.1186/1475-2891-14-6.
[11] Liu Y, Tian T, Zhang H, Gao L, Zhou X. The effect of homocysteine-lowering therapy with folic acid on flow-mediated vasodilation in patients with coronary artery disease: a meta-analysis of randomized controlled trials. Atherosclerosis 2014;235:31–5.
[12] La'ulu SL, Rawlins ML, Pfeiffer CM, Zhang M, Robers WL. Performance characteristics of six homocysteine assays. Am J Clin Pathol 2008;130:969–75.
[13] Silvia L, Novembrino C, Ippolito S, Accini R, Galli C, Troonen H, et al.. Analytical performance and method comparison of total homocysteine fluorescence polarization immunoassay (FPIA) on the AxSYM analyzer. Clin Chem Med 2004;42:228–34.
[14] Weiss N, Keller C, Hoffman U, Loscalzo J. Endothelial dysfunction and atherothrombosis in mild hyperhomocysteinemia. Vasc Med 2002;7:227–39.
[15] Pitla S, Nagalla B. Gender-related differences in the relationship between plasma homocysteine, anthropometric, and conventional biochemical coronary heart disease risk factors in middle-aged Indians. Ann Nutr Metab 2009;54:1–6.
[16] Bianca RE, Mitidieri E, Di Minno MN, Kirkby NS, Warner TD, Di Minno G, et al.. Hydrogen sulphide pathway contributes to the enhanced human platelet aggregation in hyperhomocysteinemia. Proc Natl Acad Sci USA 2013;110:15812–7.
[17] Catena C, Colussi G, Url-Michitsch M, Nait F, Sechi LA. Subclinical carotid artery disease and plasma homocysteine levels in patients with hypertension. J Am Soc Hypertens 2015;9:167–75.
[18] Pang X, Liu J, Zhao J, Mao J, Zhang X, Feng L, et al.. Homocysteine induces the expression of C-reactive protein via NMDAr-ROS-MAPK-NF-κB signal pathway in rat vascular smooth muscle cells. Atherosclerosis 2014;236:73–81.
[19] Veeranna V, Zalawadiya SK, Niraj A, Pradhan J, Ference B, Burack RC, et al.. Homocysteine and reclassification of cardiovascular disease risk. J Am Coll Cardiol 2011;58:1025–33.
[20] Zhang S, Bai YY, Luo LM, Xiao WK, Wu HM, Ye P. Association between serum homocysteine and arterial stiffness in elderly: a community-based study. J Geriatr Cardiol 2014;11:32–8.
[21] Mendis S, Ranatunga P, Jayatilake M, Wanninayake S, Wickremasinghe R. Hyperhomocysteinaemia in Sri Lankan patients with coronary artery disease. Ceylon Med J 2011;47:89–92.
[22] Homocysteine Studies Collaboration. Homocysteine and risk of ischemic heart disease and stroke: a meta-analysis. JAMA 2002;288:2015–22.
[23] Babu J, Malkiwodeyar PK, Tarikere S, Cholenahalli NM. Myriad cardiac manifestation of hyperhomocysteinemia. Cardiol Res 2015;6:263–5.
[24] Yeh JK, Chen CC, Hsieh MJ, Tsai ML, Yang CH, Chen DY, et al.. Impact of homocysteine level on long-term cardiovascular outcomes in patients after coronary artery stenting. J Atheroscler Thromb 2017;24:696–705.
[25] Moustapha A, Gupta A, Robinson K, Arheart K, Jacobson DW, Martin J. Prevalence and determinants of hyperhomocystenemia in hemodialysis and peritoneal dialysis. Kidney Int 1999;55:1470–5.
[26] Iqbal MP. Hyperhomocysteinemia and coronary artery disease in Pakistan. J Pak Med Assoc 2006;56:282–5.
[27] Zafar F, Idress MM, Aimed Z. Prevalence of enteric infection in suburban Karachi: a study focused on Landhi-Kolangi area. International Nutritional Conference 2004. Nutrition Association of Pakistan, Lahore, 157(abstract). https://www.ejmanager.com/mnstemps/27/27-1301828620.pdf.