Original Article

Association of higher circulatory levels of homocysteine with additional risk factors in CAD patients

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Abstract

Introduction: Coronary artery disease (CAD) is a leading cause of mortality worldwide. In developing countries it has been observed that stressful life style is mainly associated with CAD. Inflammatory environment has been produced due to formation of various oxidant and proinflammatory molecules which keeps inflammatory pathways active. **Aim:** The present study aimed to compare the circulatory levels of homocysteine in CAD patients and normal controls. **Methodology:** The study protocol was approved by institutional ethical committee. Total 198 subjects (107 CAD patients and 91 age sex matched controls) have been included in the present study. Blood sugar, lipid profile levels were estimated in both CAD patients and controls. **Results:** High levels of homocysteine have been observed in CAD subjects [17.41± (10.27)] compared to the controls [7.38± (3.67)]. No significant difference have been observed in LDL levels in controls and CAD respectively [105.32(±33.66)/ 108.93(±41.51)]. However HDL levels were found to be significantly lower in CAD patients than controls [38.71 (±13.73)/ 44.94(±11.34). Additional risk factors like Type 2 diabetes (DM) and Hypertension (HTN) in CAD group has shown high homocysteine than CAD patients without any additional risk factors. **Conclusion:** Hyperhomocysteinemia is found to be associated with CAD patients. It was also observed that increased pattern of homocysteine levels in CAD patients were associated with additional risk factors like DM and HTN factors.

Key words: Homocysteine, CAD and additional risk factors of CAD, inflammation.

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INTRODUCTION

Incidence of Coronary artrey disease (CAD) is growing in parallel with the modified life styles, dietary habits, sleeping patterns and stressful job profiles. Genetic factors has been also found to be associated with CAD. According to World Health Report 2002, CAD will be the largest cause of death and disability by 2020 in India (WHO report 2002)[1]. The prevalence of CAD in urban areas of India has been increased from 6.5% in 1970 to 10.5 % in 2000. Similarly, a significant increase in CAD patients has been observed which are from rural areas. This estimate can be roughly translated into prevalence of 30 million patients with CAD in Indian subcontinent by 2020[2-4]. It has been reported by various studies that dyslipidema have been associated with CAD and its comorbidities like Obesity, type 2 Diabetes mellitus[5,6]. Inflammatory mediators play an important role pathphysiological mechanism in Obesity, type 2 Diabetes mellitus[8] which might play an important role in atherogenesis and recognised as actual culprits[7-9,15]. Despite the significant advances made in the treatment, the risk of restenosis and further serious complications

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remains high. Hence instead of routine clinical investigations more emphasis should be given to advance markers to recognize the subclinical underlying inflammatory status. For a instant proinflammmatory status has been identified as a key feature for atherosclerosis and CAD[9,11]. Higher levels of various proinflammmatory markers stimulates inflammatory cascades which leads to stimulation of various cell adhesion molecules results in endothelial dysfunction[12]. Oxidised LDL resulted from inflammatory cascade easily penetrates the endothelial wall and starts the process of atherogenesis[13-14]. Various inflammatory molecules like IL-6, TNF-alpha, CRP as well Homocysteine have been identified which may link inflammation to atherogenesis[15]. In CAD an abnormality has been found to be associated with lipid profile. Various treatement statergies controls hyperlipidmia but the proinflammatory status keeps various inflammatory cascades active. Hence instead of routine lipid profile, determination of inflammatory status by investgating inflammatory molecules might clarify diagnosis and also help in progrnosis as well in prevention of some fatal episodes of CAD. Homocysteine is an intermediatory product of methionine metabolism (a sulphur containing amino acid). In 1969 for the first time homocysteine has been considered as an independent risk factor for CAD[16]. Later on in 1976 it has been reported that CAD patients have abnormality in homocysteine metaboilsm and as a result of it homocysteine levels increases[17]. Diminished dietary supplementation of some B-complex vitamins like Folic acid and Cobalamine found to be associated with hypehomocysteinemia. Genetic abnormalities in homocysteine metabolism also results in hyperhomocysteinemia[18-20]. Some other studies thereafter reported the association of hyperhomocysteinemia and development of atherosclerosis[21-23]. However according to some it is just a inert bystander[24]. While some studies did not show any statistical significant association between higher levels of Homocysteine in CAD patients[25]. Indian population found to be more predisposed to hyperhomocysteinemia than western. Folic acid and cobalamine play an important role in metabolizing homocysteine. According to Indian dietary paradox a good amount of folic acid has been included in diet but during Indian type food preparations most of its vitamin contents is lost. Vegeterians suffer with folic acid and mainly B12 deficiecies than omnivorous[26,27]. The present study aimed to determine the association of of Hyperhomocysteinemia in CAD patients with and with out additional risk factors like type 2 Diabetes and hypertention.

MATERIAL AND METHODS

The present study was conducted at Dr. D.Y. Patil Medical College, Nerul Navi Mumbai. Subjects recruited for this study were from outpatient department (OPD) and Indoor patient department (IPD). Toal 198 subjects which included out of which 107 cases were CAD patients and 91 age and sex matched healthy individuals without clinical evidence of coronary artery disease and with normal ECG constituted the control group.Written informed consent was obtained. The study has been approved by the Institutional Ethics Committee. Inclusion criteria: Patients with age 20 years and above with Coronary Artery disease (unstable angina, stable angina, Non ST elevation, Myocardial infarction and ST elevation Myocardial infarction) proved by history, clinical examination, blood investigations, Electrocardiogram, Echocardiography and Coronary Angiography. Healthy Controls above age of 20 yrs. Exclusion criteria: Pregnant women, patients less than 20 years of age, with Congenital Heart disease, acute or chronic infection, chronic liver and kidney disease, were excluded from this study. Fasting venous blood samples were collected from CAD patients and controls.

RESULTS

Table 1: Demographic Charateristics of study subjects

Variables	Controls (n=91)	CAD (n=107)
Males %	65	73
Females %	35	27
Vegeterians %	58	44
Omnivorous %	42	56

Fasting blood sugar and Lipid Profile were analysed on autoanlyer using commertially available kits in central clinical laboratory of D.Y.Patil Hospital and Rearch center, Nerul, Navi Mumbai. Homocysteine levels were analysed by using commertially available Kit Method.

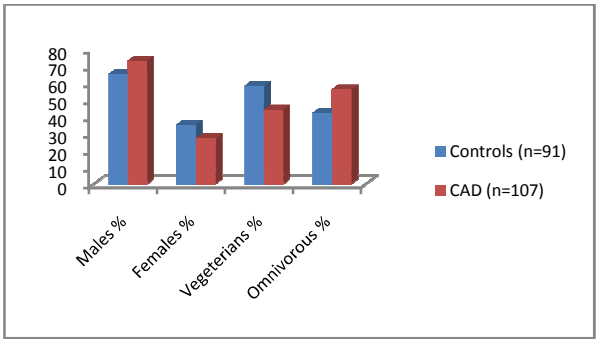

Figure 1: Demographic Charateristics of study subjects

Table 2: Clinical and Biochemical Charateristics of study subjects

Variables	Controls (n=91)	CAD (n=107)	P value
Age	55.11(± 5.16)	57.18(± 7.18)	NS
BMI	23.68(±3.26)	24.70(±3.59)	NS
FBS(mg%)	90.71(±15.35)	127.66(±55.49)	< 0.005
Triglycerides(mg%)	123.59(±49.47)	141.95(±66.01)	NS
Total cholesterol(mg%)	173.79(±37.72)	175.12(±50.81)	NS
LDL(mg%)	105.32(±33.66)	108.93(±41.51)	NS
HDL(mg%)	44.94(±11.34)	38.71(±13.73)	< 0.005
Homocysteine levels(µmol/L)	7.38(±3.67)	17.41(±10.27)	< 0.005

Data shown in the above tables indicates that, three variables have shown significant variation between control and CAD groups; while rest other variable unable to show significant difference in Control and CAD groups. Fasting blood sugar levels were significantly higher in CAD group; as some of the CAD participant were diabetic. Amongest the circulatory lipids, only HDL levels were significantly lower in CAD patients than controls [38.71(±13.73)/ 44.94(±11.34)] . However no significant difference have been observed in LDL levels in controls and CAD respectively [105.32(±33.66)/ 108.93(±41.51)]. Homocysteine levels were significantly higher among CAD cases [17.41±(10.27)] as compared to the controls [7.38±(3.67)].

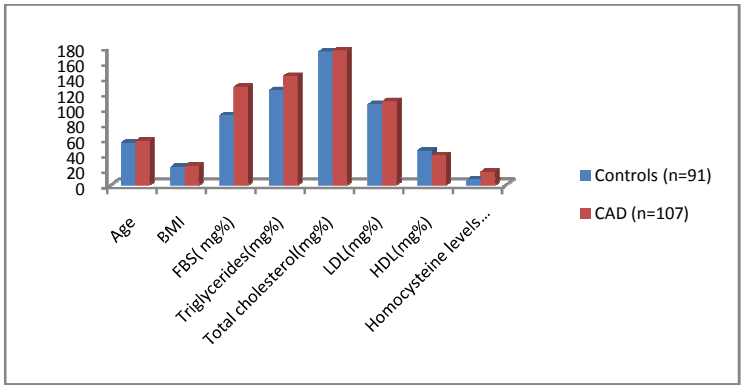

Figure 2: Comparision of clinical and Biochemical variables in CAD patients and controls

Table 3: Clinical characteristics in Control and CAD groups depending on dietary habbits

Variables	Controls (n= 91)		CAD (n= 107)	
	Vegetarians (n=53)	Omnivrous (n=38)	Vegetarians (n=47)	Omnivorous (n=60)
Age	55.08(±4.40)	55.16(± 6.13)	58.45(± 8.04)	56.18(± 7.63)
BMI	24.10(±3.13)	23.11(±3.39)	24.33(±3.50)	25.06(±3.64)
FBS (mg%)	90.10(±15.61)	91.56(±15.15)	124.78(±50.38)	129.91(± 59.51)
Triglycerides (mg%)	135.77(±54.27)	106.60(±36.12)	129.36(± 49.85)	151.81(±75.28)
Total cholesterol (mg%)	175.19(±37.89)	171.84(±37.90)	172.83(±46.73)	176.91(±54.12)
LDL (mg%)	103.92(±32.56)	107.26(±35.49)	108.87(±38.88)	108.97(±43.78)
HDL (mg%)	43.29(±8.26)	46.86(±13.98)	38.92(±14.72)	38.55(±13.03)
Homocysteine levels (µmol/L)	8.64(± 4.02)	5.6 (± 2.14)	18.33(± 9.46)	16.68(± 10.89)

Homocysteine values were compared with the vegetarians and omnivorous between both the study groups. No significant difference was observed in any of the biochemical variable. While in CAD group the mean homocysteine among the vegetarians were slightly higher [18.33(± 9.46)mmol/l] than that of non-vegetarians [16.68(± 10.89) mmol/l] but is statiastically nonsignificant.

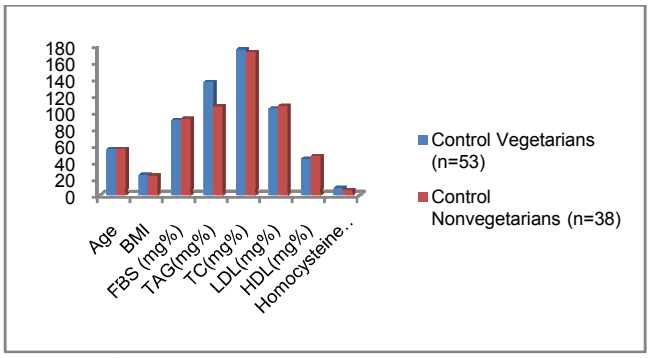

Figure 3: Comparision of study variables of CAD and Controls in Vegeterians and Omniverous subjects

Table 4: Effect of additional risk factors on circulatory levels of homocysteine

Group	Additional Risk factor	Number of Cases	Homocysteine levels (µmol/L)	P value
I	Control	91	7.38(±3.67)	
II	Only CAD	36	13.01(±9.14)	0.000
V	CAD with either or both DM and HTN	71	19.54(± 10.17)	

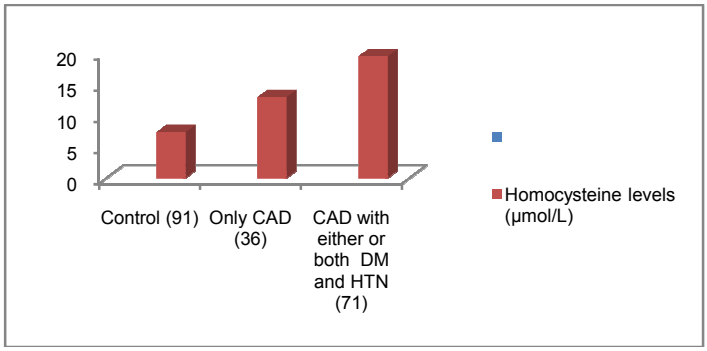

Figure 4: Comparision of circulatory homocystein levels and effect of additional risk factors

A significant difference was noted in the circulatory levls of homocysteine between the CAD patients and controls. Further additional risk factors like Type 2 diabetes Mellitus (DM) or Hypertension (HTN) or both has been shown to have an increased levels of circulatory Homocysteine.

DISCUSSION

Our study results supported previously published studies on circulatory levels of homocysteine in Indian CAD population and reported high homocystine levels in CAD patients than normal subjects. Vegeterians have higher levels of circulatory homocysteine than omnivorous, both in control and CAD groups. Higher circulatory levels of homocysteine can be considerd as an independent risk factor for atherosclerosis. Homocysteine mediates its effects the by several different mechanisms. It increases proliferation of vascular smooth muscle cells, endothelial dysfunction, oxidative damage, high collagen synthesis[28-30]. Homocysteine get auto oxidated and generates free radical which increases the burden of oxidative stress . Free radicals further oxidizes LDL (Ox LDL) which stimulates vascular cell adhesion molecule (VCAM), Monocyte chemoattractant protein-1(MCP-1), subsequently the adhesion of monocytes to endothelial cells is followed by their penetration into endothelium. Inside endothelium, monocytes are trasformed to macrophages and takes up Ox LDL and gets converted to Foam cell laden with cholesterol. Fatty streak push the endotheliam upwards and narrow down the diameter of the artey. Reduction in the blood flow or plaque ruture with thrombosis may results depending on the status of the plaque. An apparent increase have been observed in homocysteine levels among CAD patients with additional risk factors like diabetes mellitus, hypertension[31,32]. Inflammation has been identeified as a key and common feature for diabetes mellitus, hypertension and CAD. A complicated interplay between inflammatory mediators might be at a far above the ground levels in CAD patients with additional comorbidities. Highest homocysteine in these patients considered to exerts its effects by stimulating oxidative stress, LDL oxidation, endothelial damage which are the pathophysiological changes associated with atherogenesis[28-30] as well as its progression. Our results indicates that addition risk factors aggrevate the atherogenic environment supports

rise in homocysteine levels with additional risk factors. Analysis of a larger group would definitely provide an insight into the various causative factors leading to hyperhomocysteinemia and its role in CAD and related complications amongst the Indian population.

Various positive correlations with few nonsignificant observations keeps the interest of researchers high, for /in the present topic, the role of homocysteine in pathogenesis and progession of atherosclerosis and related risk.

CONCLUSION

Hyperhomocysteinemia is found to be associated with CAD patients. Further it was also observed that increased pattern of homocysteine levels in CAD patients were associated with additional risk factors like DM and HTN factors for CAD.

LIMITATIONS

Limited sample size and folic acid, cobalamine levels were not considered. It was an attempt to check the relevance of homocsteine levels with incidence of CAD and its association with additional risk factors like diabetes and hypertension.

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