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Clinical Correlation between Plasma Homocysteine Level and Coronary Artery Disease in Indian Patients

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Abstract

Objective: The aim of this study was to evaluate correlation between plasma homocysteine and coronary artery disease (CAD) in Indian patients. **Methods:** This study included 150 patients, 100 subjects in study group with angiographically diagnosed CAD and 50 subjects in control group with a normal coronary angiogram. In the study group, patients were divided into three subgroups *viz.*: CAD only, CAD with hypertension and CAD with type 2 diabetes mellitus. Plasma homocysteine, lipid profile and other risk factors were compared. **Results:** Mean homocysteine levels in study group (38.34 ± 15.25 μmol/L) were significantly higher ($p < 0.01$) than control group (9.41 ± 4.22 μmol/L). No association was found between homocysteine level and conventional risk factors. Furthermore, no significant correlation was found between plasma homocysteine and lipid components in different groups of patients. **Conclusion:** The study demonstrated that increased levels of homocysteine are independently related to CAD. However, further studies involving a larger sample size will be required to substantiate the findings of the current study.

Keywords

Homocysteine, Coronary Artery Disease, Risk Factors

1. Introduction

Coronary artery disease (CAD) has become a major public health problem in many countries. According to the World Health Organization, CAD is the most common cause of death throughout the world [1]. India has been reported to have the highest prevalence of CAD [2]. The major cardiovascular risk factors are high plasma LDL (low-density lipoprotein), low plasma HDL (high-density

lipoprotein), smoking, hypertension, diabetes, obesity and physical inactivity. An increased level of LDL is related to the development of atherosclerotic cardiovascular disease, which is the primary pathological basis of CAD [3] [4]. So, the role of newer emerging risk factors is being recognized especially homocysteine, fibrinogen, and lipoprotein.

Homocysteine is a nonprotein amino acid derived from methionine metabolism. Homocysteine converts to cysteine as a result of trans-sulfuration pathway; each pathway depends on series of biochemical enzymes such as cystathionine β synthase and methylene tetrahydrofolate reductase (MTHFR) as well as on vitamin B12 and folic acid [5]. The formation of methylene-tetrahydrofolate is catalyzed by MTHFR enzyme which has an effect on the remethylation of homocysteine and these actions depend on vitamin B12 [6]. Molecular deficiency in each of these enzymes can cause hyperhomocysteinemia. Several studies discovered that homocysteine levels are associated with a novel risk factor for CAD and premature atherosclerosis [7]. However, the mechanism of atherosclerosis plaques correlated with hyperhomocysteinemia is not clearly stated. Some studies reported that the effect of hyperhomocysteinemia is associated with increased thrombogenicity, an increase of platelet aggregation, reduction of protein C activation, oxidative damage of LDL, and endothelial dysfunction [8]. Hyperhomocysteinemia may lead to enhancement adverse effects of risk factors, lipoprotein metabolism, and development of inflammation [9]. The factors which influence the level of homocysteine may vary significantly in a population with age, genetics, and nutrition [10]. Elevated homocysteine levels are associated with some factor including increasing age, male sex, smoking, coffee consumption, high blood pressure, lipid profile, high creatinine and improper diet [7]. Low folate and vitamin B12 are related with elevated homocysteine levels [11]. The incidence of homocysteine levels is altered in ethnic groups due to different genetic conditions, nutritional factors (vitamin deficiency and folic acid deficiency) and lifestyle behaviors [12]. Therefore, the present study was undertaken to examine a possible relationship between plasma homocysteine level and coronary artery disease in Indian patients.

2. Materials and Methods

2.1. Study Design

The present study was conducted for a period of 1 year from October 2015 and October 2016 in the cardiology department, District hospital Palakkad, Kerala. The patients who subsequently underwent first elective coronary angiography and plasma homocysteine assessments were considered in this study. The present study included 150 patients, 100 subjects in study group with angiographically diagnosed CAD and 50 subjects in control with a normal coronary angiogram. Subjects in the study group were divided into three subgroups *viz.*: CAD only, CAD with hypertension and CAD with type 2 diabetes mellitus (DM).

Elective coronary angiograms were done at least two weeks following an acute

coronary event. Indications for coronary angiography were: 1) evaluation of ischemic heart disease or cardiomyopathy and 2) before coronary artery bypass grafting 3) aortic or valvular heart disease surgery or further preoperative investigation in patients with family history of CAD and positive noninvasive test results. Written informed consent was procured from every patient. Angiographic SYNTAX scorings were performed by observers blinded to plasma homocysteine measurements. The inclusion criteria for the study group were: 100 subject having ≥50% luminal narrowing in arteries, having ≥1.5 mm width in coronary artery or its major branch. 50 subjects in control were matched by age and sex individuals who had a normal coronary angiogram. Although the inclusion criteria included all age group, patients were aged above 37 years, absence of any acute disease and informed consent granted.

Patients having hypertensive emergencies, hepatic disease, renal disease, stroke, hypothyroidism, pregnancy and taking any other drugs like methotrexate, carbamaze-pine, phenytoin, theophylline or any form of vitamin supplementation were excluded from this study. All patients undergoing coronary angiography immediately following acute coronary events and all hemodynamically unstable patients were excluded from the study.

After obtaining informed consent, selected cases were subjected to detailed history, laboratory test and imaging studies like ECG, fundoscopy, Echocardiography, blood sugar, hemoglobin A_1c, lipid profile, complete blood count and other routine examinations.

2.2. Estimation of Plasma Homocysteine

Plasma homocysteine was estimated using ADVIA Centaur Homocysteine Assay (ADVIA Centaur XP Immunoassay System) which is a one-step competitive immunoassay employing direct Chemiluminescence to measure total homocysteine in EDTA plasma or serum quantitatively.

2.3. Statistical Analysis

Statistical analysis was performed using Student's t-test for paired samples using SPSS v.21.0. Values were expressed as a mean ± standard deviation or as percentages. A p value < 0.05 was considered statistically significant.

3. Results

The age of the patients ranges between 37 and 77 years. Among 150 patients, 105 (70%) were males, and 45 (30%) were females. In the study group, 75 cases (75%) were males and 25 cases (25%) were females (Table 1). Plasma homocysteine levels were significantly higher in females than males in both the study and control groups ($p < 0.001$). Plasma homocysteine level in study group (38.34 ± 15.25) was significantly higher than a control group (9.41 ± 4.22) ($p < 0.001$) [Table 2]. However, there was a significant increase in plasma homocysteine levels in group I: 36.760 ± 14.77 μmol/L, group II: 42.48 ± 18.99 μmol/L, group III:

Table 1. Plasma homocysteine levels in study and control group.

Parameter	Sex	n = 100	Study group (Mean ± SD)	n = 50	Control group (Mean ± SD)	p value
Homocysteine (μmol/L)	Male	75	36.38 ± 15.04	30	9.55 ± 1.19	<0.001
	Female	25	44.2 ± 10.07	20	9.10 ± 0.59	

Table 2. Comparison of plasma homocysteine level in different study and control group.

Group	n = 150	Homocysteine (μmol/L) Mean ± SD	p value
Study group	100	38.34 ± 15.25	0.001
Control group	50	9.41 ± 4.22	
Group-I: CAD alone	45	36.76 ± 14.77	0.05
Control without Hypertension/DM	32	9.15 ± 1.00	
Group-II: CAD with Hypertension	30	42.48 ± 18.99	0.05
Control with Hypertension	10	9.45 ± 4.93	
Group-III: CAD with Type 2 DM	25	40.35 ± 17.72	0.05
Control with DM	8	9.03 ± 2.17	

CAD—Coronary artery disease, DM—Diabetes mellitus.

Table 3. Characteristics of homocysteine in patients with conventional risk factors.

Conventional risk factors	n = 100	Homocysteine (μmol/L) Mean ± SD	p value
Hypertensives	30	42.76 ± 17.77	0.10
Non-Hypertensives	70	41.36 ± 15.20	
Diabetics	25	40.35 ± 17.72	0.33
Non-Diabetics	75	41.43 ± 15.20	
Smokers	60	43.32 ± 11.32	0.31
Non Smokers	40	44.22 ± 7.20	
Alcoholics	25	39.32 ± 11.72	0.21
Non Alcoholics	75	40.86 ±15.28	

40.357 ± 17.72 μmol/L as compared to that of a control group ($p < 0.05$), as shown in Table 2.

In our study, there was no significant statistical difference between plasma homocysteine level in study group with hypertensives and non-hypertensives (42.760 ± 17.77 μmol/L, 41.360 ± 15.203 μmol/L, respectively, $p = 0.10$) [Table 3]. Although, there was no significant difference between homocysteine level in study group with diabetics and non-diabetics (40.357 ± 17.72 μmol/L, 41.430 ± 15.203 μmol/L, respectively, $p = 0.33$) [Table 3]. In addition, there was no significant of mean homocysteine level difference between smokers and nonsmokers (43.329 ± 11.32 μmol/L, 44.229 ± 7.20 μmol/L, respectively, $p= 0.31$). Furthermore, the mean homocysteine level of alcoholics and non-alcoholics are 39.327 ± 11.72 and 40.860 ± 15.28, respectively. There was no significant correlation between the average of two groups ($p = 0.21$).

Table 4. Lipid profile parameters in different study and control group.

Group	n = 150	Serum TC (mg/dL)	Serum TG (mg/dL)	Serum HDL (mg/dL)	Serum LDL (mg/dL)
Control group	50	172.08 ± 26.80	100.88 ± 18.26	47.51 ± 14.53	110.29 ± 24.79
Group-I: CAD alone	45	202.44 ± 7.41**	122.09 ± 38.79	46.07 ± 10.01	122.37 ± 31.06**
Group-II: CAD with Hypertension	30	215.73 ± 8.56*	152.28 ± 66.36*	41.78 ± 4.63**	139.73 ± 37.04*
Group-III: CAD with Type 2 DM	25	184.23 ± 7.12	132.07 ± 49.16**	45.09 ± 16.07	95.60 ± 40.46

CAD—Coronary artery disease, TC—Total cholesterol, TG—Triglyceride, HDL—High density lipoprotein, LDL—Low density lipoprotein. Values are given as mean ± S.D. Different study group compared with control subjects. (*$p < 0.05$, **$p < 0.001$).

Table 5. Correlation between homocysteine and lipid profile parameters

Biochemical parameters	Group-I: Hypertension		Group-II: Hypertension with CAD		Group-III: Hypertension with Type 2 DM	
	r	p	r	p	r	p
Homocysteine vs TC	−0.053	0.25	−0.055	0.76	−0.083	0.66
Homocysteine vs TG	−0.008	0.96	−0.009	0.91	0.053	0.77
Homocysteine vs LDL	−0.055	0.28	−0.065	0.82	−0.080	0.70
Homocysteine vs HDL	0.219	0.21	0.221	0.23	0.196	0.17

TC—Total cholesterol, TG—Triglyceride, LDL—Low density lipoprotein, HDL—High density lipoprotein.

The lipid profile changes in control and subgroups of patients are shown Table 4. The levels of total cholesterol, triglyceride, and LDL cholesterol were significantly higher in group-II ($p < 0.001$) as compared with control group. Moreover, group-II patients had significantly decreased ($p < 0.05$) HDL cholesterol level than control group. Group-I showed significant variation ($p < 0.05$) in the levels of total cholesterol, and LDL cholesterol as compared with control. Also, group-III showed a significant increase ($p < 0.05$) in triglyceride level as compared with control. The correlations between homocysteine and lipid profile parameters are shown "r" (coefficient of correlation) and "p" value in different groups of patients (Table 5). There was no significant correlation between plasma homocysteine and lipid components in different group patients. The plasma homocysteine levels are represented in Figure 1.

4. Discussion

Homocysteine has been projected as a novel risk factor for CAD [13]. Some researchers embarked upon the mission to show plasma homocysteine as an independent risk factor for CAD. Some studies reported that correlation between high plasma homocysteine and atherothrombotic vascular disease in patients [14] [15] [16]. In this study, we demonstrated the relationship between plasma

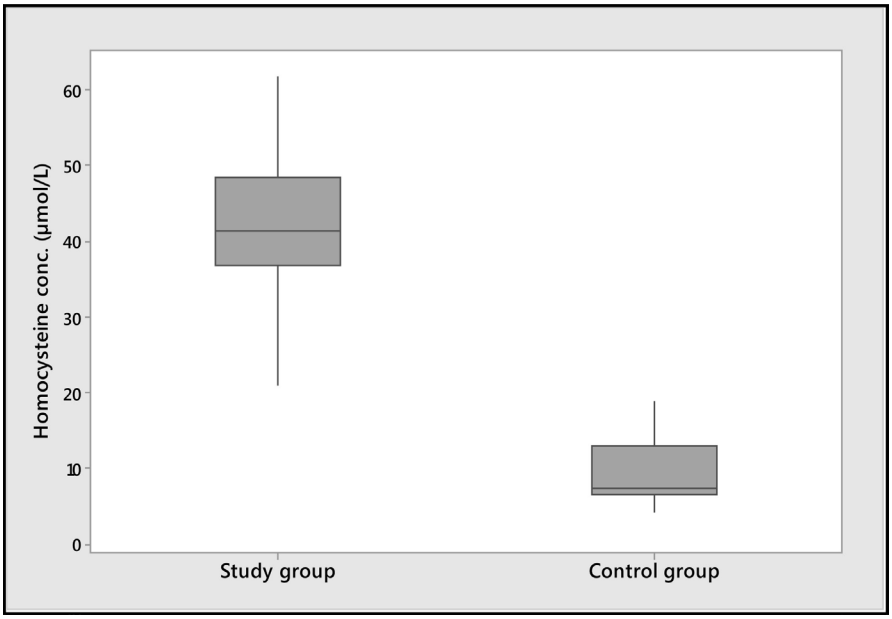

Figure 1. Plasma homocysteine levels in study and control group.

homocysteine level and CAD in Indian patients. Most prospective and retrospective studies have demonstrated that high homocysteine is an independent risk factor for CAD [7]. Previous studies have shown that high rates of CAD in Asian Indians are associated by high prevalence of conventional risk factors such as hypercholesterolemia, hypertension, and smoking [4]. Furthermore, the finding of 36.387 ± 15.04 µmol/L in male and 44.2 ± 10.07 µmol/L in female reflects a higher level of plasma homocysteine in females with CAD. Mean homocysteine levels in study group were significantly higher ($p < 0.001$) than the control group. Abraham *et al.* and Puri *et al.* also reported similar observations of plasma homocysteine level higher among study group as compared to control group [4] [13]. This finding is also consistent with western studies undertaken by Verhoef *et al.* & Taylor *et al.* [17] [18]. Most common cause of higher levels plasma homocysteine may be due to a genetic mutation (MTHFR gene) or nutritional factors. According to some studies, MTHFR gene might be associated with hyperhomocysteinemia and CAD in some populations [5] [19] [20] [21]. In this study, plasma homocysteine levels in different groups showed a significant increase ($p < 0.05$) as compared to control group. In past studies shows that similar assessment on plasma homocysteine abnormality association with hypertension and diabetes mellitus in CAD patients [19] [22] [23].

In a current study, we found no significant correlation between plasma homocysteine level and other conventional risk factors of CAD (hypertension, diabetes mellitus, smoking, and alcohol consumers). These results were similar to those reported in Deepa *et al.* study [24]. In our study, total serum cholesterol, triglycerides, LDL were significantly higher ($p < 0.001$) and HDL level significantly lower in the study group compared to control and specifically in group II (CAD with hypertension). We calculated Pearson' correlation coefficient and

"p" value in different groups for plasma homocysteine levels with lipid profile. Furthermore, there was no significant association between plasma homocysteine and lipid profile. A similar finding has been reported from Puri *et al.* [4]. This variation may be due to small sample size confounding factors in our study. Higher plasma homocysteine values in our study group indicate that our population subgroups are deficient in folate levels and require folic acid supplementation. This intervention may decrease the premature incidence of CAD and reduce the mortality rate of the population.

5. Conclusion

This study indicates that elevated plasma homocysteine is an independent risk factor associated with CAD. Further studies involving a larger sample size will be required to investigate the benefits from vitamin administration in patients with increased homocysteine levels to prevent the premature incidence of CAD.

Conflicts of Interest

The authors declare no conflicts of interest.

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