ORIGINAL ARTICLE

HOMOCYSTEINE LEVELS IN YOUNGER PATIENTS WITH CORONARY ARTERY DISEASE IN PAKISTAN

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ABSTRACT

Background: Coronary artery disease (CAD) is the leading cause of death in the world both in developed and developing countries. Pakistanis are more prone to CAD at younger ages. There are many patients with CAD who lack conventional risk factors. Hyperhomocysteinemia is considered an important modifiable risk factor for CAD. The aim of this study was to establish an association of hyperhomocysteinemia with CAD in younger Pakistani patients with CAD.

Material & Methods: It was a cross sectional comparative study conducted at Punjab Institute of Cardiology Lahore from October 2009 to June 2010. This study included 30 patients of age 20-45 years, with confirmed CAD. They were compared with 30 age matched normal healthy controls. Fasting total homocysteine levels were measured in all the study subjects by Chemiluminescent Microparticle Immunoassay technology. Data was analyzed by SPSS version 16.

Results: Statistically significant difference (p-value = 0.013) was observed in the mean concentration of total homocysteine between the CAD patients (18.1±5.3 μmol/L) and normal controls (14.7±4.93 μmol/L). In the patient group, 21 had homocysteine levels greater than normal value (15 μmol/L) while in the control group, 12 had homocysteine levels greater than normal value. Odds ratio was calculated to be 3.5.

Conclusion: The present study indicates strong association between increased levels of total Homocysteine and CAD in younger Pakistani patients.

KEY WORDS: Homocysteine; Coronary Artery Disease; Chemiluminescent Microparticle Immunoassay.

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INTRODUCTION

Coronary artery disease (CAD) has become the most common cause of death worldwide both in developed and developing countries.[1] The Indian subcontinent (including India, Pakistan, Bangladesh, Sri Lanca and Nepal) has among the highest CAD rates globally, and similarly its inhabitants experience acute myocardial infarction (AMI) at younger ages when compared with people from other countries for unclear reasons.[2] Pakistani population has high risk of CAD in the world. Every fourth middle aged Pakistani has prevalent CAD.[3]

Conventional risk factors (Smoking, Dyslipidemia, Positive family history for CAD, Diabetes Mellitus, and advancing age) do not explain all of the risk for incident CAD events. In about 20% of patients with CAD no conventional risk factors are detectable. This focused attention on other risk factors which can contribute to CAD and led researchers to the discovery of various markers. A large number of studies have identified hundreds of biochemical, clinical, or genetic markers that showed significant relationship with CAD. Out of these new risk factors Homocysteine (Hcy), C-reactive protein, Lipoprotein (a), and Fibrinogen received high attention.[4,5]

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Total Homocysteine (tHcy) is a sulphur containing, nonessential amino acid. It is not a normal dietary constituent. Its sole source is an essential amino acid methionine.[6,7] Moderate, intermediate, and severe hyperhomocysteinemia refer to level as 16 to 30, 31 to 100 and greater than 100 μmol/L respectively.[8]

Decreased circulating plasma concentration of folate, pyridoxine (Vit B6), cobalamin (Vit B12), mutation in the gene coding for the enzyme methylenetetrahydrofolate reductase (MTHFR), kidney diseases, antiepileptic drugs, and methotrexate are associated with increased plasma tHcy concentration. Ethnicity also affects plasma tHcy level.[9,10,11,12,13,14]

Although much work has been done in west in establishing homocysteine as a risk factor for CAD a few studies are available from Pakistan on this aspect. These studies are also conflicting, some showing association between tHcy and CAD while the others showing no association.[15-18]

Since tHcy concentrations are influenced by several lifestyles, genetic, and nutritional factors, studies on tHcy levels in different populations are necessary. Adequate and conclusive studies are lacking in Pakistani population to establish an association between tHcy and CAD. So in view of the conflicting observations and data we aimed to assess the significance of serum total homocysteine as a risk factor in younger Pakistani patients with CAD.

MATERIAL AND METHODS

It was a cross sectional comparative study carried out at Punjab Institute of Cardiology (PIC) Lahore from October 2009 to June 2010. The study was approved by the Advanced Studies and Research Board of University of Health Sciences, Lahore, and Ethical Committee of Postgraduate Medical Institute, Lahore. A written informed consent was obtained from all the participants before enrolling them in the study.

The subjects were divided into two groups:

Group A: comprised of 30 patients of age 20-45, both males and females, admitted with first episode of acute myocardial infarction (ST segment elevation myocardial infarction STEMI or Non ST segment elevation myocardial infarction Non STEMI) or unstable angina. Patients were diagnosed on the basis of typical history of chest pain, ECG findings, and raised levels of markers of myocardial injury, creatinine kinase (CK), CK-MB, Troponin T, and Troponin I.

Group B: Comprised of 30 normal healthy controls of age 20-45, both males and females, taken from ETT (Exercise Tolerance Test) department of PIC. They had negative ETT and normal lipid profile. In both the groups, subjects with past history of CAD, family history of premature CAD, history of diabetes mellitus, hypertension, smoking, abnormal renal functions, having chest discomfort not of cardiovascular origin, subjects using B6, B12, Folic acid, anti epileptics and methotrexate were excluded from the study.

Five ml of blood was drawn from each participant of the study after 12-14 hours overnight fast, by venepuncture using plastic disposable syringes under aseptic conditions. The blood was allowed to clot at room temperature for 30-60 minutes and was then centrifuged at 3000 rpm for 10 minutes. The serum thus separated was transferred into properly labeled vials and stored at 2-8°C for subsequent analysis. Fasting total Homocysteine levels were measured in all the study subjects by Chemiluminescent Microparticle Immunoassay (CMIA) technology using ARCHITECT analyzer.

All the collected data were entered into computer using SPSS version 16.0. To know significant difference of means between controls and patients Student's t test was used. To know significant association between Hyperhomocysteinemia and CAD odds ratio (OR) was calculated by using 2x2 contingency table. Formula used was: $OR = a \times d / b \times c$. The p-value < 0.05 was considered statistically significant.

RESULTS

In our study mean levels of tHcy were 18.1 ± 5.3 μmol/L in patients group while in control group its levels were 14.7 ± 4.93 μmol/L. It was significantly high in patients when compared with controls (p-value

Table 1: Contingency table for calculation of Odds Ratio.

	Total Homocysteine (μmol/L)	CAD patients (Number)	Controls (Number)	Odds Ratio (OR)	95% Confidence Interval	p-value
Total subjects = 60	> 15 μmol/L	a) 21	c) 12	3.5	1.20 to 10.19	0.02*
	≤ 15 μmol/L	b) 9	d) 18			

*Significant

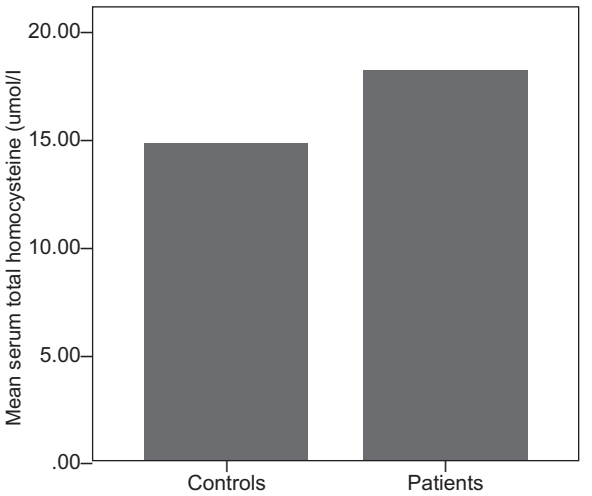

Figure 1: Comparison of homocysteine (mmol/L) levels between CAD patients and controls.

= 0.013). It was also noted in our study that out of 30 CAD patients 9 had normal tHcy levels while 20 had moderate hyperhomocysteinemia, and only 1 had intermediate hyperhomocysteinemia. Similarly out of 30 controls 18 had normal tHcy levels while 11 had moderate hyperhomocysteinemia, and only 1 had intermediate hyperhomocysteinemia. Odds ratio thus calculated was 3.5 (95% CI: 1.20-10.19) with a significance level of p-value = 0.02.

DISCUSSION

Most of the patients with CAD have one or more conventional risk factors. But there are patients with CAD who do not have any of these risk factors. This led researchers to the discovery of new risk factors for CAD with the aim of identification of new markers associated with an increased risk of CAD to provide a better insight into the pathogenesis of coronary atherosclerosis and to facilitate the development of preventive and therapeutic measures.[4,19,20]

Elevated serum tHcy level is considered to be a new risk factor for CAD. But studies on the association of tHcy levels with CAD in different ethnic groups have obtained conflicting results with some studies providing evidence for an association while the other have found none.[10,21] A few studies are available from Pakistan on this aspect. These studies are also conflicting.

Studies conducted by Ujjan et al and Shamsi et al in Pakistan showed significant higher levels of tHcy in CAD patients as compared to healthy controls. On the other hand studies conducted by Akhtar et al and Afzal et al showed no significant increase in tHcy levels in CAD patients when compared with normal controls.[16-18,22]

Since there is a dearth of sufficient data to ascertain the importance of homocysteine in Pakistan, we aimed to determine whether hyperhomocysteinemia is associated with CAD in Pakistani population or not especially in younger patients.

In our study the mean levels of serum tHcy in patients was 18.1 μmol/L and in controls was 14.7 μmol/L which is significantly high in patients than controls (p = 0.03). Our results are in accordance with those reported in other studies conducted by Puri et al. and Angeline et al. Another study conducted by Ujjan et al. has shown similar results.[6,16,23] In these studies tHcy level was much higher (27.8 μmol/L, 24.5 μmol/L, and 24.6 μmol/L respectively) in CAD patients than in CAD patients of our study (18.1μmol/L). In our study CAD patients with positive history of diabetes mellitus, hypertension, smoking, age more than 45 years and positive family history of premature CAD were excluded while in these studies such patients were not excluded. This could explain the much higher levels of tHcy in CAD patients in these studies because all these risk factors can increase tHcy level.[24-28] In our study tHcy level in normal healthy controls was 14.7 μmol/L. About 40% of our control subjects had tHcy levels greater than normal value (15 μmol/L). Similar high level of homocysteine in controls (13.2 μmol/L) was reported in study conducted by Puri et al. in India and (19.1 μmol/L) in study conducted by Akhtar et al. in Pakistan.[17,23] While studies conducted by Garcia et al. and Khurelbaatar et al. showed lower level of tHcy in control groups (9.7μmol/L and 8.7 μmol/L respectively).[29,30] These findings are in accordance with the study conducted by Chandalia et al. which showed that Asian Indians have high levels of homocysteine than European people.[11] The higher level of serum tHcy in our control group may be due to the vitamin B6, B12, and folate deficiency as there is high prevalence of deficiency of these vitamins in our healthy population. High prevalence of deficiency of these vitamins may be due to our dietary habits which include low intake of fresh fruit and green leafy vegetables and over cooking.[31]

In our study, to know significant association between Hyperhomocysteinemia and CAD, the calculated odds ratio was 3.5 reflecting that Hyperhomocystinemic persons were 3.5 times more at risk of getting CAD. Our results are in accordance with other studies conducted by Puri et al. and Yayehd et al. in which odds ratios were 6.05 and 3.03 respectively.[23,32]

CONCLUSION

This study concludes that serum tHcy level was significantly higher in CAD patients as compared to controls and there was a significant association between hyperhomocysteinemia and CAD. Therefore it can be inferred that elevated level of serum tHcy is a risk factor for CAD in younger Pakistani population. It is suggested that serum tHcy concentration should be determined, especially in younger CAD patients who do not have other conventional risk factors.

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CONFLICT OF INTEREST
Authors declare no conflict of interest.
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None declared.