Original Article

Homocysteine as a Risk Factor for Coronary Artery Disease in Pakistan

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Abstract

Pakistani people belong to an ethnic group which has the highest rate of coronary artery disease. There are a number of risk factors for developing the coronary artery disease (CAD). Homocysteine, a sulphur containing amino acid, has been reported to be an independent risk factor for CAD. The present study was done to find out the role of hyperhomocysteinemia in the development of coronary artery disease in Pakistan.

Materials and Methods: A cross sectional study was carried out. There were 40 angiographically diagnosed male patients of coronary artery disease between 30 – 40 years of age taken as cases and 40 age, sex and socioeconomically matched healthy subjects with normal carotid doppler study taken as controls. Fasting venous blood from cases and controls was taken in E.D.T.A vacationers. Plasma was analyzed for homocysteine level by enzyme immunoassay method.

Results: Mean plasma concentration of homocysteine in coronary artery disease patients i.e. cases was 13.5 ± 6.8 µmol/L and was higher than the mean for controls (10.76 ± 2.27 µmol/L) to a significant extent.

Conclusion: Hyperhomocysteinemia through interplay with the classical cardiovascular risk factors may be aggravating the risk of coronary artery disease in Pakistani people.

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Introduction

Coronary artery disease is characterized by the presence of atherosclerosis. Functional changes of endothelium, vascular smooth muscles, platelets and monocytes occur due to atherosclerosis.[1] These changes impair local blood flow and cause vascular occlusion leading to angina and myocardial infarction. Pakistani people belong to a population which has the highest risk of coronary artery disease.[2] Hyperhomocysteinemia has been reported to be common among South Asians[3] including Pakistani healthy adults. Beside other etiological factors for atherosclerosis like hypercholesterolemia, hypertriglyceridemia, high levels of low density lipoproteins (LDL), diabetes mellitus, hypertension, smoking, obesity, stress, sedentary lifestyle,[4] hyperhomocysteinemia has been reported to be an independent risk factor for atherosclerosis.[5]

A link between hyperhomocysteinemia and heart disease was first established in 1960's when it became clear that patients with inborn errors of homocysteine metabolism were prone to develop cardiovascular disease. Homocysteine is a sulphur containing amino acid formed during the metabolism of methionine. It is not a dietary constituent and its sole source is methionine.[6] The normal concentration of homocysteine in plasma

is 5 – 15 µmol/L. Typically a level less than 15 µmol/L is considered normal, between 15 – 18 µmol/L is considered as mildly elevated, between 19 – 60 µmol/L as moderately elevated and > 60 µmol/L as severely elevated.[7]

Hyperhomocysteinemia causes increased oxidetion of LDL (low density lipoproteins) side chains[8], leading to increased free radical formation which in turn may cause endothelial injury initiating the atherosclerosis.[9] Hyperhomocysteinemia also causes a dose dependent increased DNA synthesis in smooth muscle cells but 25% decreased DNA synthesis in vascular endothelial cells in humans.[10] Its inhibitory effect on endothelial cell growth, prevents re-endothelialization of the injured endothelium. Hyperhomocysteinemia is reported to cause vascular spasm by impairing the production of endothelial derived relaxing factor (E.D. R.F) and interfering with the vasodilatory and antithrombotic function of nitric oxide.[11] Homocysteine also alters the effect of many clotting proteins on endothelial surface, leading to a prothrombotic environment and it also activates factor V and inhibits thrombomodulin dependent protein C activation in vitro.[12] The odds ratio for ischemic heart disease has been estimated to be 1.4 for every 0.5 µmol/L increase in total homoysteine. That confers 6 – 7% increase in risk for having a myocardial infarction or stroke for every 1 µmol/L increase in total homocysteine.[13] The risk of heart attack is increased following mild elevations in homocysteine, with each 3 units increase in level equating to 35% increase in myocardial infarction.[14] Results of a number of clinical studies have shown that fasting homocysteine concentration in patients with vascular disease are on an average 31% higher than in normal subjects.[15] It thus appears from above discussion that hyperhomocysteinemia leads to atherosclerosis with all its deleterious effects on cardiovascular system. The present study was thus planned to ascertain the prevalence of hyperhomocyteine and its role in coronary artery disease patients in Pakistan.

Materials and Methods

Both patients and controls participated willingly with prior consent to undergo tests and examination. The criteria for inclusion for patients in the study were males between 30 – 45 years of age, who were documented cases of coronary artery disease diagnosed on the basis of angiography and who had no history of diabetes or renal disease and for controls were healthy males between 30 – 45 years of age, with no history of any disease and with normal E.C.G and carotid doppler examination. The exclusion criteria were all patients taking vitamin B_{12}, B_6 or folic acid treatment for last 6 months prior to sample collection or patients suffering from anaemia, renal disease, diabetes, malabsorption syndrome, acute infection and an age group of > 45 years. In controls in addition to above exclusion criteria, any abnormality in E.C.G or carotid doppler study were excluded.

A total of 80 subjects were taken in the study, out of which 40 subjects were taken as controls and 40 as cases after observing the above mentioned inclusion and exclusion criteria. The cases (patients) were taken from Punjab Institute of Cardiology, Lahore. The control group was selected from general population.

A 5 ml fasting sample of venous blood from all the subjects was taken in vacutainers containing E.D.T.A. The plasma was separated within half a hour,[16] and then stored at -21°C. The estimation of homocyteine was done on V – max kinetic microplate reader by Novobiolabs. Plasma homocyteine was determined by enzyme immunoassay method (E.I.A) with axis homocyteine kit manufactured by Axis Shield diagnostic Ltd, United kingdom.[17].

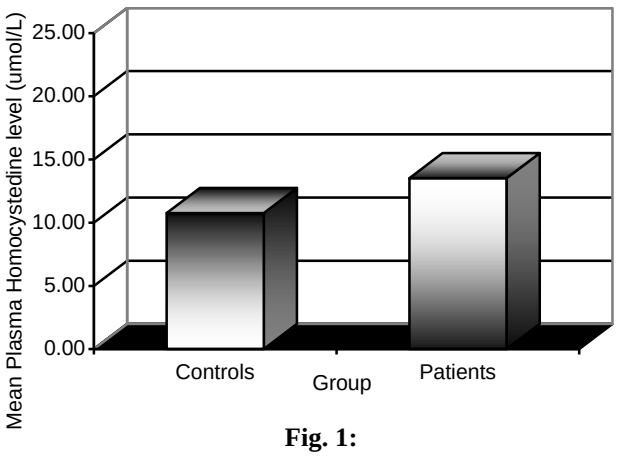

Fig. 1:

Results

In current study, 80 male subjects with age of 30 – 45 years were included. Normal reference range for plasma homocysteine is 5 – 15 µmol/L. Data revealed that mean plasma homocyteine level for 40 controls subjects (Fig. 1) was 10.76 ± 2.27 µmol/L and was within the normal range. Regarding coronary artery disease patients i.e. the cases, mean values for 40 cases was

13.52 ± 6.79. The mean values in patients were, although within the normal range, but were significantly on higher side in patients than in control group. Moreover 12 patients had values of more than upper limits of normal reference range with a range between 15.5 – 38.8 µmol/L, compared to only 3 controls with high values and even these values were not exceeding 16.0 µmol/L.

Discussion

Pakistan is facing a great challenge in combating coronary artery disease. According to the most careful estimates, based on scientific studies, nearly 100,000 individuals suffered an acute myocardial infarction in the year 2002.[18] The level of plasma homocysteine was studied in patients with coronary artery disease as well as in healthy individuals in the present research to investigate the role of hyperhomocysteinemia in coronary artery disease in Pakistan.

The current study showed significantly high levels of plasma homocysteine in patients of coronary artery disease as compared to controls. This high value suggests a causal relationship between the high homocysteine level and development of coronary artery disease. The results are in agreement with the results of Bonna[19] and the results of a large European trial.[20] The results of trial indicated that adults have 3.2 times higher risk of developing coronary artery disease, if their plasma homocyteine levels were in the top fifth of the normal range, compared with individuals with value of homocysteine in the bottom four fifth of the normal range. This risk was independent of other risk factors like high cholesterol level and smoking. Similarly a Norwagian study by Nygrad[21] revealed the risk of coronary artery disease to be directly proportional to the plasma homocysteine levels.

Although there is a great controversy regarding the relationship of homocyteine with coronary artery disease, as results of some researchers have shown a positive correlation[22] and some a negative correlation.[23,24] The previous studies were however carried out mostly in well developed countries and only very few studies have been carried in countries like Pakistan. As the lifestyle and dietary habits of this country are different from well developed countries like USA, Canada and England, so the results of present research showing a positive correlation between plasma homocysteine and coronary artery disease basically represent the low per capita income population. In this population it appears that in addition to other classical major and minor risk factors for coronary artery disease, hyperhomocysteinemia may also be an important risk factor for this disease. In order to have a broader and better insight for the implementation of the results of present study, there is a need for designing a large scale study. This would help to recognize a modifiable risk factor for coronary artery disease thereby helping to fight against this high risk disease with a high rate of mortality and morbidity.

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