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Homocysteine, folate and vitamin b_{12} status in patients with coronary artery disease in young age

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Abstract

Introduction: Rising incidence of coronary artery disease in India which is rather common causes of morbidity and mortality is not good news. Even by conservative estimate Indian spending an amount that's nearly 40% entire central Government health budget for the treatment of CAD. Hyperhomocysteinemia is considered as an independent risk factor of premature Coronary Artery Disease (CAD), but the probable role of homocysteine along with biochemically related folate & vitamin B_{12} with homocysteine in early onset of CAD is not well studied in this part of country. The aim of this study was to assess the role of hyperhomocysteinemia, folate and vitamin B_{12} deficiency in the development of premature CAD.

Materials and Methods: We have performed observational case-control study in total 200 participants having age more than 20 years in both male and female sex, out of these 100 are patients suffering from CAD who were admitted for selective coronary angiography in academic tertiary care hospital in Ahmedabad and 100 healthy controls group participants attended the said institute. The homocysteine, folate and vitamin B_{12} concentration were measured using standard reagent kit on Abbott AxSYM close system.

Result: Mean homocysteine in study group was significantly higher in study group compare to control group (33.02+/-17.41 µmol/l; 13.88+/-3.86 µmol/l). Mean homocysteine level in each age group of study group patient is significantly high than the control group participant ($P < 0.001$). Mean folate and vitamin B_{12} level is lower in each age group of study group patients than control group participants. Mean serum folate (7.13+/-3.46 ng/ml versus 12.35+/-3.36 ng/ml) and vitamin B_{12} (156.41+/-127.36 pg/ml versus 449.86+/-169.90 pg/ml) level are lower in study group than the control group participants respectively. Folate and vitamin B_{12} shows negative correlation with homocysteine. There was a significant positive correlation between homocysteine and age is observed while folate and vitamin B_{12} shows negative correlation with age.

Conclusion: We may conclude from our study that increase homocysteine level may be one of the risk factor of CAD in young age. Our study also shows that age is non-modifiable while folate and vitamin B_{12} deficiency are modifiable risk factor of hyperhomocysteinemia.

Keywords: Coronary Artery Disease; Homocysteine; Folate and Vitamin B_{12}.

Introduction

Atherosclerotic disease involving coronary, peripheral and cerebrovascular system constituent to be a major health problem in adult population in developed and developing countries. The most common manifestation of atherosclerotic disease is coronary artery disease (CAD) and is rather common causes of morbidity and mortality worldwide including India. The cost of management of CHD is a significant economic burden to the person concern, to their family, to the society and the country as well, so prevention of CAD is very important step in it's management. Even by conservative estimate India spending an amount that's nearly 40% entire central Government health budget. Recognition of its various risk factors is important to planning effective preventive measures. Homocystein (Hcy) has been linked to atherosclerosis and CAD by McCully in 1969.[1-4] He described hyperhomocysteinemia as a possible risk factor for CAD.

In 1969, Dr. Kilmer S. McCully, M.D., studied the association of acute vascular thrombotic events by comparing an 8-year-old patient with homocysteinuria who died of a stroke and an infant with an inherited defect in cobalamine metabolism who died of cardiac arrest. After completion of his study, he proposed a novel and controversial theory: elevated homocysteine (Hcy) concentration result in premature atherosclerosis.[1] After his study, many researches were done in this direction to find out the association between hyperhomocysteinemia and atherosclerotic vascular disease. Studies have suggested that elevated Hcy concentrations are associated with an increased rate of stroke, coronary artery disease (CAD), peripheral vascular disease and deep venous thrombosis.[5-8]

Hcy is a non-protein–forming sulphur containing amino acid. In human it is constantly interchanging among one of its four forms. Approximately 1% is free, 70–80% is bound to albumin via a disulfide link and the remaining 20–30% is found either as a Hcy dimer or a cysteine-Hcy-mixed disulphide.[9] The type(s) of species that contributes to the pathologic process is unknown. Hcy metabolism is at the intersection of two metabolic pathways: remethylation and transsulfuration.[10] In the trans sulferation pathway, pyridoxine (vitamin B_6) is an essential cofactor while in the remethylation pathway cobalamine (vitamin B_{12}) acts as a cofactor and folic acid provides the methyl essential for the reactions to take

place.[10,11,12] Disruption in metabolic pathway elevates the Hcy concentration in the plasma.[10] Metabolic disruption in Hcy metabolism is multifactorial: heritable enzyme deficiencies and vitamin cofactor deficiencies all play a role.[13] Other conditions such as polymorphism in the coding gene of MTHFR, consumption of folate antagonists such as carbamazepine and methotrexate and finally disorders of homocysteine metabolism during hypothyroidism and renal failure can also cause hyperhomocysteinemia. The severity of the resulting hyperhomocysteinemia dependent on the extent to which the particular disturbance affects the coordination of the two pathways of homocysteine metabolism. Dietary deficiency or disorders related to folate and/or vitamin B_{12} malabsorption lead to hyperhomocysteinaemia and an increased risk of CAD.[14] Many researcher's studies shows relation of high homocysteine levels with low intake of folic acid and vitamin B_{12}.[15-17] Mishra et al in their study of an urban population of India have observed an association between hyperhomocysteinaemia and low intake of folate and vitamin B_{12}.[18] It has been shown that high plasma homocysteine levels can be reversed with vitamin supplements.[18]

Many theories have been postulated regarding Homocysteine, Folic acid, Vitamin B_{12} on western population. Few studies also have been done on it relation with age & sex. But to our best of knowledge no studies done on population of Ahmedabad, though it is very much essential to know the facts & figure on potential population of Gujarat looking in to diversity of population and their dietary habit. Meanwhile, we do not have sufficient data regarding the role of hyperhomocysteinemia in development of premature CAD and this question needs further investigations. Here we should mention that, in most studies, premature CAD is defined as CAD in men under 55 or women less than 65 years old. However, in the Framingham risk stratification table and certain other investigations, the onset of ascending cardiovascular disease in men and women was estimated between the ages of 40–45 years. But CAD is nowadays can be seen in very young age like 30 years. This decreasing the age of onset is not clearly understood, stress & dietary habits are considered the main culprit. Based on the above mentioned points, we decided to perform this study in order to have a better evaluation of risk factors in premature CAD.

Material and Methods

This study is a case–control type of study includes total 200 participants with age more than 20 years. Out of which 100 participants (73 males and 27 females) are confirm diagnosed cases of CAD, these patients are included in study group. Diagnosis of CAD is confirmed according to the standard diagnostic criterion which includes ST elevating myocardial infarction, non-ST elevating myocardial infarction, stable angina and unstable angina. These patients were presented to the intensive coronary care unit of academic tertiary care hospital of Ahmedabad during our study period from January-2011 to December-2011 with CAD. While control group includes 100 age and sex-matched healthy individuals (67 male and 33 female) came to the same institute for routine health check-up during the same study period with normal ECG and without any clinical evidence of CAD, hypertension, diabetes mellitus, pre-existing liver and kidney disease.

The data were obtained from two different sources. First source of data collection is information gathered by interview and filling a questionnaire covering all the personal and medical history. The questionnaire was based on standard Rose questionnaire for anginal pain, known CAD risk factors and confounding factors. The second source of data is a laboratory report which includes serum homocysteine, folic acid and vitamin B_{12}.

Participants with myocardial infarction in the last 6 weeks, pre-existing renal disease, pre-existing hepatic dysfunction, pregnancy, hypothyroidism, anemia (haemoglobin < 12 g/dl) and those participants taking methotrexate, carbamazepine, phenytoin, N-Acetyl Cysteine (NAC), folic acid or vitamin B_{12} within 6 weeks of enrolment were excluded from the study.

Informed consent was taken from each participant taking part in the study according to the standard guidelines of ethics committee of the institute. Detailed history was taken regarding the duration, frequency and severity of the chest pain, exercise tolerance and history of previous Myocardial Infarction. We have also inquired about any addiction (like smoking, tobacco, alcohol). A detailed clinical examination (general and systemic) was done by proper exposure and appropriate light. A 12-lead ECG recording was taken and blood samples were also collected for the laboratory analysis.

Sample Collection and Analysis: For biochemical analysis 5-10 ml of blood sample was withdrawn and collected in vacuette containing clot activator; subsequently the samples were transported to the clinical biochemistry laboratory at 2-8°C within half an hour. Before centrifugation we have ensured that the complete clot retraction has taken place. Serum is removed carefully from the vacuette within 2 h of draw. In case of testing delayed more than 24 h, serum specimens were stored at 2-8°C and analyzed very next day. Multiple freeze-thaw cycles was avoided.

Before analysis samples were mixed thoroughly, by low-speed vortexing or by gently inverting and centrifuged to remove particulate matter and to ensure consistency in the results.

All the serum samples were subjected to assays for tHcy, folic acid and vitamin B_{12} as soon as possible after thawing at 37ºC. The measurement of all these three parameters was done on Abbott AxSYM system. Standard reagent kits from Abbott AxSYM were used for analysis of sample. The AxSYM quantitatively measure homocysteine by fluorescence polarization immunoassay, folate by ion capture assay technique and vitamin B_{12} by Microparticle Enzyme Immunoassay

(MEIA) technique. Tests were performed according to instructions suggested by the analyzer and kit manufacturer.

Care Taken for Sample Collection: Before collection of blood specimen over night fasting (8-12 h no calorie intake) was confirmed from each and every individuals, because recent food intake may considerably alter the serum folate and Hcy concentration.

Statistical analysis: Numerical data were analyzed and reported in terms of mean and standard deviation. Statistical analysis of result was done by independent 't' test. In this analysis, variable showing P- value 0.05 or less were considered to be statistically significant. For calculation of P- value SPSS software was used. To test correlation Pearson correlation test was applied.

Observation and Results

A total 200 participants were enrolled in this study. 100 patients having CAD are included as study group and 100 normal healthy participants were selected as a control group. In the study group out of 100 patient 73 (73%) were male and 27 (27%) were female. In healthy control group out of 100, 67 (67%) were male and 33 (33%) were female. The mean age of the study group is 54.77+/-11.93 years and 56.33+/-12.58 year in control group. No significant difference was noted between the patient and control group in this regard.

In both, study and control group we have maximum (41% in each group) participants in age group 50-64 yrs. While between age 20 – 34 yrs we have 5% and 4% participants in study and control group respectively. There is no significant difference in age wise distribution in study and control group.

Serum homocysteine, folate and vitamin B_{12} were measured in all participants. Mean homocysteine in study group was significantly higher (33.02+/-17.41 µmol/l) in study group compare to control group (13.88+/-3.86 µmol/l) ($p < 0.001$). We also observed significant difference in serum folate and vitamin B12 between study and control groups (Table 1).

Table 1: Mean homocyateine, folate and vitamin B_{12} in study and control group

	Mean+/-S.D		P value
	Study Group	Control Group	
Homocysteine (µmol/L)	33.02+/-17.41	13.88+/-3.86	< 0.001
Folate (ng/ml)	7.13+/-3.46	12.35+/-3.36	< 0.001
Vitamin B_{12} (pg/ml)	156.41+/-127.36	449.86+/-169.90	< 0.001

When comparing normal and above normal range homocysteine level in each age group in the study group, maximum number (*n* = 32) of patient having high homocysteine level are in age group 50-64 year. However, according to percent wise calculation in each group of CAD patients, age group 35-49 year has highest percent (82.14%) of patients. In control group, 95 patients have normal homocysteine while only 5 patients have homocysteine above normal range; most of them are above age 65 year. Calculation of Mean Homocysteine level in each age group shows that, study group patients have statically significant high Homocysteine level than the control group participant ($P < 0.001$) (Table 2). The mean plasma level of homocysteine is 35.43 ± 18.35 µmol/l in the study group male and is significantly higher ($P < 0.001$) compared to Control group male 14.31 ± 3.98 µmol/l. The same relation is observed in female participants with mean of 26.51 ± 12.30 µmol/l and 13.00 ± 3.51 µmol/l in study and control group respectively.

Laboratory finding of serum folate in our participant shows that majority of patients in study group have low serum folate level. Mean serum folate level in study group and control group is 7.13+/-3.46 ng/ml and 12.35+/-3.36 ng/ml respectively, study group have significant high serum folate level ($p<0.001$). In the study group age between 50-64 years carries maximum number (n=25) of patient having low serum folate level but when we calculate a present of patient having high and low folate level in each age group, age group 35-49 years have maximum percent (71.43%) of patient have low serum folate level. In control group most of the participant have normal folate level, only 6% participant have folate deficiency and majority of them are above 65 year age. According to age group there is significant difference in folate level in study and control group. Mean folate level in each age group is lower in study group than in control group, when we calculate p-value it is statistically significant for each age group ($p < 0.05$) (Table 2).

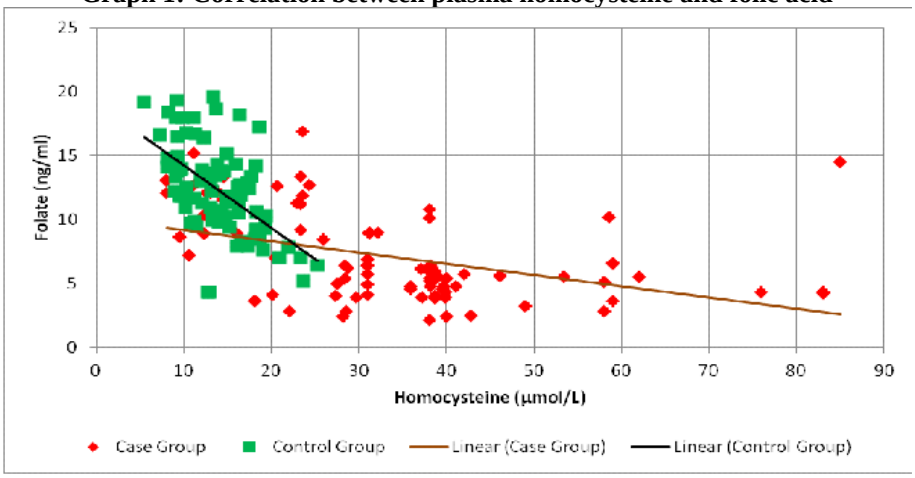
Laboratory finding of vitamin B_{12} measurement shows that vitamin B_{12} deficiency is common in patients with CAD (study group). According to age group there is significant difference in vitamin B_{12} level in study and control group. Comparing normal and below normal range vitamin B_{12} level in each age group in the study group, maximum number (*n* = 30) of patient having low vitamin B_{12} level are in age group 50-64 year. On the other hand, according to percent wise calculation in each group of CAD patients, age group 35-49 year has highest percent (89.29%) of patients. In control group vitamin B_{12} deficiency is uncommon. Mean vitamin B_{12} level in each age group is lower in study group than in control group. In study group lowest vitamin B_{12} is found in age group 35-49 years (133.45±120.69 pg/ml) while in control group it is in age group >65 years (403.91±184.25 pg/ml) (Table 2). Mean serum vitamin B_{12} level in study group is significantly lower ($p < 0.001$) than control group male, it is 158.18 +/- 135.91 pg/ml and 438.28 +/- 174.50 pg/ml respectively. The same relation is also observed in study and control group female vitamin B_{12} status in them is 151.62+/-99.77 pg/ml and 473.40+/-160.15 pg/ml respectively.

Table 2: Hcy, folate & vitamin B_{12} status in each age group of study & control group

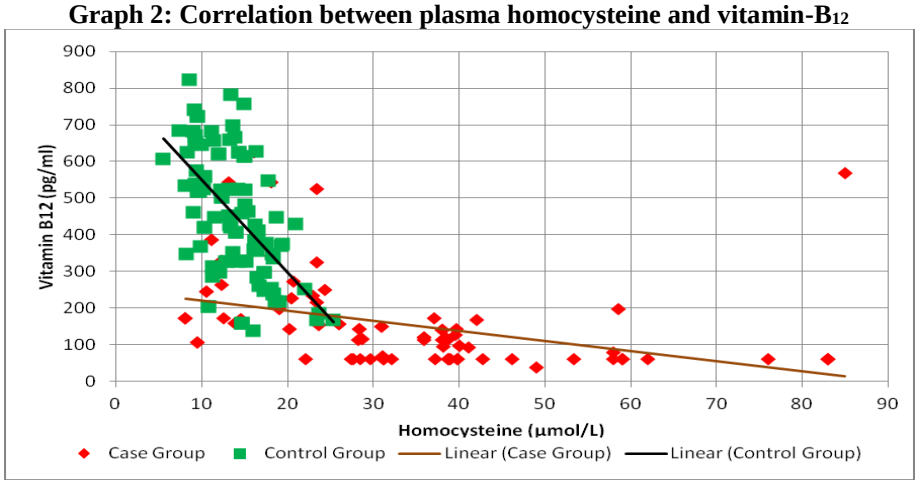
Age (Year)	Homocysteine (µmol/L) Mean ± S.D		P value	Folate (ng/ml) Mean ± S.D		P value	Vitamin B_{12} (pg/ml) Mean ± S.D		P value
	Study Group	Control Group		Study group	Control Group		Study Group	Control Group	
20 - 34	29.55 ±12.94	14.02 ±3.11	<0.05	6.86 ±3.49	12.72 ±3.10	<0.05	156 ±71.34	454.68 ±172.46	<0.001
35 - 49	34.64 ±18.32	14.02 ±4.40	<0.001	6.64 ±3.78	11.87 ±3.08	<0.05	133.45 ±120.69	456.66 ±150.90	<0.001
50 - 64	31.27 ±15.50	13.22 ±4.17	<0.001	7.30 ±3.47	12.55 ±2.99	<0.05	180.11 ±146.37	478.89 ±168.70	<0.001
> 65	34.70 ±20.08	14.64 ±4.70	<0.001	7.45 ±3.13	12.42 ±4.15	<0.05	143 ±105.26	403.91 ±184.25	<0.001

Generally speaking there is a negative correlation between plasma level of homocysteine and folic acid level in both case and control group is observed, it is – 0.44 and – 0.56 respectively (Graph 1). Homocysteine also shows negative correlation with vitamin B_{12} in study and control group, it is – 0.38 and -0.57 respectively (Graph 2).

Graph 1: Correlation between plasma homocysteine and folic acid



Graph 2: Correlation between plasma homocysteine and vitamin-B_{12}



Discussion

As a laboratory person we perform battery of investigation just to diagnose it & to facilitate the treatment. Prevention of CAD is a very important step in the management which can be done by early laboratory detection and early intervention. Alarm of the clinical clock is always late for an early intervention for patient management. In such cases laboratory has a distinctive

role for early detection and prevention of the disease and their sequel. Prevention of CHD can be approached in many ways including health promotion campaigns, specific protection strategies, life style modification programs, good control of risk factors and constant vigilance of emerging risk factors. But early detection by signature of documentary evidence of laboratory report remains the most important before the full fledge disease and its complication.

The association between raised homocysteine and thrombosis was demonstrated by many research workers worldwide. Boushy et al showed homocysteine as an independent graded risk predictor for atherosclerotic disease in coronary, cerebral and peripheral vessels.[19] He also estimated that 10% of general population CAD risk is attributed to homocysteine. In the study relation between hyperhomocysteinemia and serum level of folic acid and vitamin B_{12} shown that deficient level of folic acid and vitamin B_{12} raised the homocysteine.

Hyperhomocysteinemia increases the risk of coronary artery disease by increased thrombosis, adverse effects on endothelial function, promoting thickening of the intima and oxidative damage of low density lipoprotein.[20] The hyperhomocysteinemia was reported in 40% of patients with vascular disease and lowering plasma homocysteine level would reduce the risk of CAD by 16%.[21] Additionally, some studies showed hyperhomocysteinemia may have prognostic value in mortality of patients with CAD.[22]

Considering these facts we have undertaken this study to categorize homocysteine as a risk factor for CAD and also recognise deficiency of folic acid and vitamin B_{12} as motive for Hyperhomocysteinemia.

In our study with 200 subjects (100 study group cases and 100 control group participants) we have observed that serum homocysteine levels were increased significantly in CAD patients when compared to its controls group. It was found that mean homocysteine level in our study group was 33.02+/-17.41 µmol/l and 13.88 +/- 3.86 µmol/l in control group. When we have calculated P value it was found to be statistically significant, and rate of significance is higher in study group than in control group. It indicates that higher homocysteine level has correlation in CAD. The higher homocysteine level in CAD as we observed in our study may be because of increase homocysteine level involved in oxidative damage to vascular endothelial cells, increased proliferation of smooth muscle cells and oxidative modification of low density lipoprotein, all leading to atherosclerosis, which ultimately result in CAD.

The results of our study correlate with Saeed Sadeghian and Faramarz Fallahi study which show that serum level of homocysteine in individuals with CAD are significantly higher than participants without CAD (19.3 ± 1.7 µmol/lit versus 13.9 ± 0.9 µmol/lit, $P = 0.005$) and hyperhomocysteinemia were correlated with higher risk of CAD.[23] In our study mean level of homocysteine was higher as compared to the published data. It could be due to geographical variations, racial, genetic causes, different lifestyle, ethnic difference or dietary habit. More over limited number of subjects were included in our study which comprises mostly patient with severely high level of homocysteine.

Saeed Sadeghian and Faramarz Fallahi, have studied the relation between hyperhomocysteinemia and serum level of folic acid and vitamin B_{12}. They had observed that mean serum level of folic acid in the study group was 6.33 ± 0.29 ng/ml and mean serum level of vitamin B_{12} was 282.5 ± 9.1 pg/ml in there study, the prevalence of folic acid deficiency is 10.7% (13.1% in men and 2% in women) while vitamin B_{12} deficiency has a rate of 24.4% (26.6% in men and 16.7% in women). They found reverse relationship between homocysteine and folic acid and vitamin B_{12} level. With folic acid Pearson correlation coefficient = - 0.148, P = 0.02 and with vitamin B_{12} Pearson correlation coefficient = - 0.22, P = 0.001.[23] We have observed the same relation between homocysteine, folic acid and vitamin B_{12}. The folate and vitamin B_{12} level are low in study group compare to control group. Folate level in study group is 7.13 +/- 3.46 ng/ml while in control group it is 12.35 +/- 3.36 ng/ml and vitamin B_{12} level in study group is 156.41+/-127.36 pg/ml versus in control group it is 449.86+/-169.90 pg/ml. P-value which is statistically significant ($P < 0.05$) for folate and vitamin B_{12} shows that there is a significant difference in folate and vitamin B_{12} status in study and control group. Pearson correlation shows negative correlation of folic acid with homocysteine, – 0.44 and – 0.56 in study and control group respectively. Vitamin B_{12} also shows negative correlation with homocysteine, it is – 0.38 and - 0.57 in study and control group respectively. Jen-Shiou Lin and Ming-Ching Shen has observed serum tHcy levels tended to increase with decreasing serum levels of folate ($P < 0.05$) and vitamin B_{12} ($P < 0.029$),[24] this relation correlate with our study.

Folate and vitamin B_{12} has important role in remethylation and transsulfuration pathway of homocysteine metabolism. Defect in any of these, increase homocysteine level in circulation. Main source of vitamin B_{12} is animal food. Most of Indian population, particularly Gujaratis are pure vegetarian. Because of their dietary habit (strict vegetarian) they are more susceptible for vitamin B_{12} deficiency. So in our study group, mean vitamin B_{12} is very low. In our study vitamin B_{12} is associated with increase homocysteine level, that can be seen by calculating pearson correlating coefficient, which is – 0.38 in study & – 0.57 in control group.

Our study revealed that serum Hcy concentration tended to increase with age. Mean homocysteine level is highest in age above 65 years in both group, it is 34.70 µmol/L in study group and 14.64 µmol/L in control group. Folate and vitamin B_{12} deficiency maybe responsible for increase homocysteine level in advance

age as observed in the present study. Main cause for vitamin deficiency in advance age is due to decrease absorption from gastro intestinal tract with advancing age or it may be due to inadequate intake of vitamin B_{12} and folate, inaccurate cooking of vegetables, prolonged cooking of vegetables may destroy up to 90% of folate, improper fortification of grain products with folic acid in our country maybe responsible.

Our findings were in contrast to those found in a pilot study done by Lolin et al in 1996 (China), which demonstrated that serum Hcy concentration was independent of a deficiency of folate and/or vitamin B_{12}.[25] The data from a study in Tawanese Chinese subjects, which demonstrated that both sex and serum folate did not affect serum homocysteine. This contrast may be due to geographical variations, racial and ethnic differences, genetic causes, different lifestyle.

Our study limitations is the smaller sample size over all and also we have a small number of female participants in comparison to males. This limitation may cause our inability to precisely evaluate the role of hyperhomocysteinemia in sex. Farther study with more number of patients with more female participant is needed for more accuracy.

Conclusion

We have observed in our study that homocysteine level is significantly higher in patients suffering from CAD compare to normal healthy subjects. Our data favours that homocysteine level is affected by both non-modifiable risk factor of atherosclerosis like age and modifiable risk factors like folate and vitamin B_{12}. Finally, from our study we may conclude that hyperhomocysteinemia is an independent risk factor for CAD in young patients (below 45 year) furthermore folate and vitamin B_{12} deficiency is a preventable cause of hyperhomocysteinemia. Person with one or more other risk factor for CAD should comprise not only homocysteine but folate and vitamin B_{12} too in their routine check-up and if there is any abnormality found, treat the underlying cause which will help individual to stay away from CAD in upcoming days up to some extent and can have significant benefits with regard to cardiovascular morbidity and mortality.

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