Research Article

Homocysteine level in Coronary artery disease patients of Ahmedabad population

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ABSTRACT

Background: Coronary artery disease (CAD) is a major cause of morbidity and mortality in the modern society. The cost of managing CAD is a remarkable economic burden and so prevention of CAD is very essential step in its management. The majority of CAD patients have at least one cardiovascular risk factor, but 20% of them have no traditional risk factors. Persistent focus on newer risk factors is necessitate as they may further step forward our ability to predict future risk and determine treatment. Recently, homocysteine has been recognized as a risk factor for CAD. This study was conducted to find out the homocysteine as one of the risk factors of CAD in population of Ahmedabad. **Objective:** The aim of this study is to categorize homocysteine as a risk factor for CAD and to find out the effect of age on homocysteine level. **Materials and Methods:** Totally, 100 patients with CAD and 100 normal controls were included in study and homocysteine concentration is measured using standard reagent kit on Abbott AxSYM close system. **Result:** 78% of patients in the study group and only 5% of participants in control group have high homocysteine level above biological reference interval, and its concentration was significantly high in study group patients compared to the normal control group participants (33.02 ± 17.41 and 13.88 ± 3.86 respectively, $P < 0.001$). There was a significant positive correlation between homocysteine and age is observed. **Conclusion:** Increase homocysteine level may be one of the risk factor of CAD in young age. Homocysteine level is increases with growing age and play a role in the occurrence of CAD.

KEY WORDS: Coronary Artery Disease; Homocysteine; Novel Risk Factor

INTRODUCTION

Coronary artery disease (CAD) is also called as atherosclerotic heart disease; it is the end result of the accumulation of atheromatous plaques within the walls of the coronary arteries.[1] CAD is the leading cause of death worldwide, and forecast has been made that it will turn into the most frequent cause of death globally, counting India, by 2020.[2] The majority of patients suffering from CAD does not show any indication of the disease for many years, while the symptoms and signs of CAD are noted, the disease usually have laddered in an advanced state. CAD is the most common cause of sudden death and is also the most common reason for death of men and women over 20 years of age.[3,4]

Control of conventional risk factors (e.g., smoking, lipids, etc.) has brought a decline in the incidence of CAD. However, despite aggressive control of risk factors in the general population, it is not possible to prevent the development of CAD in all patients. Continued focus on newer risk factors is warranted as they may further improve our ability to predict future risk and determine treatment. One of the "novel risk factor" is homocysteine. Total homocysteine (tHcy) recently has been recognized as a risk factor for the presence of

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atherosclerotic vascular disease and hypercoagulability states.[4]

Homocysteine is a non-protein-forming sulfur-containing amino acid first described by Butz and du Vigneaud in 1932.[5] It is an intermediate formed during the catabolism of the essential dietary amino acid methionine. Its metabolism is at the intersection of two metabolic pathways: Remethylation and transsulfuration. Mild to moderate hyperhomocysteinemia is well-established independent risk factor for coronary, cerebral and peripheral atherosclerotic disease and venous thrombosis.[6-9]

The "homocysteine theory" of atherosclerosis came up from the observation that diseases such as homozygous homocystinuria, which are characterized by severe hyperhomocysteinemia are associated with premature vascular disease.[10,11] Epidemiological data suggest that even slight elevation of plasma homocysteine is a risk factor and possibly a causative agent for atherosclerotic disease. Support for the homocysteine theory of atherosclerosis comes from possible mechanisms of homocysteine-induced atherogenesis and thrombosis by (i) Endothelial injury, (ii) platelet activation, (iii) smooth muscle proliferation, (iv) oxidative modification of low-density lipoproteins, and (v) endothelial-leukocyte interactions.

Elevation of plasma tHcy is multifactorial: Heritable enzyme deficiencies and vitamin cofactor deficiencies all play a role.[12] Many researcher's studies show the relation of high homocysteine levels with low intake of vitamin B12 and folic acid.[13-15] The severity and type of the resulting hyperhomocysteinemia are dependent on the extent to which the particular disturbance affects the coordination of the two pathways of homocysteine metabolism.

Many theories have been postulated regarding homocysteine, folic acid, Vitamin B12 on western population. Few studies also have been done on it relation with age and sex. However, to the best of our knowledge, no studies carried out on population of Ahmedabad, though it is very much essential to know the facts and figure on potential population of Gujarat. Considering the above facts, we have undertaken this case–control, prospective study to evaluate serum homocysteine level as a risk factor for the development of CAD.

MATERIALS AND METHODS

This was a case–control study of 100 cases and 100 controls. Study (case) group includes 100 patients (73 males and 27 females) with confirm diagnosed cases of CAD made according to the standard diagnostic criteria. This includes ST elevating myocardial infarction, non-ST elevating myocardial infarction, stable angina, and unstable angina. All these patients presenting to the intensive coronary care unit of Sheth Vadilal Sarabhai General Hospital (Smt. N.H.L Municipal Medical College), Ahmedabad during the study from January-2011 to December-2011 with CAD.

Normal control group includes 100 age, and sex-matched healthy individuals (67 male and 33 female) came for regular health check-up at Sheth Vadilal Sarabhai General Hospital during the same study period, without clinical evidence of CAD, hypertension, diabetes mellitus, pre-existing liver and kidney disease, and with normal ECG.

The data obtained by interviewing all participants. The data cover detail clinical history including clinical examination, past and family history, other clinical problem, and habits.

Subjects with pre-existing renal disease, pre-existing hepatic dysfunction, pregnancy, hypothyroidism and those taking methotrexate, carbamazepine, phenytoin, Vitamin B12, and folate were excluded from the study.

Informed consent was taken from each patient, and healthy individuals participated in the study according to the standard guidelines of ethics committee.

Sample Collection and Analysis

A volume of 5 ml of blood sample collected in clot activator vacuette and transported to the laboratory at 2-8°C within half an hour. It was ensured that the complete clot retraction has taken place before centrifugation. Serum is removed from the clot within 2 h of draw. If testing was delayed for more than 24 h, serum specimens were stored at 2-8°C and analyzed next day. Multiple freeze-thaw cycles is avoided. Samples are mixed thoroughly after thawing, by low-speed vortexing or by gently inverting, and centrifuged before use to remove particulate matter and to ensure consistency in the results.

Care Taken for Sample Collection

Blood specimens were collected from fasting (8-12 h no calorie intake) individuals, because recent food intake may considerably alter the serum tHcy concentration. All samples were immediately subjected to assays for tHcy, after thawing at 37°C. The measurement of tHcy was assayed on an Abbott AxSYM system. Standard reagent kits of Abbott AxSYM were used for analysis of sample. The AxSYM analyze homocysteine by fluorescence polarization immunoassay for the quantitative measurement of homocysteine in human serum. Tests were performed according to instructions recommended by the manufacturer.

Statistical Analysis

Numerical variables were reported in terms of mean and standard deviation. Statistical analysis of result is done by

independent *t*-test. In this analysis, variable showing $P < 0.05$ and $P < 0.001$ were considered to be statistically significant and highly significant, respectively. For calculation of *P*-value SPSS software version 19 is used. Pearson correlation test was applied to test correlation.

RESULTS

In this study, 100 patients having CAD are included as study group and 100 normal healthy participants as a control group. The mean age of the study group is 54.77 ± 11.93 years and 56.33 ± 12.58 years in control group. No significant difference was noted between the patient and control group in this regard ($P = 0.37$). Distribution of participant in study and control group is as follow, between age 20 and 34 years 5% and 4%, between 35 and 49 years 28% and 25%, between 50 and 64 years 41% in both while in above age of 65 years 26% and 30%, respectively. The majority of patients are between age of 50 and 64 years age group. There is no significant difference in age wise distribution in study and control group. The mean homocysteine level in study group is 33.02 ± 17.41 μmol/L while in control group it is 13.88 ± 3.86 μmol/L ($P < 0.001$). There was a significant difference observed in tHcy concentration between study and control group.

When comparing normal and above normal range homocysteine level 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 (Table 1). According to age group, there is statically significant difference in homocysteine level in study and control group ($P < 0.001$). Mean homocysteine level in each age group is higher in study group than in control group (Table 2).

In the study group, there is a significant difference in homocysteine level in male and female ($P = 0.02$) while there is no significant difference is observed in control group male and female ($P = 0.11$) (Table 3).

DISCUSSION

Since decades homocysteine found to be an independent risk factor in pathophysiology of atherothrombotic vascular disease. Its role for prediction as a risk of CAD also has been extensively studied worldwide. Many studies have been done in this regard, but most of these studies done on western population. There is no systematic study done in Gujarat. At the same time, Gujarat is developing on fast track. Ahmedabad is the biggest city of Gujarat which caters population of different walks of life. Hence, a systematic study of homocysteine status in population with CAD of Ahmedabad is necessary.

Many studies found that homocysteine level is associated with atherosclerotic disease including CAD.[16-19] Hyperhomocysteinemia increases the risk of CAD by increased thrombosis, adverse effects on endothelial function, promoting thickening of the intima, increased platelets aggregation, 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] In addition, some studies showed hyperhomocysteinemia may have prognostic value in mortality of patients with CAD.[9]

Considering these facts we have undertaken this study to categorize homocysteine as a risk factor for CAD and also recognize age as motive for hyperhomocysteinemia.

We have studied a total number of 200 subjects (100 study group cases and 100 control group subjects) during the study. The participants selected in study and control group are age and sex match. In this study, 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 is 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 a correlation in CAD. We also observed that in study group maximum number ($n = 32$) of a patient having high homocysteine level are in age group 50-64 year.

Table 1: Distribution of the patients according to homocysteine level and age in both groups

Age group (year)	Homocysteine			
	Study group		Control group	
	In normal range *n* (%)	Above normal range *n* (%)	In normal range *n* (%)	Above normal range *n* (%)
20-34	2 (40)	3 (60)	4 (100)	0 (0)
35-49	5 (17.86)	23 (82.14)	24 (96)	1 (4)
50-64	9 (21.95)	32 (78.05)	41 (100)	0 (0)
> 65	6 (23.07)	20 (76.93)	26 (86.67)	4 (13.33)
	100		100	

Table 2: Mean homocysteine level in different age groups in both groups

Age (year)	Homocysteine (µmol/L) mean±standard deviation		*P*
	Study group	Control group	
20-34	29.55±12.94	14.02±3.11	<0.05
35-49	34.64±18.32	14.02±4.40	<0.001
50-64	31.27±15.50	13.22±4.17	<0.001
>65	34.70±20.08	14.64±4.70	<0.001

Table 3: Mean homocysteine level in both gender in both groups

Group	Sex	Homocysteine (µmol/L) Mean±standard deviation	*P*
Study group	Male	35.43±18.35	0.02
	Female	26.51±12.30	
Control group	Male	14.31±3.98	0.11
	Female	13.00±3.51	

On the other hand, if we compare normal and above normal range homocysteine level in each age group of CAD patients, highest percent (82.14%) patients having high homocysteine are in age group 35-49 year. It shows an association of high tHcy level in occurrence of CAD in early age. In each age group, study group participants have higher tHcy level than in control group. In other word, we can say that if person has higher tHcy level than he/she is more prone to develop CAD.

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/L versus 13.9 ± 0.9 µmol/L, $P = 0.005$) and hyperhomocysteinemia were correlated with higher risk of CAD.[20] Sastry et al. study in South Indian showed that the serum level of homocysteine in patients with CAD was significantly higher than the control group (18.59 ± 2.63 µmol/L versus 11.69 ± 2.80 µmol/L, $P < 0.001$).[22]

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. Moreover limited number of subjects were included in our study which comprises mostly patient with severely high level of homocysteine. Vitamin B12, Vitamin B6, and folic acid deficiency may be responsible for increased tHcy level as they are important for homocysteine metabolism. Saeed Sadeghian and Faramarz Fallahi, have studied the relation between hyperhomocysteinemia and serum level of folic acid and vitamin B12.[20]

Our study revealed that serum tHcy concentration tended to increase with age. As homocysteine shows positive correlation with age, in study group it is +0.05, and in control group, it is +0.07 (Table 3). 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. Vitamin B12 and folate deficiency may be responsible for increase homocysteine level.

Our study limitation is the smaller sample size overall, and also we have a small number of female participants in comparison to males. Further study with more number of patients with more female participants is needed for more accuracy. We also need to evaluate vitamin B12 and folic acid level as they play a key role in homocysteine metabolism. Measuring vitamin B12 level is also important to test out as we have conducted this study in Ahmedabad, Gujarat where most of the people are strict vegetarian and source of Vitamin B12 is only a non-vegetarian diet.

CONCLUSION

In this study, we explore an association between patients suffering from CAD and increase homocysteine level. Homocysteine is one of the newer risk factors identified for CAD globally in recent years, and our study results are in concordance with other studies. Our study also concludes that as age advances, homocysteine level also increases. Hence, person with increasing age (age above 35 years) and having one or more other risk factors should comprise homocysteine in their health check-up and if there is any abnormality, treat the underlying cause which will help individual to stay away from CAD in upcoming days up to some extent.

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