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Original Research Article

Study of Plasma Homocysteine Levels in Subjects with Cerebral Infarct and Myocardial Infarction

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Conflict of interest: Nil

Abstract:

Background: Numerous cross-sectional and retrospective case-control studies have linked elevated total homocysteine levels to peripheral, cerebral, and coronary vascular disease. . These investigations have also identified homocysteine as a unique risk factor that goes beyond the traditional risk factors. Because they might be influenced by a variety of factors, epidemiological findings suggesting a correlation between high HCY levels and cardiovascular risk do not establish a causative relationship. Moreover, other clinical studies revealed that vitamin supplementation had no discernible impact on cardiovascular risk, despite lowering HCY levels. Hence, our study aimed to evaluate the association between homocysteine and coronary and cerebral vascular disease without other risk factors like hyperlipidemia, diabetes mellitus, hypertension, smoking and old age.

Methods: This was a case-control study involving 100 subjects of either sex of age group between 13 and 40 years, with 50 cases with myocardial and cerebral infarct and 50 controls. Plasma total homocysteine was determined by HPLC (High Performance Liquid-Chromatography).

Results: The mean homocysteine level was 19.36±8.06090 among cases, while among controls it was 13.88±4.69. Hyperhomocystinemia was seen in a higher percentage of cases, 58%, compared to 38% among the control group. The cases had a 1.50-fold higher risk for MI or stroke than controls (the relative risk ratio), and the odds ratio is 2.25. The percentage of hyperhomocystinemia was 36% in the non-vegetarian group and 62% in the vegetarian group. The mean homocysteine was 18.92 µmol/L in stroke and 19.56 µmol/L in MI.

Conclusion: Hyperhomocystinemia is an independent risk factor for coronary artery disease and cerebrovascular disease. In the present study, the cases had a 1.50-fold higher risk for MI or stroke than the controls. It is strongly recommended to screen for hyperhomocystinemia especially among young patients with arterial occlusive disease or venous thrombosis without other risk factors.

Keywords: Homocystine, Myocardial Infarction, Stroke.

Introduction

Age, gender, smoking, lipids, hypertension, diabetes, psychological problems, inactivity, and a diet poor in fruits and vegetables have all been found to be risk factors for CVD (cardiovascular disease) in a number of epidemiological studies. Inadequate vitamin consumption has long been thought to be one of the dietary risk factors for CVD. Other vitamins are still targets for intervention, even though antioxidant vitamins have not demonstrated any advantages. Increased levels of homocysteine, an intermediary in one carbon metabolism, have been linked to high rates of folate and cyanocobalamin (vitamin B12) deficits in a number of populations at high risk for CVD.

Elevated levels of homocysteine, an amino acid, are a recognized risk factor for CVD, including heart attack, stroke, and blood clots, due to its adverse effects on blood vessels and endothelial function. While various factors can elevate homocysteine levels, such as deficiencies in B vitamins (B12, B6, folate) and certain genetic conditions like homocystinuria, its exact causal role in CVD and the effectiveness of strategies aimed at preventing cardiovascular events remain subjects of ongoing research and debate. Elevated total homocysteine levels have been linked to peripheral, cerebral, and coronary vascular disease in numerous cross-sectional and retrospective case-control studies. These investigations have also identified homocysteine as a unique risk factor

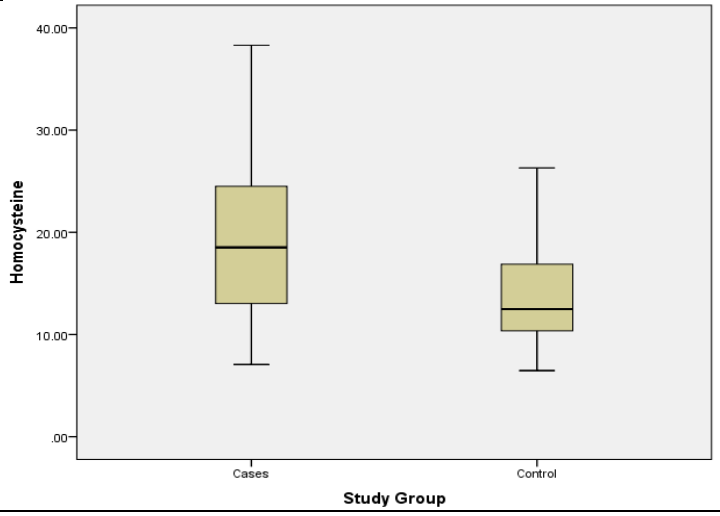
that goes beyond the traditional risk factors. Because they might be influenced by a variety of factors, epidemiological findings suggesting a correlation between high HCY levels and cardiovascular risk do not establish a causative relationship. Moreover, other clinical studies revealed that vitamin supplementation had no discernible impact on cardiovascular risk, despite lowering HCY levels. Hence, our study aimed to evaluate the association between homocysteine and coronary and cerebral vascular disease without other risk factors like hyperlipidemia, diabetes mellitus, hypertension, smoking and old age.

Materials & Methods

This was a case-control study involving 100 subjects of either sex of age group between 13 and 40 years, with 50 cases with myocardial and cerebral infarct and 50 controls. Subjects with a history of myocardial infarction or cerebral infarction, diabetes, hyperlipidemia, hypertension, old age (>40), smoking, pregnancy, renal failure, diseases of the GI tract and liver, malignancy, acute moribund diseases, chronic alcoholism, or drug substance abuse were excluded from the study. Plasma total homocysteine was determined by HPLC. Following instructions to fast and abstain from alcohol, a certified phlebotomist extracted whole blood from the antecubital vein while the subjects were fasting. The blood was then aspirated into two 4.5 ml evacuated tubes containing EDTA, mixed to prevent coagulation, chilled, and centrifuged at 2000 g for 20 minutes within an hour of the sample. After being moved to a polycarbonate vial, the plasma fractions from both tubes were quantified in an HPLC and, in the event that the measurement was delayed, frozen at 2 to 8 degrees Celsius.

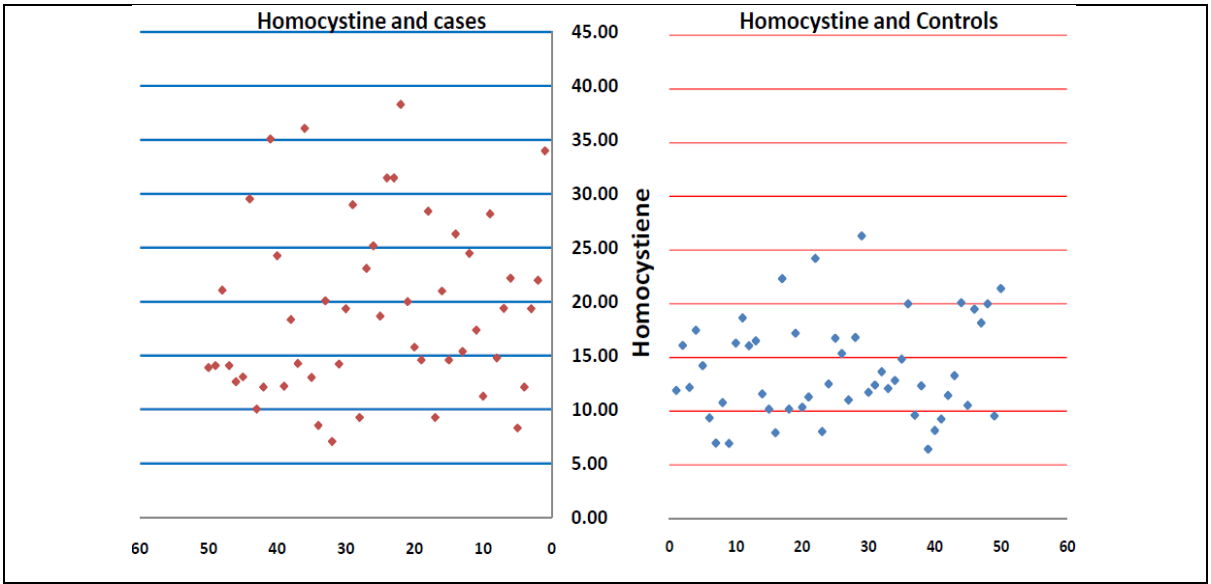
Results

The mean age among cases was 31.94±6.659 years, while among controls it was 31.40±6.098 years; hence, the two groups were comparable. The mean homocysteine level was 19.36±8.06090 among cases, while among controls it was 13.88±4.69. The graph below shows the distribution of homocysteine values between the study and control groups. The homocysteine level was in a higher range in cases.



Graph 1: Distribution Homocytine Cases and Controls

The scattered diagram of homocystine for cases and control is as shown below.



Graph 2: Scattered Diagram Homocystine Cases and Controls

Hyperhomocystinemia was seen in a higher percentage of cases, 58%, compared to 38% among the control group. The cases had a 1.50-fold higher risk for MI or stroke than controls (the relative risk ratio), and the odds ratio is 2.25 in our study.

The t-test for the difference of two groups is as shown in the table below.

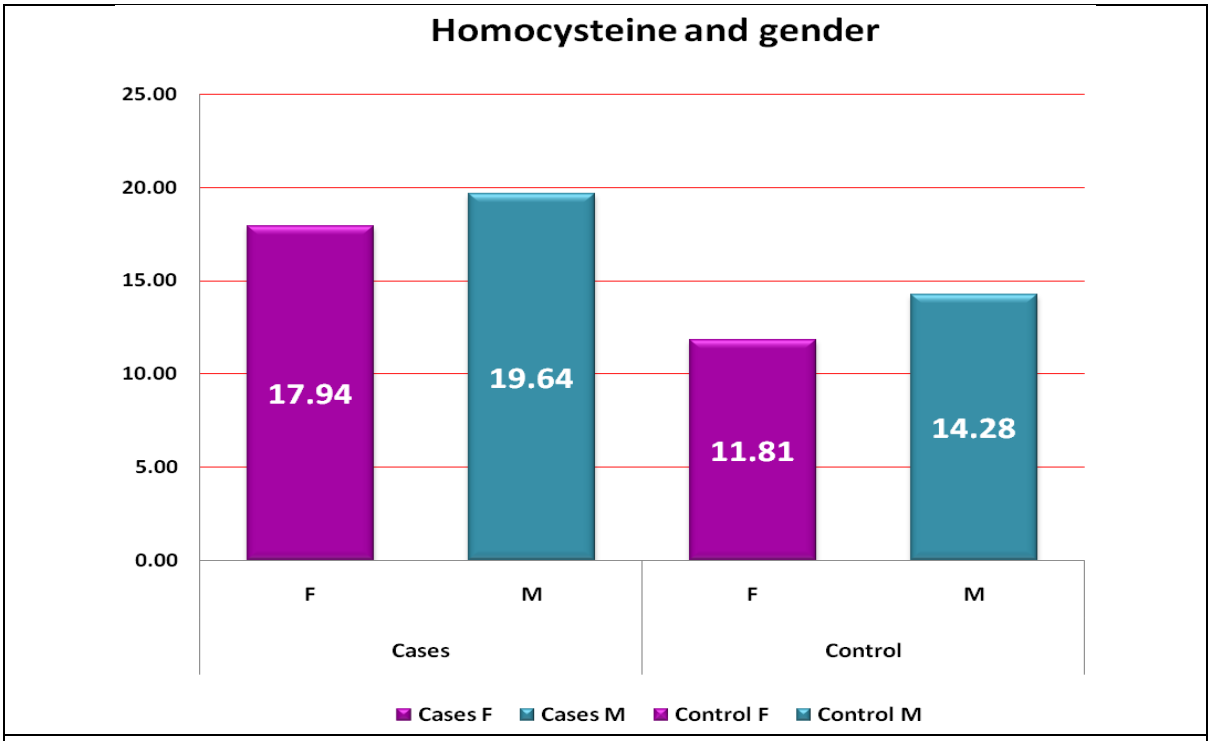
Group Statistics								
Homocysteine (µmol/L)		Group	N	Mean	Std. Deviation	Std. Error Mean		
		Control	50	13.8838	4.69490	.66396		
		Cases	50	19.3708	8.06428	1.14046		
		T-test for Equality of Means						
		t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
							Lower	Upper
Homocysteine (µmol/L)	Equal variances assumed	-4.158	98	.000	-5.48700	1.31966	-8.10582	-2.86818

Table 1: T-Test for Mean Homocysteine of Cases and Controls

H0: Null Hypothesis: There was no significant difference between the mean homocysteine of the control and case groups.

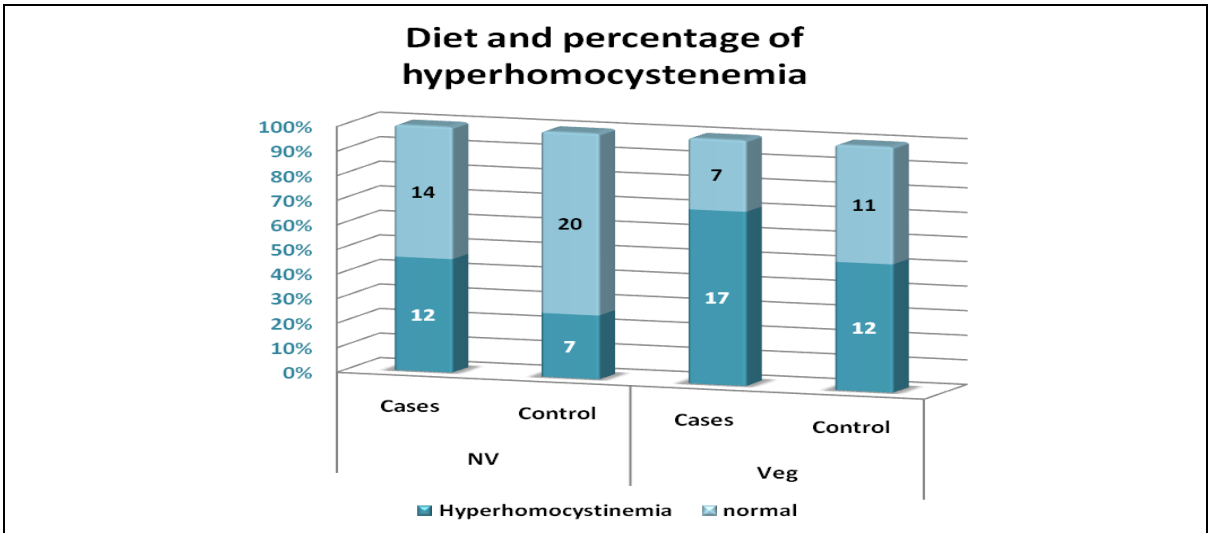
H1: Alternative Hypothesis: There was a significant difference between the mean homocysteine of the control and cases groups. Since the p-value (0.000) was less than 0.05, we rejected the null hypothesis and concluded that there was a significant difference between the mean homocysteine levels of the control and case groups.

The homocysteine level over the age in both the control and study groups was analyzed; it showed peak values were in the 15–20 age group, followed by the 35–40 age group. Otherwise, no specific correlation in age group was seen. The mean homocysteine level in females was 17.94 µmol/L in cases and 11.81 µmol/L in controls, whereas in males it was 19.64 µmol/L in cases and 14.28 µmol/L in controls. The overall mean homocysteine was 14.87 µmol/L in females and 16.96 µmol/L in males. It showed the highest value in males compared to females.



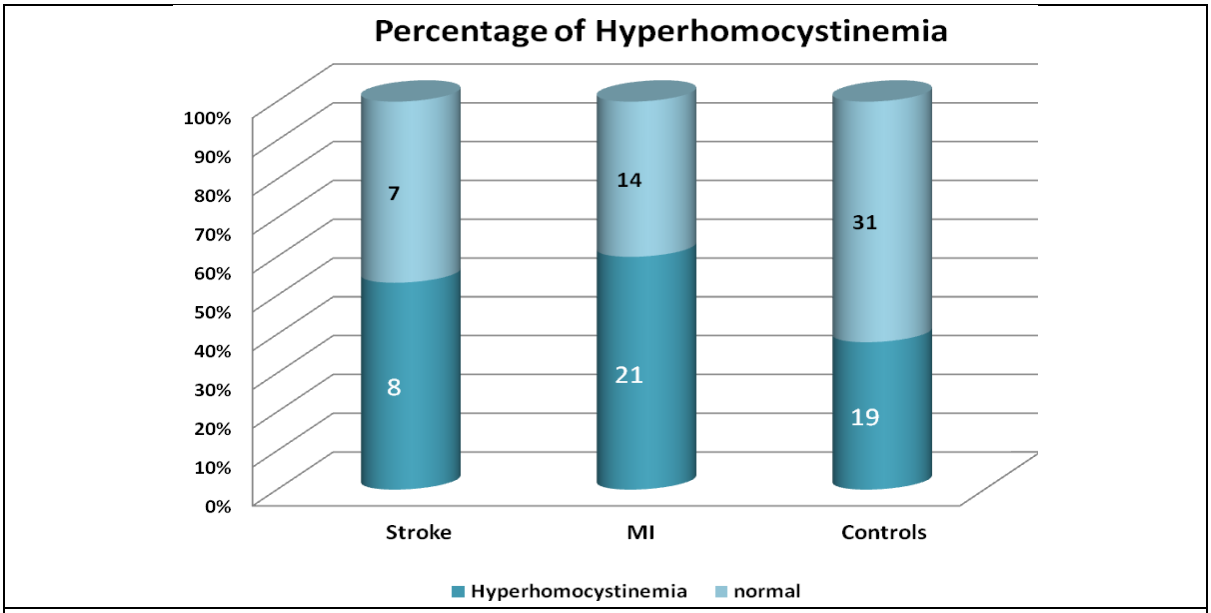
Graph 3: Mean Homocystine vs. Gender in Cases and Controls

The percentage of hyperhomocystinemia was **36%** in the non-vegetarian group and **62%** in the vegetarian group. The homocysteine level was normal in **64%** of the non-vegetarian group, whereas it was normal in **38%** of the vegetarian group.



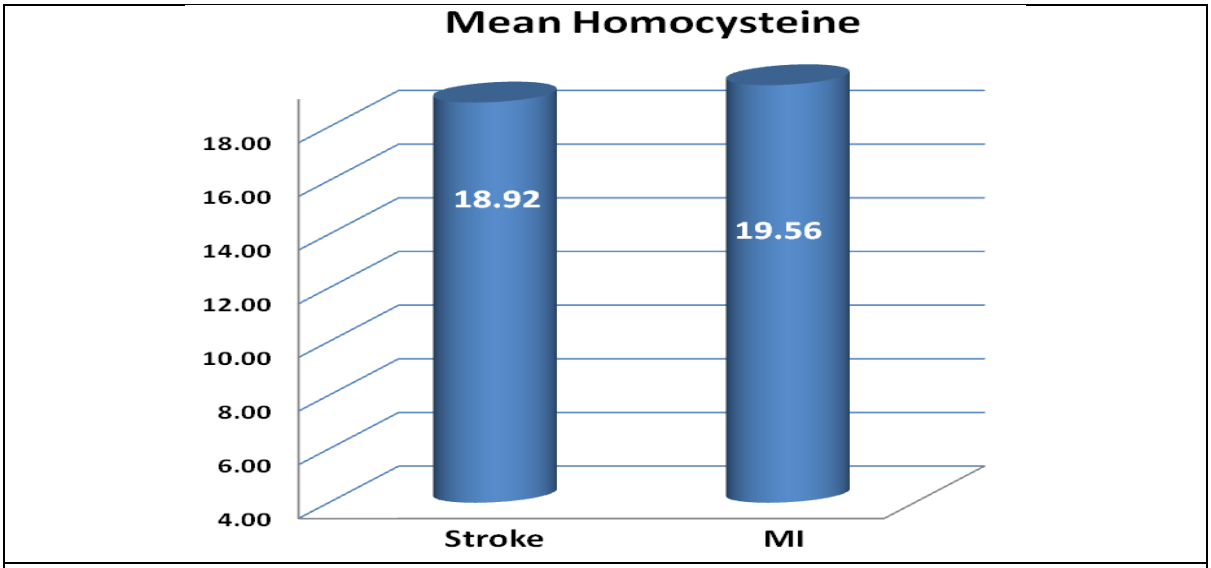
Graph 4: Diet and Percentage of Hyperhomocystinemia in Cases and Controls

The percentage of hyperhomocystinemia was 53% in stroke, 66% in MI and 38% in controls. Whereas the percentage of normal homocystine level was 47% in stroke, 34% in MI and 62% in controls.



Graph 5: Percentage of Hyperhomocytinemia in Stroke, MI, and Control

The mean homocystine was 18.92 µmol/L in stroke and 19.56 µmol/L in MI.



Graph 6: Mean Homocystine and MI vs. Stroke

Discussion

Prospective cohort studies that assessed the relationship between elevated HCY levels and CVD have shown conflicting results. A statistically significant positive correlation between high HCY and CHD (coronary heart disease) and stroke was found in some of these investigations. Other research, however, was unable to show a meaningful correlation between plasma HCY and CHD.

The majority of participants in the instances group of the current study were between the ages of 36 and 40, with 31 and 35 coming in second and third, respectively. In comparison to controls, myocardial infarction cases were more common in men, older people, and those with higher systolic blood pressure and total cholesterol, lower HDL cholesterol, and a history of myocardial infarction, according to the study by Bots ML et al. [1] Stroke was linked to advancing age, current smoking, elevated systolic and diastolic blood pressure, hypertension, diabetes mellitus, myocardial infarction history, and stroke history. Significant and positive correlations were found between HCY level and increasing age, male sex, current smoking, and history of myocardial infarction. Elevated HCY levels did not significantly correlate with other cardiovascular risk factors. An increase of 1 µmol/L in Hcy level was linked to a 7% increase in the risk of stroke (OR, 1.07; 95% CI, 1.03-1.11) and a 6% increase in the risk of myocardial infarction (OR, 1.06; 95% CI, 1.02-1.11) when

age and sex were taken into account in a logistic regression model with HCY level as a continuous variable.

The percentage of hyperhomocystinemia in the present study was 58% among cases, whereas it was 38% in the control group. This was similar to the incidence compared to 43.5% in a study by Kumar et al.,[2] 30–40% in a study by Stanger et al. [3] and 58% by Yagnik et al. [4]

The cases in the present study had a 1.50-fold higher risk for MI or stroke than controls (the relative risk ratio), and the odds ratio was 2.25. This was similar to the odds ratio of 1.6 for CAD and 1.5 for CVA in a study by Boushy et al.[5] and 2.2 by Graham et al.[6] in the European concerted action project In line with our findings, the risk was 2-fold for cardiovascular disease by Paul et al.[7] 2-fold for MI by a study by Giles et al.,[8] and 3-fold for myocardial infarction in the prospective study by Stampfer et al.[9]

Comparing the high-risk TT genotype with other genotypes in a meta-analysis by Wald et al. [10] revealed a non-significant 31% (95% CI -20% to +215%) increased risk of stroke and a 21% (95% CI 6% to 39%) greater risk of IHD. An increase in HCY of 1.93 (range 1.38–2.47) μmol/L is linked to a 1.26 (95% CI 1.14 to 1.40)-fold increase in CVA risk, which is quite similar to the 1.20 (95% CI 1.13 to 1.30)-fold increase in risk predicted based solely on plasma levels, according to a subsequent meta-analysis of 15,635 cases using Mendelian randomisation.[11]

The mean homocysteine level among vegetarians was 18.22 μmol/L in the total group, 20.95 μmol/L in cases, and 15.38 μmol/L in controls, whereas among non-vegetarians it was 15.21 μmol/L in the total group, 17.92 μmol/L in cases, and 12.61 μmol/L in controls. This was similar to the observation in the Wadia et al., study,[12] wherin higher values were observed among vegetarians; the mean value was 37.7 μmol/L in vegetarians with stroke and 25.5 μmol/L in non-vegetarians.

The percentage of hyperhomocystinemia is 62% in the vegetarian group and 36% in the non-vegetarian group. This was similar to the incidence in the Ruby Hall Study: 50% in vegetarians versus 39.5% in non-vegetarians. This may be due to vitamin B12 and folic acid in the diet, especially vitamin B12, which influences the homocysteine level. Vitamin B12 has only non-vegetarian sources. Even though vitamin therapy reduces the homocysteine level, the benefit of empirical therapy needs further investigation to confirm that such treatments will reduce the risk of future cardiovascular events, as only a few studies have shown the benefit of empirical vitamin therapy.

The mean homocysteine value among females was 14.87, whereasin males it was 16.63 in our study, similar to 13.52 in men and 8.95 in women in a study by Radha Rama Devi et al. [13] In a study by Villar and Filago[14] also, the mean homocysteine value was higher in males than females, both in cases and controls. It was 19.63 vs. 17.93 in cases and 14.27 vs. 11.80 in controls, compared to 10.7 in males and 8.5 in females. This shows that the male gender was prone to have higher values of homocysteine level than the female. It may be due to the protective effect of oestrogen and other physiological factors in females.

Inappropriate handling of the blood samples, especially if they are stored at room temperature for longer than four hours, may result in an increase in the homocysteine level.[15] The majority of research that has been able to look into this relationship has found a strong positive correlation between age and homocysteine levels.[16,17] The variation of results in various studies may be due to the method of measurement, sample preservation, sample collection, post-methionine loading results, diet habits, and local prevalence of gene mutation.

The mean homocysteine level in stroke patients was 18.92, whereas in coronary artery disease it was 19.56. The percentage of hyperhomocystinemia in stroke is 53.33% in our study, which is comparable to 59.1% in the Das R et al study,[18] 42% in cerebrovascular diseases in a study by Clarke et al.,[19] 75% of young strokes in a study by Wadia et al. in India [20] 77% by Refsum et al. [21]

The percentage of MI attributable to hyperhomocystinemia is 67% in MI in our study, when compared to 72.0% of the CAD group in the A. Puri study.[22] 7% of MI by Stamper et al., 30% of coronary atherosclerotic disease by Clarke et al.,[19] 10% of coronary artery diseases by Boushyet al.[5]

Studies conducted on middle-aged participants provide the majority of the prospective data that associates increased homocysteine levels with future risk for cardiovascular disease. In a study of US physicians, the risk of myocardial infarction was 3.1 times higher for participants with homocysteine levels above the 95th percentile of controls than for those with levels below the 90th percentile of controls 9. A graded positive correlation between tHcy and stroke risk was shown in a British Regional Heart Study report.[23] However, when individuals in the top quintile of homocysteine levels were compared to everyone else, a non-significant 1.4-fold increase in stroke risk was discovered; no correlation between homocysteine levels and the risk of coronary heart disease or stroke was found in a prospective population-based study conducted in Finland.[24]

An elevated homocysteine level is linked to an increased risk of myocardial infarction and stroke in older adults, according to the Bots ML et al. study. The idea that excessive homocysteine levels are linked to a higher risk of cardiovascular disease is

supported by these findings as well as those from other research on atherosclerotic markers, including a previous paper from our group. According to the Framingham cohort, older participants with homocysteine levels above the median had a 25% or greater chance of developing extracranial carotid artery stenosis.[25] Participants in the population-based Atherosclerosis Risk in Communities study who were middle-aged and had homocysteine levels in the top quintile were more likely than those in the bottom quintile to develop thickening of the carotid artery intima-media wall.[26]

It is believed that elevated tHcy levels cause endothelial dysfunction, potentially through increased atherosclerotic lesion formation, decreased blood platelet survival, and cell loss. Elevated tHcy levels have been linked to a higher blood platelet turnover rate. According to findings from research on animals, hypertension has a more severe and early negative impact on the development of atherosclerosis than normotension due to higher homocysteine levels. Genetic flaws or nutritional inadequacies can cause elevated tHcy levels. In comparison to other causes, the contribution of genetic abnormalities to the elevation of homocysteine levels seems to be modest as people age. Numerous studies have suggested that dietary interventions involving vitamin B12, folate, and vitamin B6 may be able to reduce homocysteine. [27,28]

Hyperhomocysteinaemia, which is defined as plasma HCY levels higher than the 90th or 95th percentile of levels in controls, was linked to an elevated risk of atherosclerotic disease in a meta-analysis of 27 observational studies with over 4,000 participants. According to analysis, a 5 μmol/L rise in basal total plasma HCY levels was linked to a 60% and 80% higher risk of CHD in men and women, respectively. This effect was comparable to that of elevating cholesterol by 0.5 mmol/L. The link between hyperhomocysteinaemia and atherosclerotic vascular disease has been consistently supported by later observational research. An increase in plasma HCY levels was found to be an independent risk factor for CVD in the largest of these, the European Concerted Action Project, which comprised 800 controls and 750 men and women with arterial vascular disease. Comparing subjects with total HCY levels > 80th percentile to those with HCY levels < 80th percentile, the former had a 2.2-fold (95% CI 1.6 to 2.9) higher risk for CVD.[6] Limitations of our study included a very small sample size, from which no generalizations can be drawn. We only studied the effects of age, sex, and diet on the levels of homocysteine and not that of other confounding factors, such as other comorbidities and medications used. Also, the effect of interventions to reduce the levels of homocysteine was not studied, which would have added valuable information to the work.

Conclusion

Hyperhomocystinemia is an independent risk factor for coronary artery disease and cerebrovascular disease. In the present study, the cases had a 1.50-fold higher risk for MI or stroke than the controls. It is strongly recommended to screen for hyperhomocystinemia especially among young patients with arterial occlusive disease or venous thrombosis without other risk factors. Standardization of measurement should be followed to avoid variation of the results due to method of measurement, sample preservation, and sample collection. Gender and dietary influences on the homocystine levels should also be considered during interpretation of the test results.

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