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Possible Ethnic Differences in Plasma Homocysteine Levels Associated with Coronary Artery Disease between South Asian and East Asian Immigrants

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Summary

Background: Hyperhomocysteinemia has been identified as a risk factor for coronary artery disease (CAD). South Asians appear to have a high incidence of CAD, while East Asians have a very low incidence.

Hypothesis: The present study was undertaken because the relative association of plasma homocysteine levels (PH) with CAD in South Asians (SA = Indian, Pakistani, Sri Lankan) and East Asians (EA = Chinese, Japanese) is not known.

Methods: Fasting PH were drawn on all patients with CAD of SA (age 62.4 ± 1.1 years, 72 men, 14 women) and EA (age 61.8 ± 3.0 years, 13 men, 4 women) descent. These were compared with PH available from Caucasian (CA) patients (age 61.1 ± 1.1 years, 89 men, 17 women) with CAD.

Results: The PH in SA, EA, and CA patients were 11.0 ± 0.5, 7.6 ± 0.5, and 10.8 ± 0.6 µmol/l, respectively ($p < 0.001$ between EA and SA/CA). Percentages of SA, EA, and CA with elevated PH (> 12.0 µmol/l) were 33.7, 5.9, and 28.2%, respectively. There were no significant differences in the lipid subfractions between the SA and EA group. History of smoking was significantly higher in the EA (52.9 vs. 26.2%), while hypertension and diabetes mellitus had similar prevalences.

Conclusion: Significant differences in PH of SA versus EA patients with CAD exist. The relative contribution of homocysteine in the development of CAD appears to be less in EA immigrants. In contrast, the association between CAD and PH in SA immigrants appears to be similar to that of Caucasians.

Key words: homocysteine, coronary artery disease, ethnicity, Asians, risk factors

Introduction

Although smoking, dyslipidemia, hypertension, diabetes mellitus, and a family history of premature ischemic heart disease are well established as risk factors for atherosclerotic coronary artery disease (CAD), taken together they are believed to account for only 40–50% of the etiology of atherosclerosis.[1] In view of this, there has been considerable research to identify other factors which may predispose to the development of CAD. Some of the factors that have been implicated are high serum fibrinogen, high plasminogen activator inhibitor level, hyperhomocysteinemia, high lipoprotein (a) level, and high total body iron stores. Nevertheless, all of these factors are not universally accepted as having an independent influence on the pathogenesis of CAD.[2,3] In recent years, there has been considerable interest in increased plasma homocysteine levels (PH) as a risk factor in view of the ease with which one might be able to lower the PH with folic acid and the known high incidence of genetic mutations contributing to high levels of homocysteine.[4–6]

Two different ethnic groups of individuals of Asian origin appear to demonstrate marked differences in the incidence of CAD. Persons of origin from the Indian subcontinent, herein called South Asians (SA) (i.e., Indians, Pakistanis, Bangladeshis, Sri Lankans), have been shown to have a very high incidence of CAD.[7–9] The high incidence of CAD is present not only in SA immigrants living in developed countries but also in SAs living in their native countries.[10] In contrast, people from Eastern Asian countries, herein called East Asians (EA) (i.e., Chinese, Japanese, Koreans), have a very low incidence of CAD, although the rates appear to increase with emigration to Western nations.[11] The present study was undertaken to assess the PH levels associated with CAD in immigrants of these two distinct populations and to compare them with the PH levels of the Caucasian (CA) population.

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Methods

All patients of SA and EA origin who were registered in the Cardiac Rehabilitation Service at the Grey Nuns Hospital were identified. Thereafter, the patients were contacted and asked for voluntary participation in terms of evaluation of their homocysteine levels. All those who agreed to come for the test were interviewed with all data in terms of their demographics and clinical history obtained in a prospective manner. Then each patient was asked to come for a lipid profile and measurement of PH level. All vitamin B preparations (including multivitamins containing vitamin B) were withheld for a period of 4 weeks prior to the PH measurement. Blood samples were taken after a 12-h overnight fasting period, with instructions given to abstain from alcohol for the preceding 72 h. Blood for PH was collected in precooled test tubes (containing ethylene diamine tetraacetic acid), with the plasma separated within 10 min of collection and stored at −20C° until analysis. In patients who were on pharmacologic therapy for dyslipidemia, the initial lipid profile that had been taken prior to beginning pharmacologic therapy was obtained and recorded. The PH level was also measured in a series of consecutive CA patients who were referred to the Cardiac Rehabilitation Service for comparison with the SA and EA groups. All these patients were assessed by the dietitian attached to the Cardiac Rehabilitation Service with regard to their nutritional intake. This was done by a personal interview and a 3-day food record. Dietary analysis included estimation of the number of portions of different food types (e.g., fruits, vegetables, meats, etc.) consumed per day for each patient. Determination of red blood cell folate levels, serum vitamin-B12 levels, and pyridoxine levels was not done as previous studies have demonstrated the deficiency of these vitamins was not a significant factor in the etiology of hyperhomocysteinemia in patients with atherosclerotic vascular disease.[12]

Statistical Analysis

All data were entered into a database formulated with the Statistical Package for Social Sciences (SPSS Inc., Chicago, Ill., USA) data management system, with the statistics performed using the same system. All discrete variables were categorized into mutually exclusive categories and all continuous variables were entered as such. Comparison between the groups for the primary hypothesis variable (the plasma homocysteine level) were made using an unpaired *t*-test. Comparison between discrete variables was made using a chi-square test. Comparisons between other continuous variables were made using one-way analysis of variance. Whenever the F value was significant ($p < 0.05$) in an analysis of variance, the significance of the differences among the three individual groups (EA, SA, CA) were further elucidated using the Bonferroni's *t*-statistic to make allowance for multiple comparisons. Correlation between continuous variables was made utilizing the Spearman's correlation coefficient. A p value of <0.05 was taken as significant. The mean ± standard errors of the mean (SEM) are quoted for continuous variables.

Results

The study included 86 SA (men = 72, women = 14, age: mean 62.4 ± 1.1 SEM year), 17 EA (men = 13, women = 4, age: mean 61.8 ± 3.0 SEM year) and 106 CA (men = 89, women = 17, age: mean 61.1 ± 1.1 SEM year) with CAD. The countries of origin for the SA and EA groups are shown in Table I. All the SA and EA patients in the present study had immigrated to Canada with none born here. The mean duration of stay in Canada was 16.9 - 1.2 (SEM) (range: 1.0–51.0 years) with no significant difference between the SA and EA groups ($p > 0.05$). The dietary analysis for the two groups is outlined in Table I. There were no significant differences in the daily consumption of vegetables, fruit, grain, meat, and dairy product portions between the SA and EA groups ($p > 0.05$). Vegetarians comprised 9.5 and 0.0% in the SA and EA, respectively. Sixty-one patients had a history of acute myocardial infarction, 40 patients with previous coronary artery bypass surgery and 34 patients with previous coronary angioplasty. The rest of the patients had a diagnosis of angina based on clinical symptoms and a positive exercise test, although some underwent nuclear perfusion imaging and/or coronary angiography in addition.

The demographics and other clinical characteristics of the patients are outlined in Table II. There was no significant difference in the prevalence of hypertension, dyslipidemia, and diabetes mellitus in the three population groups. The age and gender distribution in the three groups were not significantly different from one another ($p > 0.05$). Family history of premature CAD (defined as the diagnosis of CAD in a first degree male relative <55 years of age and/or in a first degree female relative <65 years of age) was significantly less in the EA group (7.1%) than in the SA (28.2%) and CA (35.4%) groups ($p < 0.05$). The prevalence of current smoking (i.e., smoking at present or having quit <1 year ago) as well as any smoking history was significantly lower in the SA than in the EA and CA

TABLE I Countries of origin and dietary analysis of the South Asian and East Asian patients (n = 103)

	South Asians	East Asians
Number of patients (%)	86 (83.5)	17 (16.5)
Ethnic origin		
Indian (%)	77 (89.5)	
Pakistani (%)	5 (5.8)	
Sri Lankan (%)	4 (4.7)	
Chinese (%)		16 (94.1)
Japanese (%)		1 (5.9)
Food portions per day		
Vegetable (leafy)	2.8 ± 0.2	2.0 ± 0.6
Fruit	2.1 ± 0.3	1.3 ± 0.3
Grain	5.8 ± 0.2	5.7 ± 0.7
Meat	1.8 ± 0.1	2.0 ± 0.1
Dairy product	1.6 ± 0.2	1.9 ± 0.3
Vegetable (fruity)	4.9 ± 0.3	3.3 ± 0.3

TABLE II Clinical and investigational characteristics of the patients (n = 207)

	South Asians n = 86	East Asians n = 17	Caucasians n = 106
Age (years)			
Mean	62.4 ± 1.1	61.8 ± 3.0	61.1 ± 1.1
Range	34–84	33–86	36–79
Weight (kg)	70.7 ± 1.7	63.7 ± 3.0	82.7 ± 2.0
Males (%)	83.7	76.6	84.0
Diabetes mellitus (%)	20.7	29.4	19.6
Hypertension (%)	47.6	47.1	42.2
CVD (%)	6.3	6.3	3.7
F/H CAD (%)	28.2	7.1	35.4
Smoking			
Current (%)	2.3	17.6	18.6
Ever (%)	26.2	52.9	71.6
PH (µmol/l)	11.0 ± 0.5	7.6 ± 0.5	10.8 ± 0.6
Baseline lipids (mmol/l)			
Total cholesterol	4.86 ± 0.11	4.80 ± 0.27	5.34 ± 0.12
LDL cholesterol	2.92 ± 0.08	2.93 ± 0.25	3.25 ± 0.09
HDL cholesterol	1.04 ± 0.02	1.01 ± 0.04	1.02 ± 0.02
Triglycerides	1.99 ± 0.16	1.84 ± 0.18	2.40 ± 0.20

Abbreviations: CVD = cerebrovascular disease, F/H CAD = family history of coronary artery disease, PH = plasma homocysteine level, LDL = low-density lipoproteins, HDL = high-density lipoproteins.

groups ($p < 0.001$). Baseline serum cholesterol levels as well as high-density lipoprotein (HDL) levels, low-density lipoprotein levels (LDL), and triglyceride levels were not significantly different between the SA and EA groups ($p > 0.05$). Baseline total cholesterol and LDL levels were significantly higher in the CA than in the SA ($p < 0.05$), although not significantly different from the EA. The serum triglyceride and HDL levels in the CA were not significantly different from those in the other two groups. The mean weight in the CA (82.7 ± 2.0 kg) was significantly higher than that in the EA and SA groups ($p < 0.001$). The mean weight in the SA (70.7 ± 1.7 kg) appeared to be higher than that in the EA (63.7 ± 3.0 kg), but the difference was not statistically significant ($p > 0.01$), although likely due to the small numbers of subjects in the EA group.

The PH in the SA group had a mean of 11.0 ± 0.5 (SEM) µmol/l which was not significantly different from the mean of 10.8 ± 0.6 (SEM) µmol/l observed in the CA group (Fig. 1). In contrast, the PH of the EA patients had a mean of 7.6 ± 0.5 (SEM) µmol/l which was significantly different from that observed in the CA ($p < 0.001$) and SA ($p < 0.001$) groups. The upper limit of normal for the laboratory at this institution was 12 µmol/l. On the basis of this value, 33.7 and 28.2% of the SA and CA groups were found to have abnormal PH, respectively. In contrast, only 5.9% of the EA group were found to have abnormal levels ($p < 0.05$, Fig. 2).

The PH in the entire group of patients did not appear to correlate significantly with serum cholesterol, triglycerides, and HDL and LDL levels. The PH level had a significant positive correlation with age ($p < 0.05$).

The men had a higher PH (10.8 ± 0.6 µmol/l) than women (8.6 ± 0.5 µmol/l, $p < 0.05$). In the CA group, one patient was on methotrexate (PH = 6.5 µmol/l) and two patients were on phenytoin (PH levels 12.4 and 13.1 µmol/l) therapy for psoriasis and seizure disorders, respectively. No patients in the SA and EA groups were on drugs that could affect the PH levels.

Discussion

Although there has been a decrease in the incidence of CAD in developed nations during the last three decades, there has been a concomitant increase in the incidence of CAD in developing nations. Industrialization of the developing nations has been accompanied by urbanization of the populations with increasing adaptation to Western lifestyles which may predispose to the development of atherosclerosis, although it is unclear whether other factors contributed to this change.[13, 14] The incidence of CAD in these populations appears to increase further with emigration to Western nations. This increase appears to become even more apparent with succeeding generations with greater acceptance of the Western lifestyle.[15] In spite of these changes associated with the adaption of Western lifestyles, the difference in the incidence of CAD between the EA and SA population appears to persist. This is apparent by the relative infrequency of individuals of EA origin in cardiac rehabilitation programs and cardiological practices together with

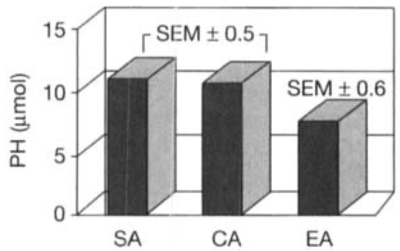

FIG. 1 Plasma homocysteine levels (PH) in South Asian (SA: n = 86), Caucasian (CA: n = 106), and East Asian (EA: n = 17) patients. SEM = standard error of the mean.

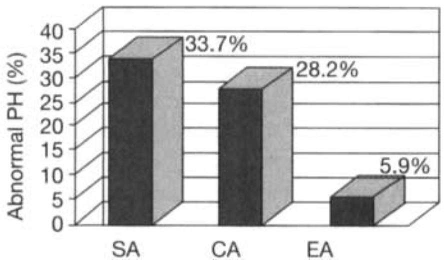

FIG. 2 Prevalence of abnormal plasma homocysteine levels (PH > 12 µmol/l) in South Asian (SA: n = 86), Caucasian (CA: n = 106), and East Asian (EA: n = 17) patients.

a relative preponderance of individuals of SA origin. In a recent study from this institution on 497 consecutive patients admitted to the Coronary Care Unit with an acute myocardial infarction, SA constituted 9.1% of the study population[16] while EA constituted only 0.8% of the population. However, in the city of Edmonton, a recent census demonstrated that the number of EA (n = 46, 130) exceeds that of SA (n = 24, 995) in terms of population demographics.[17] The reason for this difference remains unclear. It is likely that genetics play a major role in the observed differences, although dietary habits may also be quite different between these two population groups.

In the city-state of Singapore, despite an increase in the incidence of CAD during the last 30 years, the four-fold difference between the Indians and Chinese appeared to persist.[15] In Singapore, which has a free-market industrialized economy with a mixed population of Chinese, Indians, and Malays, the environmental factors are common among the different ethnic groups. Furthermore, the dietary habits also tend to come together (relative to the differences observed among China, India, and Malaysia) given the small size of the island of Singapore approximating 544 square kilometers. Given the common environmental and dietary factors, one would expect the incidences of CAD in the EA and SA populations to be approximately the same. Thus, the observed difference in the incidences between these two populations in Singapore are unlikely to be due to environmental factors.

Previous studies have demonstrated that SA have a higher prevalence of metabolic syndrome-X phenotype, lower HDL cholesterol levels, higher lipoprotein (a) levels, and higher plasminogen activator inhibitor levels compared with EA.[15] The relative prevalence of hyperhomocystenemia in these two populations groups has not been investigated to our knowledge.

The present study demonstrates that there is a marked difference in PH levels in these two patient groups. The SA group appeared to have PH levels similar to those of CA, which is in keeping with the high incidence of CAD observed in the former group similar to that observed in the Caucasians. Furthermore, the percentage of persons with abnormal PH also appears to be quite similar between the two groups. In contrast, the EA group had a very low prevalence of abnormal PH levels. The limitation of the present study is the relatively low number of patients in the EA group. This was due to the difficulty of finding EA with CAD as a result of the lower prevalence in this group, as noted in a previous study as well.[16] Thus, a larger study to confirm this conclusion would be useful. The study by Araki *et al.*[18] reported a PH level of 7.5 ± 2.1 (standard deviation) μmol/l in 57 healthy Japanese subjects. The PH levels for EA observed in the present study were also similar to those reported by Alfthan *et al.*[19] in a sample of healthy Japanese men. A study (presented in abstract form) by Anand *et al.* also reported lower levels of PH in EA than in SA individuals.[20] However, in a study by Lolin *et al.* of 45 Chinese (39 men) with CAD living in Hong Kong, a much higher level (14.9 ± 4.4 μmol/l) was noted.[21] The difference between that and the present study (as well as the studies by Araki *et al.*[18] and Alfthan *et al.*[19]) remains unclear.

The significance of the difference found in the present study also remains unclear. At first glance, one might conclude that the lower PH in EA may explain the difference in the incidence of CAD observed in these two ethnic groups. However, this does not necessarily explain the current findings as all patients including the EA patients in the present study had documented CAD. Thus, in spite of the low levels of PH, this group of EA still developed atherosclerosis with consequent clinical manifestations. The only conclusion one could make based on the findings of the present study would be that the relative contribution of PH in relation to the pathogenesis of atherosclerosis in EA appears to be negligible. In contrast, the relative contributions (if any) of homocysteine in the pathogenesis of CAD in the SA population appear to be similar to those in Caucasians.

The PH appears to be dependent on a number of intrinsic and extrinsic factors such as age, gender, and nutrition.[5, 22] The presence of a number of these extrinsic factors did not appear to be different between the SA and EA groups in the present study and thus would not explain the differences. One possibility that needs to be considered would be the difference between the two groups in the consumption of the three B vitamins (folic acid, vitamin B_{12}, and vitamin B_6) which are known to affect the PH levels. Differences in the levels of these vitamins in the body could exist because of differences in nutrition. However, the dietary analysis carried out in the present study demonstrated no significant differences between the SA and EA with respect to the daily consumption of the major components of the diet. If at all, the SA appeared to demonstrate a higher (although nonsignificant) consumption of vegetables compared to EA. This would be expected to lead to a higher intake of B vitamins and thus a lower PH level and is opposite to what was found in the present study. Furthermore, dietary sources (which would not be expected to provide more than 100 μg of folic acid per day) by themselves are unlikely to have a major influence on the PH in the absence of significant malnutrition or underlying genetic susceptibility. This is confirmed by a recent study that demonstrated that even cereals fortified with 140 μg of folic acid did not appear to change the PH appreciably.[23] A more plausible explanation for the observed difference in the PH may be genetic differences between the SA and EA groups. Although homocystinuria related to cystathionine synthase deficiency is extremely rare, with an incidence of 1:300,000 in the population, other mutations that influence the PH appear to be quite common. In particular, studies have demonstrated that mutations resulting in a thermolabile variant N^5, N^6 - methylene tetrahydrofolate reductase occurs frequently in the general population.[5] There appear to be marked ethnic differences in the mutations related to this enzyme, with an incidence of 38% in French Canadians and a 5–15% incidence in the general populations of Canada.[24, 25] It is possible that a difference in the mutation of this enzyme or other genetic influences may account for the differences observed in the present study. This needs to be elucidated further with genetic studies in the three population groups. However, there is controversy as to whether this particular mutation resulting in the thermolabile form of the enzyme increases the susceptibility to CAD. It would also be interesting to study sample populations of SA and EA without any evidence for atherosclerotic vascular disease to determine whether the differences in PH observed in the present study persist in those without manifest CAD.

Conclusion

The present study demonstrates a significant difference in the PH of EA immigrants with CAD compared with CA and SA immigrant patients, with the etiology of the difference remaining unclear at the present time. However, as the study population of EA is small, a larger study to confirm these findings would be useful.

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