

Homocysteine, folate, vitamin B12, and cardiovascular risk in Indians, Malays, and Chinese in Singapore

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Abstract

Objective—To examine the hypothesis that the higher rates of coronary heart disease (CHD) in Indians (South Asians) compared with Malays and Chinese is partly attributable to differences in blood concentrations of homocysteine, and related blood concentrations of folate and vitamin B12.

Design—Cross sectional study of the general population.

Setting—Singapore.

Participants—Random sample of 726 fasting subjects aged 30 to 69 years.

Main results—Mean plasma total homocysteine concentrations did not show significant ethnic differences; values were Indians (men 16.2 and women 11.5 $\mu\text{mol/l}$), Malays (men 15.0 and women 12.5 $\mu\text{mol/l}$), and Chinese (men 15.3 and women 12.2 $\mu\text{mol/l}$). Similarly, the proportions with high plasma homocysteine ($>14.0 \mu\text{mol/l}$) showed no important ethnic differences being, Indians (men 60.0 and women 21.9 %), Malays (men 53.9 and women 37.8 %), and Chinese (men 56.6 and women 30.6 %). Mean plasma folate concentrations were lower in Indians (men 8.7 and women 10.9 nmol/l) and Malays (men 8.5 and women 10.8 nmol/l), than Chinese (men 9.7 and women 13.8 nmol/l). Similarly, the proportions with low plasma folate ($<6.8 \text{nmol/l}$) were higher in Indians (men 44.9 and women 36.6 %) and Malays (men 45.3 and women 24.5 %) than Chinese (men 31.4 and women 12.6 %). Mean plasma vitamin B12 concentrations were lowest in Indians (men 352.5 and women 350.7 pmol/l), then Chinese (men 371.1 and women 373.7 pmol/l), and then Malays (men 430.5 and women 486.0 pmol/l).

Conclusion—While there were ethnic differences for plasma folate and vitamin B12 (in particular lower levels in Indians), there was no evidence that homocysteine plays any part in the differential ethnic risk from CHD in Singapore and in particular the increased susceptibility of Indians to the disease.

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composed of Chinese 76%, Malays 14%, Indians 7%, and Others 3%. Of the Indians, 80% originate from Southern India (Tamil Nadu and Kerala) and Sri Lanka.

In Singapore, Indians have more CHD (but not more cerebrovascular disease) than Malays and Chinese, with Malays having more CHD than Chinese.² The greater susceptibility of Indians to CHD is not explained by cigarette smoking, hypertension and hypercholesterolaemia.³ Compared with Malays and Chinese, Indians have more abdominal obesity and insulin resistance, and some components of Syndrome X (lower high density lipoprotein cholesterol, more glucose intolerance, and higher plasminogen activator inhibitor type 1), which partly explains their increased susceptibility to CHD.⁴ Also, Indians have higher levels of lipoprotein(a),⁴ and lower levels of vitamin C and selenium (both anti-oxidants),⁵ which could increase their risk of CHD. However, other mechanisms may exist.

Homocysteine is a sulphur containing amino acid formed during the metabolism of methionine, an essential amino acid derived from dietary protein. Epidemiological studies indicate that hyperhomocysteinaemia is a risk factor for atherothrombotic disease, though the molecular mechanism is not clear.⁶ Homocysteine is metabolised with folate as a co-substrate and vitamin B12 as a co-factor and low levels of these vitamins causes hyperhomocysteinaemia.⁶ A study found an association between low serum folate and cardiovascular disease mortality,⁷ and there may be benefits from increasing folate intake.⁸ This article examines plasma homocysteine, folate, and vitamin B12, among the three main ethnic groups in Singapore.

Methods

SAMPLE

The data are from the National University of Singapore Heart Study, which was a cross sectional survey of a random sample of persons aged 30 to 69 years from the general population of Singapore. The sampling has been described in detail.⁴ There was a response rate of 71.2%. Of the 961 responders, 726 persons (randomly selected) had measurements of plasma homocysteine, folate, and vitamin B12.

PROCEDURES

Morning clinics were held from June 1993 to December 1995 with the two genders and three ethnic groups seen concurrently. The

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Indians or South Asians (that is, persons from the Indian subcontinent) have been found in a number of countries to be particularly susceptible to coronary heart disease (CHD).¹ Singapore has a population of 3.3 million people

Table 1 Pearson partial correlation coefficients (*p* values), adjusted for age, between plasma homocysteine, folate, and vitamin B12, for 369 men and 357 women aged 30 to 69 years

	Homocysteine	Vitamin B12
Homocysteine		
Men		-0.02 (0.73)
Women		-0.01 (0.93)
Folate		
Men	-0.14 (<0.01)	0.30 (<0.01)
Women	-0.11 (0.04)	0.24 (<0.01)

subjects were asked to fast from 2100 the previous evening.

Venous blood specimens were taken with the subject in a sitting position using venoject vacuum containers with minimum venous stasis. Within 30 minutes, the blood was spun and plasma separated. Specimens were stored at -20°C with all measurements made at the end of the project. Measurement of plasma total homocysteine was by high performance liquid chromatography (HPLC),⁹ and of plasma folate and vitamin B12 by radioimmunoassay.¹⁰ The reproducibility (precision) of the measurements for all parameters were above 95%. The coefficients of variation (*n* = 4) for within and between assays were, homocysteine 1.3% and 5.1%, folate 2.7% and 4.5%, and vitamin B12 3.3% and 5.0%. High concentrations of plasma homocysteine were taken as >14.0 µmol/l,^{6 11 12} and low concentrations of plasma folate as <6.8 nmol/l.¹³

ANALYSIS

The distributions of homocysteine, folate and vitamin B12 were near normal with just slight skewing to the right, and hence there was no log transformation and arithmetic means have been calculated. Though the mean ages were similar by gender and ethnic group, there was age adjustment. Pearson product moment partial correlation coefficients, adjusted for age, were calculated on SPSS. Age adjustment was performed for means by analysis of covariance

Table 2 Means (95% confidence intervals), age adjusted by analysis of covariance, of plasma homocysteine, folate, and vitamin B12, for Indians (I), Malays (M), and Chinese (C), by gender, for age group 30 to 69 years

	Indians (men=129, women=119)	Malays (men=104, women=106)	Chinese (men=136, women=132)	Significance, <i>p</i> value		
				I v M	I v C	M v C
Homocysteine (µmol/l)						
Men	16.2 (15.1, 17.2)	15.0 (13.8, 16.1)	15.3 (14.3, 16.3)	0.13	0.23	0.99
Women	11.5 (10.5, 12.3)	12.5 (11.5, 13.5)	12.2 (11.3, 13.1)	0.17	0.31	0.68
Folate (nmol/l)						
Men	8.7 (7.8, 9.6)	8.5 (7.5, 9.5)	9.7 (8.8, 10.6)	1.00	0.39	0.21
Women	10.9 (9.2, 12.7)	10.8 (8.9, 12.6)	13.8 (12.1, 15.4)	1.00	0.07	0.06
Vitamin B12 (pmol/l)						
Men	352.5 (320.5, 384.4)	430.5 (395.0, 460.0)	371.1 (340.0, 402.3)	<0.01	0.41	0.01
Women	350.7 (318.7, 385.0)	486.0 (449.8, 522.2)	373.7 (341.2, 406.1)	<0.01	0.34	<0.01

Table 3 Prevalence rates % (95% confidence intervals), age adjusted by direct standardisation to the total sample, of high plasma homocysteine (>14.0 µmol/l) and low plasma folate (<6.8 nmol/l), for Indians (I), Malays (M), and Chinese (C), by gender, for age group 30 to 69 years

	Indians (men=129, women=119)	Malays (men=104, women=106)	Chinese (men=136, women=132)	Significance, <i>p</i> value		
				I v M	I v C	M v C
Homocysteine (>14.0 µmol/l)						
Men	60.0 (51.5, 68.5)	53.9 (44.4, 63.5)	56.6 (48.2, 64.9)	0.35	0.58	0.68
Women	21.9 (14.6, 29.2)	37.8 (28.6, 47.1)	30.6 (22.7, 38.5)	<0.01	0.11	0.24
Folate (<6.8 nmol/l)						
Men	44.9 (36.3, 53.4)	45.3 (35.7, 54.9)	31.4 (23.6, 39.2)	0.95	0.02	0.03
Women	36.6 (27.9, 45.2)	24.5 (16.3, 32.7)	12.6 (6.9, 18.3)	0.05	0.02	0.13

KEY POINTS

- In Singapore, coronary heart disease (CHD) is highest in Indians, then Malays, and then Chinese.
- Plasma folate is lower in Indians and Malays than Chinese and plasma vitamin B12 is lowest in Indians. Lower antioxidant activity in Indians may play a part in their higher susceptibility to CHD.
- There are no ethnic differences for plasma total homocysteine.
- Hyperhomocysteinaemia seems to play no part in ethnic differences for CHD in Singapore and in particular the increased susceptibility of Indians to the disease.

using the GLM Procedure of SAS and for prevalences by direct standardisation to the total sample population with significance testing by the Z test. All significance testing was two tailed.

Results

Table 1 shows in both genders, that there were moderate direct correlations between folate and vitamin B12, slight/moderate inverse correlations between homocysteine and folate, but no correlations between homocysteine and vitamin B12.

Table 2 shows that mean plasma homocysteine concentrations were higher in men than women for all three ethnic groups. However, homocysteine showed no significant ethnic differences, with Indians having slightly higher levels in men and slightly lower levels in women. Table 3 further shows that the only significant difference for high homocysteine was that Malay women had a higher proportion than Indian women by 15.9%.

Mean plasma folate concentrations were lower in men than women for all three ethnic groups (table 2). Concentrations were lower in

Indians and Malays than Chinese, though there was statistical significance only in women, with differences of 2.9 nmol/l and 3.0 nmol/l respectively (table 2). Table 3 shows that Indians had higher proportions with low folate than Chinese by 13.5% in men and 24.0% in women and Malays had higher proportions than Chinese by 13.9% in men and 11.9% in women. There was no difference for the proportion with low folate between Indians and Malays in men, but in women Indians had a higher proportion than Malays by 12.1%.

Table 2 shows that mean plasma vitamin B12 concentrations (which showed no important gender differences) were lower in Indians and Chinese than Malays for both genders. For men, vitamin B12 was lower in Indians by 78.0 pmol/l and in Chinese by 59.4 pmol/l, while for women the differences were 135.3 pmol/l and 112.3 pmol/l respectively. Indians had lower levels than Chinese for both genders but the differences were not statistically significant.

Discussion

The subjects were fasting as recommended for homocysteine measurement.¹⁴ For all three ethnic groups, plasma levels of total homocysteine were higher in men than women (as in other studies,¹¹), those of folate were higher in women than men, while there were no gender differences for vitamin B12. Plasma levels of folate and vitamin B12 were directly correlated, as their metabolism is linked. Folate was inversely correlated with homocysteine, but there was no correlation between vitamin B12 and homocysteine. Other studies have found that homocysteine is most strongly correlated with folate.¹⁴

Indians and Malays had lower mean plasma folate and a higher proportion with low levels than Chinese. Folic acid is present in most foods, particularly organ meats (especially liver), fresh green vegetables, and some fresh fruits.¹⁵ Folate is heat labile and largely destroyed by prolonged cooking.¹⁵ Vitamin C is similarly destroyed and diets low in vitamin C are low in folate.¹⁵ Interestingly, plasma vitamin C has also been found to be lower in Indians and Malays than Chinese in Singapore.⁵ There is more prolonged cooking at high temperatures with Indian and Malay dishes such as curries compared with the typical Chinese stir fried dishes, with probably more destruction of dietary folate and vitamin C. Lower folate levels in Indians and Pakistanis than in white populations has been found in the United Kingdom and this has been attributed to their diet.¹⁶ Overall, a high proportion of people in Singapore have low folate levels, which has been found in other populations.¹³

Mean plasma vitamin B12 was lowest in Indians, then Chinese, and then Malays, for both genders, though the differences between Indians and Chinese were not statistically significant. Vitamin B12 is mainly present in animal protein particularly organ meats (especially liver) and bivalves (clams and oysters), and to a less extent in seafood, milk and milk

products.¹⁵ Vitamin B12 is resistant to heat,¹⁵ though deficiency may occur in vegetarians, in whom small amounts in legumes may be the only dietary source.¹⁵ Lower plasma vitamin B12 in Indians is probably explained by their more vegetarian diet; as found in a food consumption survey.¹⁷

Lower levels of folate and vitamin B12, together with lower levels of vitamin C and selenium as previously reported,⁵ indicates lower anti-oxidant activity in Indians, which may play a part in their higher susceptibility to CHD.

However, there are no ethnic differences for plasma homocysteine. The small ethnic differences in folate and vitamin B12 are not translated into different homocysteine levels. In Singapore, hyperhomocysteinaemia does not seem to be part of the explanation for ethnic differences in CHD and in particular the increased susceptibility of Indians to the disease.

Bangladeshis have been found to have higher homocysteine levels than the white population in the United Kingdom and this has been put forward as a possible explanation for their higher rates of CHD.¹⁸ This study in Singapore does not support this, in so far as Indians have more CHD than Malays and Chinese despite similar levels of homocysteine.

However, it should be noted that levels of plasma homocysteine for all three ethnic groups in Singapore are higher than have been reported in the West. A study is in progress to determine the frequencies of methylenetetrahydrofolate reductase (MTHFR) genotypes as a possible explanation for these high levels. MTHFR is an enzyme in the metabolism of homocysteine and its genotypes are associated with different levels of homocysteine.¹⁹

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Conflicts of interest: none.

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