

Homocysteine, Folate and Vitamin B12 as Risk Factors for Acute Myocardial Infarction in a Southeast Asian Population

K C Ng,*^{MBBS, MRCP}, Q W Yong,**^{FAMS, MBBS, MRCP}, S P Chan,***^{MSc}, A Cheng,**^{FAMS, FRCP, MD}

Abstract

Introduction: Hyperhomocysteinaemia is an emerging risk factor for coronary artery disease (CAD) and most studies done to date are in Caucasian populations. We aimed to determine whether hyperhomocysteinaemia is a risk factor for acute myocardial infarction (AMI) in a Southeast Asian population comprising different ethnic groups and relate it to the traditional risk factors and plasma vitamin B12 and folate levels. **Materials and Methods:** This was a case-control study comprising 168 AMI patients and 141 controls with a median age of 55 years (range, 27 to 77 years), living in Singapore. Homocysteine was measured by fluorescence polarisation immunoassay and vitamin B12 and folate were measured by electrochemiluminescence immunoassay. Logistic regression analysis was used to test the association of homocysteine, vitamin B12 and folate with the occurrence of AMI. The study was approved by the Tan Tock Seng Ethics Committee. **Results:** We found that the odds of having AMI was higher for subjects with hypertension, smoking habit, lower plasma folate and vitamin B12 levels and non-Chinese ethnic group. On the other hand, plasma homocysteine level was not significantly associated with AMI. The baseline levels of plasma total homocysteine in both AMI patients and controls were higher than other studies (median values between 12 and 14 $\mu\text{mol/L}$). **Conclusion:** In our population, plasma total homocysteine levels were not associated with AMI but low plasma levels of folate and vitamin B12 were independently associated.

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Key words: Cross-sectional study, Plasma folate, Plasma total homocysteine, Plasma vitamin B12

Introduction

Since the beginning of the last decade, hyperhomocysteinaemia has been identified as a risk factor for acute coronary syndromes. A few large prospective studies have shown a clear increase in relative risk, especially for those populations with plasma homocysteine levels above 15 $\mu\text{mol/L}$.¹⁻³ Case-control studies in patients with acute myocardial infarction (AMI) have also borne out this conclusion.⁴⁻⁶ More importantly, Anderson et al⁷ showed that, in patients with angiographically-defined coronary artery disease (CAD), homocysteine was a significant predictor of mortality, independent of other risk factors. However, most of these studies are in American and Northern European populations who have different dietary habits and genetic make-up compared to the Asian population. To date, there are only a few studies which involved an indigenous Asian population. Kawashiri et al,⁸ in a study on Japanese patients with CAD, showed that only premature CAD was associated with a high level of plasma homocysteine. Joubert et al⁹ also showed that hyper-

homocysteinaemia was independently associated with CAD in Arab men. Recently, Chambers et al¹⁰ reported that hyperhomocysteinaemia was an independent risk factor for coronary heart disease (CHD) in Indian Asians who have migrated to the United Kingdom (UK).

Therefore, the aims of this study were to determine whether hyperhomocysteinaemia is a risk factor for AMI in a Southeast Asian population comprising different ethnic groups and relate it to the traditional risk factors and plasma vitamin B12 and folate levels.

Material and Methods

Patient Population

This case-control study was carried out in Tan Tock Seng Hospital between January 1998 to May 1999. The protocol was approved by the hospital ethics committee and verbal informed consent was taken from every patient recruited. A total of 168 cases and 141 controls were recruited. The cases were consecutive patients who were admitted to the coronary care unit with a diagnosis of an AMI, which was

* Registrar

** Consultant

Department of Cardiology

*** Senior Medical Statistician

Department of Clinical Epidemiology

Tan Tock Seng Hospital

Address for Reprints: Dr Kenneth Ng, Department of Cardiology, Tan Tock Seng Hospital, 11 Jalan Tan Tock Seng, Singapore 308433.

defined as including two or more of the following criteria: typical chest pain, ST elevation of equal to or more than 2 mm in two contiguous precordial leads or 1 mm elevation in the limb leads, and a raised creatinine kinase (CK) MB mass activity of 2 or more times.¹¹ After the recruitment of the cases, controls with similar age, sex and ethnic profiles were recruited from orthopaedic, geriatric, ophthalmology and otorhinolaryngology wards. Case record review was used to determine if patients had renal dysfunction, traditional risk factors of diabetes, smoking, hypertension, history of hyperlipidaemia, previous AMI, previous cerebrovascular accident, peripheral vascular disease, Q-waves on the electrocardiogram (ECG) and family history of AMI. Controls were not recruited if the case notes revealed that they had a previous AMI, previous cerebrovascular accident, peripheral vascular disease, Q-waves on the ECG and renal dysfunction. However, the usage of drugs, such as corticosteroids and cyclosporine, was not documented in the controls and these could have affected the homocysteine levels. Diabetes was defined as a fasting blood glucose of >7.0 mmol/L or a random blood glucose of >11.3 mmol/L, and hypertension was defined as two blood pressure readings of more than systolic 140 mm Hg and diastolic 90 mm Hg 2 weeks apart. Smoking was defined as current or ex-smokers within the past 20 years.

Protocol

Ten mL of venous blood was drawn on admission of AMI patients to the coronary care unit as far as possible before initiation of thrombolysis into ethylenediamine-tetraacetic acid (EDTA) tubes. The time of day of admission of AMI patients when their blood was collected was not documented in this study. For control patients, blood samples were obtained after an overnight fast. No methionine loading was done prior to obtaining of the blood samples as it offered no additional value.¹⁰ Platelet-poor plasma was immediately obtained by centrifugation at 3500 rpm for 15 minutes. The plasma obtained was aliquoted out into pyrogen-free plastic tubes and stored at minus 80°C for batch analysis.

Homocysteine, B12, Folate and Lipid Measurements

To analyse homocysteine levels, fluorescence polarisation immunoassay was done.¹² In the assay, bound homocysteine and mixed disulphide homocysteine was reduced to free homocysteine by dithiothreitol. The free homocysteine that was formed was then enzymatically converted to S-adenosyl-L-homocysteine (SAH) by the use of SAH hydrolase and excess adenosine. The coefficients of variation for intra-assay were 2.2%, 1.9% and 1.4% for concentrations of homocysteine at 5.9 μ mol/L, 10.8 μ mol/L and 21.6 μ mol/L, respectively. The coefficients of

variation for inter-assay were 5.2%, 4.1% and 3.7% at the same concentrations. Vitamin B12 and folate were analysed by electrochemiluminescence immunoassay.

Statistical Analyses

In order to determine whether homocysteine, folate and vitamin B12 were risk factors for AMI, logistic regression analysis¹³ was carried out. The list of covariates also included gender, age, race, smoking status, presence of diabetes mellitus, presence of hypertension and family history of AMI. The outcome was occurrence of AMI (0: controls, 1: cases). To validate the model further, about half of the observations were temporarily omitted in a random manner to form the test sample and the auxiliary model based on the remaining observations was constructed. The area under receiver operating curve (ROC)¹⁴ was then estimated to check whether the model was able to discriminate patients who suffered AMI from those who did not. A separate linear regression analysis¹⁵ was also carried out to check if folate and vitamin B12 might have a significant effect on homocysteine and assess the correlation between them. In order to keep analysis straightforward, no imputation techniques were applied for handling missing observations, which is a pervasive problem in most retrospective studies. All statistical tests were conducted at the 5% level of significance. The data were entered into Stata 6.0 (Stata Corporation, Texas, USA) for analysis.

Results

The majority of the patients (95.5%) in the sample were male. In terms of ethnic distribution, 65.0% were Chinese, 10.0% were Malay and 20.3% were Indian (Table I). The plasma total homocysteine, folate and vitamin B12 levels for the AMI cases and controls are shown in Table II, including the breakdown for the different racial groups. The median and range values were reported because the homocysteine, folate and vitamin B12 levels were highly skewed. Multiple linear regression analysis showed that folate and vitamin B12 were negatively related with homocysteine (both *P* values <0.001 ; all 3 variables were log-transformed in view of the skewness). There was no severe violation of the regression assumptions, such as homoscedasticity and normality, by means of the residual plot and Q-Q plot.

From the logistic regression analysis, plasma total homocysteine was found not to be significantly associated with AMI as it was not selected in the final model. The odds of having AMI was found to be higher for patients with hypertension, smoking habit, lower levels of folate and vitamin B12, and of non-Chinese origin (Table III). The selection of only 5 covariates does not mean that other factors were unimportant. Rather, their presence was not as

TABLE I: SUMMARY OF DATA

Variable	Cases with AMI (n = 168)*	Controls (n = 141)*	P value [†]
Age (y)	Median: 55 Range: 27 to 77	Median: 55 Range: 28 to 75	0.82 [‡]
Sex			
Male	142 (94.7%)	136 (96.5%)	0.58
Female	8 (5.3%)	5 (3.5%)	
Race			
Chinese	93 (62.0%)	96 (68.1%)	0.33
Malay	15 (10.0%)	14 (9.9%)	
Indian	33 (22.0%)	26 (18.4%)	
Others	9 (6.0%)	5 (3.5%)	
Diabetes mellitus			
Present	50 (33.6%)	33 (26.8%)	0.24
Absent	99 (66.4%)	90 (73.2%)	
Hypertension			
Present	63 (42.9%)	28 (22.8%)	<0.01
Absent	84 (57.1%)	95 (77.2%)	
Family history of AMI			
Present	11 (7.4%)	0 (0.0%)	<0.01
Absent	138 (92.6%)	123 (100.0%)	
Smoking			
Yes	63 (42.9%)	28 (22.8%)	<0.01
No	84 (57.1%)	95 (77.2%)	
Homocysteine (umol/L)	Median: 13.6 Range: 6.1 to 45.0	Median: 12.9 Range: 4.6 to 42.4	0.21 [‡]
Vitamin B12 (pmol/L)	Median: 297 Range: 53 to 1476	Median: 398.5 Range: 25 to 1476	<0.01 [‡]
Folate (nmol/L)	Median: 12.0 Range: 2.4 to 55	Median: 11.2 Range: 3.8 to 90	0.97 [‡]

AMI: acute myocardial infarction

* Discrepancies in the table were due to missing observations

† Based on Fisher's exact test unless otherwise stated

‡ Wilcoxon rank sum test

significant as those selected. The Hosmer-Lemeshow goodness-of-fit statistic was statistically non-significant ($\chi^2_8 = 9.48$; $P = 0.30$), implying that the estimated logistic regression model fitted the data well. To check for external validity, an auxiliary model was estimated based on half of the sample and it was noted that the estimates fell within two standard errors of those obtained from the original sample. Since the area under the ROC turned out to be around 0.78, there was sufficient evidence suggesting that the proposed model was able to discriminate patients who suffered AMI from those who did not. There was a fairly large amount of incomplete observations (25.2%), but imputation technique¹⁶ was not carried out in order to keep the analysis simple. When both folate and vitamin B12 were omitted from the logistic regression analysis, homocysteine was still not significantly associated with AMI ($P = 0.35$), after adjusting for race, hypertension and smoking habits.

TABLE II: HOMOCYSTEINE, VITAMIN B12 AND FOLATE BY RACE

Variable	Cases median (range)	Controls median (range)
Homocysteine (umol/L)		
Chinese	13.4 (6.1-44.5)	12.9 (5.7-42.4)
Malay	12.0 (7-20.9)	12.3 (4.6-19.1)
Indian	14.3 (6.9-45.0)	12.1 (4.7-23.2)
Others	16.1 (9.1-23.3)	14.3 (12-15.7)
Vitamin B12 (pmol/L)		
Chinese	297.5 (107-1476)	372 (25-1414)
Malay	354 (259-584)	585 (177-1476)
Indian	269.5 (53-1053)	585 (201-1476)
Others	244 (57-491)	405.5 (187-643)
Folate (nmol/L)		
Chinese	12.3 (4.6-55)	11.3 (3.8-90)
Malay	14.3 (5.1-34.6)	12.5 (5-90)
Indian	10.8 (2.4-35.5)	10.3 (6.6-21.6)
Others	13.4 (6.3-18.4)	10.2 (6.7-12.8)

Discussion

We have shown that in our case-control study, plasma total homocysteine was not significantly associated with AMI. This is supported by 2 studies in Norwegian and Finnish populations which showed no difference in total plasma homocysteine levels between patients with AMI and their controls.^{17,18} A recent study by Voutilainen et al,¹⁹ in a Eastern Finnish population, showed no statistical difference in the mean total homocysteine concentration between patients with acute coronary syndromes and controls. The Physicians' Health Study in men who suffered an AMI failed to demonstrate a significant association between plasma homocysteine levels and risk for AMI after a follow-up of 7.5 years.²⁰ However, there is a wealth of data from both cross-sectional, retrospective case-control and prospective cohort studies^{1-4,6,21} supporting the association between hyperhomocysteinaemia and CAD. There are several possible reasons for the lack of association. Our study population is predominantly Asian and there is a paucity of data in Asians with regards to the association between hyperhomocysteinaemia and AMI, and most of the positive studies are from the West. Only 1 study in Arab men reported an independent association of hyperhomocysteinaemia with CAD.⁹ It has been shown in a study by Hughes et al²² that there were no differences in plasma homocysteine levels between the various races in the Singapore population, so the predominance of the Chinese race in our study could not have masked any association. Secondly, compared to the studies that showed a positive association, our study sample was smaller. Thirdly, we did not control for all known risk factors of atherosclerosis, especially the lipid levels in the controls were unknown, and that could have caused the lack of association between plasma total homocysteine levels and

TABLE III: MULTIVARIATE LOGISTIC REGRESSION MODEL

Covariate	Odds ratio	Standard error	Wald statistic	95% CI	P value
Folate (pmol/L)	0.964	0.014	-2.574	0.937–0.991	0.01
Vitamin B12 (nmol/L)	0.997	0.001	-3.480	0.995–0.999	0.001
Homocysteine (umol/L)	0.982	0.027	-0.679	0.930–1.036	0.497
Race					
Chinese					
Non-Chinese	2.408	0.862	2.454	1.194–4.856	0.009
Gender					
Male					
Female	3.621	3.051	1.527	0.694–18.881	0.127
Age (y)	0.988	0.017	0.672	0.955–1.023	0.501
Hypertension					
Without					
With	3.328	1.192	3.357	1.649–6.715	0.001
Diabetes mellitus					
Without					
With	1.222	0.449	0.547	0.595–2.509	0.584
Smoking status					
Non-smoker					
Smoker	4.973	1.651	4.832	2.595–9.532	<0.001

No. of observations: 229; Log-likelihood: -121.22; Hosmer-Lemeshow statistic: 9.48 ($P = 0.30$)

AMI. Fourthly, the elimination of homocysteine in the selection process of the logistic regression analysis could be due to the fact it was highly correlated with folate and vitamin B12. However, when both folate and vitamin B12 were omitted, homocysteine was still not significantly associated with AMI in the model. Two other factors which were not controlled for in the study were the influence of the AMI and the circadian rhythm on homocysteine levels. Egerton et al²³ found that plasma protein and non-protein bound homocysteine was lower in the first 3 days post AMI and rose to basal levels after day 7. This could account for an artificially low homocysteine level in our AMI patients. Circadian rhythm also affects homocysteine levels with levels lowest between 1000 hours and 1200 hours, which covers only part of the time of day when we obtained the blood samples for our AMI patients.²⁴

We noticed that the plasma total homocysteine levels in our study population, both for AMI patients and controls, were higher than those reported in studies where significant associations were found. Our population had higher median levels of total plasma homocysteine (cases, 13.6 umol/L and controls, 12.9 umol/L) as compared to the median total plasma homocysteine levels of 10 to 12 umol/L in studies by Arnesen et al⁶ and Chambers et al.¹⁰ The median levels of total plasma homocysteine in our population would be placed in the 70th to 80th percentiles in the Physician's Health Study.¹ The reason is unclear as we had followed precautions in the collection and processing of blood samples and our study population was not elderly and the

majority do not smoke nor have diabetes mellitus. However, we did not document any pre-existing renal dysfunction, acute illnesses or concomitant drug therapy which may have caused elevated homocysteine levels. The relatively higher plasma total homocysteine levels could be due to low plasma folate and vitamin B12 levels, as we have shown a strong negative correlation between plasma homocysteine and folate and vitamin B12 levels in our study population.

We found in our study population that plasma folate and vitamin B12 were strongly associated with AMI. The AMI patients had significantly lower levels of plasma folate and vitamin B12. This is supported by data from large epidemiological studies done in the United States and Europe, where the relative risk for cardiovascular disease ranges from 1.37 to 1.69 for the lowest compared to highest quarters of folate level.^{25–28} There is still no strong data for the association of cardiovascular disease and vitamin B12, although 1 study did not find any significant association between vitamin B12 levels and AMI.²⁶ Our study shows that the association of lower folate and vitamin B12 levels with AMI seems to be independent from plasma total homocysteine levels. At present, it is not known whether there are any other pathways in which low folate and vitamin B12 can promote atherosclerosis without involving the methylene tetrahydrofolate reductase or the methionine synthase pathways. It has been mentioned that a low level of these vitamins may be a marker of diets poor in fruits and vegetables, which have been independently associated

with a higher risk of cardiovascular disease.²⁹ It is unfortunate that we were unable to obtain any socio-economic or dietary data from our study population which may have helped elucidate this. Recently, Newman³⁰ has postulated that deficiencies in serum levels of folic acid, vitamin B12 and vitamin B6 could lead to hypomethylation of deoxyribonucleic acids in cells in the arterial intima, resulting in the proliferation of smooth muscle cells which lead to the formation of atheroma.

In conclusion, plasma total homocysteine was not significantly associated with an increased risk of AMI in our study population. We did find, however, that low levels of plasma folate and vitamin B12 were significantly associated with AMI and further studies are needed to determine the exact pathophysiological mechanisms.

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