Association of Homocysteine Levels to Traditional Risk Factors in Unstable Angina and Non-ST Elevation Myocardial Infarction: Implications from the FINEST study

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ABSTRACT

Background: Increased blood homocysteine level is a risk factor for unstable angina and non-ST elevation myocardial infarction (NSTEMI). Studies associate homocysteine levels with conventional cardiovascular risk factors - smoking, diabetes, hypertension, advanced age, and dyslipidemia – in subjects with evidence of having coronary artery disease.

Objective: This study seeks to determine the effect of conventional cardiovascular risk factors on homocysteine levels of patients with intermediate to high-risk unstable angina and non ST elevation myocardial infarction (NSTEMI).

Results: Data were taken from 219 patients with unstable angina and non ST elevation myocardial infarction (NSTEMI) with onset within the past two weeks and who were included in a folic acid supplementation trial (ISRCTN30249553). Using univariate analysis, baseline homocysteine levels are positively correlated with age (p=0.008) and significant smoking history, both current and past (p=0.028), but negatively correlated with body mass index (BMI) (p=<0.001). No significant association was detected with diabetes mellitus, hypertension, dyslipidemia and sex.

Conclusion: Homocysteine levels increase with age and smoking, but decrease with BMI, in patients with unstable angina and NSTEMI.

Key Words: homocysteine, risk factors, unstable angina, myocardial infarction

Introduction

Non-ST elevation myocardial infarction (NSTEMI) and ustable angina (UA) belong to the spectrum of acute coronary syndromes (ACS) that implies partial occlusion of the coronary arteries leading to ischemic events. These conditions are significant indicators of further myocardial infarction that may be fatal or may require intensive hospital care.[1] Unstable angina and NSTEMI also have high recurrence rates for ACS and mortality six months after an event.[2] This makes research in the assessment of its risk factors imperative so that monitoring and control could be optimized.

Specific conditions are already identified to be traditional risk factors for coronary artery disease, namely: smoking, diabetes, hypertension, advanced age, and dyslipidemia. Though several epidemiologic studies have been done to determine the risk factor profile of subjects with coronary artery disease, studies that exclusively focus on unstable angina and NSTEMI have been scarce. In a large cross-sectional study of patients with ACS, about four-fifths had at least one of the risk factors.[3] In addition, compared to ST elevation myocardial infarction (STEMI), only smoking was noted to be less common in subjects with unstable angina or NSTEMI. Moreover, subjects with unstable angina and NSTEMI had a lower age than those with STEMI. Obesity and family history of coronary heart disease were present in about half of the remaining subjects with none of the other five risk factors. Notably, this pattern was noted to persist in several geographic locations: North and Central America, Europe, Middle East, Africa and Australasia.[3] In addition, in the CRUSADE study, younger age and lack of smoking history were noted to be associated with the occurrence of insignificant coronary heart disease, and of lesser subsequent mortality, in patients with non ST elevation acute coronary syndrome (NSTE-ACS).[4]

Increased plasma homocysteine has been recently recognized as another risk factor for cardiovascular diseases. A study showed that blood homocysteine levels are higher in patients with unstable angina compared with healthy controls.[5] Besides being a potential risk factor for unstable angina and NSTEMI, increased homocysteine levels have been implicated in poorer outcomes and greater myocardial injury.[6] Moreover, low homocysteine levels confer better long-term outcomes among patients with coronary heart

disease.[7,8]

The growing evidence that an increased homocysteine level is a risk for cardiovascular disorder suggests possible association with other risk factors. Studies have shown that blood homocysteine levels are affected by the presence of the traditional risk factors. Age has been positively correlated with blood homocysteine levels.[9,10] An association with insulin resistance syndrome has also been noted.[11] In the Framingham Offspring study, smoking and hypertension increase with homocysteine levels.[10] The Hordaland study, involving 7,053 subjects, showed that homocysteine levels increase with advancing age, male sex, smoking, dyslipidemia, less physical activity and increasing blood pressure.[12] However, recent studies noted that homocysteine levels seem to be more correlated to coronary artery disease than the conventional risk factors of coronary artery disease (CAD) – age, diabetes mellitus, smoking, hypertension and dyslipidemia. In a study on elderly subjects, the association between age and homocysteine is evident.[13] In a study in Thailand among 301 men and women with suspected CAD, homocysteine is significantly associated with angiography-proven CAD, and the correlation persists even after adjusting for age, sex, diabetes mellitus, hypertension, hyperlipidemia, and smoking.[14] Hence, a dilemma exists if homocysteine is affected by these risk factors. Though at present, the mechanism of vascular injury associated with homocysteine is still not clear, investigation of its association with the traditional risk factors could suggest potential mechanisms for its injurious effects that may direct future studies. This warrants further study to determine the relationship of homocysteine with the conventional risk factors. The authors know of no previous study on NSTEMI and unstable angina with regard to this relationship.

The objective of this study is to determine the relationship between the traditional risk factors and the blood homocysteine levels of patients with intermediate- and high-risk unstable angina, and NSTEMI. The results may be helpful especially in the assessment of the role of homocysteine in CAD as a possible modifiable risk factor. This study follows from a randomized controlled trial investigating the use of folic acid on subjects with non ST elevation acute coronary syndromes to be published elsewhere.[15] The article at hand uses the baseline data of subjects included in the previous RCT to determine the association of homocysteine levels with traditional cardiovascular risk factors.

Methodology

Subject selection and randomization

From August 2003 to September 2006, subjects diagnosed with unstable angina (intermediate- and high-risk) or NSTEMI within the past two weeks were screened for inclusion in the study *Folate intervention in non-ST elevation myocardial infarction and unstable angina* (FINEST) (ISRCTN30249553), a randomized controlled trial on the supplementation of folic acid, vitamin B6 and vitamin B12 for the secondary prevention of death, nonfatal acute coronary syndromes and serious rehospitalization within six months after treatment. The subjects were recruited from five hospitals in the Philippines, namely: Philippine Heart Center (PHC), Philippine General Hospital (PGH), Quirino Memorial Medical Center (QMMC), Ospital ng Maynila Medical Center (OMMC), and East Avenue Medical Center (EAMC). The Ethical Review Board of the Research Implementation and Development Office of the University of the Philippines College of Medicine approved the protocol and the informed consent. All subjects were able to provide informed consent independently.

Being primarily an interventional trial, the study used the following exclusion criteria: hemodynamic instability (cardiogenic shock, ongoing chest pain, unresolved and new onset end-organ damage, and unstable congestive heart failure in the past two weeks), significant liver disease (classical signs and symptoms, or liver enzyme levels 3x the normal upper limit, or a PT at 1.5x normal), significant renal disease (with creatinine levels more than 180 umol/dl or requiring dialysis), hemoglobin less than 1 g/dl, high output failure, inability to provide adequate self care, malignancy or any terminal illness, age <18 years old, pregnancy, and residence outside Metro Manila or the adjacent provinces of Cavite and Rizal. In line with the finding that folic acid supplementation increases the rate of in-stent restenosis, all patients with previous revascularization procedures were excluded from the study.[16]

Upon signing the informed consent, subjects were interviewed to establish baseline characteristics. Important features included age, sex, and presence of other risk factors for ACS (smoking, diabetes mellitus, dyslipidemia with total cholesterol, LDL, triglycerides levels, menopause and obesity as measured by BMI). Dyslipidemia was defined as the presence of the following: total cholesterol >190 mg/dl, LDL >160mg/dl or the use of a cholesterol-lowering drug. Significant smoking was defined as >20 pack years of cigarette use. Previous smoking denoted discontinuation of smoking at least one month prior to onset of present NSTEMI or unstable angina. Homocysteine levels were determined using Abbott IMx. Since no normative level has been established for Filipinos, arbitrary cutoffs at 9.0 umol/li and at 16 umol/li were used. The 9.0 umol/li cutoff was based on the Hordaland study which demonstrated an increased risk for coronary artery disease at 9.0 umol/li.[12] The 16.0 umol/li cutoff was based on a previous study involving post-coronary event patients who showed a significant increase in mortality and recurrent coronary events[17]. In addition, in several studies that correlate homocysteine levels with cardiovascular outcomes, including our own,[18] a threshold of 16 umol/li was found to be associated with significant cardiovascular lesions compared with controls. Thus, those with <9 umol/li will be designated as the low level group; 9-16 umol/li, the moderate level group; and >16 umol/li, the high level group.

Statistical analysis

Data were analyzed with appropriate tests using STATA ver. 6.0 software. Descriptive statistics and univariate analyses were done to evaluate the risk factor profiles, ANOVA for parametric test for means, chi square test for

Table 1. Risk factor profile of subjects included in the study

Characteristics	n=219
Age	
in years, mean (SD)	59.2 (10.1)
male>45,female>55%	173 &79.0)
Sex (% male)	128 (58.4)
Diabetes mellitus (%)	50 (22.8)
Hypertension (%)	143 (63.5)
Smoking history *(%)*	
Nonsmokers	93 (42.5)
Ex-smokers	93 (42.5)
Occasional smokers	10 (4.6)
Current smokers	23 (10.5)
Dyslipidemia (%)	69 (31.1)
Body Mass Index*	
mean (SD)	24.2 (4.3)
<18	10 (5.2)
18-23 (%)	69 (35.9)
23.1-30 (%)	101 (52.6)
>30 (%)	12(6.3)
Menopause (%)†	81 (88.0)
Homocysteine levels‡	
umol/l, mean (SD)	12.8 (11.3)
<9 umol/l	62 (28.3)
9-16 umol/li	121 (55.3)
>16 umol/li	36 (16.4)

* 192 data for BMI
† For female subjects only
‡ If subject with skewed levels is (159.5 umol/li) not considered, mean is 12.1 (5.25)

parametric comparison of proportions, and Kruskal-Wallis test for nonparametric comparison of means and proportions. Scatter plots and Pearson correlation tests were constructed to demonstrate significant relationships with homocysteine levels.

Results

Subject characteristics

Two hundred and forty subjects were assessed for the FINEST study. However, only 219 homocysteine results were available for the study because of hemolysis of the blood samples. A second sample would not be reliable because of the initiation of folic acid intake. The risk factor profile is presented in Table 1. Only 192 subjects had available data for BMI since some subjects refused or were unable to weigh themselves. In order to prevent significant deviation that may result in lack of normality and homogeneity, a subject with a homocysteine level of 159.5 umol/li was removed from analysis involving means of homocysteine level. However, the subject was included in categorical studies of homocysteine levels, as well as in descriptive studies. Significant differences in results due to this maneuver are discussed.

The mean age was 59.2±10 years, with 79% in the at-risk group (male >45, female >55). The majority of the subjects were male (58.4%), hypertensive (63.5%), and, for women, menopausal (88.0%). Only 11.2% were current smokers, but 42.5% were ex-smokers, all of whom had a significant smoking history. The mean homocysteine level was 12.8 umol/li. The majority of the subjects (55.3%) had moderate homocysteinemia. Thirty-six subjects (16.4%) were found to have high homocysteine levels (>16 umol/li).

Association of homocysteine to risk factors

Increased mean homocysteine levels were found to be significantly associated with advanced age, increased use of tobacco, and decreased body mass index (Table 2). However, in stratified analysis of homocysteine levels, the significance was decreased. This may be due to two reasons: the smaller samples in the non-risk group, and the inclusion of the outlier, who was a non-risk 42-year old male. Nevertheless, the trend persisted suggesting the association of age with homocysteine levels.

Age and homocysteine

Homocysteine levels were found to correlate positively with age (Fig. I). This relationship was more evident in the lower levels (<16 umol per liter). Of the subjects with high homocysteine levels, only four out of 36 (11%) of them belong to the non-risk age group. Extremely high homocysteine levels (>30 umol/li) were noted in two out of four subjects in the non-risk age group.

Smoking and homocysteine

Homocysteine levels were lower in nonsmokers than in smokers (Fig. II). Homocysteine levels of <10 umol/li were more common in nonsmokers. Moreover, there was a higher proportion of high homocysteine levels in significant smokers than in nonsmokers, including occasional smokers. Homocysteine levels were comparable between current and previous smokers, with a trend towards higher homocysteine levels compared with nonsmokers, both quantitatively and qualitatively (Table 3).

Body mass index and homocysteine

Homocysteine levels were inversely correlated with body mass index (Fig. III). This relationship seems to involve both low and high levels of homocysteine, but significance was not evident probably due to inadequate sample size. Most of the cases with high homocysteine levels had a BMI ranging between 12 and 30, including the outlier (BMI=24.3). No obese subject had a homocysteine level >16 umol/li. About 61% of the subjects had normal or low BMI. Extremely high homocysteine levels (>30 umol/li) occur only with BMI <25.

Discussion

The results of the FINEST trial,[15] as well as the results of various randomized controlled trials, on the use of folic acid to lower homocysteine levels did not demonstrate conclusive benefit for cardiovascular diseases, except probably for stroke. In fact, there have been trends for increased potential harm with the use of the supplement. However, modifiable risk factors that are associated with homocysteine might be points of control to alleviate the potential risk from homocysteine through lifestyle modification, aggressive therapy and prognostic stratification, and secondary prevention. Moreover, all risk factors, including age or previous smoking history, could be relevant to our findings that homocysteine could independently predict adverse

Table 2. Relationship of traditional risk factors to blood homocysteine levels

Traditional risk factors	Homocysteine levels*		Homocysteine categories, frequency (% per row)			
	Mean, umol/li	p value	<9 mmol/li	9-16 mmol/li	>16 mmol/li	p value
Age		0.006†				0.088
Male >45, female >55	12.41 (4.88)		43 (25)	100 (58)	30 (17)	
No	11.08 (6.43)		19 (41)	21 (46)	6 (13)	
Sex		0.380				0.280
Male	12.40 (5.48)		31 (24)	75 (59)	22 (17)	
Female	11.76 (4.92)		31 (34)	46 (51)	14 (15)	
Diabetes mellitus		0.531				0.731
Yes	11.72 (4.55)		16 (32)	25 (50)	9 (18)	
No	12.25 (5.45)		46 (27)	96 (57)	27 (16)	
Hypertension		0.737				0.472
Yes	12.05 (5.32)		39 (27)	83 (58)	21 (15)	
No	12.30 (5.15)		23 (30)	38 (50)	15 (20)	
Smoking history		0.012				0.028
Yes	12.77 (5.56)		24 (21)	70 (60)	22 (19)	
No	11.42 (4.81)		38 (36)	51 (50)	14 (14)	
Dyslipidemia		0.218				0.290
Yes	12.79 (5.04)		15 (22)	39 (57)	14 (21)	
No	11.84 (5.33)		47 (31)	82 (54)	22 (15)	
Body Mass Index		0.003				<0.001
<18	19.03 (7.31)*		1 (10)	2 (20)	7 (70)	
18-23 (%)	12.49 (5.15)		18 (26)	38 (55)	13 (19)	
23.1-30 (%)	13.10 (15.55)		31 (31)	57 (56)	13 (13)	
>30 (%)	9.73 (2.67)		5 (42)	7 (58)	0 (0)	
Menopause		0.179				0.189
Yes	11.84 (4.70)		26 (32)	43 (53)	12 (15)	
No	10.50 (5.37)		6 (54)	3 (27)	2 (18)	

* tests performed without one outlier variable (Hcy = 159.5 umol/li)
† nonparametric (Kruskal-Wallis)

outcomes in subjects with non ST elevation ACS.[18] In this case, the traditional risk factor profile and homocysteine levels could serve as criteria to select patients that may need more aggressive therapy and monitoring.

The study found that blood homocysteine levels are associated with the age, cigarette smoking and body mass index (BMI) of survivors of non ST elevation myocardial infarction and unstable angina. The increase in homocysteine levels with age could be due to the degenerative loss of renal function[19,20] or the greater prevalence of blood folate deficiency in the elderly.[21,22] Since homocysteine is more common in the elderly, selective homocysteine determination to select at-risk patients should prioritize them. Moreover, very high homocysteine levels (>30 umol/li) were found in half of young patients, implying an important cause of coronary disease in the young. This subset of patients could have genetic conditions such as homocysteinuria. All of the subjects were not tested before they died.

Tobacco use was associated with increased homocysteine levels. It seems in our results that there was a trend for higher homocysteine levels in all groups of significant smokers, both current and past. However, statistical significance was only observed in the previous smokers probably because of the greater sample size (93 previous versus 23 current smokers). Thus, the groups were pooled to increase statistical sensitivity. The association between smoking and increased homocysteine levels were observed previously in both subjects with cardiovascular disease and in apparently healthy adults.[23,24] Both smoking and homocysteinemia are considered independent risk factors for coronary artery disease (CAD).[25,26] However, an amplifying effect of the two factors is implied in the findings of another study.[27] Hence, the association between smoking and homocysteinemia could be involved in the alleged synergistic effect of homocysteine and smoking in individuals with cardiovascular disease. It is also speculated that smoking and homocysteine have a significant overlap in their mechanisms of vascular injury - endothelial dysfunction, smooth muscle cell proliferation, etc. - that results in facilitation of one factor's effects by the other.

Smoking cessation does not lower homocysteine levels compared with significant smokers. This was also observed in smokers who ceased smoking for 10-11 weeks.[28] However, another study observed a mean 11.6% reduction in homocysteine levels in healthy smokers (36 cigarettes/day) if they discontinued smoking.[29] Considering that the FINEST subsample of smokers were older and had longer smoking histories, it may be that homocysteinemia is more difficult to lower by mere cessation in these subjects. Moreover, a bias is likely since several of these subjects discontinued smoking after a previous acute coronary event, which, by itself, is significantly associated with high homocysteine levels.

For body mass index (BMI), some studies had recorded similar negative correlation with homocysteine levels.[9,30] BMI

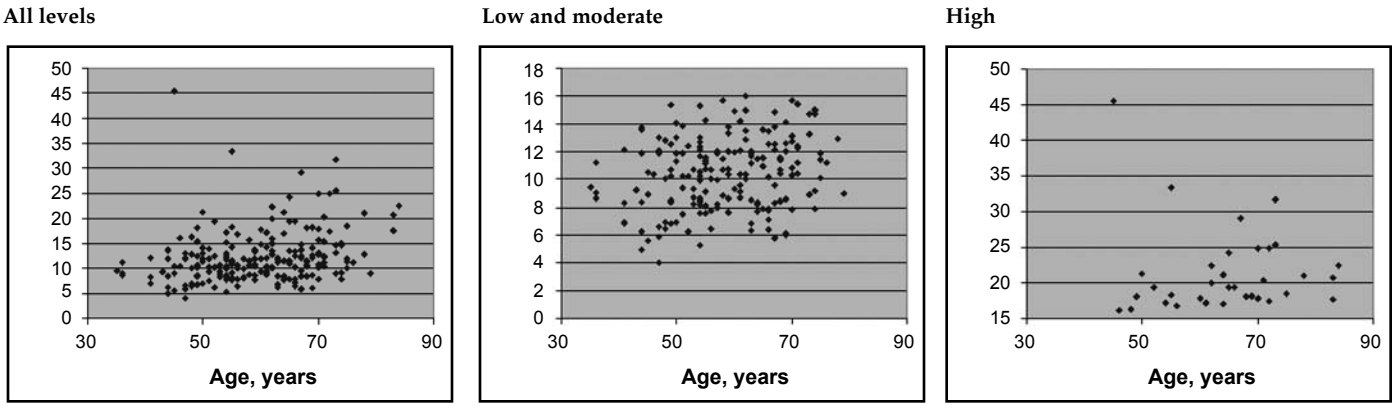

Figure I. Graphs show plots of homocysteine levels versus the age of subjects. There is a significant positive correlation of age and homocysteine levels (left; R=0.250; p=<0.001). This association is more evident in low and moderate homocysteine level (middle; R=0.228; p=0.002) than in the high homocysteine level (right; R=0.087; p=0.618).

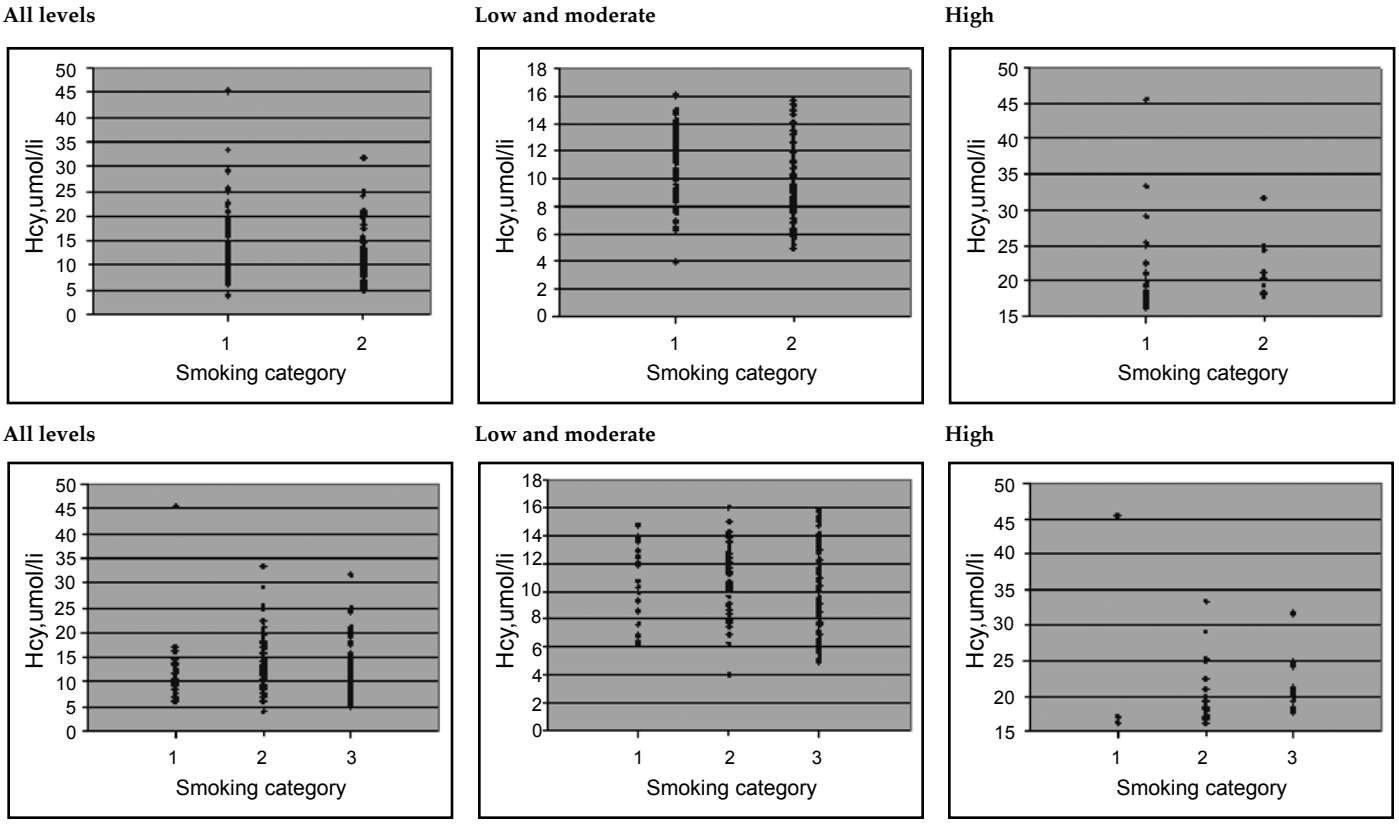

Figure II. Graphs show plots of homocysteine levels versus the smoking history of subjects. Upper graphs compare significant smokers (1) and nonsmokers (2), while lower graph further subgrouped smokers to current (1) and previous (2) with the nonsmokers (3). There is a significant positive correlation of smoking and homocysteine levels in the low and moderate homocysteine levels (upper middle; R=0.272; p=<0.001). This association is less evident in the high homocysteine levels (upper right; R=0.056; p=0.750) showing an overall trend (upper left; R=0.129; p=0.057). There is a trend of higher homocysteine levels in both current and previous smokers (lower left; R=0.116; p=0.089). This is more evident in low to moderate homocysteine levels (lower middle; R= 0.142; p=0.056) than in high homocysteine levels (lower right; R=0.161; p=0.356).

is a risk factor for CAD independent of other conventional risk factors.[31] Despite the negative correlation to body mass, a high homocysteine level is still associated with CAD in both obese and non-obese patients,[32] suggesting additional risk independent of BMI status. Since homocysteine is a possible risk factor for mortality and serious outcome in the first six months after a non ST elevation coronary event, the negative relationship with BMI could explain the fewer adverse events (death, occurrence of nonfatal ACS or serious re-hospitalization) noted in obese subjects compared with nonobese subjects after an acute coronary syndrome. This effect was called the obesity paradox.[33] The observation that homocysteine levels are inversely related to obesity should remain as is, an observation. The justification of the use of obesity to lower homocysteine or of indulgence in food to apparently lower the adverse effect of homocysteine

Table 3. Homocysteine levels according to smoking history

Smoking history	Homocysteine levels*		Homocysteine categories, frequency (% per row)			
<9	Mean, umol/li					
	9-16 mmol/li	>16 mmol/li	p value			
Current, mean (SD)	12.75 (7.90)	0.163	5	14	4	
Previous, mean (SD)	12.78 (4.91)		19	56	18	0.124
Insignificant, mean (SD	11.42 (4.81)		62	121	36	

*tests performed without one outlier variable (Hcy = 159.5 umol/li)

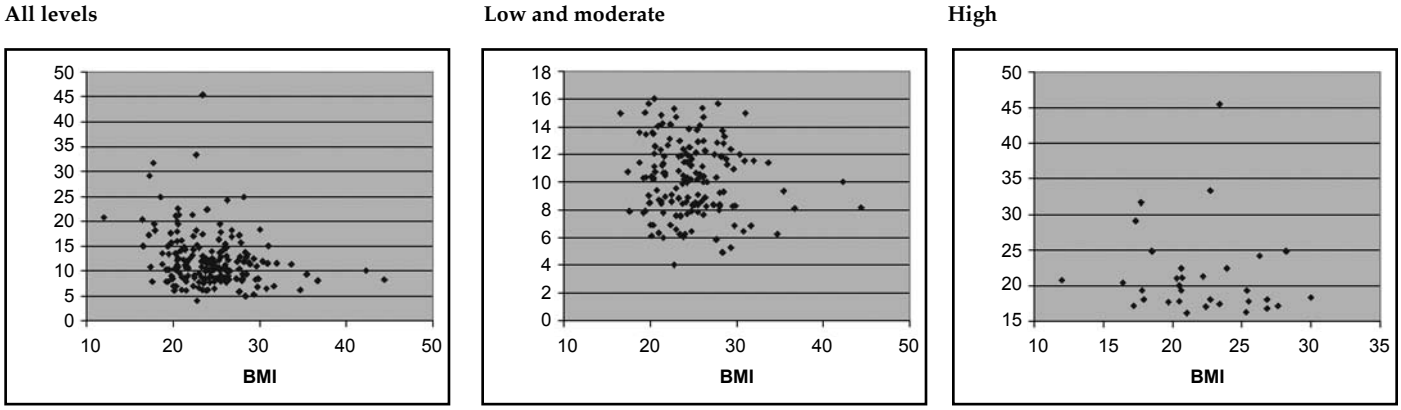

Figure III. Graphs show plots of homocysteine levels versus the BMI of subjects. There is a significant negative correlation of BMI and homocysteine levels (left; R=-0.247; p=0.001). This association is not evident in low and moderate homocysteine level (middle; R=-0.121; p=0.128) than in the high homocysteine level (right; R=-0.093; p=0.614).

is still discouraged. However, the above finding could be important in the elucidation of the mechanism of action of homocysteine. Moreover, contrary to the above observation, positive correlation was also recorded between body mass and homocysteine in some studies.[34,35] This needs to be validated in subsequent studies. For this study, about 13% of the subjects have no BMI records. For this study, a systematic selection bias could have existed since the less active and more severely ill subjects could not be included due to refusal; subjects with higher homocysteine levels and BMI could have been missed.

In summary, homocysteine levels are positively associated with age, smoking and low BMI in subjects with NSTEMI and unstable angina in subjects. No significant association was noted with diabetes mellitus, hypertension, dyslipidemia and sex. However, a larger sample size is recommended to establish the association between these factors and high levels of homocysteine. The findings imply that the potential detrimental effect of age and smoking could be enhanced by consequent higher homocysteine levels.

Because of the lack of evidence to support an effective direct pharmaceutical treatment in homocysteine lowering,[36] controlling modifiable risk factors, such as smoking, may have an impact in controlling the risk due to elevated homocysteine levels. And since homocysteinemia can predict adverse clinical outcomes but is still costly to assess, this study may help in the profiling of patients who may warrant homocysteine determination for prognostication and may require more aggressive conventional therapeutic intervention and/or monitoring, and direct future research for such approaches.

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