Journal of Cardiology 64 (2014) 290–296

Contents lists available at ScienceDirect

Journal of Cardiology

journal homepage: www.elsevier.com/locate/jjcc

Original article

Homocysteine and metabolic syndrome: From clustering to additional utility in prediction of coronary heart disease

Alireza Esteghamati (MD)*, Nima Hafezi-Nejad (MD, MPH), Ali Zandieh (MD, MPH), Sara Sheikhbahaei (MD, MPH), Maryam Ebadi (MD), Manouchehr Nakhjavani (MD)

Endocrinology and Metabolism Research Center, Tehran University of Medical Sciences, Tehran, Iran

ARTICLE INFO

Article history:
Received 4 October 2013
Received in revised form 8 January 2014
Accepted 3 February 2014
Available online 14 March 2014

Keywords:
Homocysteine
Metabolic syndrome
Coronary heart disease
Clinical utility
Biomarkers

ABSTRACT

Background: The association between homocysteine (Hcy) and metabolic syndrome (MetS)-related disorders remains to be unveiled. First, the role of Hcy–MetS interaction in prediction of coronary heart disease (CHD) was assessed. Next, we investigated whether serum Hcy improves CHD risk-prediction beyond MetS and traditional risk factors (TRFs).
Design: A prospective study of 5893 community-dwelling participants (two sub-cohorts, 3286 diabetic and 2607 non-diabetic; ~8.5 years of follow-up).
Methods: Clustering of Hcy with MetS components was assessed using exploratory factor-analysis. Cox regression hazard ratio (HR) was used to predict CHD using Hcy level and MetS status. Baseline model included MetS and TRFs. Addition of Hcy and hyper-homocysteinemia (HHcy) to the baseline model was evaluated in two separate models.
Results: Hcy was correlated with MetS components, especially with systolic blood pressure. The factor linking MetS to CHD is the factor through which Hcy is linked to MetS. HHcy and MetS interacted as risk factors for CHD.
Conclusion: Hcy adds to the value of MetS and TRFs for CHD risk-prediction by reclassifying around 47.3–49.0% of the overall and 21.6–28.1% of the intermediate-risk population.
© 2014 Japanese College of Cardiology. Published by Elsevier Ltd. All rights reserved.

Introduction

Coronary heart disease (CHD) is among the leading causes of mortality worldwide [1]. Traditional risk factors (TRFs) contributing to a CHD event have been recognized [2]. Preventive strategies altering these modifiable risk factors can play a major role in controlling the incident cases [3]. Prediction of susceptible or at-risk subjects is the prior step to any preventive program. Scoring systems predicting the risk of CHD in prospective time frames have been designed [4,5]. Despite predictive strategies, a great number of cases remain unpredicted [3]. Moreover, among the at-risk stratified population, a majority fall into the intermediate-risk group, for whom the clinical decision remains controversial [6]. Novel strategies are mandated to unveil indeterminate cases and to enhance the precision of CHD risk-prediction models that rely on conventional risk factors [7].

Novel markers have been introduced in recent decades which can either anticipate or be associated with an incident CHD event [8]. C-reactive protein, B-type natriuretic peptide, lipoprotein (a), and homocysteine (Hcy) are to name a few [9]. Hcy is a non-essential amino acid that is known as a marker of endothelial injury [10]. Hyper-homocysteinemia (HHcy) is regarded as a risk factor for development of atherosclerotic vascular injuries including CHD [11].

Metabolic syndrome (MetS) is an entity that clusters a number of metabolic abnormalities, including abdominal obesity, impaired glucose metabolism, dyslipidemia, and hypertension [12]. MetS and its components have been strongly correlated to CHD and other thrombotic events [13]. Control of MetS can be the mainstay of preventive programs [14].

MetS components and Hcy have both been subjects of studies focused on CHD prevention [14–16]. Nonetheless, the existing relationship between them is a contentious issue in the literature [17–19]. Debates on whether they share a common linkage or not persists [17]. It seems appealing to indicate if Hcy is part of

* Corresponding author at: Endocrinology and Metabolism Research Center (EMRC), Vali-Asr Hospital, School of Medicine, Tehran University of Medical Sciences, P.O. Box 13145-784, Tehran, Iran. Tel.: +98 21 88417918; fax: +98 21 64432466.
E-mail address: esteghamati@tums.ac.ir (A. Esteghamati).

http://dx.doi.org/10.1016/j.jjcc.2014.02.001
0914-5087/© 2014 Japanese College of Cardiology. Published by Elsevier Ltd. All rights reserved.

MetS-related disorders and if MetS components and Hcy interact in an incident CHD event.

The purpose of this study was to investigate the association between Hcy and MetS components, and to reveal their clustering pattern. Next, we assessed whether Hcy improves CHD risk prediction beyond MetS and TRFs. Finally, the extent by which Hcy measurement could assist in the prediction and prevention of a CHD event, beyond the capability of MetS and the TRFs, was estimated.

Materials and methods

Study population

This study uses the data from an Iranian prospective open cohort. Organized subject recruitment for research purposes started in 2005. Strategic preparations of the structure were performed in the years before 2005. The primary aim of the study was to investigate the natural history and the outcome of MetS and its correlates. Participants were at least 15 years old (by the time of inquiry) and were randomly selected with the aid of four local primary health surveillance centers located in the center, east, west, and south of Tehran. Details on the recruitments and extrapolation of the data to the Tehran general population are as previously described [12]. Multiple cross-sectional studies revealed characteristics of the study population from 2006 to 2012 [12,20–22]. The original cohort consists of two sub-cohorts of healthy, community dwelling participants with and without diabetes. The diabetic sub-cohort consisted of subjects who were newly diagnosed with diabetes in their entry workup. All subjects were investigated prior to their inclusion and those with recognized benign or malignant pathologies of internal organs were not included. Details on examinations and included individuals were as previously described [12]. Overall, 5893 subjects with available follow-ups until fall 2013 were included in the study. Missing values accounted for 3.4% of the values. Model-based expectation maximization method was used in handling the missing data. Hcy's main effect was confirmed to be similar to the obtained results from complete case analysis and multiple imputations. The study protocol was approved by the institutional review board of the Endocrinology and Metabolism Research Center, Tehran University of Medical Sciences. All participants provided informed consent on their entry.

Measurements and laboratory tests

Individuals' demographics including age, gender, and medications were recorded by history taking. Weights and heights were measured in light clothing and without shoes. Waist circumference was measured in the middle point of iliac crest and rib cage. After resting in supine position for 10 min, systolic and diastolic blood pressures were measured twice with a 15-min interval. The average of these measurements was used for the analysis. After 12 h overnight fasting, blood samples were collected for laboratory testing. Fasting plasma glucose (FPG) and 2-h post-prandial plasma glucose levels were assayed by glucose oxidase method. Lipid profile including total cholesterol, triglycerides (TGs), low-density lipoprotein cholesterol (LDL-C), and high-density lipoprotein cholesterol (HDL-C) were measured by direct enzymatic method (Parsazmun, Karaj, Iran). Fasting insulin levels were assessed by radioimmunoassay (the antibody had no cross-reactivity with pro-insulin and C-peptide; Immunotech, Prague, Czech Republic). C-peptide was measured by radioimmunoassay (Immunotech), and serum creatinine level was determined by the Jaffe method (Parsazmun). Hemoglobin A_{1c} was determined using high performance liquid chromatography (HPLC; DS5 Pink kit; Drew, Marseille, France). Hcy level was measured using HPLC by our designated laboratory.

Definitions and outcome measures

For the current study, first CHD was set as the primary outcome; definitions and ascertainments were done according to established protocols [23] and our previous investigations [20,22]. CHD was defined as evident episodes of myocardial infarction, angina pectoris, coronary insufficiency, or CHD death. All events were adjudicated by our center's physicians. All subjects were scheduled to be visited every 3 months. Trained research assistants completed the follow-up visits in case of missed visits. Date of each event was recorded. In non-event cases, date of the last visit was recorded. Data updates are performed annually. Cigarette smoking was defined by self-reported use of cigarettes in the year preceding the entry. Diabetes was defined according to the American Diabetes Association guidelines [24]. Diabetic subjects began initial treatment with metformin, glibenclamide, or both. Subjects requiring use of insulin for glycemic control at the time of diagnosis as well as subjects diagnosed with type I diabetes mellitus or pancreatitis-related diabetes, were not included in the cohort. Body mass index (BMI) was computed using the weight (kg)/height^2 (m) equation. The homeostasis model assessment-insulin resistance (HOMA-IR) index indicating insulin resistance was estimated as FPG (mg/dL) × fasting insulin (U/L)/405. According to the nationally modified version of the International Diabetes Federation criteria [12], patients with abdominal obesity (waist circumference ≥90 cm in both genders) along with any two or more of the following were considered to have MetS: FPG ≥ 100 mg/dL or previously diagnosed diabetes; elevated blood pressure [systolic blood pressure (SBP) ≥ 130 mmHg and/or diastolic blood pressure (DBP) ≥ 85 mmHg]; TG ≥ 150 mg/dL; low HDL-C levels (<50 mg/dL in females and <40 mg/dL in males).

Statistical analysis

One-way ANOVA was used to describe the baseline characteristics of the study population. A *p*-value for trend was derived to compare each variable in consecutive Hcy levels (<10 μM/L, 10–15 μM/L, and ≥15 μM/L).

Clustering of Hcy with MetS components

Correlation of MetS components with Hcy was assessed using Pearson bivariate correlation analysis. An exploratory factor analysis was performed to extract the principal components of MetS, with and without addition of Hcy. The choice of MetS components was based on a number of previous studies [12,25]. Waist circumference, HOMA-IR, TG, HDL-C, and SBP were chosen as MetS components. Factors with an eigenvalue ≥ 1 were included as principal components. Components with a factor loading of >0.40 were considered to be significantly enrolled on the corresponding factor. A Varimax rotation was applied. Receiver operating characteristic (ROC) curve demonstrated the prediction of CHD by each factor. Area under the curve (AUC) was used to compare the derived factors.

Prediction and reclassification of CHD risk

Analysis of the CHD-free survival was performed using Cox regression analysis. The assumptions of proportionality were tested using Shoenfeld residuals. TRFs other than MetS components were considered as possible confounders including age, sex, BMI, smoking, LDL-C, family history of CHD, and therapeutic interventions [2]. Therapeutic interventions including the use of lipid-lowering agents were coded as nominal variables, similar to the Framingham risk score for prediction of 30-year risk of cardiovascular disease [26]. The baseline model was considered to include MetS and TRFs for CHD. Afterwards, Hcy and HHcy were added in two separate models, to investigate whether they add a significant

hazard to the full model. Calibration of each model was assessed using Hosmer–Lemeshow's χ^2 statistics, which evaluates the correspondence between model predictions and actual observations. Intrusions of the null hypothesis ($p < 0.01$, $\chi^2 > 20$) are in means of lack of calibration. Global goodness-of-fit was evaluated for each model using Akaike and Bayesian Information Criterion [27]. These measures of model entropy evaluate the likelihood of a model against its complexity. Likelihood ratio test compares a model's global fit before and after the addition of Hcy and HHcy [27]. To evaluate the discriminatory power of models before and after addition of Hcy and HHcy, we used ROC curve and AUC statistics. Due to drawbacks of AUC in detecting the incremental value of an additional marker, newer methods were used including integrated discrimination improvement (IDI) and net reclassification improvement (NRI) [28]. Two main considerations were taken into account to control for the inflation of type I error due to multiple testing. First, a p-value less than 0.1% was adopted as the statistical significance threshold. Moreover, where applicable, we used Bonferroni adjustments for multiple comparisons to avoid the inflation of type I error caused by multiple comparisons. Analyses were performed using SPSS Software v.18 (Chicago, IL, USA) and STATA Software, v.12 (College Station, TX, USA).

Results

The mean follow-up time was 8.8 years; approximating for a maximum of more than 45,000 person-years of follow-up. First CHD was documented in 523 cases including 318 hard events. Among non-diabetic subjects, the first event was angina pectoris in 205 (39.2%), coronary insufficiency (documented by angiography) in 159 (30.4%), and myocardial infarction or CHD death in 159 (30.4%) subjects. The metabolic profile of the included subjects had a worsening trend across the Hcy subgroups. Subjects with higher levels of Hcy were more likely to fulfill a MetS criterion including abdominal obesity, impaired glucose tolerance, hypertriglyceridemia, and elevated blood pressure (Table 1).

Clustering of Hcy with MetS components

Hcy was correlated with MetS components. SBP was the strongest correlate ($r = 0.293$, $p < 0.001$) (Table 2). One principal component was extracted for MetS components to explain the whole entity (Table 3). When Hcy values were added to the MetS components, a second factor consisting of Hcy, SBP, and waist circumference appeared and increased the total explained variance (39.42–53.24% without and with Hcy, respectively). Hcy clustered with MetS components through SBP (Fig. 1). The factor which Hcy was loaded on, predicted CHD more precisely (Fig. 2).

Prediction and reclassification of CHD risk

When adjusted by TRFs, MetS was still associated with a hazard ratio (HR) of 1.76 (1.42–2.18). In categorical modeling, presence of HHcy was associated with a HR of 3.62 (2.38–5.50), which was

Table 1
Baseline characteristics of the study cohort across homocysteine categories.

	Homocysteine (μM/L)		
	<10 ($N = 2804$)	10–15 ($N = 2960$)	≥15 ($N = 129$)
Age (years)	39.2 ± 12.2	54.6 ± 11.3	57.1 ± 11.5*
Male (%)	16	45	58*
Waist circumference (cm)	90.8 ± 12.4	99.4 ± 11.5	101.6 ± 14.0*
Abdominal obesity (%)	52.7	81.8	83.7*
Fasting plasma glucose (mg/dL)	112.5 ± 50.6	135.2 ± 51.7	141.8 ± 55.1*
Impaired fasting glucose (%)	40.1	74.2	84.5*
Insulin (U/mL)	9.67 ± 6.46	10.47 ± 7.31	10.85 ± 6.97*
HOMA-IR	2.66 ± 2.08	3.45 ± 2.69	3.89 ± 3.34*
Triglyceride (mg/dL)	139.3 ± 83.6	180.7 ± 114.5	177.2 ± 94.9*
Triglyceride ≥ 150 (%)	33.3	53.4	51.2*
HDL-C (mg/dL)	50.8 ± 12.5	46.1 ± 11.5	46.9 ± 19.0*
Low HDL-C (%)	47.9	54.8	55.0*
Systolic blood pressure (mmHg)	114.0 ± 15.2	127.1 ± 16.8	128.9 ± 15.2*
Diastolic blood pressure (mmHg)	76.4 ± 16.5	80.2 ± 8.4	80.5 ± 7.2*
Elevated blood pressure (%)	18.7	49.5	54.3*
Body mass index (kg/m^2)	28.8 ± 6.1	29.5 ± 5.0	29.5 ± 4.9*
LDL-C (mg/dL)	112.3 ± 32.5	114.0 ± 35.6	119.7 ± 121.5
Total cholesterol (mg/dL)	192.1 ± 40.5	194.9 ± 42.6	189.3 ± 46.0
Creatinine (mg/dL)	0.90 ± 0.15	1.00 ± 0.22	1.20 ± 1.13*
Smoking (%)	17.3	17.3	18.6
Family history of CHD (%)	29.0	34.0	29.0
MetS, modified IDF (%)	30.0	65.1	75.2*

HOMA-IR, homeostasis model assessment-insulin resistance; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; CHD, coronary heart disease; MetS, metabolic syndrome; IDF, International Diabetes Federation.
* p-Value < 0.001.

Table 2
Correlation matrix of metabolic syndrome components and homocysteine.

Variable	Waist circumference	HOMA-IR	TG	HDL-C	SBP
Non-diabetics					
HOMA-IR	0.410				
TG	0.261	0.282			
HDL-C	−0.195	−0.175	−0.301		
SBP	0.316	0.180	0.180	−0.060	
Homocysteine	0.273	0.125	0.135	−0.166	0.293

All p-values are <0.001.
HOMA-IR, homeostasis model assessment-insulin resistance; TG, triglyceride; HDL-C, high-density lipoprotein cholesterol; SBP, systolic blood pressure.

Table 3
Factor loadings of MetS components and prediction of coronary heart disease; with and without addition of homocysteine.

Variable	MetS components	MetS components + homocysteine	
	Factor 1	Factor 1	**Factor 2**
Waist circumference	0.738[a]	0.446[a]	0.594[a]
HOMA-IR	0.689[a]	0.588[a]	0.304
TG	0.652[a]	0.735[a]	0.107
HDL-C	−0.506[a]	−0.729[a]	0.056
SBP	0.520[a]	0.021	0.789[a]
Homocysteine	NA	0.065	0.701[a]
Explained variance (%)	39.42	27.02	26.22
AUC	0.672 (0.652–0.693)	0.561 (0.536–0.587)	0.744 (0.724–0.763)
Total explained variance (%)	39.42	NA	53.24

Factors with an eigenvalue ≥1 were selected for analysis. Factor loadings are calculated after Varimax rotation with Kaiser normalization of variables in each extracted factor. All p-values are <0.001.
MetS, metabolic syndrome; HOMA-IR, homeostasis model assessment-insulin resistance; TG, triglyceride; HDL-C, high-density lipoprotein cholesterol; SBP, systolic blood pressure; AUC, area under the curve.
[a] Factor loadings ≥ |0.40|.

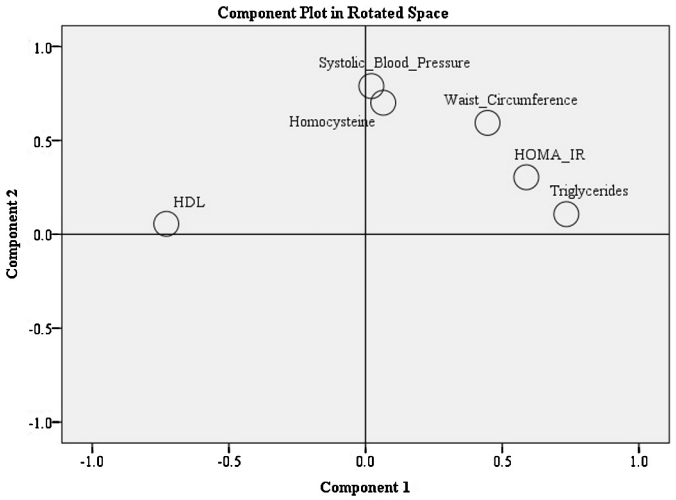

Fig. 1. Components plot in rotated space. HOMA-IR, homeostasis model assessment-insulin resistance; HDL, high-density lipoprotein.

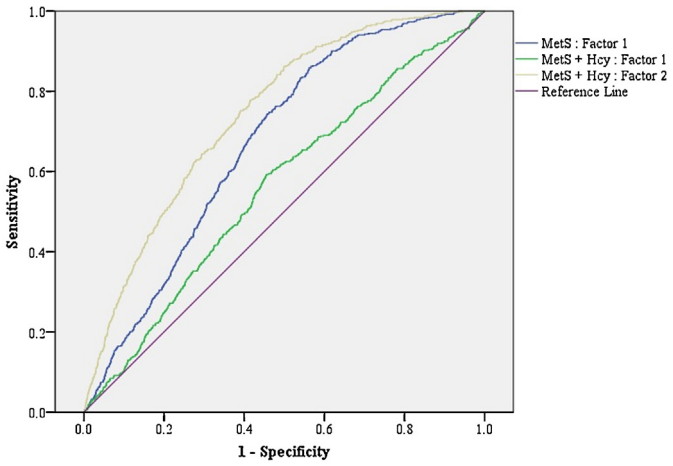

Fig. 2. Receiver operating characteristic curve statistics comparing principal components of metabolic syndrome (MetS) with and without homocysteine (Hcy).

independent from MetS and TRFs. In continuous modeling every one-unit increase in Hcy level was associated with 7% increase in the risk of CHD (Table 4). Results of the analyses for outcome subtypes held similar results as for all events. By considering hard events as the outcome of interest, every one-unit increase in serum Hcy had an effect size (HR) of 1.07 (1.05–1.09) while HHcy was associated with HR of 1.37 (0.98–1.90) and 3.42 (2.15–5.44) for the values greater than 10 and 15 μM/L, in the fully adjusted model, respectively.

Table 5 displays the summary of the statistics evaluating properties of adding Hcy and HHcy to MetS and TRFs. All three models had acceptable calibrations. Addition of HHcy and Hcy increased the concordance between the predictions of the model and the actual observation (decreased Hosmer–Lemeshow χ^2 statistics). By adding HHcy, the global fit increased and the information criteria measuring the model entropy decreased significantly (likelihood ratio test, p-value < 0.05). All baseline models had AUC ranging from 0.853 to 0.858. The incremental AUC after addition of Hcy was >1 for HHcy. IDI was also greater for Hcy, in comparison with HHcy. Addition of HHcy and Hcy successfully reclassified 47.3% and 49.0% of the whole population, respectively. However, intermediate-risk groups were reclassified in approximately 21.6–28.1% of cases.

Discussion

We evaluated the incidence of CHD with its determinants including Hcy, MetS, and TRFs. Our goal was to evaluate the clustering pattern of Hcy and MetS components. We questioned the extent by which Hcy measurement could assist in the prediction and prevention of a CHD event, beyond the capability of MetS and

Table 4
Incidence estimates of MetS and Hcy for the first coronary heart disease.

Variable	Adjusted MetS (baseline model)	+HHcy (categorical model)	+Hcy (continuous model)
MetS	1.76 (1.42–2.18)*	1.61 (1.30–2.00)*	1.63 (1.31–2.03)*
Hcy ≥ 10	NA	1.89 (1.45–2.46)*	NA
Hcy ≥ 15	NA	3.62 (2.38–5.50)*	NA
Hcy (per 1 μM/L)	NA	NA	1.07 (1.05–1.09)*

Values are hazard ratios (95% confidence interval) from Cox proportional hazard analysis. All models are adjusted for age, gender, body mass index, low-density lipoprotein cholesterol, smoking, family history of coronary heart disease, interventions and drugs.
MetS, metabolic syndrome; Hcy, homocysteine; HHcy, hyper-homocysteinemia. NA, not applicable.
* $p < 0.001$.

Table 5
Summary of the statistics evaluating the addition of Hcy to MetS.

	Adjusted MetS (baseline model)	+HHcy (categorical model)	+Hcy (continuous model)
Model calibration, HL χ^2 (p-value)	13.27 (0.10)	10.56 (0.23)	12.63 (0.12)
Model complexity, AIC	2936.38	2914.55	2938.21
Model complexity, BIC	2989.83	2974.68	2998.34
Likelihood ratio test, p-value		<0.001	0.680
AUC	0.853 (0.839–0.867)	0.855 (0.841–0.869)	0.858 (0.844–0.872)
Incremental AUC		0.002	0.005
IDI		0.005	0.009
NRI – overall (%)		47.3	49.0
NRI – intermediate risk (%)		21.6	28.1

All models are adjusted for age, gender, body mass index, low-density lipoprotein cholesterol, smoking, family history of coronary heart disease, interventions, and drugs. MetS, metabolic syndrome; Hcy, homocysteine; HHcy, hyper-homocysteinemia. AIC and BIC, Akaike and Bayesian Information Criterion; measures of model complexity, weighting the additional information of a model against its entropy; a lower value indicates an increased global fit. AUC, Area under the curve using receiver operating characteristic curve statistics; measures of discrimination; a higher value indicates better discrimination of events vs. non-events. IDI, integrated discrimination improvement. NRI, net reclassification improvement; measuring the percent of reclassified subjects, among events and non-events; a higher value indicates better implication of the added marker.
The calibration of the models was assessed using Hosmer–Lemeshow (HL) χ^2 statistics ($p > 0.01$).

the TRFs. A recent study by Veeranna et al. [29], evaluated the addition of Hcy to TRFs in two American cohorts. In agreement with our results, Hcy was found to be an independent predictor of CHD. The adjusted HR for hard CHD events (according to Hcy of ≥15) equaled 2.00 and 2.53 in MESA (Multi Ethnic Study of Atherosclerosis) and NHANES (National Health And Nutrition Examination Survey) cohorts, respectively. By contrast, we report HHcy as an independent predictor of CHD in diabetic and non-diabetic West Asian sub-cohorts. They also indicated a maximum of 21.2% of individuals with intermediate risk who can be correctly reclassified using Hcy levels. In comparison, in this study, by adding Hcy to TRFs and MetS, the authors successfully reclassified 28.1% of the intermediate-risk group.

Previous studies unveiled the CHD–Hcy association in various non-diabetic subgroups [30,31]. However, this prospective association was not well studied in Asian populations. Some authors had questioned [32,33] and even denied the role of Hcy in CHD prediction in the region [34]. This is the first prospective report regarding the associated hazard of HHcy in a West Asian population. An increase in every unit of Hcy was independently associated with 7% increase in CHD risk. Similarly, a previous meta-analysis suggested a 20–50% increase in the risk of CHD for every five-unit increase in Hcy level [35]. It was proposed that plasma Hcy level is an independent risk factor for CHD. In the current study, the authors described HHcy as a relatively stronger predictor of CHD 3.62 (2.38–5.50). Even mild increase in Hcy level (≥10) is associated with a 1.89-fold increased risk of CHD. Only a limited number of prior studies have indicated the increased risk of CHD and CHD mortality in HHcy diabetic patients [36].

CHD prevention in high-risk patients had been validated thoroughly [5,37]. Besides traditional treatments, Hcy-lowering therapies were identified as strategies in preventing CHD events [15], knowing that folate and vitamin B supplementation can reduce the Hcy level [38]. However, recent investigations failed to demonstrate the efficacy of Hcy-lowering therapies in preventing CHD events in healthy individuals [16]. We suggest that the missing link lies in patient selection. Those who will be reclassified in higher-risk groups due to an elevated Hcy level are the subgroup that would benefit from Hcy-lowering therapies. Nevertheless, further clinical trials should elucidate this issue.

Both Hcy and MetS have been allied to a number of metabolic abnormalities [39]. Insulin resistance had been proposed as the main regulator of HHcy [40]. Even Hcy-lowering therapies were shown to reduce insulin resistance [41]. In fact, there has been a tight connection between insulin resistance, endothelial injury, and HHcy, especially in diabetic subjects [39,42]. Nevertheless, the relation between Hcy and either MetS [43,44] or its components is a matter of debate [18,45]. Even if related, it is not clearly understood whether an increased Hcy level in subjects with MetS will add to the risk of CHD [19]. The authors revealed that the factor that links MetS to CHD is the factor through which Hcy is linked to MetS. In other words, we contradict the idea that leaves no common mechanism for MetS and HHcy [17]. In the present study, Hcy and MetS interacted as hazards and risk factors for CHD. Hcy added to the predictive value of MetS and TRFs by reclassifying almost 50% of the population. We insist that the presence of MetS should be considered when using Hcy for the prediction of an incident CHD [19].

We found metabolic abnormalities to be more prevalent in subjects with higher serum Hcy levels. Hcy was correlated with MetS components, especially through SBP. As a prior study suggests, Hcy and SBP load on the same factor, which had the greatest predictability for CHD [46]. HHcy has been correlated to other MetS components including insulin resistance, obesity, and waist circumference as well [47]. Our result is in agreement with clustering of Hcy with MetS components, including waist circumference and HOMA-IR.

Most of our current understanding of the CHD risk factors is derived from studies on Caucasians of European origin. However, it is well recognized that the ethnicity has a remarkable modulating effect on the CHD risk factors effect [48]. Specifically, Asian populations are recognized to be more susceptible to the adverse effects from certain risk factors including abdominal obesity and other MetS components [20,49]. Call for studies assessing the effect of ethnic differences on CHD risk factors has been made [48]. Various studies have revealed MetS–CHD association in Middle-Eastern populations [12,49]. However, the Hcy–MetS relation in West Asian populations remains a topic to be studied. Regarding the role of ethnicity in Hcy–CHD association, it was reported that HHcy is associated with an approximate 2-fold increase in the CHD risk regardless of ethnicity [50]. Moreover, it was proposed that ethnicity has no role in modifying the effect of Hcy-lowering therapies despite nutritional and genetic differences [51]. Recent studies have evaluated this issue among South Asians. They suggested that dietary factors may affect the Hcy–CHD association [52]. On the other side, contradicting results have pointed to ethnicity as a determinant of serum Hcy [53]. In all, what is mainly accepted is that the role of Hcy–CHD association remains to be closely clarified, especially with regard to the insulin resistance status [54]. Our study reports a close correlation between Hcy and MetS components.

The additional reclassification power of Hcy was notable in our study. In comparison, other additional markers have provided lower reclassification improvements [6,9]. For instance, the calculated NRI was 9.35% for MetS components [55], 25% for coronary artery calcification [56], and up to 13.8% for a panel of genetic markers [57]. In the same line with a previous study, we emphasize the remarkable benefits of assessing Hcy [29]. As an implication, testing Hcy can help us in clinical decision making. Further studies including economic evaluations are mandated to elucidate the feasibility of Hcy testing in preventing CHD events.

Limitations

One limitation of our study is the fact that our sample size was not be large enough to stratify our analyses for all different subtypes of events without losing the statistical power adopted in our study. Interval gaps and time differences among the assumed intervals of entries and events are one of the shortcomings of open cohort studies. We tried to overcome that by more frequent visits/follow-ups. We used adjudicated CHD events as the outcome of our study. However, considering more robust (though complex) procedures including prospective changes in echocardiographic parameters, will add valuable information to our current research gaps in the field.

Conclusion

Serum Hcy level is correlated to MetS components, especially to SBP. The factor linking MetS to CHD is the same factor through which Hcy is linked to MetS. Hcy and MetS interacted as hazards for CHD. Hcy successfully clustered with MetS components in predicting CHD. Hcy added to the predictive value of MetS and TRFs in predicting a CHD event by reclassifying around 50% of the at-risk population.

Conflict of interest

The authors declare no conflicts of interest.

References

[1] Lozano R, Naghavi M, Foreman K, Lim S, Shibuya K, Aboyans V, Abraham J, Adair T, Aggarwal R, Ahn SY, Alvarado M, Anderson HR, Anderson LM, Andrews KG, Atkinson C, et al. Global and regional mortality from 235 causes of death for 20 age groups in 1990 and 2010: a systematic analysis for the Global Burden of Disease Study 2010. Lancet 2012;380:2095–128.

[2] Go AS, Mozaffarian D, Roger VL, Benjamin EJ, Berry JD, Borden WB, Bravata DM, Dai S, Ford ES, Fox CS, Franco S, Fullerton HJ, Gillespie C, Hailpern SM, Heit JA, et al. Heart disease and stroke statistics—2013 update: a report from the American Heart Association. Circulation 2012;127:e6–245.

[3] Ebrahim S, Taylor F, Ward K, Beswick A, Burke M, Davey Smith G. Multiple risk factor interventions for primary prevention of coronary heart disease. Cochrane Database Syst Rev 2011:CD001561.

[4] Hippisley-Cox J, Coupland C, Vinogradova Y, Robson J, May M, Brindle P. Derivation and validation of QRISK, a new cardiovascular disease risk score for the United Kingdom: prospective open cohort study. BMJ 2007;335:136.

[5] Wilson PW, D'Agostino Sr R, Bhatt DL, Eagle K, Pencina MJ, Smith SC, Alberts MJ, Dallongeville J, Goto S, Hirsch AT, Liau CS, Ohman EM, Röther J, Reid C, Mas JL, et al. An international model to predict recurrent cardiovascular disease. Am J Med 2012;125:695–703.e1.

[6] Yeboah J, McClelland RL, Polonsky TS, Burke GL, Sibley CT, O'Leary D, Carr JJ, Goff DC, Greenland P, Herrington DM. Comparison of novel risk markers for improvement in cardiovascular risk assessment in intermediate-risk individuals. JAMA 2012;308:788–95.

[7] Helfand M, Buckley DI, Freeman M, Fu R, Rogers K, Fleming C, Humphrey LL. Emerging risk factors for coronary heart disease: a summary of systematic reviews conducted for the U.S. Preventive Services Task Force. Ann Intern Med 2009;151:496–507.

[8] Wang TJ, Wollert KC, Larson MG, Coglianese E, McCabe EL, Cheng S, Ho JE, Fradley MG, Ghorbani A, Xanthakis V, Kempf T, Benjamin EJ, Levy D, Vasan RS, Januzzi JL. Prognostic utility of novel biomarkers of cardiovascular stress: the Framingham Heart Study. Circulation 2012;126:1596–604.

[9] Wang TJ, Gona P, Larson MG, Tofler GH, Levy D, Newton-Cheh C, Jacques PF, Rifai N, Selhub J, Robins SJ, Benjamin EJ, D'Agostino RB, Vasan RS. Multiple biomarkers for the prediction of first major cardiovascular events and death. N Engl J Med 2006;355:2631–9.

[10] Hayden MR, Tyagi SC. Homocysteine and reactive oxygen species in metabolic syndrome, type 2 diabetes mellitus, and atherosclerosis: the pleiotropic effects of folate supplementation. Nutr J 2004;3:4.

[11] Welch GN, Loscalzo J. Homocysteine and atherothrombosis. N Engl J Med 1998;338:1042–50.

[12] Esteghamati A, Zandieh A, Khalilzadeh O, Meysamie A, Ashraf H. Clustering of metabolic syndrome components in a Middle Eastern diabetic and non-diabetic population. Diabetol Metab Syndr 2010;2:36.

[13] Malik S, Wong ND, Franklin SS, Kamath TV, L'Italien GJ, Pio JR, Williams GR. Impact of the metabolic syndrome on mortality from coronary heart disease, cardiovascular disease, and all causes in United States adults. Circulation 2004;110:1245–50.

[14] Sattar N, Gaw A, Scherbakova O, Ford I, O'Reilly DS, Haffner SM, Isles C, Macfarlane PW, Packard CJ, Cobbe SM, Shepherd J. Metabolic syndrome with and without C-reactive protein as a predictor of coronary heart disease and diabetes in the West of Scotland Coronary Prevention Study. Circulation 2003;108:414–9.

[15] Qin X, Huo Y, Xie D, Hou F, Xu X, Wang X. Homocysteine-lowering therapy with folic acid is effective in cardiovascular disease prevention in patients with kidney disease: a meta-analysis of randomized controlled trials. Clin Nutr 2013;32:722–7.

[16] Huang T, Chen Y, Yang B, Yang J, Wahlqvist ML, Li D. Meta-analysis of B vitamin supplementation on plasma homocysteine, cardiovascular and all-cause mortality. Clin Nutr 2012;31:448–54.

[17] Vaya A, Carmona P, Badia N, Perez R, Hernandez Mijares A, Corella D. Homocysteine levels and the metabolic syndrome in a Mediterranean population: a case–control study. Clin Hemorheol Microcirc 2011;47:59–66.

[18] Godsland IF, Rosankiewicz JR, Proudler AJ, Johnston DG. Plasma total homocysteine concentrations are unrelated to insulin sensitivity and components of the metabolic syndrome in healthy men. J Clin Endocrinol Metab 2001;86:719–23.

[19] Hajer GR, van der Graaf Y, Olijhoek JK, Verhaar MC, Visseren FL. Levels of homocysteine are increased in metabolic syndrome patients but are not associated with an increased cardiovascular risk, in contrast to patients without the metabolic syndrome. Heart 2007;93:216–20.

[20] Esteghamati A, Abbasi M, Nakhjavani M, Yousefizadeh A, Basa AP, Afshar H. Prevalence of diabetes and other cardiovascular risk factors in an Iranian population with acute coronary syndrome. Cardiovasc Diabetol 2006;5:15.

[21] Esteghamati A, Ashraf H, Rashidi A, Meysamie A. Waist circumference cut-off points for the diagnosis of metabolic syndrome in Iranian adults. Diabetes Res Clin Pract 2008;82:104–7.

[22] Nakhjavani M, Morteza A, Jenab Y, Ghaneei A, Esteghamati A, Karimi M, Farokhian A. Gender difference in albuminuria and ischemic heart disease in type 2 diabetes. Clin Med Res 2012;10:51–6.

[23] Ives DG, Fitzpatrick AL, Bild DE, Psaty BM, Kuller LH, Crowley PM, Cruise RG, Theroux S. Surveillance and ascertainment of cardiovascular events. The Cardiovascular Health Study. Ann Epidemiol 1995;5:278–85.

[24] Sacks DB, Arnold M, Bakris GL, Bruns DE, Horvath AR, Kirkman MS, Lernmark A, Metzger BE, Nathan DM, National Academy of Clinical Biochemistry; Evidence-Based Laboratory Medicine Committee of the American Association for Clinical Chemistry. Guidelines and recommendations for laboratory analysis in the diagnosis and management of diabetes mellitus. Diabetes Care 2011;34:e61–99.

[25] Wijndaele K, Beunen G, Duvigneaud N, Matton L, Duquet W, Thomis M, Lefevre J, Philippaerts RM. A continuous metabolic syndrome risk score: utility for epidemiological analyses. Diabetes Care 2006;29:2329.

[26] Pencina MJ, D'Agostino Sr RB, Larson MG, Massaro JM, Vasan RS. Predicting the 30-year risk of cardiovascular disease: the Framingham Heart Study. Circulation 2009;119:3078–84.

[27] Steyerberg EW, Van Calster B, Pencina MJ. Performance measures for prediction models and markers: evaluation of predictions and classifications. Rev Esp Cardiol 2011;64:788–94.

[28] Pencina MJ, D'Agostino RB, Vasan RS. Statistical methods for assessment of added usefulness of new biomarkers. Clin Chem Lab Med 2010;48:1703–11.

[29] Veeranna V, Zalawadiya SK, Niraj A, Pradhan J, Ference B, Burack RC, Jacob S, Afonso L. Homocysteine and reclassification of cardiovascular disease risk. J Am Coll Cardiol 2011;58:1025–33.

[30] Sun Y, Chien KL, Hsu HC, Su TC, Chen MF, Lee YT. Use of serum homocysteine to predict stroke, coronary heart disease and death in ethnic Chinese. 12-year prospective cohort study. Circ J 2009;73:1423–30.

[31] Chambers JC, Obeid OA, Refsum H, Ueland P, Hackett D, Hooper J, Turner RM, Thompson SG, Kooner JS. Plasma homocysteine concentrations and risk of coronary heart disease in UK Indian Asian and European men. Lancet 2000;355:523–7.

[32] de Bree A, Verschuren WM, Blom HJ, Nadeau M, Trijbels FJ, Kromhout D. Coronary heart disease mortality, plasma homocysteine, and B-vitamins: a prospective study. Atherosclerosis 2003;166:369–77.

[33] Troughton JA, Woodside JV, Young IS, Arveiler D, Amouyel P, Ferrieres J, Ducimetière P, Patterson CC, Kee F, Yarnell JW, Evans A, PRIME Study Group. Homocysteine and coronary heart disease risk in the PRIME study. Atherosclerosis 2007;191:90–7.

[34] Al-Nozah M, Al-Daghri N, Bartlett WA, Al-Attas O, Al-Maatouq M, Martin SC, Kumar S, Jones AF. Serum homocysteine concentration is related to diabetes

mellitus, but not to coronary heart disease, in Saudi Arabians. Diabetes Obes Metab 2002;4:118–23.
[35] Humphrey LL, Fu R, Rogers K, Freeman M, Helfand M. Homocysteine level and coronary heart disease incidence: a systematic review and meta-analysis. Mayo Clin Proc 2008;83:1203–12.
[36] Hoogeveen EK, Kostense PJ, Jakobs C, Dekker JM, Nijpels G, Heine RJ, Bouter LM, Stehouwer CD. Hyperhomocysteinemia increases risk of death, especially in type 2 diabetes: 5-year follow-up of the Hoorn Study. Circulation 2000;101:1506–11.
[37] Steyerberg EW, Vickers AJ, Cook NR, Gerds T, Gonen M, Obuchowski N, Pencina MJ, Kattan MW. Assessing the performance of prediction models: a framework for traditional and novel measures. Epidemiology 2009;21:128–38.
[38] De Bree A, Verschuren WM, Kromhout D, Kluijtmans LA, Blom HJ. Homocysteine determinants and the evidence to what extent homocysteine determines the risk of coronary heart disease. Pharmacol Rev 2002;54:599–618.
[39] Hellgren M, Melander A, Ostgren CJ, Rastam L, Lindblad U. Inverse association between plasma homocysteine, sulphonylurea exposure and physical activity: a community-based sample of type 2 diabetes patients in the Skaraborg Hypertension and Diabetes Project. Diabetes Obes Metab 2005;7:421–9.
[40] Fonseca V, Keebler M, Dicker-Brown A, Desouza C, Poirier LA, Murthy SN, McNamara DB. The effect of troglitazone on plasma homocysteine, hepatic and red blood cell S-adenosyl methionine, and S-adenosyl homocysteine and enzymes in homocysteine metabolism in Zucker rats. Metabolism 2002;51:783–6.
[41] Setola E, Monti LD, Galluccio E, Palloshi A, Fragasso G, Paroni R, Magni F, Sandoli EP, Lucotti P, Costa S, Fermo I, Galli-Kienle M, Origgi A, Margonato A, Piatti P. Insulin resistance and endothelial function are improved after folate and vitamin B12 therapy in patients with metabolic syndrome: relationship between homocysteine levels and hyperinsulinemia. Eur J Endocrinol 2004;151: 483–9.
[42] Meigs JB, Jacques PF, Selhub J, Singer DE, Nathan DM, Rifai N, D'Agostino Sr RB, Wilson PW, Framingham Offspring Study. Fasting plasma homocysteine levels in the insulin resistance syndrome: the Framingham Offspring Study. Diabetes Care 2001;24:1403–10.
[43] Rhee EJ, Hwang ST, Lee WY, Yoon JH, Kim BJ, Kim BS, Kang JH, Lee MH, Park JR, Sung KC. Relationship between metabolic syndrome categorized by newly recommended by International Diabetes Federation criteria with plasma homocysteine concentration. Endocr J 2007;54:995–1002.
[44] Rosolova H, Simon J, Mayer Jr O, Racek J, Dierze T, Jacobsen DW. Unexpected inverse relationship between insulin resistance and serum homocysteine in healthy subjects. Physiol Res 2002;51:93–8.
[45] Guven A, Inanc F, Kilinc M, Ekerbicer H. Plasma homocysteine and lipoprotein (a) levels in Turkish patients with metabolic syndrome. Heart Vessels 2005;20:290–5.
[46] Neugebauer S, Tarnow L, Stehouwer C, Teerlink T, Baba T, Watanabe T, Parving HH. Total plasma homocysteine is associated with hypertension in Type I diabetic patients. Diabetologia 2002;45:1315–24.
[47] Vaya A, Rivera L, Hernandez-Mijares A, de la Fuente M, Sola E, Romagnoli M, Alis R, Laiz B. Homocysteine levels in morbidly obese patients: its association with waist circumference and insulin resistance. Clin Hemorheol Microcirc 2012;52:49–56.
[48] Forouhi NG, Sattar N. CVD risk factors and ethnicity—a homogeneous relationship. Atheroscler Suppl 2006;7:11–9.
[49] Esteghamati A, Abbasi M, Rashidi A, Meysamie A, Khalilzadeh O, Haghazali M, Asgari F, Nakhjavani M. Optimal waist circumference cut-offs for the diagnosis of metabolic syndrome in Iranian adults: results of the Third National Survey of Risk Factors of Non-Communicable Diseases (SuRFNCD-2007). Diabet Med 2009;26:745–6.
[50] Giles WH, Croft JB, Greenlund KJ, Ford ES, Kittner SJ. Association between total homocyst(e)ine and the likelihood for a history of acute myocardial infarction by race and ethnicity: results from the Third National Health and Nutrition Examination Survey. Am Heart J 2000;139:446–53.
[51] Kasiman K, Eikelboom JW, Hankey GJ, Lee SP, Lim JP, Lee JH, Chang HM, Wong MC, Chen CP. Ethnicity does not affect the homocysteine-lowering effect of B-vitamin therapy in Singaporean stroke patients. Stroke 2009;40:2209–11.
[52] Sosin MD, Patel JV, Bhatia GS, Hughes EA, Davis RC, Lip GY. Effects of white European, African Caribbean and South Asian ethnicity on homocysteine levels in patients with systolic heart failure. Int J Cardiol 2008;129:69–75.
[53] Albert MA, Glynn RJ, Buring JE, Ridker PM. Relation between soluble intercellular adhesion molecule-1, homocysteine, and fibrinogen levels and race/ethnicity in women without cardiovascular disease. Am J Cardiol 2007;99:1246–51.
[54] Wijeyaratne CN, Nirantharakumar K, Balen AH, Barth JH, Sheriff R, Belchetz PE. Plasma homocysteine in polycystic ovary syndrome: does it correlate with insulin resistance and ethnicity? Clin Endocrinol 2004;60:560–7.
[55] Rachas A, Raffaitin C, Barberger-Gateau P, Helmer C, Ritchie K, Tzourio C, Amouyel P, Ducimetière P, Empana JP. Clinical usefulness of the metabolic syndrome for the risk of coronary heart disease does not exceed the sum of its individual components in older men and women. The Three-City (3C) Study. Heart 2012;98:650–5.
[56] Kullo IJ, Li G, Bielak LF, Bailey KR, Sheedy 2nd PF, Peyser PA, Turner ST, Kardia SL. Association of plasma homocysteine with coronary artery calcification in different categories of coronary heart disease risk. Mayo Clin Proc 2006;81:177–82.
[57] Hughes MF, Saarela O, Stritzke J, Kee F, Silander K, Klopp N, Kontto J, Karvanen J, Willenborg C, Salomaa V, Virtamo J, Amouyel P, Arveiler D, Ferrières J, Wiklund PG, et al. Genetic markers enhance coronary risk prediction in men: the MORGAM prospective cohorts. PLoS ONE 2012;7:e40922.