Original Article

Hyperhomocysteinemia as a Risk Factor for Coronary Atherosclerotic Diseases in the Elderly

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OBJECTIVE

To investigate whether hyperhomocysteinemia is an independent risk factor for atherosclerotic disease in elderly individuals.

METHODS

A case-control study with 172 elderly individuals, 88 belonging to control group and 84 to case group, who showed coronary angiography requested for clinical indications. Quantitative coronary angiography was performed in 91% of the patients. Homocysteinemia was assessed in a continuous and categorized way, through univariate and multivariate analysis.

RESULTS

When analyzed continuously, in univariate analysis, it was verified that case group elderly individuals showed an average homocysteinemia levels significantly higher than the control group individuals' (14.33±4.59 µmol/l against 11.99±4.59 µmol/l, p=0.015). In multivariate analysis, continuous homocysteinemia was associated to the risk rate for coronary artery disease of 1.07 for each 1 µmol/l increase of homocysteine level. Na increase of 5 µmol/l corresponded to the risk rate of 1.40. When analyzed in categorized way, the values found over percentile 75 of control group (14 µmol/l) were defined as hyperhomocysteinemia. Hyperhomocysteinemia was found in 34% of elderly individuals, being 37.3% in control group and 62.7% in case group (p= 0.009). In multivariate analysis, hyperhomocysteinemia constituted an independent risk factor for coronary atherosclerotic disease for elderly individuals, with a risk rate for coronary artery disease of 2.03, confidence interval 95%, 1.02-4.03.

CONCLUSION

Hyperhomocysteinemia was an independent risk factor for coronary artery disease in elderly individuals.

KEY WORDS

hyperhomocysteinemia, homocysteine, elderly, octogenarian, risk factor, coronary artery disease

The main cause of mortality and morbidity among elderly individuals is cardiovascular disease[1] and, particularly, coronary atherosclerotic disease, responsible for half of deaths and deficiencies in the group of elderly individuals over 80 years old[2]. Although only 20 to 30% among elderly individuals show clinical manifestations of coronary disease, approximately 70% of people over 70 years old show coronary atherosclerosis at necropsy[3]. There are many factors that can contribute to coronary artery disease, such as hypertension, Dyslipidemia, smoking, obesity, sedentary lifestyle, and diabetes. However, new risk factors have been detected. Among those we find hyperhomocysteinemia.

Homocysteine is an amino acid that contains sulfur, formed during methionine. It follows two end paths: part of it returns to methionine formation and the other part is excreted in urine[4] (fig. 1). So, homocysteine, which is part of methionine cycle. Contribute simultaneously to its maintenance (methionine and homocysteine are precursory to each other). A disorder at any stage of such metabolism induces to the increase of homocysteine[5].

In 1969, Mc Cully et al. identified the direct clinical importance of hyperhomocysteinemia, by suggesting for the first time the connection between genetic metabolic disturbance, caused by homozygotic deficiency of cystationine B-synthase, and appearance of early atherosclerosis in necropsy-submitted infants[6]. The homozygotic deficiency of cystationine B-synthase was associated to high levels (>100 µmol/l) of plasma and urine homocysteine, followed by diffuse atherosclerosis, which led to the appearance of coronary atherosclerotic disease, peripheral vascular disease and cerebrovascular accident in infants. It was also observed that individuals with moderate increase of homocysteine levels also showed an increased risk of cardiovascular disease. Such moderate increase resulted from heterozygotic deficiency of cystationine B-synthase (CBS) or of methylenetetrahydrofolate reductase (MTHFR), an enzyme involved in the homocysteine remethylation in methionine.

In addition to congenital factors (homozygotic or heterozygotic deficiency of CBS, and heterozygotic of MTHFR), other factors may cause increase of homocysteinemia in a smaller proportion, such as nutritional factors (deficit of vitamins B6, B12 and folate), physiological factors (age, sex)[7], lifestyle (smoking[8], excessive ingestion of coffee[9], alcoholic beverage consumption[10], sedentary lifestyle[10]), the action of some medicines (nitric oxide, isoniazide, theophylline, carbamazepine, methotrexate, niacin, cholesty-ramine)[11,12], some diseases (chronic renal failure[13], psoriasis). It is also considered that an aging-related decrease in the activity of enzymes responsible for the metabolism of homocysteine[14] may occur. It was also suggested that vascular changes are induced by homocysteine itself[15].

Action mechanisms by which hyperhomocysteine would cause injurious effects are not clear yet. It is believed that endothelial dysfunction, smooth vascular cell proliferation and coagulation disturbances[16-19], constitute important factors in that process.

The association between plasma homocysteine concentration and atherosclerosis has been investigated in some clinical studies[20-27], with controversial results. Such disagreement existent in medical literature attracted the attention to the research of hyperhomocysteinemia as an independent risk factor for coronary disease in elderly individuals.

Another point of disagreement among the authors is the limits of normal variation of homocysteine plasma concentration among the general population and the definition of hyperhomocysteinemia. Some authors
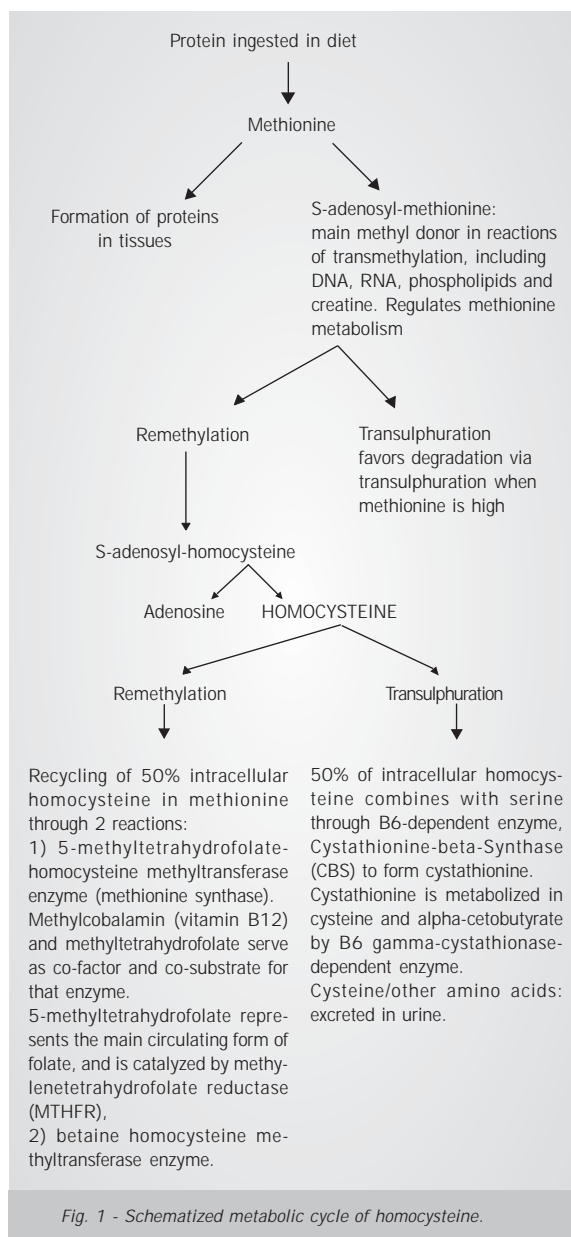

Fig. 1 - Schematized metabolic cycle of homocysteine.

suggested that such values may vary in several countries, being recommendable that each country determines its own normality values. In Japan, for example, average values of homocysteine, in health individuals, are 6 µmol/l, and in South Africa, they are 13 µmol/l[28].

The definition of hyperhomocysteinemia in a population is performed by using an arbitrary cut-off point, for example, above percentile 95, a method similar to the initial assessment methods for cholesterol and hypertension levels[29,30]. According to some authors, the "normal" blood homocysteine limits oscillate between 5 and 15 µmol/l[31,32]. According to others, the average value for plasma homocysteine lies between 9 to 10 µmol/l[33,34]. Values that are equal to or greater than 16 µmol/l are, arbitrarily called hyperhomocysteinemia, which can be moderate (16-30 µmol/l), intermediate (31-100), or severe (>100 µmol/l). Among elderly individuals, the limits of normal variation have not been established yet.

METHODS

A case-control-type study was performed, with data collection in 172 patients with age equal to or higher than 65 years old and recent coronary angiography. Data were collected by means of coronary angiography, anamnesis, questionnaire, physical exam, anthropometric measurements and blood tests. "Off-line" quantitative coronary angiography, by using Quantitative Coronary Angiography-Cardiovascular Measure System (QCA-CMS) program of *Medical Imaging System*, version 5.1 and 3.0, was performed in 91% of elderly individuals.

The inclusion criteria were as follows: a) Cases: represented by elderly individuals who showed coronary angiography-proven CAD, with lesion from 70% to 100% in one or more main arteries (left coronary trunk, anterior descending artery, right coronary artery, circumflex artery). The lesion magnitude was confirmed by a duly qualified observer by means of an "off-line" quantitative analysis. b) Controls: represented by elderly individuals who did not show obstructive lesions at coronary angiography or who showed, in secondary branch, discreet parietal irregularities or single inferior lesion at 30%, measured by off-line quantitative angiography.

The quantitative angiography analysis was performed in initial, middle and distal portions of left coronary trunk, anterior descending artery, septal artery, diagonal arteries, circumflex artery, marginal arteries, circumflex artery atrioventricular branch, right coronary artery, posterior ventricular artery, posterior descending artery and left ventricular fraction of ejection.

The elderly individuals were submitted to coronary angiography for several reasons, such as investigation of precordialgia, valvopathies or arrhythmias.

The exclusion criteria were as follows: regular use of vitamins; neoplasias lack of compression of the protocol;

history of myocardial revascularization or angioplasty; acute coronary insufficiency in the last 30 days; serum creatinine higher than 2 mg/dl; cerebrovascular accident.

Homocysteine, glucose, triglycerides, total cholesterol, HDL cholesterol (calculation of LDL cholesterol through Friedewald formula), creatinine, blood count, albumin, TSH, T4 free were dosed in the blood.

Homocysteinemia was dosed through fluorescence polarization immunoassay (FPIA), by using AXSYM equipment from Abbott, duly validated through correlation with Imx® Homocysteine assay, by using linear regression analysis[35]. For its turn, Imx® Homocysteine assay was duly validated with the traditional method of high performance liquid chromatography (HPLC)[36].

The material collected for homocysteinemia dosage was placed in a dry tube, without anticoagulant, and centrifuged in a period shorter than 30 minutes[37], at the collection site, in order to separate red blood cells from serum. The resulting serum was placed in 2ml cryotubes. It was then transported to the central laboratory and stored in a refrigerator for a further analysis, at a temperature of -80°C. After defrosting, the samples were completely homogenized through agitation, in low speed, and centrifuged again, to release the sample from any interference and assure the consistency of results. Homocysteine dosage was repeated in all analyzed samples, with an excellent reproducibility, confirming the results found in accordance to the variation coefficient described for the method[38].

Variables of the study: anamnesis, physical exam, analysis of risk factors and serum dosages allowed for establishing diagnoses of dyslipidemia, hypertension, diabetes mellitus, hypertriglyceridemia, obesity, smoking and sedentary lifestyle, which are necessary for the assessment of hyperhomocysteinemia as an independent risk factor. The elderly individuals who made use of antihypertensive medication and those who showed high blood pressure on the day of the appointment were considered as hypertensive, according to consensus of Diretrizes em Cardiogeriatria da Sociedade Brasileira de Cardiologia[39] (systolic blood pressure ≥140 mmHg and/or diastolic blood pressure ≥90 mmHg). The elderly individuals who made use of hypolipemiants and those who showed a high serum level of total cholesterol (≥200 mg/dl), of reduced HDL cholesterol (≤40 mg/dl), of high LDL cholesterol (≥100 mg/dl) or hypertriglyceridemia (≥150 mg/dl) were regarded as dyslipidemia carriers. Smoking was assessed according to the classification of the smoker elderly individual at the time of the appointment (regardless of the number of cigarettes smoked), ex-smokers (who has stopped smoking at least 3 months ago), and non-smokers (who has never smoked). The elderly individual was also assessed as a smoker (smoker and ex-smoker) against non-smoker (never smoked). Obesity was defined by three indexes: body mass index (BMI)[40], abdominal circumference

Table I - Distribution of age, sex and risk factors

	Control Group n (%)	Case Group n (%)	Total	p
Age (years old)	65-88	65-86	65-88	0.405
Mean±SD	72.2±0.469	72.77±0.495	72.48±0.34	
Sex				
Male	27 (30.7)	45 (53.6)	72 (41.9)	0.002*
Female	61 (69.3)	39 (46.4)	100 (58.1)	
Hypertension	77 (87.5)	66 (78.6)	143 (83.1)	0.118
Dyslipidemia	27 (30.7)	44 (52.4)	71 (41.3)	0.004*
Diabetes	19 (21.6)	29 (34.5)	48 (27.9)	0.059
Smoking				
Non-smoker (never smoked)	59 (67)	32 (38.1)	91 (52.9)	<0.001*
Smoker (ex-smoker/smoker)	29 (33)	52 (61.9)	81 (47.1)	
Physical activity				
Yes	69 (78.4)	55 (65.5)	124 (72.1)	0.031*
No	19 (21.6)	29 (34.5)	48 (27.9)	
Obesity				
BMI ≥30 kg/m^2	29 (33)	24 (28.6)	53 (30.8)	0.534
Waist-hip ratio Man >0.98 cm Woman >0.90 cm	25 (28.4)	26 (31)	51 (29.7)	0.715
Abdominal circumference Man >102 cm Woman >88 cm	53 (60.2)	51 (60.7)	104 (60.5)	0.948
*P<0.05.				

measurement[41] and waist-hip ratio measurement[42]. For the elderly individual not being regarded as sedentary, the practice of at least 15 minutes of programmed physical activity, 3 times a week[43], was necessary. Diabetes mellitus was diagnosed through feast glycemia >126 mg/dl, through the use of hypoglycemic medication or previous diabetes diagnosis.

For the comparison of the groups, the chi-square test, for qualitative variables, and the t-test of Student, for quantitative variables, were used in univariate analysis. In cases in which there was no normality in data distribution, the non-parametric test of Mann-Whitney was used. For the comparison of more than two means (as with "age") the non-parametric test of Kruskall-Wallis was used. In multivariate analysis the logistic regression method was used. The inclusion criteria of independent variables in regression model were the results from univariate analysis and clinical relevance. The independent variables included in the model were sex, age classes (65-74 years old, 75-79 years old, and ≥80 years old), sedentary lifestyle, smoking (smokers versus non-smokers), high LDL cholesterol, low HDL cholesterol, high total cholesterol, hypertension, diabetes, obesity (according to BMI), homocysteinemia, serum creatinine >1.4 mg/dl (yes versus no).

The observation of homocysteine levels, displayed continuously on the accumulated frequency curve in the 2 groups, allowed for the identification of the point at which the homocysteinemia of the patients of case and control groups were most different, percentile 75. So, we selected the value of homocysteinemia found in percentile 75 of the sample under study, 14 µmol/l, as cut-off point for categorization in hyperhomocysteinemia. That value coincides with the value of percentile 80 of Robinson's sample[44], arbitrarily chosen as cut-off point to calculate the prevalence of hyperhomocysteinemia in the population he studied. Then, a second univariate analysis with chi-square test, and a second multivariate analysis, with logistic regression model, were carried out, in which hyperhomocysteinemia was assessed as a categorical variable (≤14 and >14 µmol/l). The inclusion criteria of independent variables were the results from the univariate analysis and clinical relevance. The independent variables included in the previous analysis were repeated.

The results whose descriptive levels (p values) were lower than 0.05 were regarded as statistically significant.

RESULTS

One hundred and seventy-two (172) patients with age ranging between 65 and 80 years old, with 84 belonging to case group and 88 to control group. The distribution of age, sex and risk factors is exhibited in table I.

Patients from case group, in addition to show severe lesion in main arteries, showed other different intensity lesions in secondary branches. Control group patients showed coronaries free of atherosclerotic process at coronary angiography in 83% of the cases; only 15 patients showed discreet parietal irregularities or single lesion of discreet level (<30%) in secondary arterial branch.

Quantitative coronary angiography was performed in 91% of the patients, confirming coronary angiographic data. The main diagnoses found were HAS (83.1%), coronary insufficiency (50%, from which 25.6% have already had myocardial infarction), dyslipidemia (41%), diabetes mellitus (27.9%), arrhythmia (13.4%), segmental or diffuse left ventricular dysfunction (57.1%), congestive heart failure (4.1%), atrial fibrillation (5.2%), mitral insufficiency (3.5%), aortic insufficiency (2.9%), aortic stenosis (5.2%), mitral stenosis (1.2%), syncope (2.3%).

The main medicines under use were antiplatelet (66.3%), conversion enzyme inhibitors (58.1%), diuretics (53.5%), beta-blockers (47.1%), nitrates (43%), hypolipiemians (24.4%), hypoglycemic (23.8%), calcium channel antagonists (18%), digital (10.5%), aldosterone II receptor antagonists (5.2%), anticoagulants (3.5%)

The mean and standard deviation of homocysteinemia levels found in elderly individuals were 11.99 µmol/l ±4.59 in control group, and 14.33 µmol/l ±6.84 in case group, p=0.015 (fig. 2). The accumulated frequency
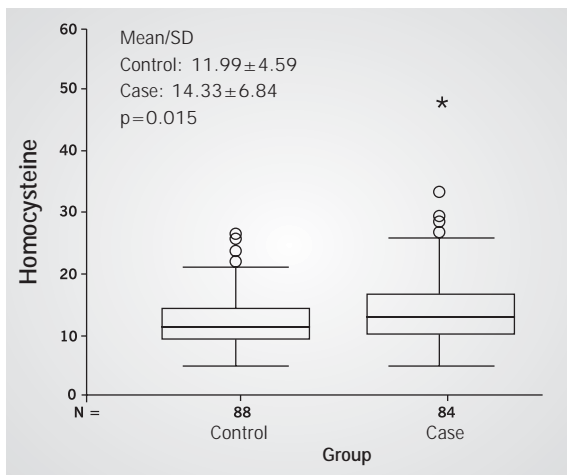
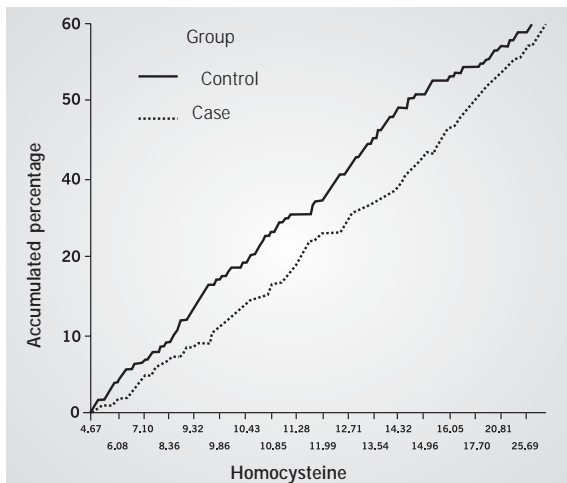Fig. 2 - Statistical analysis of homocysteinemia according to the group.

Fig. 3 - Accumulated frequency curve of homocysteinemia levels between case and control groups.

Table II - Results from logistic: homocysteinemia as a continuous variable			
Independent variables	Value p	Risk Rate	Confidence interval
Female sex	0.1069	0.5650	0.2822 - 1.1310
Age 75-79 years old	0.3601	1.4221	0.6690 - 3.0230
Age ≥80 years old	0.6157	0.6883	0.1601 - 2.9594
Smoking*	0.0050	2.7254	1.3533 - 5.4887
Homocysteinemia*	0.0385	1.0700	1.0057 - 1.1384

Fig. 4 - Prevalence of hyperhomocysteinemia (>14 µmol/l) second group.
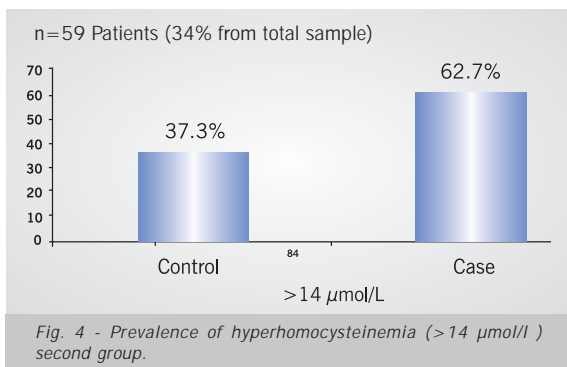
curve of homocysteine levels demonstrated that, along the whole sample, more control group patients showed lower values than the patients from case group (fig. 3).

In multivariate analysis, by using the logistic regression model, the stepwise forward method for the final selection of independent variables for obstructive atherosclerotic disease was employed. Variables that remained in the final equation, after the use of stepwise forward method, were smoking, and homocysteinemia. Sex and age had their entry forced in the final model, in spite of not showing statistical significance after the use of stepwise forward method. Those variables were included in a special way, for being frequent questionings when a relation between a variable and a clinical condition is trying to be established. Continuously analyzed homocysteinemia showed a risk rate of 1.07, with confidence interval from 1.0057 to 1.1384, p=0.0385. This means that for every increase of a unit (1 µmol/l) of homocysteine, the elderly individual showed 7% more chance of being case, which means, of showing coronary disease. An increase of 5 µmol/l of homocysteine was associated to the risk rate of 1.403 (which means, 40% more chance of being case).

Smoking showed a risk rate of 2.7254, confidence interval from 1.3533 to 5.4887, p=0.0050 (tab. II).

Categorized homocysteinemia analysis (hyperhomocysteinemia): from 172 elderly individuals, 59 (34%) showed hyperhomocysteinemia (homocysteine levels >14 µmol/l). From those, 37.3% were in control group and 62.7% in case group; p=0.009 (fig. 4). In multivariate hyperhomocysteinemia analysis, again, homocysteine and smoking remained as independent variables. Sex and age, although non-significant, were included in the final equation. Smoking showed a risk rate of 2.7644, with a confidence interval from 1.3709 to 5.5744, p=0.0045. Hyperhomocysteinemia showed a risk rate of 2.0297, with a confidence interval from 1.0207 to 4.0361, p=0.0435. This means that elderly individuals with homocysteinemia higher than 14 µmol/l showed 2.03 times more chance to be CAD than elderly individuals with plasma levels of homocysteine lower than 14 µmol/l (tab. III).

DISCUSSION

The results from this clinical-epidemiological

Table III - Results from logistic regression: hyperhomocysteinemia as categorized variable (>14 mmol/l)			
Independent variables	Value P	Risk Rate	Confidence interval
Female sex	0.1387	0.5911	0.2947 - 1.1855
Age 75-79 years old	0.3288	1.4563	0.6848 - 3.0968
Age ≥80 years old	0.7775	0.8164	0.1999 - 3.3347
Smoking*	0.0045	2.7644	1.3709 - 5.5744
Hyperhomocysteinemia*	0.0435	2.0297	1.0207 - 4.0361

study, with case-control plan, demonstrated that hyperhomocysteinemia is an independent risk factor for obstructive atherosclerotic disease in elderly individuals (risk rate of 1.07 for each increase of 1 μmol/l of homocysteinemia). An increase of 5 μmol/l of homocysteinemia corresponded to an estimate risk (risk rate) of 1.40. Elderly individuals with hyperhomocysteinemia showed a risk rate of 2.03.

Stampfer et al. performed blood collection and a 5-year follow-up of 14,916 apparently healthy physicians. The main outcome analyzed was myocardial infarction (AMI) or death due to coronary artery disease. Blood samples from 271 physicians who developed AMI were analyzed for homocysteinemia dosage, with matching and adjusted controls for age and smoking. The authors concluded that moderately high levels of homocysteine were associated with subsequent AMI risk, regardless of other factors[45]. Meta-analysis carried out by Boushey showed that an increase of 5 μmol/l of homocysteine was associated to an increase in risks of coronary artery disease of 60% in men and 80% in women[46]. Glueck et al. dosed homocysteine in 482 consecutive patients, sent for diagnosis and treatment of dyslipidemia, and concluded that it is an independent risk predictor of coronary disease[47]. A revision made by Van der Griend[48] presented a growing epidemiological evidence that hyperhomocysteinemia is an "independent cardiovascular risk factor, although the cause and effect relationship is not proven yet."

However, other studies did not show the same conclusion. Folsom et al., in a prospective case-cohort-type study, with a duration of 3.3 years, asserted that their findings give rise to doubts to conclusions that hyperhomocysteinemia be an independent risk factor for coronary artery disease. Those authors suggested that atherosclerosis could alone rise homocysteine levels, which would result in an association between hyperhomocysteinemia and coronary artery disease (CAD), through a reverse casualty mechanism (hyperhomocysteinemia could be the consequence and not the cause of CAD). It was also suggested that, in CAD patients, hyperhomocysteinemia could forecast a bad prognosis, reflecting severity of CAD and the possibility of a risk of thrombosis[49].

Stampler, in a previously mentioned article[46], anticipates that question and asserts that the doubt whether high homocysteine could be the consequence and not the cause of acute myocardial infarction, is rejected by the prospective planning of his study.

Similarly, that reverse casualty hypothesis was not accepted by Bostom and Selhub[50], who asserted, in an editorial published in 1999, it was not the same supported by epidemiological evidence of many works and by the findings in studies in humans and animals, such as the presence of atherothrombotic events at 30 years of age, in 50% of infants with non-treated

hyperhomocysteinemia, and in young adults, without traditional risk factors and with homocystinuria due to deficiency of cystationine synthase. The reduction of homocysteinemia levels in those patients showed a decrease of the incidence of cardiovascular events[51,52]. Randomized and controlled studies revealed that diet-induced hyperhomocysteinemia resulted in abnormal vascular reactivity in primates[53]. Among young individuals, without atherosclerosis or risk factors for coronary disease and with normal flow reactivity of brachial artery[54], a stressed reduction of flow reactivity of brachial artery after acute hyperhomocysteinemia, caused by an oral dose of L-methionine, was observed.

Studies in the literature used apparently healthy individuals, with absence of clinical symptoms of coronary insufficiency or electrocardiographic changes[55], as inclusion criteria in control group. However, due to the scarce anginous symptomatology, which may occur among elderly individuals, the option in this work was for not choosing control group among apparently healthy elderly individuals. So, despite the difficulty to detect elderly individuals, with age equal to or higher than 65 years old, with coronary angiography demonstrating absence of lesions or ischemia, such criterion was used for considering it stricter and to allow for a greater reliability in the comparison between groups.

The findings of the present study agree on the fact that hyperhomocysteinemia constitutes a risk factor for coronary disease in elderly individuals. It suggests, for example, that the average value of homocysteine among Brazilians may be similar to that among Americans: the Hordaland Homocysteine Study[9] (carried out in 3,318 elderly individuals from 65 to 67 years old) detected average levels of homocysteine of 12.3 μmol/l in men and 11 μmol/l, in women; the Framingham study[56] (with 1160 elderly individuals from 67 to 96 years old) showed an average homocysteine concentration of 11.9 μmol/l, which coincides exactly with the one in this study, 11.99 μmol/l. Studies with a greater number of Brazilian elderly individuals are necessary to confirm such suggestion.

It was also observed that differences between the means of homocysteine levels among elderly individuals with and without the disease were small, 2.34 μmol/l or 16%. That finding is in accordance to the one found by other authors. Verhoef[57] (who evinced hyperhomocysteinemia being an independent risk factor for myocardial infarction, with risk rate of 1.35 for each increase of 3 μmol/l of homocysteine), found an 11% difference. In the Physicians Health Study[46], the cases had an average homocysteine level only 5.7% higher than the one in controls. The authors considered that, although the differences had been modest, they were similar to the differences of means for many established risk factors, such as cholesterolemia and blood pressure.

Finally, data from this work suggest that the normal higher limit of homocysteinemia levels should be reduced,

as the values found in this study, over 14 µmol/l, were associated to an estimate risk of 2.03 for coronary disease.

It was not observed significant difference in traditional risk factors in both groups, except smoking. Such fact is justified for the own structure of the study model used here, in which, although control group patients were free from coronary disease, they showed many risk factors. Besides, risk factors were being controlled. The same happened to Robinson's study[45], regarding dyslipidemia, and to Tromso's study[58], regarding hypertension and hypertriglyceridemia. The authors considered such findings as resulting from the treatment of risk factors and change in lifestyle.

In conclusion, data obtained in this study allow for concluding that elderly individuals with obstructive coronary atherosclerotic disease showed significantly higher homocysteinemia levels than elderly individuals without obstructive coronary atherosclerotic disease, and that hyperhomocysteinemia was an independent risk factor for coronary atherosclerotic disease in elderly individuals.

ACKNOWLEDGEMENTS

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